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**Opening Adress**

Dear Participants,
It gives me great pleasure to welcome you all to the 15th International Conference on Organic Fruit Growing “Ecofruit. The IFOAM EU Group is delighted to be the patron of this biennial event for the fourth consecutive period. Ecofruit is an important date in the calendar for the organic researchers, consultants and growers to address problems and exchange solutions for organic fruit production now and into the future
Your contribution to food and farming systems not only helps to develop more robust and resilient organic fruit farming in Europe, but ensures that the organic fruit sector continues to secure and expand its market share and in the process play a strategic part in realising more sustainable food production worldwide. While there has been a notable decline in organic fruit cultivation in some EU countries, Europe continues to record growth across key fruit groups, including grapes and temperate and citrus fruit. This is a testament to the results you and colleagues has delivered over the decades and the valuable contribution you make to the organic researcch as a whole.

The organic research community continues to demonstrate its ability and potential to respond proactively to the impending environmental and socio-economic challenges we face in terms of climate change, biodiversity loss and water scarcity. Over the last 20 years public policymakers have slowly begun to acknowledge the added value that the sector delivers in the area of research and innovation, with several research projects funded by the European Commission since the mid-1990s. Current figures show that research funding for organic farming has increased seven-fold from 5m between 1990 and 1994 under FP3 to 41m between 1990 and 2002 under FP6. Despite the sector’s proven track record funding under FP6 only represented 0.23% of the overall budget.

The new EU framework programme for EU Research and Innovation Horizon 2020 provides a decisive opportunity for the organic research community to sustain and build upon its achievements to date. With its proven track record the time has come for our sector to be recognised in its entirety by the EU as a key actor within the Union’s research programmes. This recognition must be backed up with the necessary financial support in order to realise the full potential of the sector.

Through the TP Organics technology platform the IFOAM EU Group in partnership with other members and supporters is strongly committed to strengthening the position of organic research and development now and into the future. Further, IFOAM EU will strengthen its efforts to improve knowledge transfer from research to practice, a challenge you are very much aware of.

Over the past four years we have worked consistently to promote the strategic importance of organic research in making Europe a world leader in ecologically sustainable food production. We have confronted many barriers in working to secure public financial support and I am sure we will face new challenges ahead, but I am confidence with your cooperation and support we can ensure that organic research has a bright and promising future.

Marco Schlüter
Director IFOAM EU Group

## Reviewing Process of Conference Contributions

In order to maintain a high and consistent scientific quality, the contributions for the *Proceedings of the 15th International Conference on Organic Fruit-Growing* were subjected to a reviewing process.

The Ecofruit Conference aims at bringing together results and experiences on organic fruit growing. Thus, not only scientific papers with results of trials completed over many years and/or of many different sites that allow a proper evidence-based conclusion are of interest for the conference but also smaller contributions with the description of one year experiments or other more limited trial set ups.

Thus, the Conference Proceedings are divided in two parts:

### 1. Reviewed papers

This part contains papers of scientific quality which were subject of a full anonymous peer review process. Each contribution was sent to at least two reviewers with expertise in the relevant field of study.
The editorial decision of acceptance of a paper in the Conference and in the part "reviewed papers" of the Proceedings was done by the Conference Organisers based on the referees' reports.

### 2. Short contributions

This part of the Proceedings is reserved for smaller contributions. They were subject to an abbreviated reviewing process (only one reviewer, conclusions must not be based on trials over more years or locations).

We would like to thank the following colleagues for reviewing one or more of the papers submitted to the *Proceedings of the 15th International Conference on Organic Fruit-Growing*:

Bastian Benduhn, OVB Jork (D); Sascha Buchleither, KOB Bavendorf (D); Philipp Haug, Konstanz (D); Johannes Jehle, JKI Darmstadt; Markus Kelderer, Research Station Laimburg (I); Jutta Kienzle, Kernen (D); Karsten Klopp, OVB Jork (D); Stefan Kunz, University of Konstanz (D); Ulrich Mayr, KOB Bavendorf (D), Barbara Pfeiffer, LVWO Weinsberg (D); Franz Ruess, LVWO Weinsberg (D); Andreas Spornberger, Institute of Horticulture and Viticulture, University of Natural Resources and Applied Life Sciences, Vienna (A), Bart Timmermans, Louis Bolk Institute (NL); Peter Triloff, Marktgemeinschaft Bodenseeobst eG (D); Francois Warlop, GRAB Avignon (F); Franco Weibel, FIBL Frick (CH); Heidrun Vogt, JKI Dossenheim (D); C.P.W. Zebitz, University of Hohenheim (D).

# Reviewed Papers

# Breeding apples to withstand infection pressure by fire blight and other diseases

I.O. Baumgartner[1]*, L.R. Leumann[1], J.E. Frey[1], M. Joos[2], R.T. Voegele[2] and M. Kellerhals[1]

## Abstract

*Commercial cultivars, old varieties and wild species with known resistance against fire blight, caused by the bacterium* Erwinia amylovora*, were selected as breeding parents. Due to strong fire blight quantitative trait loci (QTLs), the crab apples 'Robusta 5' and 'Evereste' confer virtual immunity against the disease. However, these crab apples lack the characteristics of good fruit quality. In a multilevel phenotypic and molecular selection process, progeny plants were selected based on additional breeding objectives such as agronomic tree features and scab and mildew resistance.*

*'Ladina' is a newly named variety and 'ACW 14995', and 'ACW 11303' are advanced selections with interesting fruit quality. They all carry Rvi6 (Vf) scab resistance and have a low susceptibility against fire blight based on shoot and flower inoculation tests in the greenhouse. Latent* E. amylovora *presence was analysed in shoots of plants grown in the greenhouse without visible disease symptoms after artificial inoculation by using quantitative polymerase chain reaction (qPCR). The development of apple cultivars with marketable fruit quality and low susceptibility to* E. amylovora *and other major apple diseases is especially valuable for organic growing and fruit growing in general.*

**Keywords:** *Malus x domestica*, *Erwinia amylovora*, multiple disease resistance, marker-assisted selection, plant inoculation

## Introduction

Fire blight, caused by the bacterium *Erwinia amylovora* (Burrill) Winslow *et al.*, is one of the most important plant diseases in pome fruit production. Recently, the disease has become a considerable economic problem in high-density orchards. Bacteria infect blossoms, fruit, vegetative shoots, and rootstock crowns. During flowering under optimal conditions *E. amylovora* is actively distributed through pollinating insects. After fast multiplication on stigma and migration to the nectarthodes (Spinelli *et al*., 2005) bacteria invade the floral cluster as well as petiols and migrate further through the stem to the roots (Bogs *et al.,* 1998).

Planting of resistant cultivars is potentially the most promising disease control strategy (Peil *et al*., 2009). However, classical apple breeding of resistant cultivars with high fruit quality is a costly and time consuming process due to the long juvenile stage (Kellerhals *et al*., 2008). The most promising breeding parents are selected based on literature, phenotypic or molecular testing results. Different sources of QTLs against fire blight are known. In cultivars such as 'Enterprise', 'Rewena', 'Free Redstar' and 'Fiesta' the susceptibility towards fire blight is reduced compared to the susceptible cultivar 'Gala Galaxy' (Fischer & Richter, 1999; Calenge *et al*., 2005; Khan *et al*., 2006; Sobiczewski *et al*., 2006). The QTL of 'Fiesta' (*FBF7*) is flanked by the SCAR (sequence-characterized amplified region) markers AE10-375 and GE-8019 (Khan *et al*., 2006; Khan *et al*., 2007). In 'Robusta 5' and 'Evereste' strong fire blight QTLs (*FB_MR5* and *Fb_E*, respectively)

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have been found (Peil *et al.,* 2007; Peil *et al*., 2008; Durel *et al.,* 2009; Fahnentrapp et al., 2011). Due to low fruit size and poor quality of these crab apples the QTL cannot be directly introduced while breeding for marketable fruit quality. This leads to pseudo-backcrosses with qualitative cultivars over several generations to eliminate most of the negative fruit traits of these wild apples.

For the development of new disease resistant apple varieties, progeny plants are selected based on the breeding objectives for multiple disease resistance, tree features and fruit quality. By performing crosses multiple disease resistances against fire blight (*FR*), apple scab (*Venturia inaequalis*; *SR*) and powdery mildew (*Podosphaera leucotricha, MR*) can be combined. For a more sustainable and stable situation, polygenic resistances and resistance pyramids are developed, combining several resistance genes or QTLs for the same disease. For example the scab resistance *Rvi6* (= *Vf*) of *Malus floribunda* 821 can be combined with *Rvi4* (= *Vh4*) of the Russian Seedling R12740-7A. Disease resistance can be determined with molecular markers or phenotypic inoculation tests. Marker-assisted selection (MAS) is an established procedure to confirm the presence of the desired resistance genes (Kellerhals *et al*., 2009) and has the advantage, that it can be applied at an early seedling stage. In phenotypic tests fire blight susceptibility can be investigated with shoot and blossom inoculations. Real Time PCR is a fast and sensitive method to quantify the bacteria within plant tissue (Higuchi *et al*., 1993). Voegele *et al.* (2010) have shown that *E. amylovora* can even be present in tissue without visible disease symptoms.

This paper highlights results of the project ZUEFOS, breeding fire blight resistant fruit varieties. We describe breeding activities including phenotyping and molecular selections and present new cultivars with low susceptibility to fire blight, good fruit quality and tree features.

## Material and Methods

*Plant material*

Parents for crosses were chosen based on literature, phenotypic and molecular testing results.

Pollen of the selected father trees was collected at balloon stage and dried. Branches of mother trees were bagged, flowers manually pollinated and re-bagged until petal fall. From the harvested fruits, seeds were extracted and stratified in humid sand for 2 months at 2°C. The crosses of ‘58/06’ x ‘ACW 11301’ and ‘01/05’ x ‘ACW 11301’ were established in 2010. ‘58/06’ and ‘01/05’ both originate from a cross of ‘Resi’ x ‘Julia’ obtained by a ZUEFOS project partner, with low fire blight susceptibility and carrying the *FBF7* resistance QTL. ‘ACW 11301’ is an advanced selection with low fire blight susceptibility carrying *Rvi4* and *Rvi6* scab resistances.

‘Ladina’ (= ‘ACW 14959’), a newly named variety, ‘ACW 14995’, and ‘ACW 11303’ are advanced selections with interesting fruit quality. ‘Ladina’ and ‘ACW 14995’ originate from a cross of ‘Topaz’ x ‘Fuji’, ‘ACW 11303’ from a cross of ‘ACW 6104’ (‘Arlet’ x ‘Gloster’) x ‘Rewena’. All of them carry *Rvi6* scab resistance, ‘ACW 11303’ carries additional *Rvi4* scab resistance and ‘Ladina’ and ‘ACW 14995’ the *FBF7* fire blight resistance QTL. All three selections were investigated upon their fire blight susceptibility using inoculation tests.

*Scab inoculation*

After germination, seedlings were raised in a greenhouse. After artificial scab inoculation plants were scored for scab symptoms according to Chevalier *et al*. (1991). A control progeny with scab susceptible parents was included, in 2011 ‘Scifresh’ x ‘La Flamboyante’.

Seedlings rated in classes 0 to 3b were considered as scab resistant. Seedlings with high susceptibility and heavy sporulation (class 4) were excluded from further analyses.

*Marker analyses*

For MAS, phenotypically scab resistant seedlings of the progeny populations '58/06' x 'ACW 11301' (n = 61) and '01/05' x 'ACW 11301' (n = 42) were individually labelled as well as leaf samples collected for molecular analysis. DNA was extracted according to Frey *et al.* (2004), followed by multiplex PCRs with fluorescently labelled primers assembled according to Patocchi *et al.* (2009). Seedlings were genetically analysed for the two SCAR markers AE10-375 and GE-8019 (Khan *et al*., 2007) and for the two microsatellite markers, (simple sequence repeat, SSR) CH02C02a linked to the *Rvi4* scab resistance and CHVf1 linked to the *Rvi6* scab resistance. Fragment analysis was carried out on a 3130xl Genetic Analyzer (Applied Biosystems) and data were analysed using GeneMapper$^{TM}$ v.4.1 Software (Applied Biosystems).

*Fire blight inoculation tests of shoots and flowers*

Fire blight inoculation tests of shoots and flowers were conducted in a quarantine greenhouse. 'Gala Galaxy' was included as a susceptible control, 'Enterprise' as tolerant control. Prior to inoculation plants were grown for four to six weeks in a greenhouse.

For shoot inoculations scion wood was grafted on M9 rootstock for twelve replicate trees per genotype. One or two shoot(s) per plant were inoculated at the tip with a syringe containing an *E. amylovora* suspension of $10^9$ cfu/ml Swiss strain FAW610 (Rezzonico & Duffy, 2007). The length of necrotic lesion (cm) was measured 7, 14 and 21 days after inoculation. Susceptibility of the genotypes was estimated by calculating the percent lesion length (PLL (%) = lesion length (cm) divided by shoot length (cm)).

For flower inoculations perennial wood was grafted on M9 rootstock. 'Enterprise' was included as three year old trees grafted on M9. One day old floral clusters were reduced to three flowers per cluster and pollinated. The subsequent day flowers were injected with an *E. amylovora* solution of $10^8$ cfu/ml strain FAW610 in PBS (200-250 µm, i.e. $10^7$ cfu/ml per flower). Infection symptoms were scored 4, 7, 14 and 28 days after inoculation.

*qPCR analyses*

Thirteen weeks after shoot inoculation three random shoots including new secondary shoots and rootstock of 'Ladina', 'ACW 14995' and 'Enterprise', and eight weeks after inoculation three random shoots of 'Gala Galaxy' were cut into 5 cm long pieces (starting from the shoot tip) and analysed with Real Time PCR according to Voegele *et al.* (2010).

## Results

In 2008, 2009, 2010 and 2011, between 3,500 and 11,000 seeds were produced each year for the project ZUEFOS. Selected examples of fire blight relevant crosses are shown in Table 1.

In the scab inoculation test, 268 out of 433 '58/06' x 'ACW 11301' and 273 out of 465 '01/05' x 'ACW 11301' progeny plants were rated as scab resistant (Fig. 1). In the control progeny 'Scifresh' x 'La Flamboyante' 9 out of 53 plants were rated as scab resistant.

Scab resistant seedlings of all progenies were selected for tree features and powdery mildew resistance in the first autumn. From the crosses of 2008, 2009 and 2010 17.7% of the plants were selected for further investigation of fruit and trees, and grafted on 'M27' rootstock with 'Golden Delicious' as interstem. These selection steps allowed for the reduction of 10,744 primal seedlings to 1,000 grafted plants.

The results of the marker analyses of scab resistant seedlings are shown in Table 2. Eleven (18%) of the progeny seedlings ‘58/06’ x ‘ACW 13001’ and 13 (34%) of the progeny seedlings ‘01/05’ x ‘ACW 11301’ analysed at the molecular level carry markers for *FBF7* as well as the markers for *Rvi4* and *Rvi6*.

Table 1: Examples of fire blight relevant crosses and progeny size in years 2008, 2009 and 2010.

| **Year** | **Mother (parents; resistances)** | **Father (parents; resistances)** | **Progeny size** |
|---|---|---|---|
| 2008 | ACW 11303 (ACW 6104 x Rewena; *FR*, *Rvi4*, *Rvi6*, *MR*) | Evereste (*Fb_E*, *Rvi6*) | 39 |
| 2008 | La Flamboyante – Mairac® | DA02. 2.7 (Idared x *Malus robusta* 5; *FB_MR5*, *Rvi4*) | 196 |
| 2008 | Milwa – Junami® | Free Redstar (*FR*, *Rvi6*) | 539 |
| 2009 | Ariane (*Rvi6*) | Ladina (= ACW 14959; Topaz x Fuji; *FBF7*, *Rvi6*) | 1383 |
| 2010 | 58/06 (Resi x Julia; *FBF7*) | ACW 11301 (ACW 6104 x Rewena; *FR*, *Rvi4*, *Rvi6*) | 310 |
| 2010 | 01/05 (Resi x Julia; *FBF7*) | ACW 11301 (ACW 6104 x Rewena; *FR*, *Rvi4*, *Rvi6*) | 308 |

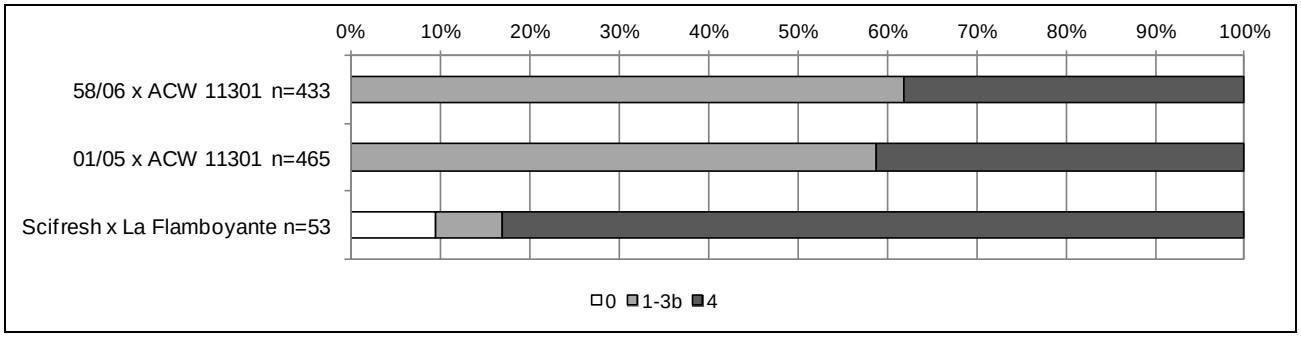


Figure 1: Scab screening of selected crosses 2010. Seedlings rated in classes 0 to 3b were considered as scab resistant and seedlings in class 4 with heavy sporulation as highly susceptible.

Table 2: Molecular analyses of phenotypically scab resistant progeny seedlings ‘58/06’ x ‘ACW 11301’ (n=60) and ‘01/05’ x ‘ACW 11301’ (n=38). Percentage of observed and from segregation expected progeny population carrying the markers for *FBF7*, *Rvi4*, *Rvi6* resistance.

| **58/06 (*FBF7*) x ACW 11301 (*Rvi4*, *Rvi6*)** | | | |
|---|---|---|---|
| Resistances | *FBF7* | *Rvi4* | *Rvi6* |
| Observed (n) | 26 | 30 | 53 |
| Observed (%) | 43 | 49 | 87 |
| Expected (%) | 50 | 50 | 50 |
| **01/05 (*FBF7*) x ACW 11301 (*Rvi4*, *Rvi6*)** | | | |
| Resistances | *FBF7* | *Rvi4* | *Rvi6* |
| Observed (n) | 29 | 21 | 35 |
| Observed (%) | 76% | 55% | 92% |
| Expected (%) | 50% | 50% | 50% |

The results of the tested advanced selections ‘Ladina’, ‘ACW 14995’ and ‘ACW 11303’ revealed some differences in susceptibility towards *E. amylovora* between the different years (Fig. 2). Control cultivar ‘Enterprise’ was as expected very low susceptible with a mean of 12.9% percent lesion length relative to ‘Gala Galaxy’. ‘Ladina’ was in the same range as ‘Enterprise’ with a mean of 14.2% percent lesion length. ‘ACW 11303’ and ‘ACW

14995' reached a mean percent lesion length relative to 'Gala Galaxy' of 33.6% and 37.1%, respectively.

Figure 3 illustrates the levels of fire blight susceptibility after flower or shoot inoculation. The highest infection symptoms were observed with 'Gala Galaxy' with 61.7% of bourse shoots or shoots with fire blight symptoms four weeks after flower inoculation and 87.0% of total shoot length three weeks after shoot infection. 'Ladina', 'ACW 11303' and 'ACW 14995' were less susceptible with 10.0%, 20.0% and 32.7% of bourse shoots or shoots with fire blight symptoms four weeks after flower inoculation and 12.6%, 19.7% and 13.5% of total shoot length three weeks after shoot infection. Control cultivar 'Enterprise' is very low susceptible with 2.9% of bourse shoots or shoots with fire blight symptoms four weeks after flower inoculation and 5.9% of total shoot length three weeks after shoot infection.

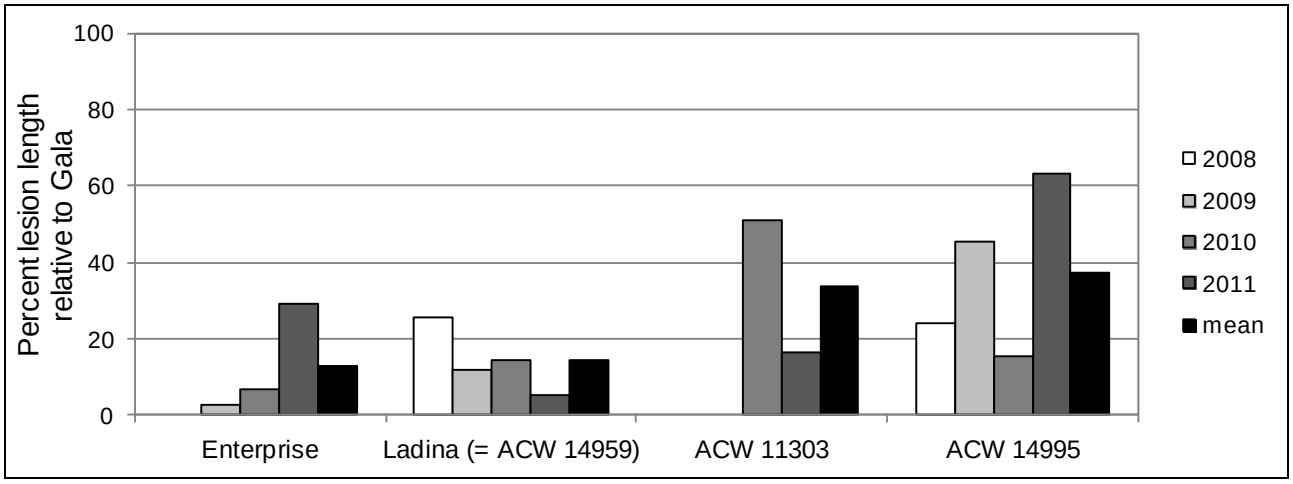


Figure 2: Mean lesion length in percent of total shoot length relative to 'Gala' for advanced selections 3 weeks after shoot infection in years 2008 to 2011 compared to 'Enterprise' (tolerant control).

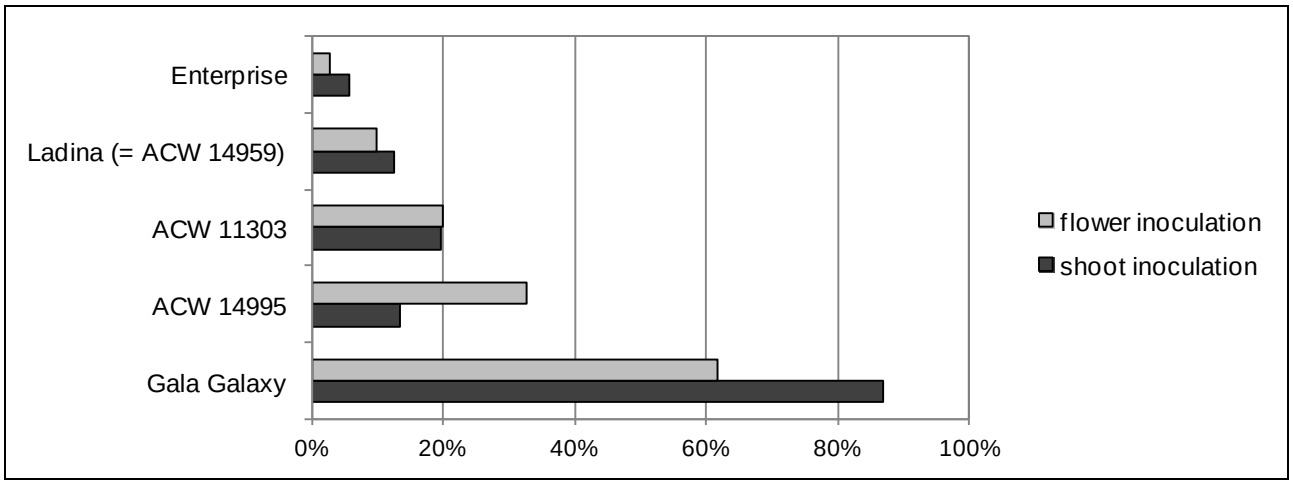


Figure 3: Fire blight symptoms after flower or shoot inoculations for advanced selections compared to 'Enterprise' (tolerant control) and 'Gala Galaxy' (susceptible control). The upper bar shows the percentage of bourse shoots or shoots with fire blight symptoms 4 weeks after flower inoculation in year 2011. The mean lesion length in percent of total shoot length 3 weeks after inoculation in year 2010 is shown in the lower bar.

Real Time PCR results of shoots after artificial fire blight inoculation show that significant amounts of bacteria, up to $10^8$ cells per gram of tissue, were detected in samples without

visible symptoms. Spread of bacteria down the shoot and to the rootstock seems to proceed faster than upwards towards new shoots. In symptomatic tissue of 'Gala' shoots highest amounts of bacteria with up to $10^{12}$ cells per gram of tissue were measured. In new shoots and rootstocks only minor differences between cultivars were found.

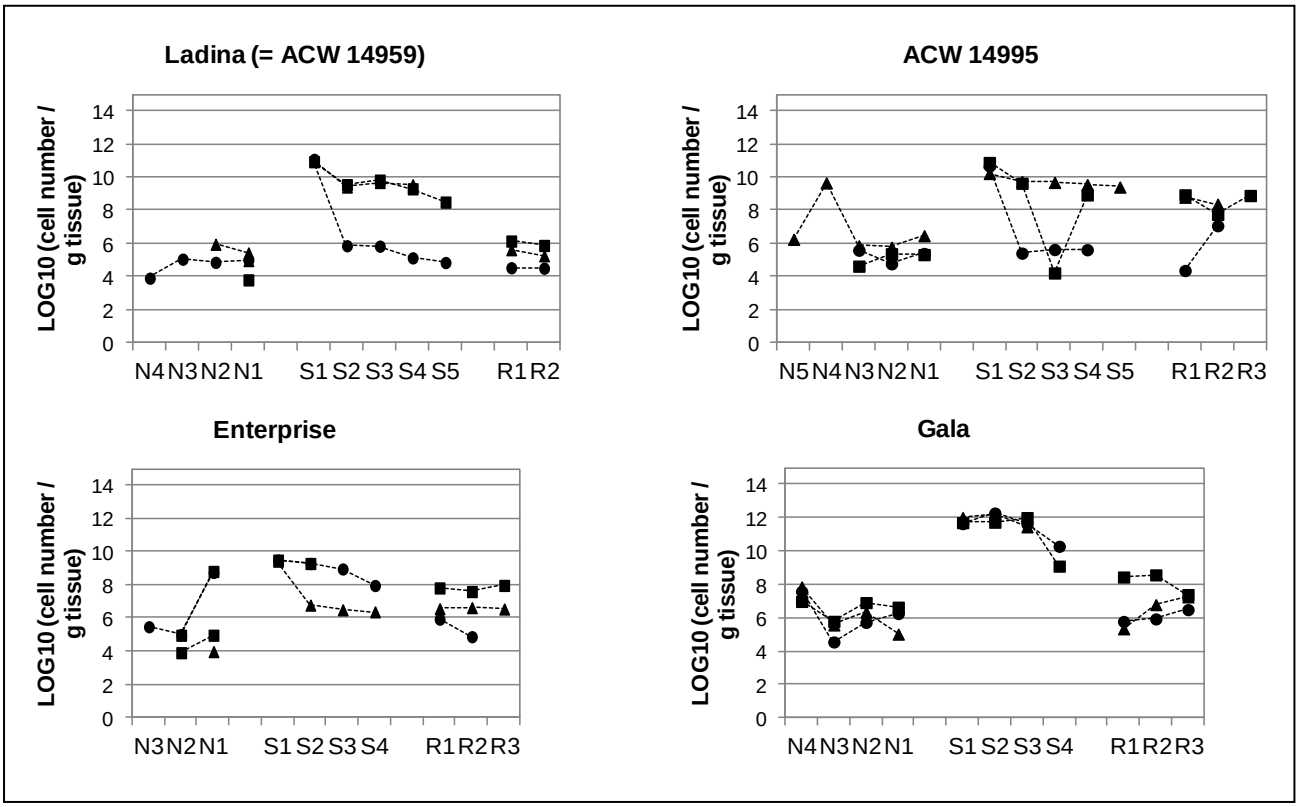


Figure 4: Spread of *E. amylovora* after artificial shoot inoculation in new secondary shoots (N), shoots (S) and rootstock (R).

## Discussion

In this paper we show that crosses within the project ZUEFOS lead to a wider genetic diversity pool in respect to fire blight resistance in the ACW breeding program. By scab inoculation, molecular analyses, evaluation of tree features and powdery mildew the seedling number was considerably reduced. Fruit quality and tree features of selected plants, as well as fire blight susceptibility with different inoculation tests will be further evaluated within the next years.

Screenings with molecular markers for the *FBF7* QTL can provide some information about susceptibility to fire blight but the association between marker presence and tolerance is not absolute (Nybom *et al*., 2011). Seedlings with fire blight resistance of 'Evereste' or 'Robusta 5' need four to five generations of pseudo-backcrossing due to the small fruit size and poor fruit quality of the parental resistance donors. In 2012 we expect plants of the third pseudo-backcross-generation with *FB_MR5* resistance and first flowers in second generation with *Fb_E* resistance. Within the $F_2$ seedling progeny plants, while using a set of molecular markers covering the whole genome, some individuals might be identified with a high proportion of genome inherited from the high quality parents (Volz *et al*., 2009). Discovery of further QTLs from other parent sources (Peil *et al*., 2007) and development of markers will improve germplasm screenings and enable the selection of plants with pyramided fire blight resistance QTLs for durable resistance.

The newly named variety ‘Ladina’ carries *Rvi6* scab resistance and was low susceptible in fire blight inoculation tests of both shoots and flowers. Fire blight resistance evaluations under controlled conditions have been shown to correlate well with field resistance (Quamme *et al*., 1976). Our results have shown, that flower and shoot resistances are often correlated, but there are occasions reported where this is not the case (Le Lezec *et al.,* 1987). However, even in asymptomatic tissue, *E. amylovora* was detected. Such latent infections could explain fire blight infections in orchards where no fire blight had ever been seen before (Vanneste & Eden-Green, 2000). Further testing of advanced selections under field conditions is planned from 2012 onward.

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# Strategy to reduce the investment of copper for control of apple scab in organic apple growing

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**Abstract**

*Apple scab (Venturia inaequalis) is still a major problem for organic apple growers. The control of apple scab requires a rigorous control strategy especially in the phase of primary scab infection. To develop a save scab control strategy it is necessary to work out the parameter of alternatives and their combinations thoroughly. In consideration of the ascospore potential, the phenological growth stage of the host plant and the potential of the plant protection products, an advice for a control strategy without or with reduced copper amount should be established in all situations.*

*In the context of the BÖLN project 'Establishing a strategy to reduce the investment of copper for scab control in organic apple growing' different products were tested in laboratory and in field trials.*

*In laboratory alternative products against apple scab such as potassium bicarbonates showed good efficacies. But in the field the products were not efficient enough (rain stability, UV stability…). Depending on the weather potassium bicarbonates in combination with wettable sulphur can be an additional option for scab control. Lime sulphur with specific treatment in the germination period proved to be the most effective and the most reliable alternative to copper. A significant reduction of copper can be achieved through the new generation of copper products in the form of copper hydroxide.*

**Keywords:** apple scab, *Venturia inaequalis*, copper, lime sulphur

## Introduction

The apple scab project "Establishing a strategy to reduce the investment of copper for scab control in organic apple growing", which is funded by the "Bundesprogramm Oekologischer Landbau und andere Formen nachhaltiger Landwirtschaft" (BÖLN) started in spring 2008. During the project period the beneficial effect of several plant protection products against apple scab is tested in an in-vivo test system by Bio-Protect GmbH (Dr. Stefan Kunz) in Konstanz. Products which promised good efficacy are then tested under field conditions at various locations of Germany. The field trials for scab control during the primary and secondary scab period are carried out at four different sites: Dienstleistungszentrum Ländlicher Raum Rheinpfalz, Öko-Obstbau Norddeutschland, Kompetenzzentrum Obstbau Bodensee and Sächsiches Landesamt für Umwelt, Landwirtschaft und Geologie. The geographical distribution ensured that regional climatic differences in west, east, north and south Germany were considered. The regional climatic differences as well as the intensity of scab infestation are important to detect side effects of the products such as fruit russeting.

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## Material and Methods

At the DLR Rheinpfalz in Klein-Altendorf the trial was carried out in the variety ‘Gala’. The testing variants have been repeated four times with always fifteen trees per testing plot. During the whole trial the control remained untreated. In the months April and May in treatment 2 three preventive treatments with copper and eight treatments with lime sulphur into the germination period were applied.

With the product ‘Cuprozin progress’ in total an amount of 800 g copper per hectare was used. Treatment 3 was sprayed three times with lime sulphur before rain and 8 times with lime sulphur into germination period. 9 treatments only with lime sulphur into germination period were applied in treatment 4. For details about treatments, application rate and dates see table 1. After the end of primary infection period all treatments were treated the same way.

Table 1: Treatment, application rate and date at DLR Rheinpfalz Klein-Altendorf

| Treatment | | Application rate per ha | Date |
|---|---|---|---|
| 1 | untreated control | --- | --- |
| 2 | preventative **copper** covering before rain<br>+<br>lime sulphur into germination period | 200 g – 300 g Cu/ha<br>from bloom without copper as variant 3<br><br>20 l/ha till bloom<br>15 l/ha from begin of flowering | **16.03.*** / **29.03. *** / 31.03. / 07.04. / **11.04.*** / 12.04. / 21.04. / 27.04. / 08.05. / 17.05. / 30.05.<br>(11 treatments) |
| 3 | preventative **lime sulphur** covering before rain<br>+<br>lime sulphur into germination period | 20 l/ha till bloom<br>15 l/ha from begin of flowering | **16.03.** / **29.03**. / 31.03. / 07.04. / **11.04**. / 12.04. / 21.04. / 27.04. / 08.05. / 17.05. / 30.05.<br>(11 treatments) |
| 4 | lime sulphur into germination period | 20 l/ha till bloom<br>15 l/ha from begin of flowering | 16.03. / 31.03. / 07.04. / 12.04. / 21.04. / 27.04. / 08.05. / 17.05. / 30.05. (9 treatments) |

***** treatment with “Cuprozin progress” before high infection risk: 16.03. (300 g Cu/ha), 29.03. (300 g Cu/ha), 11.04. (200 g Cu/ha) = 800 g Cu/ha

The ÖON experiment was carried out in the Jork area during the primarily scab season in a randomized ‘Elstar’ block with four replicates. Additional to 11 wettable sulphur and 7 lime sulphur applications in all blocks, 960 g copper were sprayed in the treatments 3, 5 and 7. “TS-Forte” (Treatment 4, 5) and “Nu-Film-P” (Treatment 6, 7) were used as surfactant. Dosage and timing of applications are shown in table 2.

Table 2: Treatment, application rate and date at ÖON Jork

| Treatment | | Application rate per ha | Date |
|---|---|---|---|
| 1 | untreated control | --- | --- |
| 2 | preventative sulphur covering before rain<br>+<br>lime sulphur into germination period | 3000 g S/ha<br><br>37,5 l/ha before blossom<br>25,0 l/ha as of blossom | 30.03. / 06.04. / 09.04. / 20.04 / 22.04. / 25.04. / 27.04. / 02.05. / 05.05. / 08.05. / 11.05. / 14.05. / 19.05. / 23.05. / 26.05. / 29.05. / 30.05. / 06.06.<br>(18 treatments) |
| 3 | preventative **copper** & preventative sulphur covering before rain<br>+<br>lime sulphur into germination period | 105 g – 405 g Cu/ha before blossom<br>3000 g S/ha<br><br>37,5 l/ha before blossom<br>25,0 l/ha as of blossom | **30.03.*** / 06.04. / **09.04.*** / 20.04 / 22.04. / 25.04. / 27.04. / 02.05. / **05.05.*** / 08.05. / 11.05. / 14.05. / 19.05. / 23.05. / 26.05. / 29.05. / 30.05. / 06.06.<br>(18 treatments) |
| 4 | preventative sulphur covering before rain<br>+<br>lime sulphur into germination period<br>+<br>TS Forte | 3000 g S/ha<br><br>37,5 l/ha before blossom<br>25,0 l/ha as of blossom<br><br>2,5 l /ha | 30.03. / 06.04. / 09.04. / 20.04 / 22.04. / 25.04. / 27.04. / 02.05. / 05.05. / 08.05. / 11.05. / 14.05. / 19.05. / 23.05. / 26.05. / 29.05. / 30.05. / 06.06.<br>(18 treatments) |
| 5 | preventative **copper** & preventative sulphur covering before rain<br>+<br>lime sulphur into germination period<br>+<br>TS Forte | 105 g – 405 g Cu/ha before blossom<br>3000 g S/ha<br><br>37,5 l/ha before blossom<br>25,0 l/ha as of blossom<br><br>2,5 l /ha | **30.03.*** / 06.04. / **09.04.*** / 20.04 / 22.04. / 25.04. / 27.04. / 02.05. / **05.05.*** / 08.05. / 11.05. / 14.05. / 19.05. / 23.05. / 26.05. / 29.05. / 30.05. / 06.06.<br>(18 treatments) |
| 6 | preventative sulphur covering before rain<br>+<br>lime sulphur into germination period<br>+<br>Nu-Film-P | 3000 g S/ha<br><br>37,5 l/ha before blossom<br>25,0 l/ha as of blossom<br><br>0,3 l/ha | 30.03. / 06.04. / 09.04. / 20.04 / 22.04. / 25.04. / 27.04. / 02.05. / 05.05. / 08.05. / 11.05. / 14.05. / 19.05. / 23.05. / 26.05. / 29.05. / 30.05. / 06.06.<br>(18 treatments) |
| 7 | preventative **copper** & preventative sulphur covering before rain<br>+<br>lime sulphur into germination period<br>+<br>Nu-Film-P | 105 g – 405 g Cu/ha before blossom<br>3000 g S/ha<br><br>37,5 l/ha before blossom<br>25,0 l/ha as of blossom<br><br>0,3 l/ha | **30.03.*** / 06.04. / **09.04.*** / 20.04 / 22.04. / 25.04. / 27.04. / 02.05. / **05.05.*** / 08.05. / 11.05. / 14.05. / 19.05. / 23.05. / 26.05. / 29.05. / 30.05. / 06.06.<br>(18 treatments) |

***** treatment with “Cuprozin progress” before high infection risk: 30.03. (405 g Cu/ha), 09.04. (405 g Cu/ha), 05.05. (150 g Cu/ha) = 960 g Cu/ha

At KOB Bavendorf the presented trial was carried out 2010 in a randomized block design with the variety ‘Jonagold’. During the phase of primary scab infection all treatments were sprayed seven times with lime sulphur (treatments 2, 3, 4) or sulphur (treatment 5) in the window of germination. In Treatment 2 and 3 four additional preventive treatments with copper (2) respectively sulphur (3) were applied. Application rates and dates are shown in table 3. Two treatments with sulphur + Vitisan were applied in case of high infection risk and rain periods longer than 48 hours in all variants. For modelling and forecast of scab infections RimPro apple scab model was used. After the primary infection period all

treatments were treated equally with a standard fungicide management based on sulphur and bicarbonate. The control treatment remained untreated until the end of the primary infection phase.

Table 3: Treatment, application rate and date at KOB Bavendorf

| Treatment | | Application rate per ha | Date |
|---|---|---|---|
| 1 | untreated control | --- | --- |
| 2 | preventative **copper**<br>covering before rain<br>+<br>lime sulphur into<br>germination period | 100 g – 300 g Cu/ha<br>till bloom<br>+<br>20 l/ha till bloom<br>15 l/ha from begin of flowering | **30.03.*/** 04.04. / **09.04.*** / 14.04. /<br>**20.04.*** / **25.04.*** / 27.04. / 01.05. /<br>02.05. / 05.05. / 10.05.<br>(11 treatments) |
| 3 | preventative **sulphur**<br>covering before rain<br>+<br>lime sulphur into<br>germination period | 5 kg/ha till bloom<br>+<br>20 l/ha till bloom<br>15 l/ha from begin of flowering | **30.03.** / 04.04. / **09.04.** / 14.04. /<br>**20.04.** / **25.04.** / 27.04. / 01.05. /<br>02.05. / 05.05. / 10.05.<br>(11 treatments) |
| 4 | lime sulphur into<br>germination period | 20 l/ha till bloom<br>15 l/ha from begin of flowering | 04.04. / 14.04. / 27.04. / 01.05. /<br>02.05. / 05.05. / 10.05.<br>(7 treatments) |
| 5 | sulphur into<br>germination period | 5 kg/ha | 04.04. / 14.04. / 27.04. / 01.05. /<br>02.05. / 05.05. / 10.05.<br>(7 treatments) |

***** treatment with “Funguran” before high infection risk: 30.03. (250 g Cu/ha), 09.04. (300 g Cu/ha), 20.04. (100 g Cu/ha), 25.04. (100 g Cu/ha) = 750 g Cu/ha

## Results

At the DLR Rheinpfalz in Klein-Altendorf the apple scab infestation on the rosettes and on the young fruits of ‘Gala’ was evaluated on 28$^{th}$ of June. At this time the rosettes and the fruits of the untreated control showed a scab infestation of 82 % and 78 % (Figure 1). All treatments were highly effective with efficiencies of 90 % and more.

Nine treatments with lime sulphur into the germination period reduced the apple scab to 8 % and 4 % (rosettes and fruits). A further preventative lime sulphur covering led to a reduction of the scab infestation to 7 % (rosette) and 4 % (fruits), which equates to an efficiency of 92 % and 95 %.

The best result was achieved in the treatment protective application with copper before rain plus lime sulphur application in the germination period. The three copper applications (in total 800 g copper) with a following treatment with lime sulphur in the germination period reduced the infestation of the rosettes to 3 % and the infestation of the young fruit to 2 %. This corresponds to an efficiency of 96 % and 98 %.

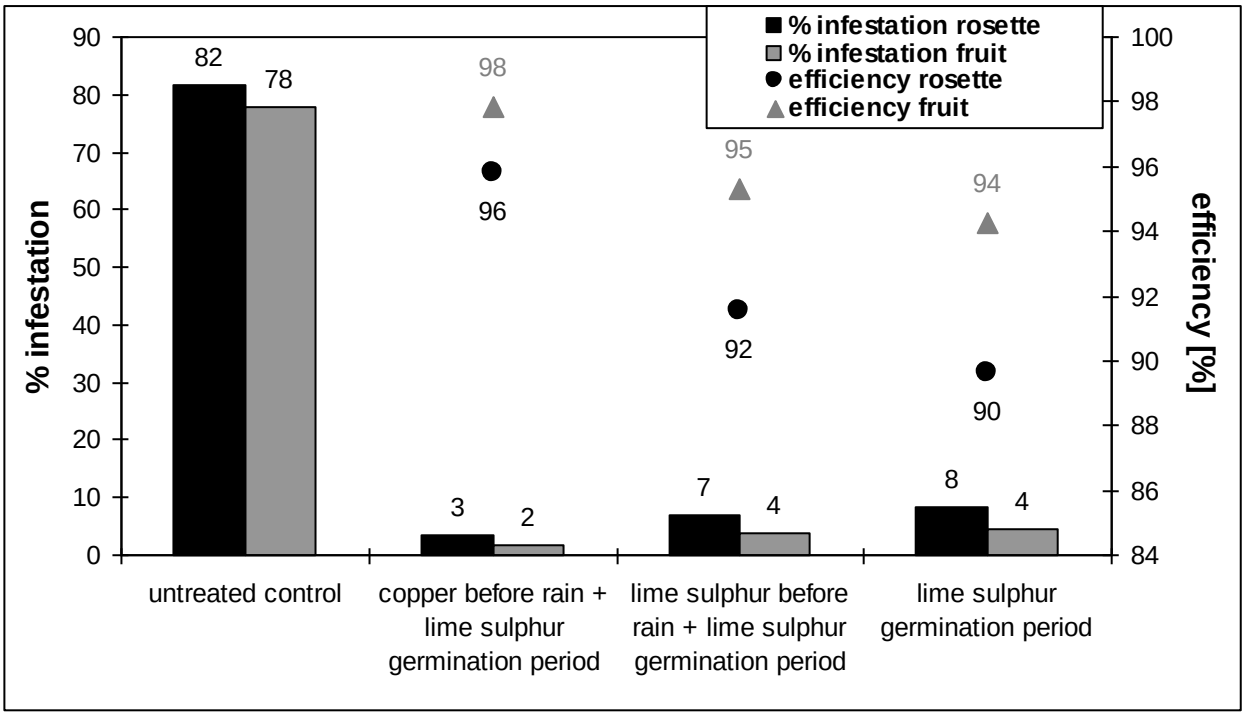


Figure 1: Percentage infestation and efficiency of the different treatments for the variety 'Gala', DLR Rheinpfalz Klein-Altendorf (2011)

Evaluations of apple scab infection in the Jork area were carried out at the 3rd of June for infection on the rosette leaves and on 13th of July for fruit infections. Rosette leaves and fruits in the untreated control were totally infected with apple scab and scored with 100 % and 98 %, respectively.

Apple scab infections on leaves and on fruits were significant reduced in all treatments compared to the untreated control. In between the different treatments the highest infection rate of leaves (3 %) and on fruits (11 %) was observed in treatment 2 (Figure 2).

The use of "Nu-Film-P" and "TS-Forte" lead to a reduction of the infection rate with apple scab both on leaves and fruits (treatment 4, 6).

A lower level of infections on leaves and fruits were observed in all treatments that included copper as a protectant coverage (treatments 2, 5 and 7); efficacies here varied in between 98 % and 100 %.

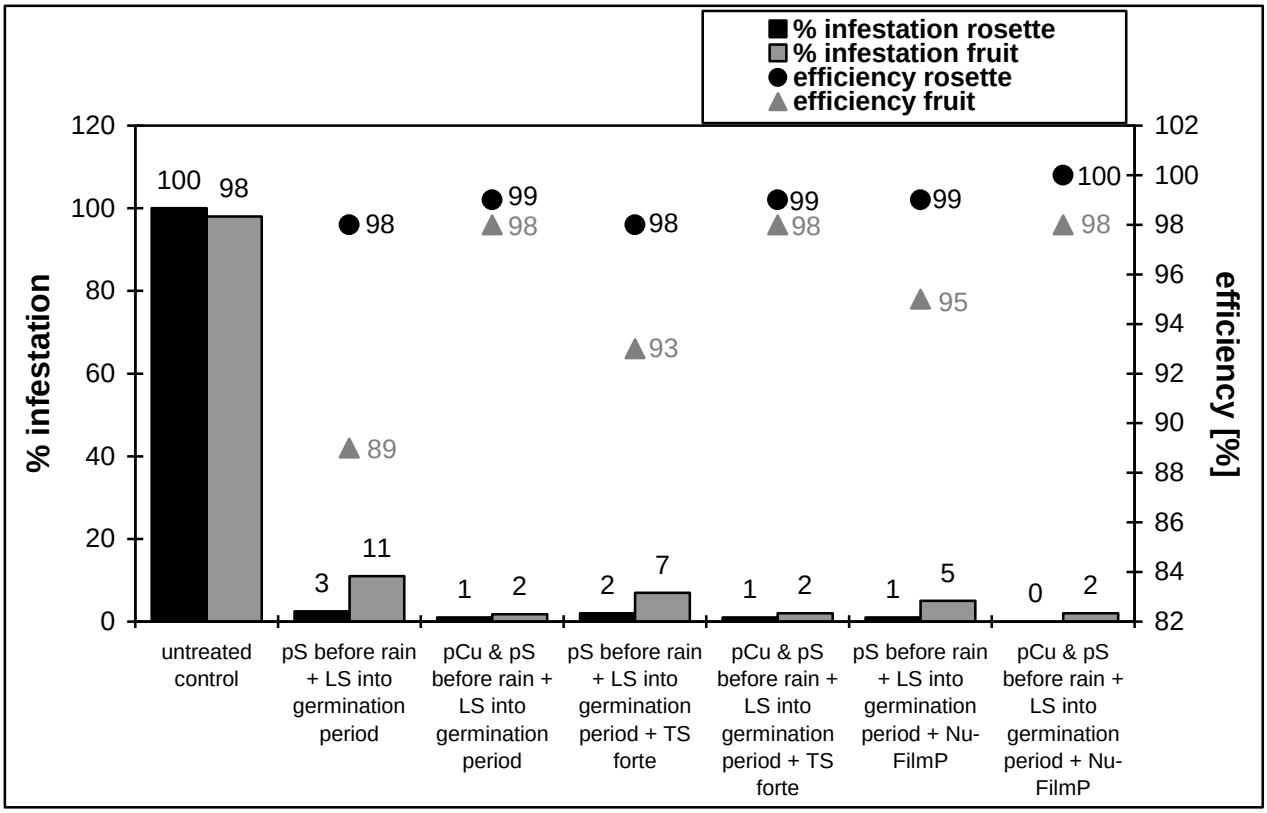


Figure 2: Percentage infestation and efficiency of the different treatments for the variety 'Elstar', ÖON Jork (LS = lime sulphur ; pS = preventative sulphur ; pCu = preventative copper)

At the KOB Bavendorf the apple scab infestation on the rosettes was assessed on 8th of June and accordingly on the fruits on the 9th of August 2010. Compared with the high infestation of the untreated control with 65 % affected rosettes and 91 % affected fruits, all treatments lead to a clear reduction of the scab infestation (Figure 3).

Seven treatments with lime sulphur into the window of germination reduced scab infestation to 6 % (rosettes) and 20% (fruits) which corresponds to an efficacy of 90 % and 78 % respectively.

Alternative treatments with sulphur in the germination period lead to a higher infestation with 15 % infested rosettes and 69 % scabbed fruits. Compared to lime sulphur applications exclusively in the germination period, four additional preventive treatments with sulphur lead to a further reduction of scab infestation to 2 % (rosettes) and 18 % (fruits).

The best results were achieved with lime sulphur in the germination period and four additional preventive copper treatments. In this treatment the infestation of the rosettes could be reduced to 1 % and on the fruits to 12 %. This corresponds to an efficacy of 98 % (rosettes) and 87 % (fruits).

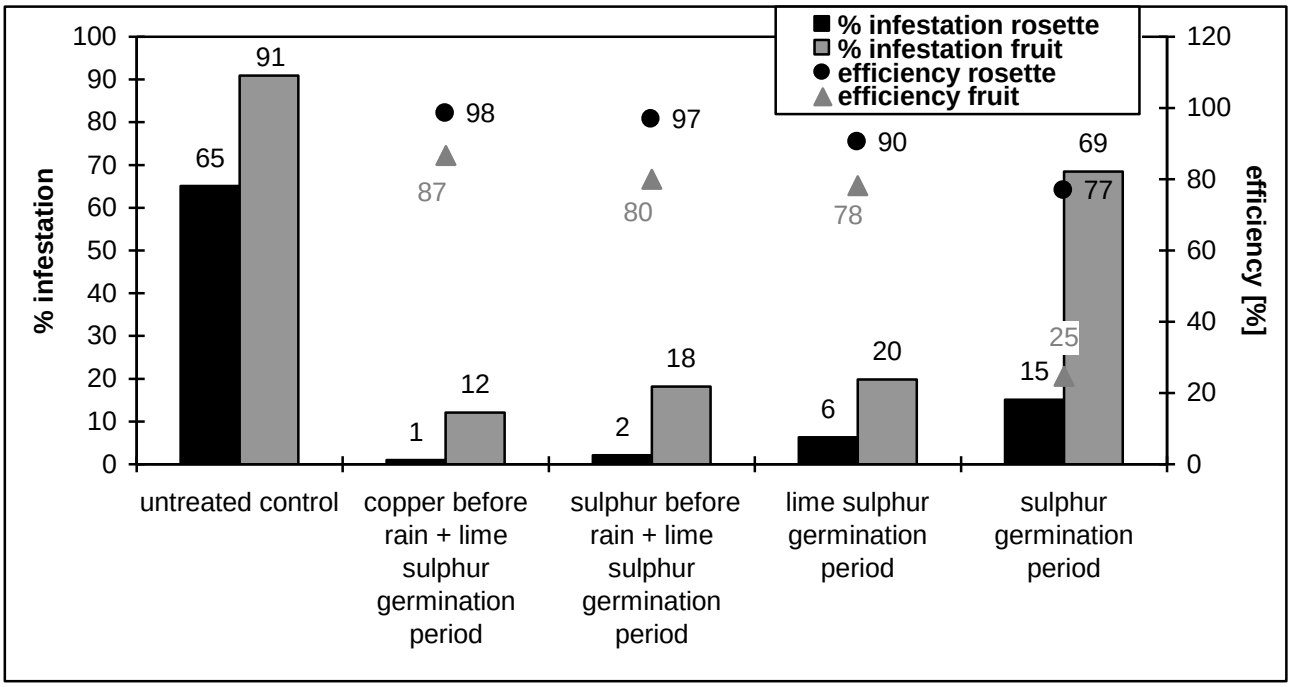


Figure 3: Percentage infestation and efficiency of the different treatments for the variety 'Jonagold', KOB Bavendorf (2010)

## Discussion

Summarising all field trial results a reduction of the copper application rate (application per hectare and year) is possible by optimization and substitution of copper. But a total substitution of copper products for the control of the apple scab is not possible yet. Lime sulphur with specific treatment in the germination period seems to be a good and safe alternative, because the best potential of reducing copper applications was reached with this method. A further alternative for the control of the apple scab is the combination of wettable sulphur with a potassium bicarbonate during the germination period at the time of infection or within 24 hours after the infection on the dry leaf (Benduhn *et al*., 2011). But in the field the efficiencies were not constant during trial years. An explanation for the different efficiencies is the rain intensity and quantity. At the moment the exclusive use of wettable sulphur with potassium bicarbonate could only be recommended during minor infections. As an extra treatment for middle and serious infections, especially under difficult weather conditions during the primary apple scab period, this combination is a good opportunity for an effective apple scab control. With an additional application within 24 hours after infection the efficiency of the first lime sulphur application can be increased. The addition of additives to the copper compound or wettable sulphur adduced a slight increase in the efficiency.

## Acknowledgements

We thank BLE for the financial support of the project and all project participants.

## References

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# Apple Scab: Experiences from 2009 to 2011 in Trentino

L. Mattedi[1], F. Forno[1], R. Maines[1] and M. Varner[2]

**Abstract**

*The scab is, in many years, the "key problem" of the apple production. After several years generally quiet, since the summer of 2008, it returned with an aggressiveness requiring major attention for its containment. The scab control in organic farms has highlighted the potential of the known lime sulphur and its use requires, in some conditions, an integration with other fungicides, particularly copper (in low doses) to be used before the rain,( in order to differentiate the defence in the larger reality), with sulfur (to be included in certain stages and certain varieties) in the window of germination and potassium bicarbonate used curative in combination with sulphur.*

*A series of experiments started in 2009 in order to find some answers to these questions. The choice of the optimal timing was performed with the help of RIMPRO (scab - model from Marc Trapman). The model has been validated in Trentino during the last twenty years and it is now used by a good group of organic producers.*

*Curious to note is that in recent years, scab - management showed more interesting results in the organic farms than in conventional ones.*

**Keywords**: apple, apple scab, *Venturia inaequalis*, organic, disease management

## Introduction

After a relatively quiet period in the years from 2000 to 2007, severe apple scab attacks started again from the summer 2008. The evolution of the disease can be summarised as follows:

- 2008: secondary infections were very serious during summer even where the situation was calm after primary infections. The secondary infections favoured the formation of a very high inoculum level for the next season;
- 2009: very severe primary infections resulting from the inoculum of 2008. Secondary infections were not very aggressive due to the summer weather conditions.
- 2010: the inoculum of 2009 and the weather conditions of spring 2010 caused grave primary infections. Very serious secondary infections, favoured by the summer weather conditions, resulted in severe damages at harvest, especially on Golden Delicious.
- 2011: primary infections were moderate. The short leaf wetting recorded during spring 2011 hampered severe primary infections, in spite of the great inoculum of the previous year. Also secondary infections were not very severe, with only one worrying period in the first decade of June.

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Several experiments aimed at continuing and deepening the knowledge of apple scab behaviour in our environmental conditions are ongoing at “Maso delle Part” (one of the experimental farms belonging to FEM-IASMA), located in Mezzolombardo municipality (Valle dell’Adige-Trentino). These activities are focused on:

- evaluation of pseudothecia development,
- survey of the ascospores flight,
- exposure of “bait plants” in order to follow the seasonal evolution of primary infections,
- monitoring of control plots in areas with different inoculum levels and assessment of the primary infections and duration of spots appearance within the same infection,
- confirmation of the RIMpro model developed by Marc Trapman (Bio Fruit Advies-Zoelmond, Netherlands) and preliminary attempts to fix threshold levels for the RIM values,
- evaluation of vegetative growths.

Furthermore, some studies have started since 2009 to support apple scab management in organic fruit farming. The main targets were to improve the knowledge of fungicide use and, on the other hand, to find answers to some practical requirements. These activities are conducted in collaboration with the Gruppo Produzione Biologica of the Stazione Sperimentale di Laimburg and funded by the Ufficio delle Produzioni Biologiche della Provincia.

As to show the results of three years (2009-2011) would be too long, only the year 2010, when scab attacks were very aggressive, and the results of the control experiments are presented here.

**Material and Methods**

Apple scab evolution on Golden Delicious (very susceptible to either primary or secondary infections) is described by the survey of:

- “bait plants”: potted plantlets exposed, in groups of three, to one rainfall during the primary infection period (from new growth to the end of May). Afterwards these plantlets are left under a tunnel and the presence of scab spots on flower fascicles or buds of each plant is surveyed before the end of June. With time, this survey allows establishing the duration of primary infections and the severity of every attack;
- “untreated controls”, regularly monitoring the development of scab spots on both leaf sides and on fruits. Observations are carried out in several control plots located in different microclimatic areas, which often show different inoculum levels due to the previous season. If possible, also the duration of spot appearance within the same infection is assessed;
- development of the pseudothecia, obtained by diseased leaves collected in fall and left in the orchard under natural conditions. From the half of February, every week, the developmental stage of 50 pseudothecia is observed by the microscope, classifying them as primordial, immature or mature;
- flight of ascospores with the “Marchi captaspores” during every rainy period.

Efficacy trials with newly introduced molecules for the apple scab management in organic fruit farming are conducted on Golden Delicious/M9 (7-year old in 2010) in an about 1100 $m^2$ wide plot where the efficacy of every treatment is compared to an untreated control.

For the different treatments considered, the single molecules are distributed in the key-moments of the primary infection period. Following the specific features of each molecule, they are used (Kelderer, M., Casera,C., Lardschneider, E., 2006; Trapman, M., 2008; Hinze, M., Kunz, S., 2010):

- **preventive** before rainfall (copper at 10 g/hl of active ingredient mixed with liquid and wettable sulphur),
- **"stop spray"** (sulphur in liquid formulation),
- **as curative** (potassium bicarbonate at 1000 g/hl mixed with wetting sulphur),
- the reference standard is represented by the "key molecule" for the practice: lime sulphur used as **stop -spray** at 1500 g/hl,
- sulphur used in the different treatments was standardised with the dosage for the reference lime sulphur (1500 g/hl of lime sulphur corresponds to 334 g/hl of sulphur).

Treatments are made by hand, distributing about 10 hl/ha in big parcels (without repetitions) and controls are conducted with three repetitions/variant. During each control, at least 300 shoots and 1500 fruits are observed (every variant).

Furthermore, in each strategy the side effects are evaluated by considering the equilibrium mite-fauna on leaves (100 leaves every variant were examined in July for the presence of phytoseiid mites) and the rustiness (all the fruits collected from three trees/treatment are visually inspected at harvest).

## Results

Seasonal evolution of apple scab in 2010

In bottom-valley areas the actual primary infections observed can be ascribed to the rainfalls of the end of March-beginning of April, but especially to the weather conditions of the period 2$^{nd}$–6$^{th}$ of May (in some bottom-valley locations, depending on the wetting duration, a further infection was observed the 16$^{th}$-17$^{th}$ of April).

The season 2010 was characterized by a very serious series of secondary infections (the 2$^{nd}$-6$^{th}$ of May, the 15$^{th}$-18$^{th}$ of June, the 14$^{th}$-17$^{th}$ of August) that caused severe attacks on both leaves and fruits.

"Bait plants" exposure

Figure 1 illustrates the historical evolution of scab primary infections. In 2009 and 2010 the severity of the disease increased, while in 2011 the infection level slimmed down.

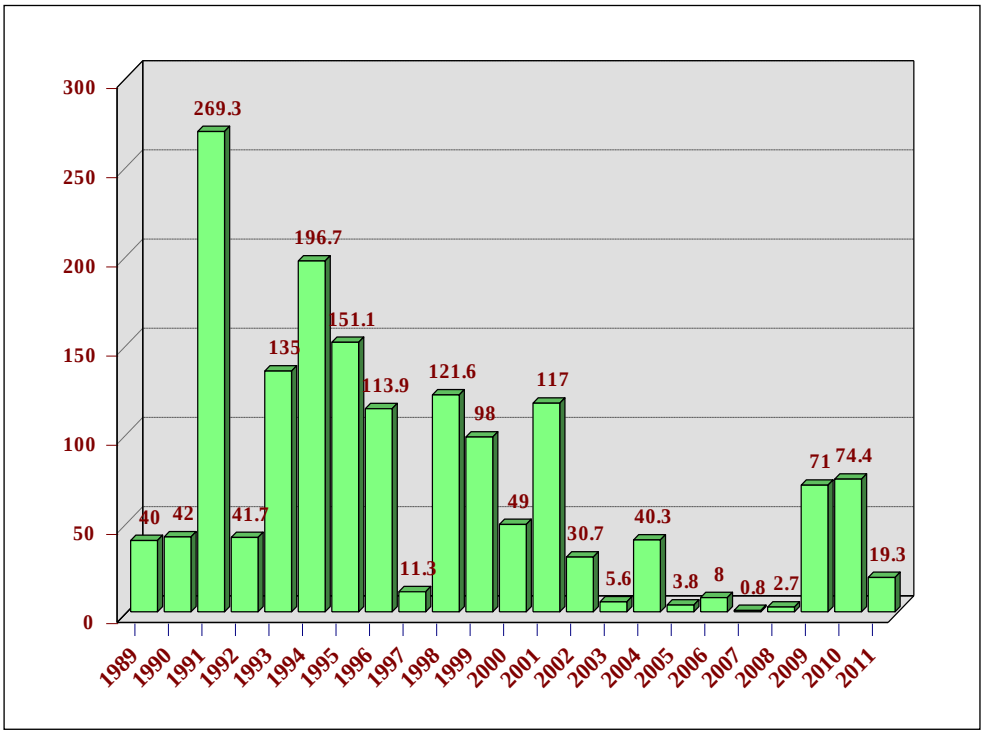


Figure 1: Potted plants – Maso delle Part. Sum of the scabbed shoot percentages during primary infections from 1989 to 2011.

Untreated controls

The infection evolution is followed at the same time in 4 untreated plots in the farms of Istituto Agrario; they are substituted every year and normally different inoculum levels are observed in the different areas (Figure 2). Interestingly, at the end of primary infections, all climatic conditions being equal, the damage level recorded is definitely different.

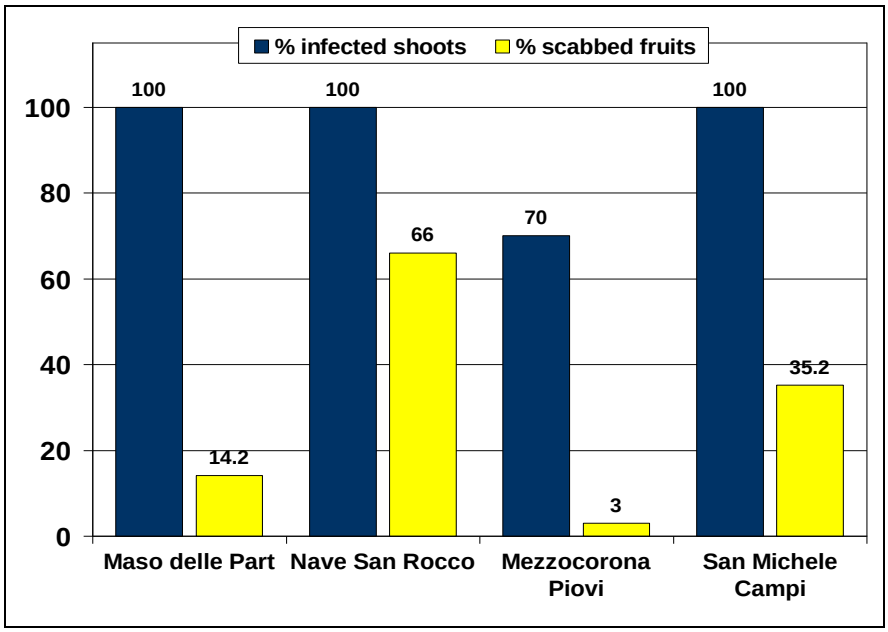


Figure 2: Apple scab presence on shoots and fruits in the untreated controls in the farms of Istituto Agrario (control at the end of primary infections-10$^{th}$ June 2010).

Survey on pseudothecia development and ascospores flight

The evolution of pseudothecia development and the flight of ascospores followed in a representative bottom valley area of Trentino are shown in Figures 3 and 4, respectively.

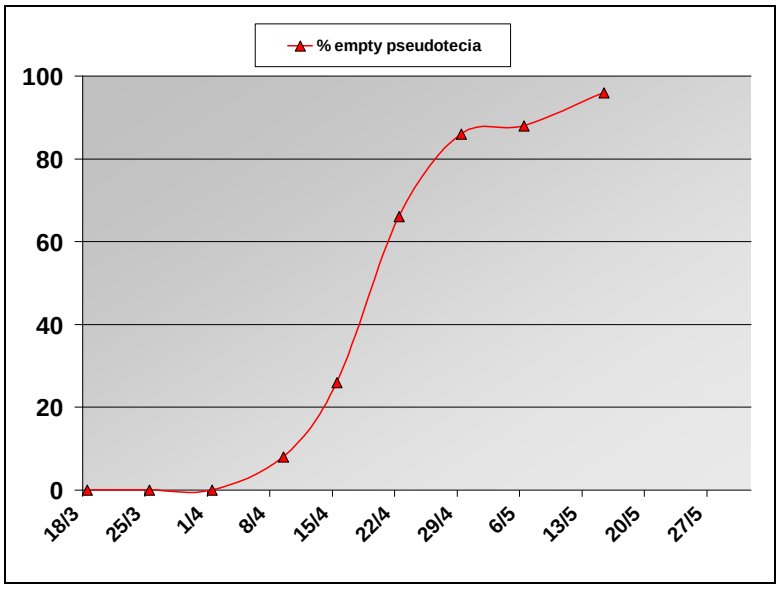


Figure 3: evolution of the pseudothecia development in 2010 at “Maso delle Part”. The percentage of empty pseudothecia is represented.

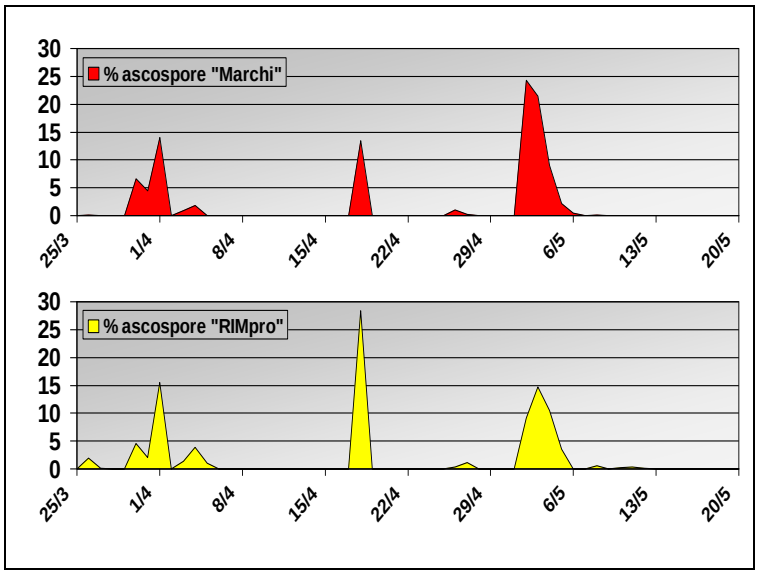


Figure 4: comparison between the ascospores air dispersal in 2010 at “Maso delle Part”. In the upper graph the flight is simulated with the “Marchi captaspores”, while in the lower one it is simulated by the “RIMpro” model.

RIMpro model confirmation

To confirm the effectiveness of the RIMpro model in our environmental conditions, at “Maso delle Part” the comparison between the observed severity of the infections and the simulation by the program is still ongoing (Figure 5). A twenty-year study with simulations and comparisons with field collected data points out very interesting results.

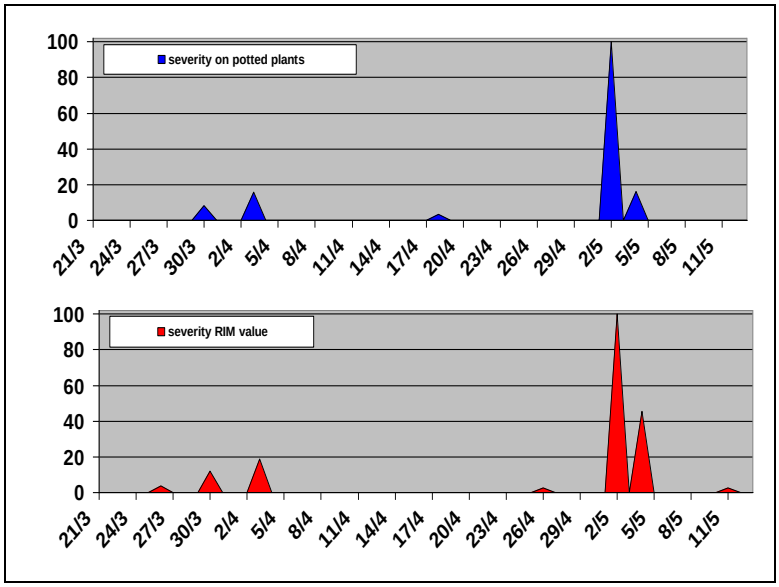


Figure 5: Comparison between the primary infection severity recorded on potted plants at "Maso delle Part" (Valle dell'Adige) and that simulated by the RIMpro model in 2010.

Some additional observations

Thanks to the observations carried out, much knowledge can be built up; some very interesting points concern:

- the duration of spot comparison within the same infection, which in our environment was observed lasting 11 days; such a duration can be precisely simulated also by RIMpro using the increase in the number of spots shown by the model for each infection (Figure 6).

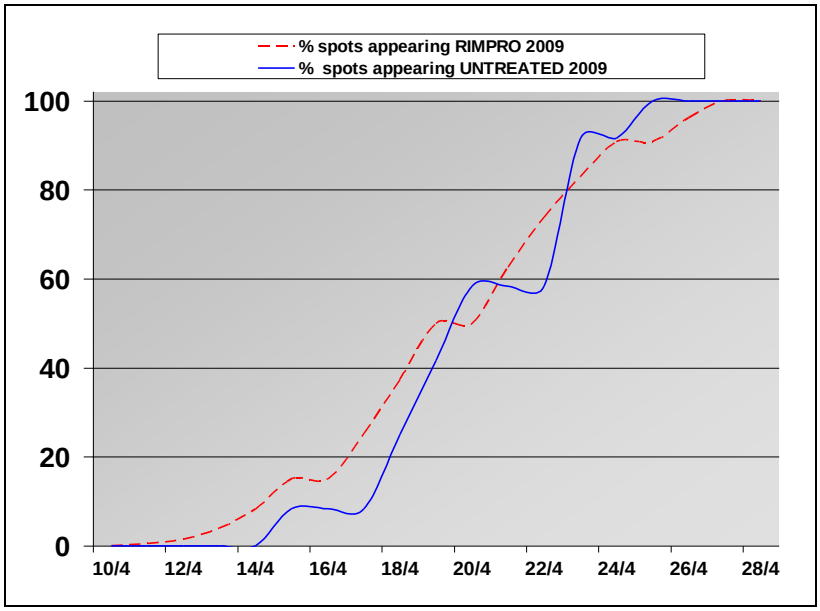


Figure 6: % of appearing of apple scab spots predicted by the RIMpro model (dotted line) and verified in an "untreated control" (continuous line). The infection considered took place April the 1st-2nd 2009 and 11 days went by between the beginning of spot appearance and their stabilization (April 15th-25th).

- During 2009 and 2010, while surveying the untreated control, it was possible to evaluate the varietal susceptibility that concerns, all climatic conditions being equal, Golden Delicious and Granny Smith. A higher damage level was found in the former variety (Table 1), which showed also more diseased fruits in plants with an average productive power than in plants with alternation (Table 2).

Table 1: harvest survey on two varieties planted in the same untreated control plot

| Variety | % of diseased shoots 2009/2010 | % of diseased fruits 2009/2010 |
|---|---|---|
| Golden Delicious | 100/100 | 79/99 |
| Granny Smith | 37/41 | 16/12 |

Table 2: harvest survey on Golden Delicious with different production power

| Production power | % of diseased fruits harvest 2009 | % of diseased fruits harvest 2010 |
|---|---|---|
| Plants with average production | 89 | 99 |
| Plants in alternation | 27 | 24 |

Efficacy of products

The control strategies and the time schedule for treatments are listed in Table 3. In the trial we had five variants (2 Preventive, 2 in Germination window and 1 Curative)

Table 3: treatment schedule in 2010

| **Preventive**<br>Copper 10 g/hl + Thiopron 550 g/hl<br>Copper 10 g/hl + Sulphur 420 g/hl | **Germination window**<br>Lime-sulphur 1,5 kg/hl<br>Thiopron 550 g/hl | **Curative**<br>Bicarbonate 1 Kg/hl<br>+ sulphur 420 g/hl |
|---|---|---|
| March 26th | March 27th | March 27th |
| March 29th | March 30th | |
| March 31st | | March 31st |
| April 2nd | April 2nd (Lime-sulphur) | April 2nd |
| April 12th | | |
| April 18th (Lime-sulphur) | April 18th | April 19th |
| April 22nd | | |
| April 26th | April 27th | April 27th |
| May 1st | | |
| May 3rd | May 3rd (Lime-sulphur) | May 3rd |
| May 5th (Lime-sulphur) | May 5th (Lime-sulphur) | May 5th (Lime-sulphur) |
| May 7th | | |
| | May 11th | May 11th |

The results of these trials are represented in Figure 7, which shows the damaged shoots and leaves at the end of primary infections (3rd of June), and in Figure 8, which shows the damage level on fruits in two different moments (3rd of June and 16th of July).

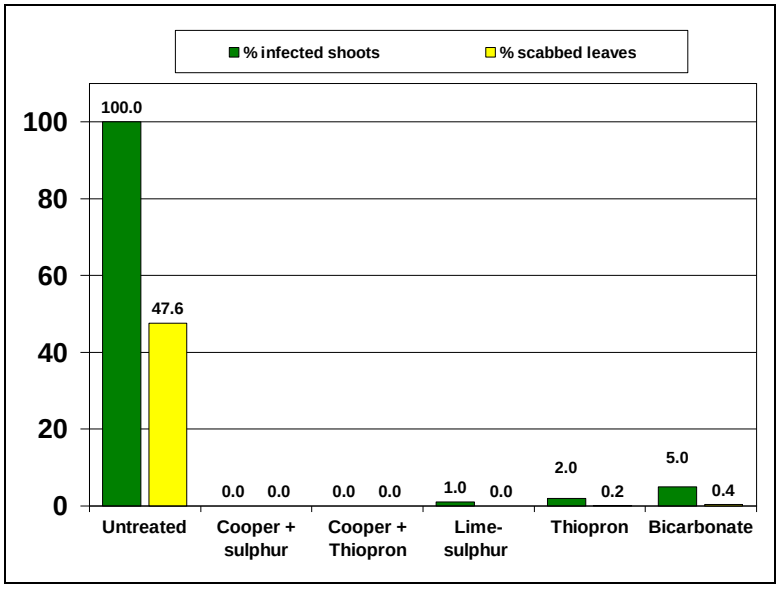


Figure 7: % of diseased shoots and leaves the 3rd of June. Trial for "Bio" control of apple scab in 2010.

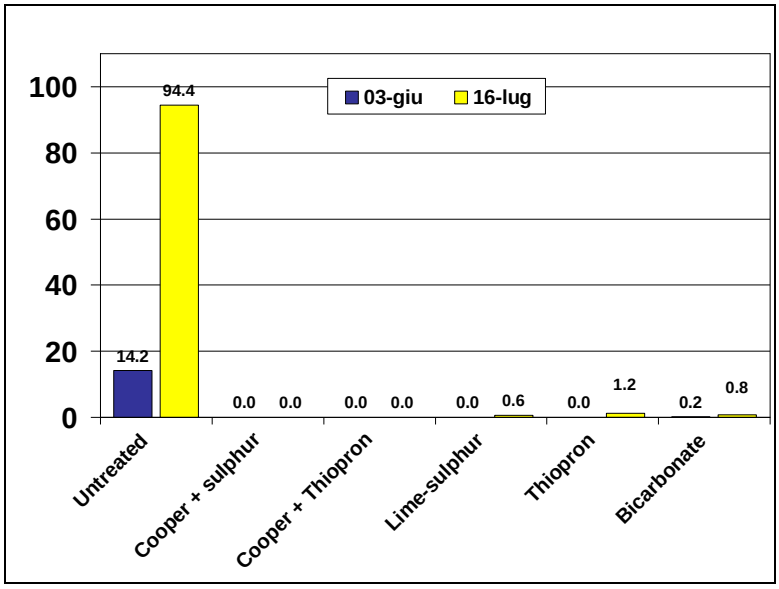


Figure 8: % of scabbed apple fruits surveyed the 3rd of June and the 16th of July. Trial for "Bio" control of apple scab in 2010.

As depicted in Table 3, the last treatment was done the 7th or the 11th of May. Afterwards, in spite of climatic conditions that favour secondary infections, treatments were stopped. The survey conducted at the half of July to evaluate the interference of secondary infections of June (Figure 9) and, at harvest, to evaluate the incidence on fruits (Figure 10).

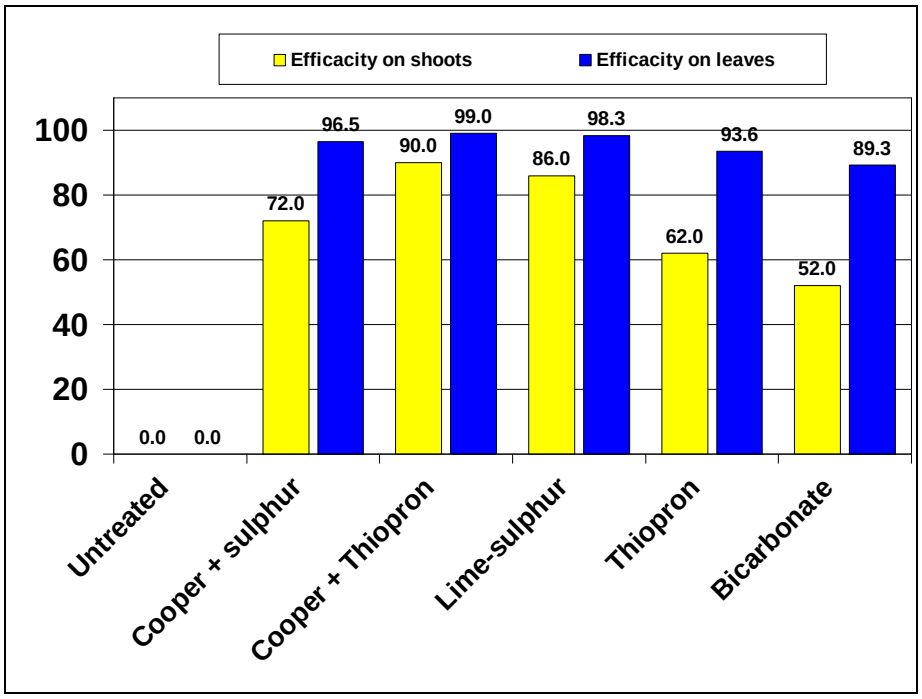


Figure 9: efficacy of the different strategies evaluated the 16th of July on shoots and leaves. Trial for "Bio" control of apple scab in 2010.

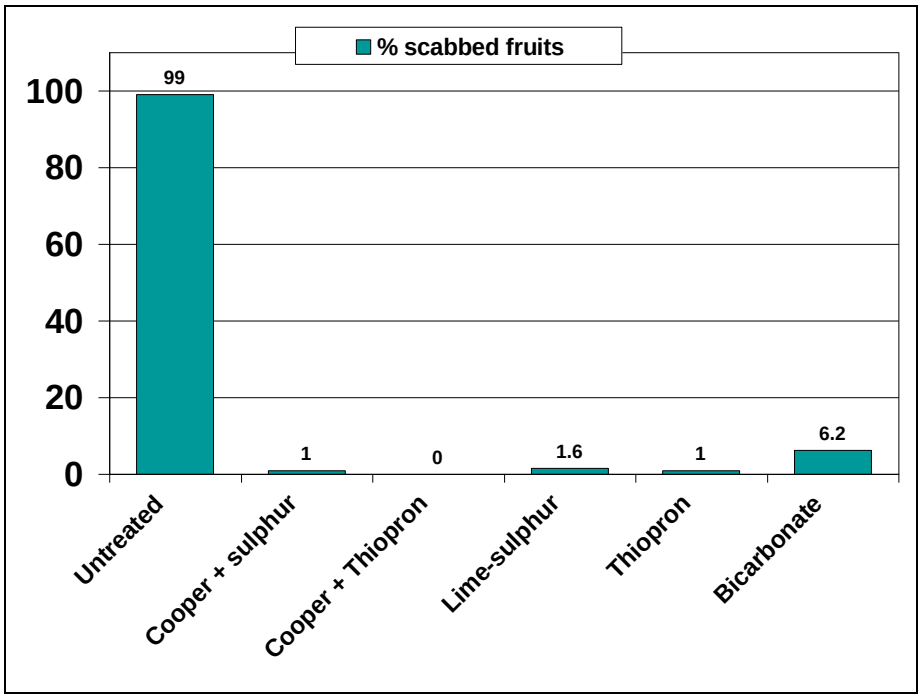


Figure 10: % of scabbed fruits evaluated the 10th of September in the different control strategies. Trial for "Bio" control of apple scab in 2010.

Side effects

A survey was conducted in July to evaluate the side effects on phytoseiid mites: the different treatments sowed no differences with the untreated control (Table 4).

Table 4: side effects in phytoseiid mites

| Control strategies | N° phytoseiids/leaf |
|---|---|
| untreated control | 1,76 |
| thiopron + copper | 1,08 |
| sulphur + copper | 1,08 |
| polysulphide | 1,16 |
| thiopron | 1,16 |
| bicarbonate + sulphur | 1,08 |

The results of the evaluation of rustiness conducted at harvest is represented in Figure 11; as shown by the untreated control, the climatic conditions were particularly favourable. The comparison between the different strategies results in an increase in the rustiness in the copper treatment.

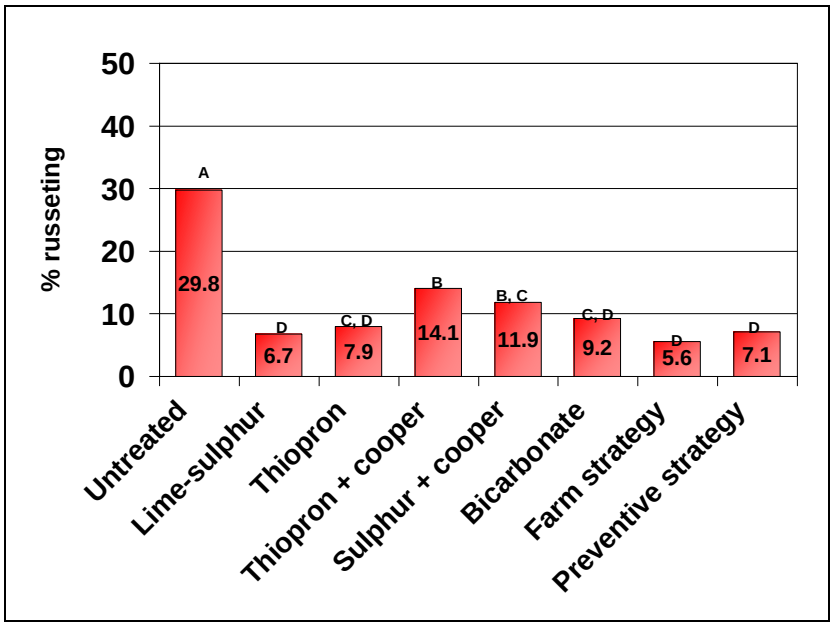


Figure 11: evaluation of fruit russeting at harvest. Trial for “Bio” control of apple scab in 2010.

## Discussion

As the knowledge of apple scab biology in different environmental conditions is the key factor for its management, it is very important to follow the historical evolution of this disease but also its behaviour year by year. The reduction of the primary infection level allows, in our environments, eliminating or at least holding down summer treatments.

The use of tools as RIMpro model results very useful for supporting decisions in the management of the disease.

Calcium polysulphide is the most important compound in apple scab management not only in organic fruit farming: also IPM shows an increasing interest for it due to the small number of new molecules available. Another reason to be considered is the probable reduction in number of the treatments with products as “dithianon”, following a new European guideline for the sustainable use and marketing of pesticides.

The products tested for apple scab management in organic fruit farming seem very interesting, even though they need further experimental validations. Anyway, these results are confirmed also in the years 2009 and 2011, although the pathogen evolution was quite different.

In 2010 at the end of primary infections (3rd of June) all the products and the management strategies resulted very efficacious compared to the infection level of the untreated control (initially higher on leaves and shoots than on fruits). At the half of July, after the attack on fruits by the secondary infection of June, a very interesting difference emerged between the control and all the treatments, in spite of the lack of specific summer treatments. This difference lasts until harvest even if a stronger attack of fruits is recorded in the bicarbonate treatment compared to the other products.

Copper is confirmed effective at low doses and the addition of sulphur seems to reduce its phytotoxicity. It should be worth to use it in wide farms, where it could be difficult to treat the entire surface with polysulphide during rainfall. Moreover, it could be used with other varieties different from Golden Delicious and its efficacy and persistence should be tested even at lower doses.

The good results obtained with the mixture potassium bicarbonate + sulphur have to be confirmed in further trials where treatments are done with atomizer in wider experimental plots. This mixture, at the doses used, does not show severe phytotoxicity problems; on the contrary, a slight “growth retardant” effect can be observed in 2010 and 2011.

The evaluation of side effects picks out no interference either as favouring agent for phytophagous mites or as disturbing agent against phytoseiids, while copper causes more rustiness problems than the other molecules on Golden Delicious.

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# Potassium bicarbonate: a conceivable alternative control measure towards scab on pome fruits

W. Van Hemelrijck[1], E. Croes and P. Creemers[1]

**Abstract**

*Scab on apple and pear, caused by the ascomycete* Venturia inaequalis *and* V. pirina*, respectively, is one of the most important diseases in fruit growing, due to high economic losses if no control measurements are undertaken. Seventy five % of the pesticide use in apple production is related with control of fungal diseases, in which apple scab has a share of 70 %. With the new regulation replacing Directive 91/414 and the Framework Directive on the sustainable use of Pesticides (2009/128/EC), products will disappear from the European market which will give a new challenge in IPM strategies to control pests and diseases. Besides there is the pressure of environmental and consumer groups on supermarkets to reduce residues on fruits. As such, the use of pesticides is at the moment under debate. Reduction in pesticide use is possible, for example, by taking alternative control measures against scab. Pear scab, and to a lesser extent apple scab, does not only infect fruits and leaves, but gives rise to twig lesions as well. The presence of twig scab represents a major problem, especially in organic pear growing, as fruit growers lack satisfying measurements to put an end to the disease. Furthermore, as the use of copper is under discussion, new alternative control strategies are a must in the organic farming. To this end, the efficacy of potassium bicarbonate (pbc) to control scab was investigated. The first experiments on the efficacy of kbc towards scab were performed on apple. The mode of action of pbc on conidial infection and the timing of applications in function of the infection period was determined. Out of the first trials it was concluded that pbc has a fungistatic activity and has the best activity towards apple scab when it is applied curatively, shortly after the infection around 300 degree hours.*

**Keywords:** Scab, potassium bicarbonate, conidia, fungistatic

## Introduction

The most important disease on pome fruits is scab. This disease can be present on apple (*V. inaequalis*) and pear (*V. pirina*) and if no control measurements are undertaken, high economic losses will appear. At this moment about 50 % of the pesticide use in apple production is related with control of scab disease. With the new regulation replacing Directive 91/414 and the Framework Directive on the sustainable use of Pesticides (2009/128/EC), chemical products will disappear from the European market which will give a new challenge in IPM strategies to control diseases. Besides that, a competition between retailers is present, which is based on residue levels that are much stronger compared with the legally MRL (Maximum Residue Level) and eventually are combined with a maximum of active ingredients present on the fruit. Furthermore there is the public concern about pesticide residues present on the fruits. This will all lead to a reduced use of fungicides starting from the post bloom period. As such, the challenge for the present and the future research will be how to manage pest and diseases starting from the post blossom period until harvest. The basis to achieve this goal in scab control will be directed in sanitation measures and in obtaining a total control during the primary scab season. In the secondary season alternative control measures such as biological control organisms, salts or elicitors could be contribute to manage the disease. The past decade much

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research concerning alternative control of scab by means of biological antagonists or elicitors has been performed (Köhl *et al.*, 2009; Spinelli *et al.,* 2010). Besides those alternative techniques also salts can be used to control fungal diseases in vegetables and fruits (Deliopoulos *et al*., 2010). The past years different carbonates have been tested for the control of apple scab with varying efficiencies (Jamar *et al*., 2007; Tamm *et al*., 2006; Trapman, 2008; Kunz *et al.,* 2008). In this article the potential of Vitisan as an alternative control measure to manage scab on apple will be discussed. Much attention has been paid to determine the best application time of the product and the influence of additives on the efficacy of the product and this both in *in vitro* and *in vivo* trials.

## Material and Methods

*In vitro germination tests*

A *V. inaequalis* spore solution of 300.000sp/ml was made out of infected seedling leaves. The solution was prepared by cutting the leaves in small pieces and shaking them in a solution containing 0.5ml/L Tween20. Out of this stock solution a concentration range was prepared for the different tests. The *in vitro* germination tests were performed in microtiter plates. In each well 100µl of a specific spore concentration was added in combination with 100µl of a specific concentration of Vitisan, or captan, which was included as a control treatment. The plates were incubated at room temperature for specific time periods whereafter the percentage of spore germination was counted. To test whether Vitisan has a fungitoxic or fungistatic activity the spore solution, after a specific contact time with Vitisan, was plated out on PDA medium to test if the spores would continue germinating once again. The percentage of germination was counted 24h after incubation of the plates at room temperature.

*Seedling trials*

Preventive or curative efficacy of Vitisan

For this trial, apple seedlings were inoculated with a spore solution of 150.000sp/ml, prepared as described above, and treated with Vitisan (0.5% or 1%) at specific time points prior to or after infection. As a control treatment preventive application of captan (0.15%) was included. Seedlings were then incubated for 48h at 100% RH and room temperature. Thereafter, they were placed in climate chambers (20°C, 90% RH, 12h light/dark cycle) for up to 2 weeks. Two and three weeks after inoculation seedlings were analysed for the presence of scab symptoms. The degree of infestation and the efficacy were determined with the formula of Townsend-Heuberger and Abbott (see below).

Townsend-Heuberger formule: $TH_{v\,max} = \frac{\Sigma\,(n \times v)}{v_{max} \times N} \times 100$

($TH_{v\,max}$ = degree of infestation (%), v = infection classes (0,1,2,3), $v_{max}$= highest infection class, n = amount leaves/fruits in each class, N = total amount of evaluated leaves/fruits)

Abbott formule: $ABB = \frac{C - T}{C} \times 100$

(C = degree infestation untreated object, T = degree infestation treated object)

Rain fastness and efficacy of additives

For this trial, apple seedlings were inoculated with a spore solution of 150.000sp/ml, prepared as described above, and treated with Vitisan (0.5% or 1%) 24h after inoculation. As a control treatment curative application of lime sulphur (1.5%) was included in the test. To test the rain fastness of the product a rain event was created at specific moments after

application of the fungicide by means of a spraying device (<1.0l/$m^2$, flow rate: 0.1l/$m^2$). In order to test the efficacy of additives different products were applied in combination with Vitisan (0.5%). The incubation of the seedlings and the evaluation of the symptoms was done as described above.

*Field trial*

The objective of this trial, performed in 2010, was to check the optimal timing and the effect of Vitisan in comparison with sulphur, copper and lime sulphur treatments (Table 4) towards scab on leaves and fruits of apple trees cultivar Jonagold. This trial was performed during the primary scab season (08/04/2010-19/05/2010) and covered 4 ascospore releases (8-9/04; 03-05/05; 12-14/05 and 17-19/05). Object 2 was treated on the following dates: April 8, 26 and 30, May 5, 8, 11 and 17. For object 3 the treatment dates were April 8 and 26, May 3, 11, 12 and 17. Object 4 till 10 was treated on April 9 and May 4, 14 and 19 and object 11 and 12 were treated on April 8, 26 and 30 and May 4, 8, 14, 17 and 19. Four times 9 trees were included per object. Assessment of scab on leaves and fruits was done by analyzing 100 different leaves at the sun and shadow side and 100 fruits per plot in the field. The degree of infestation and the efficacy were determined with the formula of Townsend-Heuberger and Abbott.

*Statistical analysis*

Statistical analyses were performed on TH-value using the Unistat Statistical Package, version 5.5 (Unistat Ltd. 1998). Means were compared using Duncan's multiple range test.

## Results

*In vitro* germination tests

The first trials pointed out that Vitisan can inhibit the germination of apple scab conidia and that the efficacy of the product is dependent on the concentration of the spore solution and of the product. For example, addition of Vitisan (13.7g/L) resulted in 50% inhibition of spore germination when a concentration of 100.000sp/ml was used. On the other hand, addition of Vitisan at a concentration of 6.85g/L was enough to get the same results when the spore solution was diluted till 25.000sp/ml. In a second trial the fungistatic or fungitoxic activity of Vitisan was determined (Table 1).

After addition of Vitisan for a specific time, all spore solutions were able to restart germination when the spores were incubated on PDA. On the contrary, when the spores were in contact with Captan for a specific time prior to incubation on PDA medium, no spores could germinate any more. Based on these results it can be stated that Vitisan has a fungistatic activity towards scab conidia.

Table 1: Fungistatic activity of Vitisan

| object | incubation time (h) | % germination ( after 24h on PDA) |
|---|---|---|
| Vitisan (13,7 g/L) | 1,5 | 88 |
| | 3 | 87 |
| | 6 | 84 |
| | 12 | 68 |
| | 24 | 73 |
| | 32 | 74 |
| | 48 | 86 |
| Captan (1,5 g/L) | 1,5 | 0 |
| | 3 | 0 |
| | 6 | 0 |
| | 16 | 0 |
| | 24 | 0 |
| | 32 | 0 |
| | 48 | 0 |

Preventive or curative efficacy of Vitisan

The results of the seedling trial to reveal the preventive or curative efficacy are shown in Table 2. Overall, captan as a preventive treatment had the highest efficacy. In comparison, the use of Vitisan prior to infection only had an efficacy of 35 to 45%. However, when Vitisan was applied in a curative way better efficacies were obtained. Out of this trial it can be concluded that curative application of Vitisan at 24h after inoculation resulted in the best control of scab on the seedlings. A dose range effect of the product could only be observed when the product was applied at suboptimal time points.

Table 2: Preventive and curative efficacy of Vitisan on apple seedlings

| objects | | % infestation | TH13 | | ABB |
|---|---|---|---|---|---|
| **1** | **untreated** | 68,8 | 22,8 | d | |
| **2** | **0,5% Vitisan (preventive)** | 50,0 | 14,7 | d | 35,8 |
| **3** | **1,0% Vitisan (preventive)** | 37,5 | 12,5 | d | 45,3 |
| **4** | **0,15% captan (preventive)** | 9,4 | 0,7 | a | 96,8 |
| **5** | **0,5% Vitisan (8hpi)** | 59,4 | 10,3 | d | 54,7 |
| **6** | **1,0% Vitisan (8hpi)** | 40,6 | 8,2 | c,d | 64,2 |
| **7** | **0,5% Vitisan (16hpi)** | 21,9 | 5,5 | b,c | 75,8 |
| **8** | **1,0% Vitisan (16hpi)** | 15,6 | 3,4 | a,b | 85,3 |
| **9** | **0,5% Vitisan (24hpi)** | 21,9 | 3,8 | b,c | 83,2 |
| **10** | **1,0% Vitisan (24hpi)** | 15,6 | 3,4 | a,b,c | 85,3 |
| **11** | **0,5% Vitisan (32hpi)** | 62,5 | 17,3 | d | 24,2 |
| **12** | **1,0% Vitisan (32hpi)** | 46,9 | 11,3 | d | 50,5 |

* TH13: degree of infestation; ABB: efficacy of the product, hpi: hours post inoculation; different letters indicate significant differences in degree of infestation.

Rain fastness of Vitisan and effect of additives towards efficiency of Vitisan

The results of the seedling trial to reveal the rain fastness of Vitisan and the effect of additives are shown in Table 3. As can be seen in this table, the efficacy of Vitisan declines even after a small (<1.00l/$m^2$) rain event (ABB:73% vs ABB 37-55%). On the other hand, addition of additives, like T/S Forte, Cocana and Trend had a synergistic effect towards the efficacy of Vitisan. Although the infestation on the seedlings was rather low, differences between objects could be observed. The combination of Vitisan with additives

performed in general much better than Vitisan alone, however nog significant differences were observed. Between the different additives applied also no significant differences has been observed.

Table 3: Rain fastness of Vitisan and effect of additives towards efficiency of Vitisan towards scab on apple seedlings

| | | % A | TH13* | ABB |
|---|---|---|---|---|
| **1** | **Vitisan 0,5% curative** | 4,7 | 1,4 abc | 73,0 |
| **2** | **Vitisan 1% curative** | 3,6 | 0,3 ab | 94,9 |
| **3** | **Lime sulphur 1,5% curative** | 1,6 | 0,2 a | 95,5 |
| **4** | **untreated + rain (after 15 min)** | 15,1 | 5,3 c | |
| **5** | **Vitisan 0,5% curative + rain (after 15 min)** | 7,8 | 2,4 abc | 55,0 |
| **6** | **untreated + rain (after 2u)** | 9,4 | 2,5 abc | |
| **7** | **Vitisan 0,5% curative + rain (after 2u)** | 11,0 | 3,4 c | 36,8 |
| **8** | **untreated + rain (after 24u)** | 11,8 | 4,6 bc | |
| **9** | **Vitisan 0,5% curative + rain (after 24u)** | 12,7 | 3,7 c | 31,2 |
| **10** | **Vitisan 0,5% curative + T/S Forte** | 1,6 | 0,5 ab | 91,0 |
| **11** | **Vitisan 0,5% curative + cocana** | 0,0 | 0,0 a | 100,0 |
| **12** | **Vitisan 0,5% curative + trend** | 1,6 | 0,6 ab | 88,8 |

* different letters indicate significant differences in degree of infestation.

Field trial

The results of the field trial are shown in Table 4. In the untreated object, the scab infestation on the leaves was very high (92%). The best efficacy towards scab on leaves were obtained with sulphur applied during the germination window (object 3) and with schedule 12 in which sulphur was used preventively according to the weather forecast, in complement with a curative treatment with Vitisan about 300 degree hours (DH) after start RIM-curve.

Table 4: Assessment of scab symptoms on leaves and fruits in field trial

| Objects | | leaves 11/08 | | | | fruits 15/09 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | %A | TH3 | ABB | | %A | TH3 | ABB | |
| 1 | Check | 91,88 | 78,46 | | | 42,90 | 24,30 | | |
| 2 | preventive Cu (0,025%)/after bloom S (0,3%) | 21,00 | 11,29 | 85,61 | ab | 3,60 | 1,40 | 94,20 | ab |
| 3 | S (0,3%) in germination window | 14,75 | 7,00 | 91,08 | a | 3,50 | 1,40 | 94,20 | ab |
| 4 | lime sulphur (1%) 300 DH | 26,88 | 16,13 | 79,45 | bc | 5,60 | 2,40 | 90,20 | b |
| 5 | Vitisan (0,33%) 300 DH | 23,75 | 13,88 | 82,32 | bc | 12,00 | 5,60 | 77,00 | c |
| 6 | Vitisan (0,33%) + S (0,3%) 300 DH | 25,50 | 14,38 | 81,68 | bc | 6,10 | 2,50 | 89,70 | b |
| 7 | Vitisan (0,33%) + T/S Forte (0,133%) 300 DH | 34,88 | 22,96 | 70,74 | d | 13,30 | 6,00 | 75,40 | c |
| 8 | Vitisan (0,5%) 300 DH | 35,00 | 23,00 | 70,69 | d | 14,80 | 6,50 | 73,20 | c |
| 9 | Vitisan (0,5%) + S (0,3%) 300 DH | 19,75 | 10,96 | 86,03 | ab | 2,80 | 1,00 | 95,90 | ab |
| 10 | Vitisan (0,5%) + T/S Forte (0,133%) 300 DH | 28,88 | 19,00 | 75,78 | cd | 11,30 | 4,80 | 80,20 | c |
| 11 | optimal schedule S or lime sulphur 300DH* | 19,50 | 10,25 | 86,94 | ab | 1,60 | 0,50 | 97,80 | a |
| 12 | optimal schedule S and Vitisan 300 DH** | 14,75 | 6,96 | 91,13 | a | 1,90 | 0,70 | 97,30 | a |

* S (sulphur) preventive or in germination window (RIM<150) or Vitisan 300DH (RIM between 150 and 300) or lime sulphur 300 DH (RIM >300), DH: degree hours, different letters indicate significant differences in degree of infestation.

** idem object 11 but without lime sulphur; so also Vitisan at 300DH with RIM>300

In general a good efficacy (70-82%) was obtained with the curative application of Vitisan at about 300 DH. Although not significantly different, the application of Vitisan at 300 DH seems to have a better efficacy compared with the application with Lime sulphur at the same time point. When sulphur was added to Vitisan applied at the highest dose rate, a slightly better efficacy was obtained compared with application of Vitisan alone, although results were not significantly different. However, when T/S forte was added, a decline in efficacy was observed, although results out of the *in vitro* trial on seedlings indicated a synergistic effect when this product was added.

At harvest time about 43% of the fruits in the untreated object were infested with scab. The best efficacy towards scab on fruits was also obtained with schedule 11, where sulphur was applied preventively combined with a curative application of lime sulphur or Vitisan according to the RIM value and with schedule 12 in which sulphur was used preventively according to the weather forecast, in complement with a curative treatment of Vitisan at about 300 degree hours (DH) after start of the RIM-curve. No significant differences have been observed between the two dose rates of Vitisan that were tested. Again, when sulphur was applied in a tank-mixture with Vitisan, at the highest dose rate applied, an increase in efficacy towards scab on fruits was obtained. As observed for the leaves, the synergistic effect when an additive was applied was lost. Overall, it can be concluded that Vitisan has a potential as an alternative control measure against scab on apples.

**Discussion**

With the new regulation replacing Directive 91/414 and the Framework Directive on the sustainable use of Pesticides (2009/128/EC), chemical products are under discussion and some of them will disappear from the European market. These facts will give a new challenge in IPM strategies to control diseases. One solution for this problem is to look for alternative techniques or strategies as a substitution for the use of fungicides. In this work, the potential of unformulated potassium bicarbonate (Vitisan) to control scab on apples was investigated. A lot of research on the potential of bicarbonates to control fungal diseases is already performed. The activity of (sodium) bicarbonates towards scab on apples was reported earlier (Ilhan *et al*., 2006). *In vitro* trials pointed out that sodium bicarbonate could strongly reduce the germination of *V. inaequalis* conidia. Here, we reported that Vitisan has a fungistatic activity towards conidia of *V. inaequalis*. Inhibitory activity of carbonates towards micro-organisms is mostly fungistatic (Smilanick *et al.,* 1999). Therefore, it is probable that a residue of carbonate or bicarbonate must remain on the fruit, or at least within the wound infection courts occupied by this pathogen to be able to control it.

Furthermore, field trials based on a 10 days schedule revealed that efficacy of sodium bicarbonate (1%) was comparable with that of tebuconazole (Ilhan et al., 2006). Sodium bicarbonate applied at a concentration of 2% resulted in phytotoxicity. In our trials, no phytotoxicity on leaves was observed after application of Vitisan (0.5% and 1%). On the other hand, it was also reported previously that a during-infection application of Armicarb resulted in a good efficacy towards scab on leaves and fruits of apple trees (Jamar *et al.,* 2008). Besides that, Trapman (2008) reported a varying activity depending on the orchard when Vitisan was applied shortly before a rain event or during the germination window of the spores.

However, in the trials described here, it was pointed out that for the control of scab, potassium bicarbonate can best be applied as a curative treatment at 240 - 320 degree hours. The same results were also obtained by Kunz et al. (2008). Furthermore, it was stated that the efficacy of Vitisan could be enhanced when it was applied in combination with sulphur (Kelderer *et al*., 2008) and this was also confirmed in our trials. However, it

was observed that not only addition of sulphur but also tank-mixtures with additives enhanced the efficacy of Vitisan. A possible explanation is that due to the tank mixture a better formulation of the product is obtained. Previous studies with Armicarb (Kelderer *et al.* 2006; 2008) have shown that the formulation can definitely affect the efficacy of the product. Overall, it can be concluded that Vitisan has a potential as an alternative control strategy towards scab on apple, when applied as a curative treatment. Further research concerning a correct timing of the product (in relation to its rain fastness) and the efficacy of this product when it is applied in combination with other alternative techniques is still needed.

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# Pest status of the sooty blotch and flyspeck complex in Asturian (NW Spain) apple orchards

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**Abstract**

*Sooty blotch and flyspeck (SBFS) fungi occur on apple surface in regions with moist climate, such as Asturias, in the Northwest coast of Spain. Due to the lack of knowledge on SBFS in our region, research has been conducted to determine (1) the timing of appearance of symptoms, (2) the incidence of these fungi on different cultivars, orchards and years and (3) the effectiveness of several fungicides for controlling this disease. Development of SBFS signs was followed in the 2011 season from early July to harvest in several cultivars and orchards. SBFS signs were observed from July 20 onwards. Signs classically related to SB and those of FS appeared on the same date. Some variation in symptom development was observed among cultivars and orchards. Cultivar susceptibility was recorded just before harvest in 2010 and 2011. The severity of SBFS depended greatly on the cultivar but also on the orchard. Damage level in individual cultivars and orchards was rather similar in the two years of observation. Lime sulphur and potassium bicarbonate (Armicarb) sprayed alone or combined with wettable sulphur provided good control of SBFS when they were applied fortnightly from early June to late September (9 sprayings). The clay Myco-San was not as efficient at controlling fruit blemish although diminished the symptoms compared to untreated trees. The use of potassium bicarbonate had, however, side effects increasing lenticel spotting to intolerable limits and reducing fruit weight and yield. This study allowed us to determine the importance of the disease and the cultivar susceptibility in our conditions. Further research is needed to develop a forecasting model and to use fungicides more efficiently.*

**Keywords:** SBFS complex, cultivar susceptibility, organic control, symptom development

## Introduction

Sooty blotch and flyspeck (SBFS) fungi occur worldwide in regions with moist climate, including Asturias, in the Northwest coast of Spain. The development of these fungi on apple surface causes the appearance of black spots that produces an aesthetical damage and reduces consequently the yield value. Sooty blotch symptoms are diffuse dark areas of different size while flyspeck ones are small dots closely clumped. These two different types of symptoms led to a two-disease paradigm (that sooty blotch was caused by a single species and that flyspeck was caused by a different single species) that remained until recent years. However, nowadays, it has been shown that SBFS is caused by a complex of fungi comprising more than 60 different species (Gleason *et al*., 2011). SBFS complex is likely not composed of the same species assemblage at each region (Gleason *et al*., 2011) and so SBFS may have a different local response to factors such as temperature or fungicides. Therefore there is a need to conduct local studies on the species assemblage and their environmental biology at each apple-producing region (Gleason *et al*., 2011).

Apple in Asturias is mainly devoted to cider production and thereby aesthetical damage is not as important as in the case of dessert apple. However, there is an increase in the

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surface of orchards guided to produce organic apple for fresh consumption. SBFS may be a constraint for apple production in those orchards, but there is a lack of knowledge on the SBFS status in our region. To fill this gap, research was conducted to determine (1) the timing of appearance of symptoms, (2) the incidence of these fungi on different cultivars, orchards and years and (3) the effectiveness of some fungicides for controlling this disease.

## Material and Methods

In all the essays disease occurrence was scored following Mayr & Späth (2008): 0=no symptoms; 1=small spots; 2= symptoms on <10% fruit surface; 3= on 10-25%; 4= on 25-50% and 5= on >50%. Disease incidence was the percentage of fruits with symptoms while severity was calculated as: $(\Sigma(n*c)/(N*5))*100$, where n is the number of fruits of each class, c is the evaluation class (0-5), N is the total number of fruits and 5 is the highest class of the evaluation scale. In all the cases we differentiated between the classical SB symptoms and those of FS. Occurrence of lenticel spotting was recorded in the same scale.

*Timing of appearance of SBFS signs*

The occurrence of disease symptoms on apples was recorded weekly in four untreated orchards from the first week of July to the end of September 2011. Two or three cultivars were evaluated at each orchard. Ten trees per cultivar were selected and marked at the end of June. Then ten apples per tree were randomly selected at each sampling date and disease occurrence was recorded for each apple.

*Cultivar susceptibility*

Occurrence of SB and FS signs was recorded in 2010 and 2011 just before the respective harvest of the cultivars. Eight dessert cultivars and 22 cider cultivars were observed. When possible, each cultivar was observed in five organically-conducted orchards and then ten apples of five trees of each cultivar were observed at each orchard.

*SBFS control with organic products*

An experiment was conducted in 2011 on the cultivar *Goldrush* to evaluate the effectiveness of four treatments: potassium bicarbonate (Armicarb, 0.5%), potassium bicarbonate (Armicarb, 0.5%) combined with wettable sulphur (Bayer, 0.4%), lime sulphur (Sulfoluq, 3%) and the clay Myco-San (0.8%) mixed with an adjuvant (Nu-film 17, 0.1%). Treatments were applied exactly every two weeks from early June to late September (9 sprayings) using a backpack sprayer. Treatments were applied on dry leaf and never with rainy weather. Results were compared with untreated trees. A randomized complete block design with three replicates was used. Each replicate consisted of a five-tree subplot, in which sampling was done on the three central trees.

## Results and discussion

*Timing of appearance of SBFS signs*

Both signs of SB and FS were observed for the first time on July 20, specifically on cultivar *Regona* in orchard 3 (Figure 1). In both cases fungi developed at a different rhythm depending on the cultivar. A correlation was found between the date at which the first symptoms were observed and the final severity recorded for both SB and FS signs: the sooner the cultivars showed first signs, the higher was the severity they suffered (Figure 2). According to the disease development on the different cultivars, an orchard effect may be also appreciated in the case of FS: pressure seemed higher in orchard 3 followed by

orchard 4 and then orchards 1 and 2 (Figure 1B). In other studies, between-year variability in the development of SBFS infection has been observed and related to rainfall variables (Mayr *et al*., 2010; Spolti *et al*., 2011). Therefore, new observations taking into account weather variables should be performed in our case in order to develop a forecasting model.

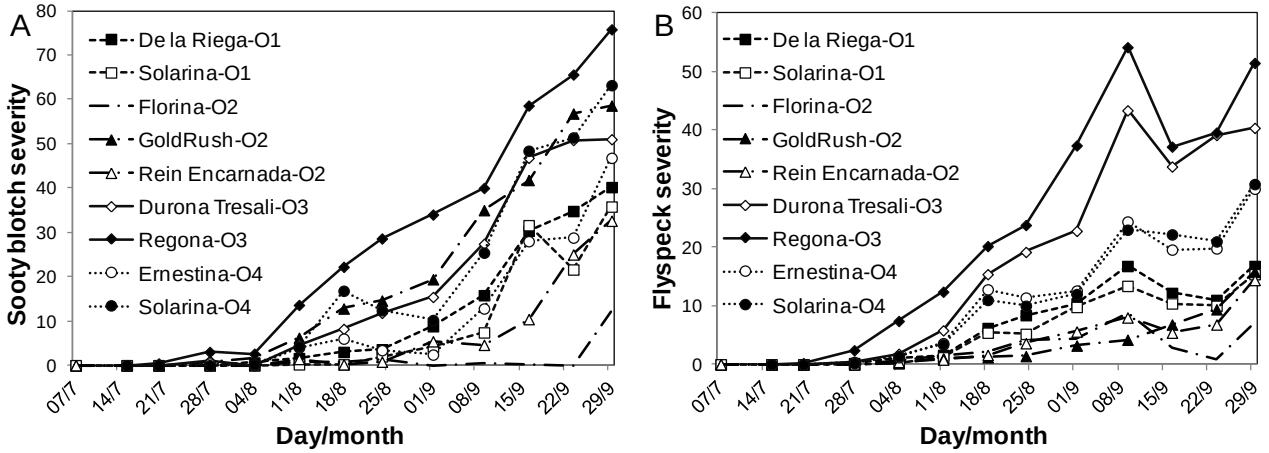


Figure 1: Temporal progress of SB (A) and FS (B) severity in 2011 in different cultivars from four orchards (O1 to O4).

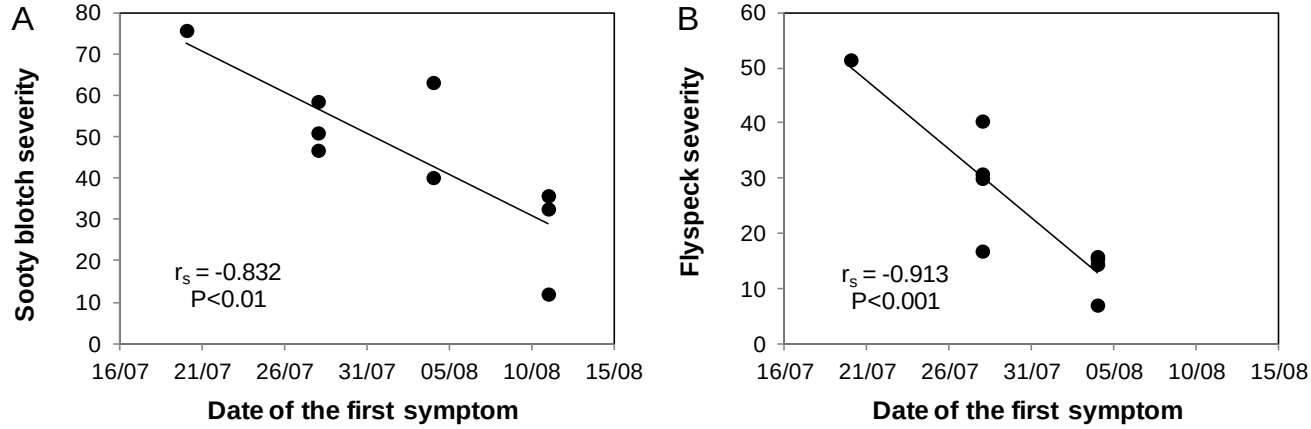


Figure 2: Correlation between the date when first symptom was observed for each cultivar and the final severity recorded for those cultivars.

*Cultivar susceptibility*

Susceptibility to SBFS varied widely among cultivars (Table 1). For all the cultivars but *Florina* SB severity was higher than FS severity. The four cultivars with higher SB severity (*Goldrush*, *Regona*, *Verdialona* and *Durona de Tresali*) produce yellow or light green apples that ripen late in the season, supporting previous findings (Biggs *et al*., 2010, Mayr *et al*., 2010). Notable differences among orchards were also recorded. For example, SB severity in *Goldrush* in different orchards ranged between 51.2 and 84.4 and FS severity between 4.4 and 28.0. In general, lower fruit blemish was observed in orchards placed in sunny slopes and/or orchards with lower leaf and branch density in the tree canopies.

Table 1: Cultivar susceptibility to the sooty blotch & flyspeck complex. Data from different orchards in 2010 and 2011 were pooled for calculations (mean ± SE are shown).

| Cultivar | SB severity | FS severity | Cultivar | SB severity | FS severity |
|---|---|---|---|---|---|
| **Dessert cultivars** | | | **Cider cultivars** | | |
| *Florina* | 14.6 ± 1.9 | 18.5 ± 3.2 | *Durona de Tresali* | 51.4 ± 1.7 | 31.9 ± 1.7 |
| *Galarina* | 14.4 ± 3.9 | 3.2 ± 1.3 | *Ernestina* | 39.5 ± 4.0 | 21.7 ± 2.8 |
| *GoldRush* | 65.0 ± 2.0 | 15.4 ± 1.6 | *Fuentes* | 48.1 ± 2.1 | 18.8 ± 1.4 |
| *Liberty* | 18.3 ± 3.2 | 16.4 ± 3.7 | *Limón Montés* | 32.5 ± 3.7 | 13.6 ± 2.0 |
| *Priscilla* | 28.1 ± 4.0 | 16.1 ± 2.9 | *Meana* | 21.8 ± 2.9 | 20.2 ± 2.9 |
| *Reineta Roja Canadá* | 5.0 ± 3.0 | 0.0 ± 0.0 | *Panquerina* | 31.7 ± 4.2 | 15.3 ± 1.8 |
| *Reineta Blanca Canadá* | 31.4 ± 2.6 | 7.2 ± 1.6 | *Perezosa* | 19.5 ± 3.4 | 8.2 ± 1.6 |
| *Reineta Encarnada* | 22.7 ± 3.2 | 15.4 ± 1.6 | *Perico* | 30.1 ± 3.2 | 9.7 ± 1.2 |
| | | | *Prieta* | 19.6 ± 4.4 | 12.4 ± 3.2 |
| **Cider cultivars** | | | *Raxao* | 34.6 ± 2.9 | 24.6 ± 1.9 |
| *Blanquina* | 25.0 ± 4.3 | 12.5 ± 1.8 | *Regona* | 62.3 ± 2.9 | 40.0 ± 1.8 |
| *Carrió* | 21.2 ± 3.8 | 19.5 ± 3.1 | *San Roqueña* | 19.5 ± 3.9 | 6.6 ± 1.8 |
| *Clara* | 34.1 ± 1.9 | 9.8 ± 1.5 | *Solarina* | 24.7 ± 2.8 | 17.1 ± 1.9 |
| *Collaos* | 42.9 ± 2.2 | 29.3 ± 2.2 | *Teórica* | 5.5 ± 1.8 | 4.5 ± 0.9 |
| *Coloradona* | 21.2 ± 2.8 | 4.3 ± 1.2 | *Verdialona* | 60.6 ± 4.1 | 20.8 ± 2.6 |
| *De la Riega* | 38.6 ± 3.4 | 23.8 ± 2.7 | *Xuanina* | 35.6 ± 3.4 | 20.1 ± 2.5 |

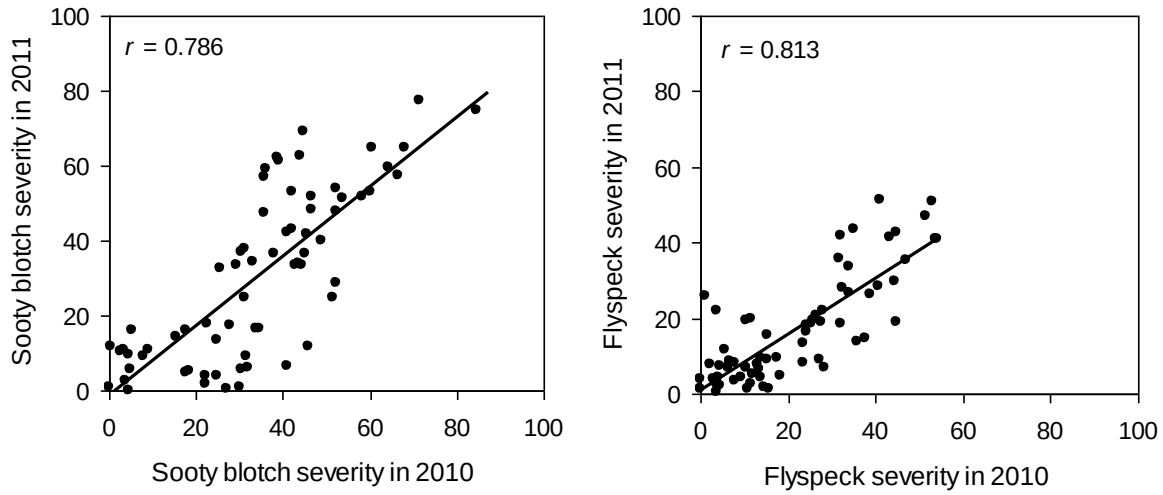


Figure 3: Correlation between sooty blotch (A) and flyspeck (B) severity in 2010 and 2011 (data for each cultivar-orchard pair are plotted).

The overall disease severity stayed in the same range for the two years of observation (SB severity in 2010 and 2011 was respectively 36.3 and 33.1, whereas FS severity was 21.8 and 17.1. In addition, there was a high correlation between years in the disease severity observed from each cultivar at each orchard (Figure 3). All these results support previous observations indicating that orchards have more importance than years at explaining disease occurrence (Trapman, 2006).

*Control with organic products*

*Goldrush* apples were harvested on 14$^{th}$ of November. Accordingly to the results in the untreated trees, the SB pressure was very high (Table 2) whereas the occurrence of FS symptoms was, by contrast, very low (Severity index = 3.3; results not presented). All the

treatments reduced the SB severity although in a different degree. Armicarb (alone and mixed with wettable sulphur) and lime sulphur provided an excellent control of SB signs, with 100% of marketable apples (level damage lower than 2, i.e. less than 10% of the fruit surface blemished) (Table 2). Spraying with Myco-San resulted in an intermediate level of blemish and marketable apples (Table 2): most of the apples showed symptoms but in general only occupying a small fruit surface, usually the less exposed. Lime sulphur and Myco-San left visual residues on the apples. Excellent protection against SBFS by Armicarb (alone or with wettable sulphur) has been previously shown (Tamm *et al*., 2006; Mayr *et al*., 2010).

Table 2. Treatment effect on the occurrence of sooty blotch signs: percentage of apples in each infection class, severity index and percentage of marketable apples.

| Treatment | Infection class | | | | | | Severity index | Marketable apples (%) |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | Mean ± SE | (classes 0, 1 & 2) |
| Control | 0.0 | 1.1 | 12.2 | 34.4 | 24.4 | 27.8 | 73.1 ± 5.4 **a** | 13.3 |
| Armicarb | 66.7 | 21.1 | 12.2 | 0.0 | 0.0 | 0.0 | 9.1 ± 2.3 **c** | 100.0 |
| Armicarb + sulphur | 65.6 | 28.9 | 5.6 | 0.0 | 0.0 | 0.0 | 8.0 ± 2.1 **c** | 100.0 |
| Myco-San | 6.7 | 25.6 | 25.6 | 30.0 | 10.0 | 2.2 | 43.6 ± 5.2 **b** | 57.8 |
| Lime sulphur | 45.6 | 40.0 | 14.4 | 0.0 | 0.0 | 0.0 | 13.8 ± 1.9 **c** | 100.0 |

The latest spraying was done on 23rd of September, that is, almost two months before harvest. During this period there was a considerable increase in SB severity in all the treatments (Figure 4). The high increase in symptoms when spraying ceased supports the results of studies that show that infection can take place throughout the fruit development period (Mayr *et al*., 2010; Spolti *et al*., 2011), and suggests the need of covering the whole season with spray applications. Therefore, in cases of late-maturing cultivars and in regions where apples are harvested relatively late, such as Asturias, a high number of sprayings could be necessary to protect apples over their development period.

As a negative side-effect, the use of potassium bicarbonate increased lenticel spotting (sometimes known as “Topaz spots”) to intolerable limits, exceeding even the results in the control trees (Figure 5). Other products, including, curiously, the combination of Armicarb with wettable sulphur, diminished lenticel spots in comparison to the untreated trees. Tamm *et al*. (2006) also recorded an increase in lenticel spotting using Armicarb in Switzerland. Considering as marketable fruits only those without or with small lenticel spots (classes 0 and 1), only 4.4% of the Armicarb-treated apples could have been sold *versus* 65.6% in the control, 80% in the Armicarb + sulphur, 92.2% in the lime sulphur and 95.6% in the Myco-San treatments (data not shown).

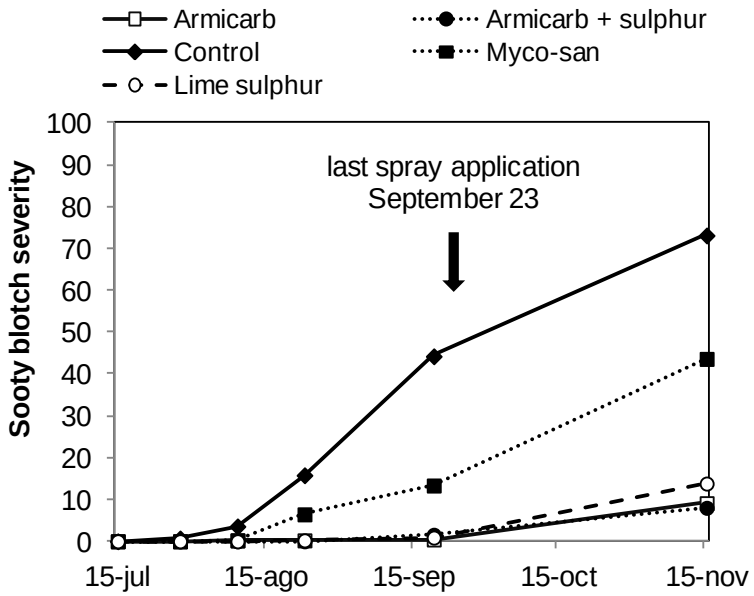


Figure 4: Treatment effect on the temporal progress of sooty blotch severity.

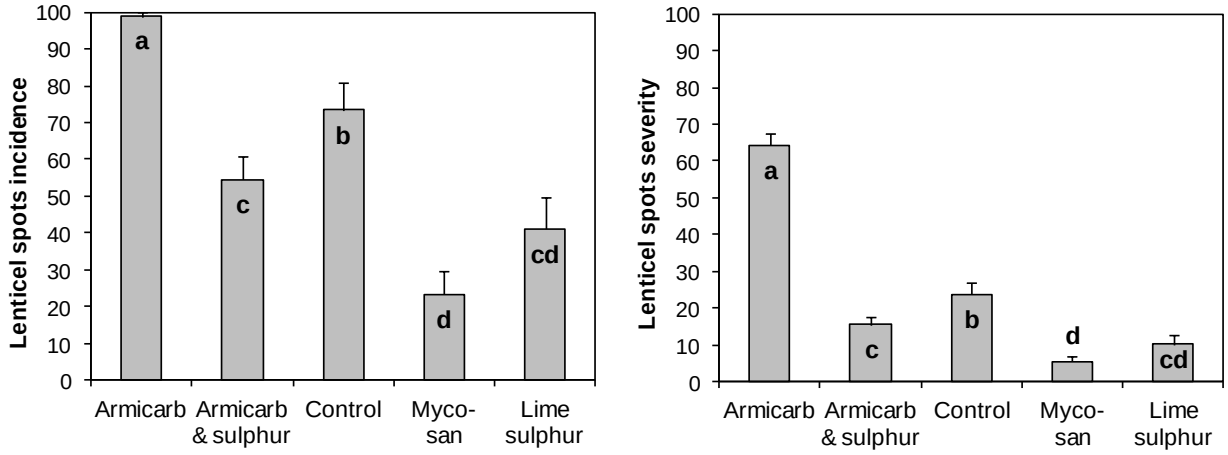


Figure 5: Treatment effect on the incidence and severity of lenticel spotting (mean + SE is shown; columns with the same letter are not significantly different).

Moreover, a reddening in foliage was observed from mid September in some treatments, mainly in Armicarb-treated and in untreated trees. The increase of both lenticell spotting and leaf-reddening may be related to some extent to a toxic effect of Armicarb and may explain the lower fruit weight and total yield observed in Armicarb plots (Figure 6), which may be mediated by a reduction in the photosynthetic capability of trees. Therefore, the use of Armicarb may not be generally recommended before testing the effect of other doses in this and other cultivars. Further research is also required to reduce the number of spray applications.

This first approach to the SBFS status in Asturias allowed us to know the importance of the disease and the cultivar susceptibility in our conditions. Further research is needed to develop a forecasting model and to use fungicides more efficiently.

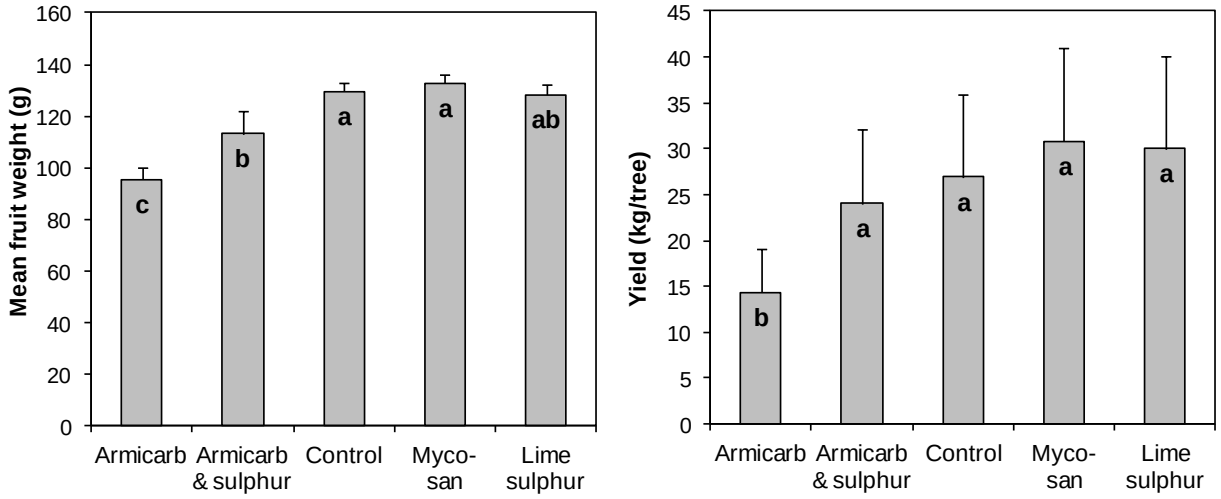


Figure 6: Treatment effect on fruit weight and yield (mean + SE is shown; columns with the same letter are not significantly different).

## Acknowledgements

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# Disease development of sooty blotch and its correlation to wetness hours

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**Abstract**

*Since 2005 research has been done on the biology of the sooty blotch complex at the Research station for fruit growing (Kompetenzzentrum Obstbau Bodensee) at Lake Constance area. In several trials, for example bagging trials, disinfection of apples on the trees and monitoring of disease development over 7 years, we find a correlation between disease development and leaf wetness. The appearance of the first symptoms in the field but also the further development of infestation seems to be linked with a certain number of wetness hours. Thereby the development stages of the fruits seem to have no influence on the appearance of sooty blotch symptoms.*

**Keywords:** sooty blotch, disease development, bagging experiments, wetness hours

## Introduction

During the past years sooty blotch disease received a strong proliferation particularly in scab resistant varieties due to the diminished fungicide treatments. Especially in regions with a high amount of rain fall and humidity like the Lake Constance area, frequent summer treatments become necessary to avoid substantial losses of crops. Knowledge about the specific pathogens of the sooty blotch complex is limited. Although results of the research from North America are available, the transfer to German apple production is limited. Suitable knowledge about the identity of the local pathogens as well as their physiology relevant for infection is lacking. Since 2006 research on the infection biology of the sooty blotch complex has been done at the KOB Bavendorf. Results of bagging trials with focus on infection periods, efficiency of different plant protection strategies or susceptibility of different varieties were already presented within the scope of this conference (Mayr *et al*., 2008, Mayr *et al.,* 2010). Based on a deeper understanding of the infection biology under western European conditions, the control of sooty blotch should be optimized in future by focusing on definable key events in the infection cycle. To reach this aim it is necessary to be able to describe several parameters which are relevant for infection, i.e. incubation period, more detailed. By using different methods, e.g. bagging trials, disinfection of fruit surface and monitoring of the infestation development, we could show that the duration of incubation as well as the further infestation development is influenced directly by the duration of wetness.

## Material and Methods

To examine the influence of wetness on the first symptom expression and the further infestation development, different methods which are described below were applied. Infestation was assessed by using a six class scheme (0= without, 1= small spots 2= up to 10%, 3= 10-25%, 4= 25-50%, 5= >50% of the surface with symptoms).

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Disease severity can be calculated as an index using the following formula which takes into account the number of apples in each disease severity class.

$$P = \Sigma (n*v) / (5*N) *100$$

(P= disease severity index (%), N= total number of fruits, v = numerical value of the severity class: 0,1,2,3,4, 5, n: number of fruits in each severity class)

The weather data were determined with a weather station from the company “Thies”. For recording leaf wetness a sensor measuring the electric conductivity of a hemp string was used. The leaf wetness sensor was positioned inside the tree.

*1. Monitoring of the disease development*

To examine the disease development over the years, untreated ‘*Topaz*’ apple trees were regularly monitored with regard to date of first symptoms and the further development of sooty blotch spots. Two times per week, beginning from the end of June, 250 marked and numbered apples were assessed for symptoms and classified in 6 severity categories. To establish a relationship between disease and fruit development as well as climatic parameters, phenological development and weather conditions were recorded. This disease survey has been carried out since 2005 and allows for seasonal comparisons of disease development.

To examine the correlation between fruit development stage and the appearance of first symptoms, starting with a fruit diameter of 25 mm, weekly thirty fruits of untreated ‘*Topaz*’ trees were picked and laid out on a grass plain apart from apple trees to reach a higher amount of wetness. In contrast to incubation trials carried out in the years before under controlled conditions in incubator and moist chamber, this method led to the desired symptom expression.

*2. Bagging trials*

To find out when infections occur during the season, 8 x 30 fruits were put separate into waterproof bags starting middle of May with a fruit diameter of 25 mm. This allows a temporary protection against rain and infections. Subsequently the apples in the several experimental units were bagged out weekly. The infestation was assessed on the 9$^{th}$ of August and at harvest time in September by using the described assessment scheme.

In the opposite direction, in each case 30 apples were put into bags in a weekly interval starting middle of May. The apples remained in the bags until harvest in the middle of September. After harvest the bags were removed and the infestation on the apples was assessed. Subsequently the apples were laid out on a grass plain over a period of 4 weeks.

*3. Disinfection of the fruit surface in the field*

For each experimental unit 30 apples were chosen and marked in untreated trees of the variety ‘*Topaz*’. On the 22$^{nd}$ of June, before the appearance of the first symptoms in the field, the surface of the marked fruits was disinfected by dipping the fruits into alcohol and sodium hypochloride. Thereby we used the following dipping sequence: alcohol (70%) – sodium hypochloride – alcohol (70%) – distilled water. The fruits were dipped 15 sec. into each solution. Fruits of the control unit were dipped only into distilled water. Disease infestation was assessed every 14 days starting on the 21$^{st}$ of July.

## Results

*1. Monitoring of the disease development*

In the last years we find a trend in the correlation between the date first symptoms occurred and the sum of leaf wetness hours between petal fall and first symptoms, according to the method of Brown & Sutton (1995). After this method all moist periods beginning from the first rain 10 days after petal fall with a duration of more than 4 hours were accumulated. During the past 7 years a sum of 240 to 285 wetness hours was necessary for the appearance of the first symptoms in the field. These values are very similar to the amount calculated by Sutton for Northern America (Sutton *et. al*., 2002).

Table 1: Summary of temporal appearance of different growth stages and sooty blotch symptoms as well as the corresponding accumulated wetness hours in the years 2005-2011 at KOB, Bavendorf.

| **Parameter** | **2005** | **2006** | **2007** | **2008** | **2009** | **2010** | **2011** |
|---|---|---|---|---|---|---|---|
| Full bloom | 30.04. | 06.05. | 18.04. | 02.05. | 25.04. | 29.04. | 19.04. |
| “Petal fall" | 09.05. | 16.05. | 27.04. | 08.05. | 04.05. | 12.05. | 24.04. |
| T-stage | 18.06. | 28.06. | 31.05. | 14.06. | 27.05. | 17.06. | 03.06. |
| Harvest time | 04.10. | 27.09. | 13.09. | 18.09. | 22.09. | 24.09. | 20.09. |
| **First symptoms of sooty blotch** | **20.07.** | **05.08.** | **20.06.** | **15.07.** | **07.07.** | **22.07.** | **05.07.** |
| All apples with symptoms | 08.09. | 19.09. | 31.07. | 05.09. | 24.08. | 26.08. | 05.08. |
| Number of days between full bloom and first symptoms | 81 | 91 | 63 | 74 | 73 | 84 | 77 |
| **Accumulated wetness hours (till first symptoms)** | **278** | **285** | **241** | **205** | **240** | **287** | **275** |

The observations allow the conclusion that the appearance of the first symptoms is primarily correlated with the wetness duration. The annual weather conditions as well as the phenological development stages of the fruits seem to play a minor part. If young fruits are transferred into a more humid environment, the incubation time can be shortened compared to the natural development in the tree. Exemplarily for the years 2009-2011 the results from the actual experiment year are shown in table 2.

The fruits which were laid on the grass weekly, starting from the 19$^{th}$ of May with a fruit diameter of 25 mm, developed symptoms much earlier than the fruits that remained in the trees. Thus the fruits laid out on the 3$^{rd}$ of June showed the first symptoms already on the 17$^{th}$ of June, 19 days before the appearance of the first symptoms in the control trees (5$^{th}$ of July). The more wetness hours the apples reached on the tree before laying out on the grass, the faster the symptoms developed under the more humid conditions.

The symptoms appeared in each case on the part of the fruit which had contact to the ground, no matter with which side they were laid on the grass. The incubation duration could be clearly shortened by transferring the apples into a more humid environment. In addition, it could be shown that infections are already possible before the fruit reaches “T-stage” and thus also before the wax layer on the fruit surface is fully developed.

Table 2: Disease severity index of apples laid out on grass at different points in time.

| Nr. | laid out in grass | leaf wetness hours | disease severity index P (%) | | | | | | | | days till symptoms |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 10.6. | 17.6. | 24.6. | 1.7. | 8.7. | 15.7. | 21.7. | 29.7. | |
| 1 | 19.05. | 41 | 0,0 | 0,0 | 7,0 | 32,0 | | 40,0 | | | 37 |
| 2 | 26.05. | 67 | 0,0 | 0,0 | 12,1 | 24,1 | 39,3 | 42,2 | 62,2 | 62,9 | 30 |
| 3 | 03.06. | 120 | 0,0 | 2,1 | 11,0 | 31,0 | 37,9 | 47,6 | 60,7 | 58,6 | 14 |
| 4 | 10.06. | 159 | 0,0 | 0,0 | 4,7 | 17,2 | 27,9 | 37,2 | 45,3 | 45,3 | 14 |
| 5 | 17.06. | 204 | | 0,0 | 2,0 | 19,3 | 43,3 | 59,3 | 53,5 | 63,3 | 8 |
| 6 | 24.06. | 255 | | | 0,0 | 5,9 | 24,0 | 44,1 | 51,7 | 67,3 | 8 |
| 7 | 01.07. | 274 | | | | 0,0 | 7,6 | 33,1 | 57,2 | 79,3 | 8 |
| 8 | 08.07. | 288 | | | | | 0,0 | 33,3 | 60,0 | 87,6 | 8 |

*2. Bagging trials*

In the bagging trials, a correlation between wetness duration and the appearance of symptoms could also be observed. On the apples which were temporarily protected against spores and rain by bags, the first symptoms appeared after bagging out also after more than 200 wetness hours. Apples of treatment “I”, which were bagged out on the 15$^{th}$ of July, still showed no symptoms on the 9$^{th}$ of August after 202 accumulated wetness hours (table 3). However, apples of treatment “H”, which have been bagged out one week earlier and reached a total of 272 wetness hours till assessment date, showed first symptoms. Furthermore, the disease severity index (P%) of the apples bagged out at different dates increased nearly linearly to the sum of wetness hours accumulated till assessment date.

Table 3: Disease severity index (P%) of different bagging variants on the 9$^{th}$ of August 2011.

| No. | bagging period | P (%) on August 9 | leaf wetness hours |
|---|---|---|---|
| A | 13.05.-19.05. | 75,6 | 519 |
| B | 13.05.-26.05. | 75,2 | 493 |
| C | 13.05.-03.06. | 60,0 | 440 |
| D | 13.05.-10.06. | 44,0 | 401 |
| E | 13.05.-17.06. | 23,0 | 356 |
| F | 13.05.-24.06. | 16,2 | 305 |
| G | 13.05.-01.07. | 8,5 | 286 |
| H | 13.05.-08.07. | 2,3 | 272 |
| I | 13.05.-15.07. | 0,0 | 202 |

In contrast to the monitoring, the apples of the single bagging treatments were directly surrounded by already infested (unbagged) apples after bagging out. At the time when the apples of variation “I” were bagged out, a disease severity index (P%) of 20% was assessed on the unbagged apples which increased to 70% until the first assessment date on 9th of August. Even with high inoculum in immediate vicinity it seems as if a certain wetness duration was necessary for incubation.

In the counter-rotating trial beginning middle of May apples without visible symptoms were bagged continuously in a weekly chronology. The apples of the 8 bagging periods remained in bags till harvest. Bagging periods between 118 and 68 days were given. At harvest time apples were taken out of bags and were transferred in the humid environment in the grass. Symptom development could be prevented by avoiding wetness temporarily with bags. Only after reentry into humid conditions visible sooty blotch symptoms appeared. Figure 1 shows the disease severity index (P%) of the fruits 17 days after they have been laid out on the grass. The more wetness hours were already reached before bagging the apples, the higher was the disease severity index (P%) after laying out on the grass.

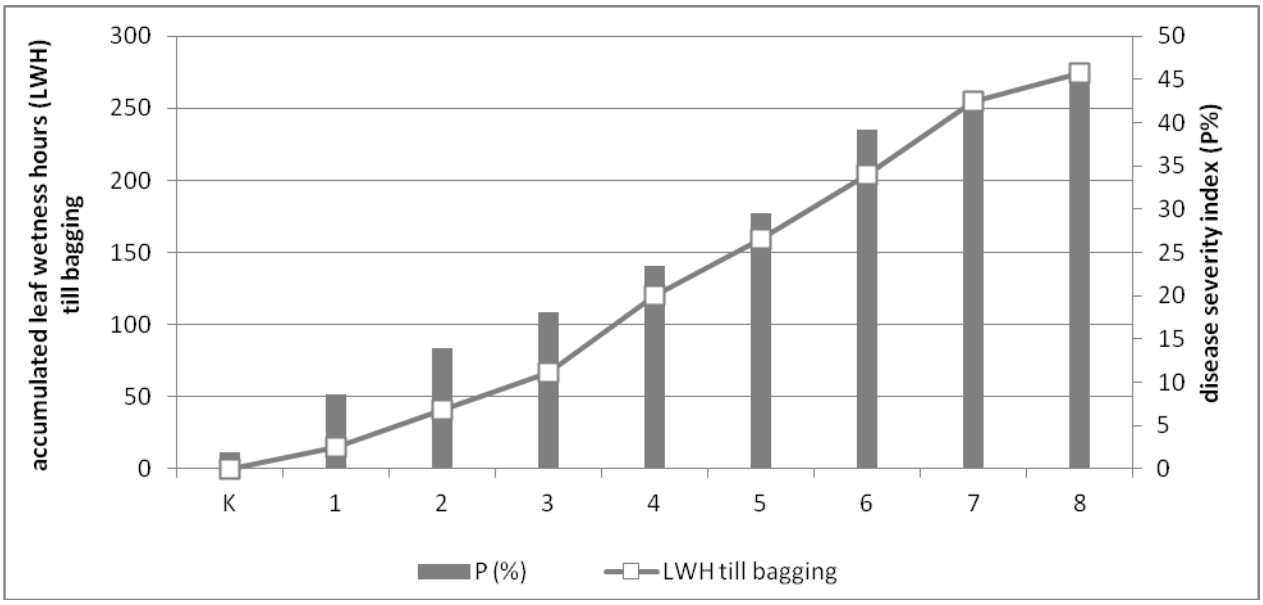


Figure 1: Disease severity index (P%) of different bagging variants after 17 days on the grass.

*3. Disinfection of fruits*

The apples which have been surface-disinfected on the tree on the 22nd of June showed a clearly lower disease severity index than the control apples on the assessment dates 21st of July, 1st of August and 15th of August (fig. 2). On the 21st of July and after an accumulated sum of 170 wetness hours, the disinfected fruits still showed no symptoms (fig. 3). At this time a severity index of 28,7% was assessed in the control apples. The first visible spots on the disinfected apples were observed on 1st of August, 40 days and 240 accumulated wetness hours after disinfection date. A disease severity index (P%) of 2,8% was assessed on this date. 13% of the apples showed first symptoms (severity class 1), 87% were still free of symptoms. At this time the untreated control already had a severity index of 49,3%.

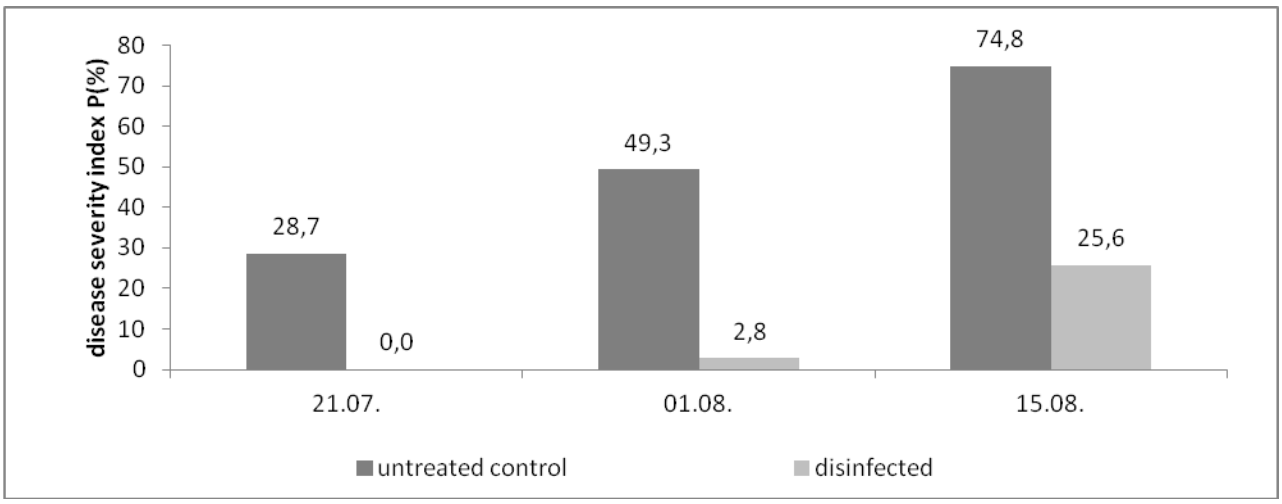


Figure 2: increase of the disease severity index (P%) of disinfected and untreated apples between 21st of July and 15th of August.

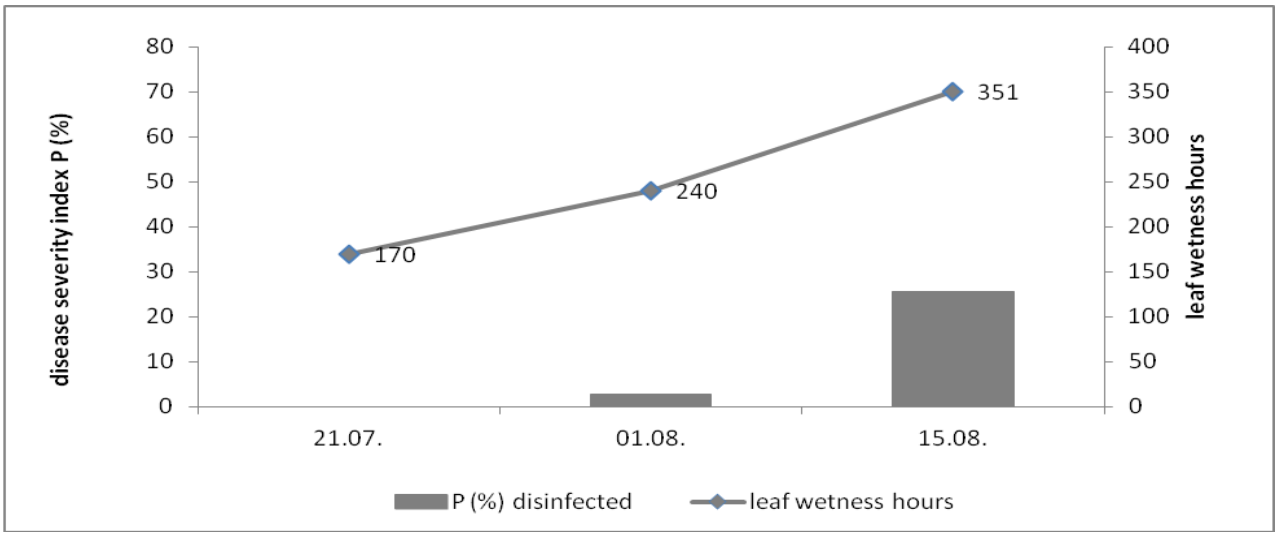


Figure 3: development of leaf wetness hours and disease severity index (P%) of disinfected apples between 21st of July and 15th of August.

## Discussion

Working with wetness hours, the methodology of the leaf wetness measurement plays a central role. A calibration of leaf wetness sensors is not possible due to a lack of appropriate norms. Thus, the presented threshold values for leaf wetness can only be seen in relation with the leaf wetness sensor used in our investigations. By using different leaf wetness sensors and divergent positioning of the sensors these values can deviate due to different methodology. By continuous use of the same leaf wetness sensors and positioning over the years the results have been reproduced in our trials. The following conclusions can be derived from our investigations:

For symptom development a certain sum of wetness hours seems to be necessary. In the Lake Constance area during the past 7 years a range of 240 – 285 accumulated wetness hours according to the method of Brown & Sutton (1995) was necessary for the appearance of the first symptoms in the field. Even if a high inoculum already exists in the orchard, a comparable threshold value seems to be necessary for the incubation. The earlier the wetness duration which is necessary for the incubation was achieved, the

earlier the first symptoms appeared. If humidity is prevented, the symptom development can be inhibited or delayed. Also after time periods of more than 110 days without wetness, symptoms develop after reentry into humid conditions within a few days and even apart from apple orchards. Under humid conditions symptoms appear even on young fruits with a diameter < 25 mm. Thus, the development stage of the fruits does not seem to play a role for symptom appearance. In the last years it was supposed that the T-stage might play a role in the pathogens biology as from around this stage a process known as 'leaching' starts when soluble substances are released out through the cuticle. Sooty blotch pathogens live only on the cuticula surface and feed on the substances released from the fruit. Thus the leaching process was supposed to be linked with the disease infection cycle.

**Acknowledgements**

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# Efficacy evaluation of plant protection products for Alternaria blotch (*Alternaria spp*) control in organic apple production

M. Kelderer[1], E. Lardschneider[1], N. Bellutti[1]

**Abstract**

*An increased occurrence of the fungal disease* Alternaria *blotch of apple, sometimes resulting in severe production losses, has been observed in South Tyrol in recent years. In integrated production, up to now the disease could be effectively contained with the fungicides currently available on the market. The studies conducted in 2010 and 2011 aimed at evaluating the efficacy of several plant protection products allowed in organic farming against* Alternaria *blotch of apple. Lime sulphur and acid clay were tested in both 2010 and 2011. Potassium bicarbonate was tested only in 2010, while algae extract and copper sulphate were tested only in 2011. Up to now, satisfactory results were obtained only with the acid clay-based product Ulmasud.*

**Keywords:** Apple*,* Alternaria, Acid clay, Ulmasud, Lime sulphur

## Introduction

The fungal species belonging to the genus Alternaria are pathogenic fungi affecting plants of worldwide distribution (Jones *et al*., 1997). Several different cultivated crops are affected. Especially hazardous for fruit growing, and thus also for the fruit growing area South Tyrol, is the species *Alternaria alternata* apple pathotype (*Alternaria mali* Roberts). In the orchards in South Tyrol, the first records of this disease occurred at the beginning of the 1990-ies. Initially the disease appeared only in isolated orchards, but soon it spread over the entire area. Infections are most severe on the cultivars Gala, Golden Delicious, and Crips Pink, and occasionally also Granny Smith can be affected (Marschall *et al*., 2004).

*A. alternata* is commonly regarded as a weakness parasite. It develops and overwinters saprophytically on non-living organic matter and in soil, and spreads by forming numerous spores producing host-specific toxins during germination, which are responsible of its pathogenicity.

Affected fruits exhibit brown or black circular, pinpoint-sized spots, frequently with brown or red borders, on lenticels. Affected leaves exhibit brown spots that later enlarge and finally result in leaf drop. First symptoms of infection usually appear in spring on leaves and/or fruits, from end of flowering to mid June. Under conditions of warm temperatures and adequate humidity, damage increases over time up to harvest (Marschall *et al.,* 2006).

In integrated production systems, acceptable levels of control can be achieved with the fungicides currently available on the market. In organic production, instead, studies on adequate and effective control strategies are still in progress.

## Material and methods

The studies on the efficacy evaluation of plant protection products for Alternaria blotch control in organic production were conducted in 2010 and 2011. The trials were conducted in an apple cultivar Golden Delicious orchard (rootstock: M9) located in Auer (South Tyrol, Italy). The study orchard had been planted in 2000. In addition to the treatments for Alternaria blotch control, all treatments commonly applied in organic orchards were carried out during the primary season (from June to September), while no additional fungicide

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sprays were applied during the secondary season. The active ingredients of the formulated products tested in 2010 and 2011, product names, distributors, and tested field rates are reported in Table 1 and 2.

Table 1: Description of the plant protection products tested in 2010

| Active ingredient | Product name | Distributor | Applied rate (/100 l) |
|---|---|---|---|
| Lime sulphur | Polisolfuro di Calcio | Polisenio | 1 kg, after July 0,8 kg |
| Acid clay | Ulmasud | Biofa | 1 kg |
| Potassium bicarbonate | Vitisan | Biofa | 1 kg |
| Untreated Control | - | - | - |

Table 2: Description of the plant protection products tested in 2011

| Active ingredient | Product name | Distributor | Applied rate (/100 l) |
|---|---|---|---|
| Lime sulphur | Polisolfuro di Calcio | Polisenio | 1 kg, after July 0,8 kg |
| Acid clay | Ulmasud | Biofa | 1 kg |
| Alga extract | RB1 | ICAS | 400 ml |
| Copper sulphate (20%) | Poltiglia Disperss | Cerexagri | 100 g |
| Untreated Control | - | - | - |

In both study years, a randomized block design with 4 replicates of 10 trees each per treatment was used. To prevent biasing of data due to border effects, each experimental plot was shielded from the other plots by additional trees and rows. Treatments were applied using a motorized sprayer for experimental trials and a spray volume of 1500 l/ha. All treatments were applied at weekly time intervals from June to September in 2010 and from June to mid August in 2011.

In both study years, assessments on Alternaria blotch were conducted at the end of August by counting the number of infected fruits and leaves on respectively ca. 200 fruits and 40 shoots. The percentage of infected fruits and leaves was then calculated.

The percentages of infected fruits and leaves recorded in the two study years were compared across treatments using 1-way ANOVAs, followed by Tukey's test for post-hoc comparisons of means.

## Results

In 2010, 25% of fruits were infected by *Alternaria* blotch in the untreated control. Ulmasud considerably reduced the percentage of infected fruits (10%), but differences among treatments failed significance due to high variability among plots (Table 3). In 2011, the percentage of diseased fruits in the untreated control amounted to 14%, and was significantly lower in plots treated with Ulmasud (1.4%) than in untreated control plots (Table 5).

In 2010, the percentage of infected leaves was significantly lower in plots treated with lime sulphur and Ulmasud than in untreated control plots (Table 4). The efficacy of Ulmasud in reducing percent leaf infection was confirmed also in 2011 (Table 6): in the plots treated with the acid clay-based product, 57% of leaves showed disease symptoms, while leaf infection was highest in the plots treated with the RB1-based product (81%).

Trial 2010

Table 3: Percentage of fruits infected by Alternaria blotch in the different treatments in 2010

| Treatment | % affected fruits | N | Std. Deviation | Std. Error of Mean | Tukey HSD* |
|---|---|---|---|---|---|
| Lime sulphur | 25.2 | 4 | 11.0 | 5.5 | a |
| Ulmasud | 10.0 | 4 | 6.5 | 3.3 | a |
| Vitisan | 23.4 | 4 | 4.7 | 2.4 | a |
| Untreated control | 25.2 | 4 | 5.7 | 2.8 | a |

* Different letters indicate statistically significant differences (Tukey HSD test: P=0.05)

Table 4: Percentage of leaves infected by Alternaria blotch in the different treatments in 2010

| Treatment | % affected leaves | N | Std. Deviation | Std. Error of Mean | Tukey HSD* |
|---|---|---|---|---|---|
| Lime sulphur | 93.5 | 4 | 3.2 | 1.6 | a |
| Ulmasud | 93.2 | 4 | 3.7 | 1.8 | a |
| Vitisan | 99.0 | 4 | .1 | .0 | b |
| Untreated control | 98.9 | 4 | .9 | .5 | b |

* Different letters indicate statistically significant differences (Tukey HSD test: P=0.05)

Trial 2011

Table 5: Percentage of fruits infected by Alternaria blotch in the different treatments in 2011

| Treatment | % affected fruits | N | Std. Deviation | Std. Error of Mean | Tukey HSD* |
|---|---|---|---|---|---|
| Lime sulphur | 11.0 | 4 | 4.8 | 2.4 | ab |
| Ulmasud | 1.4 | 4 | 1.8 | .9 | a |
| RB1 | 9.5 | 4 | 4.7 | 2.4 | ab |
| Copper | 5.9 | 4 | 4.0 | 2.0 | ab |
| Untreated control | 13.9 | 4 | 6.2 | 3.1 | b |

* Different letters indicate statistically significant differences (Tukey HSD test: P=0.05)

Table 6: Percentage of leaves infected by Alternaria blotch in the different treatments in 2011

| Treatment | % affected leaves | N | Std. Deviation | Std. Error of Mean | Tukey HSD* |
|---|---|---|---|---|---|
| Lime sulphur | 67.3 | 4 | 9.5 | 4.8 | ab |
| Ulmasud | 56.9 | 4 | 10.1 | 5.0 | a |
| RB1 | 81.0 | 4 | 4.7 | 2.3 | b |
| Copper | 72.9 | 4 | 9.6 | 4.8 | ab |
| Untreated control | 71.0 | 4 | 10.9 | 5.5 | ab |

* Different letters indicate statistically significant differences (Tukey HSD test: P=0.05)

## Discussion

Alternaria blotch is considered one of the major problems on apple in South Tyrol. In recent years, in addition to infection on leaves, disease symptoms appeared with increased frequency also on fruits, often resulting in up to 100% fruit damage.

In the trials conducted in 2010 and 2011, the fungicides most commonly used in organic farming, that is lime sulphur, copper, potassium bicarbonate and acid clay, were tested for Alternaria blotch control. The products were applied during summer, in some cases by using high field rates and application frequencies. The acid clay-based product Ulmasud was the only fungicide showing acceptable levels of disease control, with a mean efficacy according to Abbott (1925) on fruits of approximately 60% in 2010 and 90% in 2011.

Additional studies are needed to evaluate the efficacy of copper, because in the trials herein reported this active substance has been tested at a relatively low field rate (20 g copper/100 l). In previous studies conducted on pear in Emilia Romagna (Italy), copper applied at higher field rates provided extremely promising control of *Stemphylium vesicarium* (Antoniacci *et al.,* 2006), a fungus with a disease progression very similar to that of Alternaria blotch.

In addition to the evaluation of new biological plant protection products, future studies focusing also on the efficacy of sanitary measures for the control of Alternaria blotch, are warranted.

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# Monitoring of pear rust (*Gymnosporangium sabinae*) in Austria and implications for possible control strategies

M. Filipp[1], A. Spornberger[1] and B. Schildberger[2]

**Abstract**

*In recent years pear rust* (Gymnosporangium sabinae*) caused serious damages in organically managed pear orchards, on different sites in Eastern Austria, especially in the surroundings of St. Pölten. Therefore the appearance and development of the fungus was monitored over three years 2009-2011 in this area. The infection period and phase of spore discharge were estimated with a spore trap and with observations of symptoms on potted pear seedlings. The results of this monitoring campaign showed a moderate infestation level in pear orchards over the three years with low damage on fruits. In all three years, the main infection period was found to be from end of April to early May. Light infections were observed also from mid of April until the end of May. Later spores were flying until mid of June but did not lead to infections. In an organically managed orchard a reduction of the infestation dependent on the distance to an infected host plant and on treatments with fungicides used in organic growing could be found.*

**Keywords:** pear rust, *Gymnosporangium sabinae,* organic farming.

## Introduction

In 2007 and especially in 2008, pear rust (*Gymnosporangium sabinae)* caused severe damage on leaves, fruits and twigs of pears in many orchards in the area of St. Pölten. In this area most of the organically managed pear orchards in Austria were planted during the last 8 years.

*G. sabinae* is a host changing fungus which is overwintering on twigs of some cedar species *(Juniperus sabina, J. chinensis, J. x media*). It is infecting pear trees in spring, mostly on leaves and less frequent also on twigs and fruits. Infections from pear to pear are not possible, whereas infections on cedars can reappear every year. Spring precipitation cause a swelling of telial horns on *Juniperus sp*.; in this process the teliospores are germinating and release basidiospores which are transported by wind to pear leaves. Clearing of cedars in the neighbourhood of pear orchards is a well-known method to reduce the infection pressure (Hilber & Siegfried, 1990). However, not in all cases the source (i.e., the infected cedars) can be found, and also quite frequently the owners of the cedars are not willing to cut the plants. Till now there is only few data available on the spreading distance of pear rust in literature, and estimates are reaching from a few hundred meters up to a few kilometres. The effect of fungicides used in organic growing like copper, (lime) sulphur or potassium bicarbonate pear rust is not precisely understood so far and subject of continuous research.

This paper is reporting on the effort to bridge this gap reporting on lessons learned from a detailed field campaign investigating biology, development and possibilities of control of the host changing fungus in 2009-2011.

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## Material and Methods

From 2009 to 2011, the occurrence of pear rust symptoms was monitored in 19 pear orchards in the surroundings of St. Pölten at the end of August. In each orchard the occurrence frequency and the leaf position of pear rust symptoms was recorded on 10 long shoots and 10 short shoots of each cultivar ('Uta' and 'Conference'). At the same time the farmers filed a survey on how serious they see the damage in the actual and the previous year. The survey comprised 5 categories, reaching from 0 (= no damage) to 4 (4=very serious).

A Burkard 7-day recording spore trap was set up in 3 m distance from an infected *Juniperus sabina* in Klosterneuburg, Haschhof. The recording was done from April, 21st to June, 30th in 2010 and from April, 5th to June, 12th in 2011. The sum of collected spores was counted on daily basis.

In spring 2010 and 2011, for each week three (in 2010) or two (in 2011) small potted seedlings of *Pyrus communis 'Bartlett'* were placed outdoor to monitor natural infections. The remaining time those were placed indoors protected from rain and infectious spores. In 2010, the trees were placed in the garden of the University of Natural Resources and Life Sciences Vienna where the next known infected cedar was within about 150 m distance. In 2011, the seedlings were put at Klosterneuburg, Haschhof next to the spore trap and an infected *J. sabina*. The pear seedlings were placed outdoor from 3 Mai 2010 to 26 June 2010 and 12 April to 6 June 2011. The amount of infestations was counted either at the whole tree (2011) or at each tree at 1 to 3 long shoots (2010).

In the years 2010 and 2011 the infestation development on pear trees was also monitored in Vienna (10 shoots – 2010) and St. Pölten (5 shoots – 2011). This data was analysed on dependence with the occurrence and amount of precipitation. On each twig the sum of leaves and the sum and position of pear rust symptoms was investigated once a week.

In an orchard in St. Georgen the influence of the distance (50m, 110m and 170m) between pear trees and an infected host plant (*Juniperus sabina*) was examined. In each distance five pear trees of the cultivar 'Uta' were assessed, infections on two short shoots and two longshots were counted at each tree. Additionally to these three variants with fungicide applications a control variant without fungicide application located in 50m distance to the infected host plant was monitored in the same way.

## Results

Monitoring of symptoms on pears on the organically managed farms

The results of the three year field campaign (2009-2011) on 19 farms showed a moderate infestation level with symptoms mainly located on leaves and rarely on twigs. The farmers participating in the survey estimated the infestation level in the years 2009 to 2011 between light and medium, in comparison they conceived the infections in the year 2008 as very severe. (data not shown).

The largest number of infestations was found in 2010 on short shoots and long shots (25.72 and 11.05 per 100 leaves, respectively, see Table 1). In general the frequency of infestations symptoms was always higher on short shoots than on long shoots. Inter-cultivar comparison showed that the cultivar 'Uta' often had fewer infestations than the cultivar 'Conference'. However the statistical analysis of the data showed that significant difference between the cultivars was only found for 2010 on long shoots ($p=0.035$; independent sample T-Test).

Table 1: Average infestation level with *G. sabinae* on pear leaves in the observed orchards (N=19) in the area of St.Pölten

| | | Symptoms/100 leaves | | |
|---|---|---|---|---|
| | | Uta | Conference | Mean |
| 2009 | long shoots | 5.39 | 6.20 | 5.73 |
| 2010 | long shoots | 9.41 | 13.3 | 11.05 |
| 2011 | long shoots | 5.98 | 7.32 | 6.60 |
| 2010 | short shoots | 28.27 | 23.03 | 25.72 |
| 2011 | short shoots | 11.5 | 14.31 | 12.81 |

Temporal development of infections in 2009-2011

2009 was a quite early year regarding the vegetative development in spring and no precipitation occurred from budding to flowering. The first springtime rainfall occurred at the end of April (29th and 30th April) and caused first infections. Pear rust symptoms appeared in this year especially on the 7th to 9th developed leaf on long shoots.

In 2010, first infection symptoms appeared on the 10th of May (Figure 1). As the incubation time of *G. sabinae* is about 3 to 4 weeks (Hilber & Siegfried, 2003), the infection can be related to the rainfall events of April 12th and 15th or the later event of the 20th of April. Our data shows that the main infection period was between early and mid of May when also the highest number of spores could be monitored in the spore trap at Klosterneuburg (Figure 2). Most symptoms occurred at the end of May, and only a small number of new symptoms could be counted in June. From July onwards no new pear rust spots occurred. However, spores were discharged until 21st of June. Infections especially happened on the 3rd to 6th leaf of long shoots. Pear rust symptoms appeared in 2010 on earlier built leaves then in 2009.

The alternately outdoor placed pear seedlings got infected only in the first two settings (3rd Mai to 17th May). The later exposed trees had already stopped growing, and no symptoms did appear (data not shown).

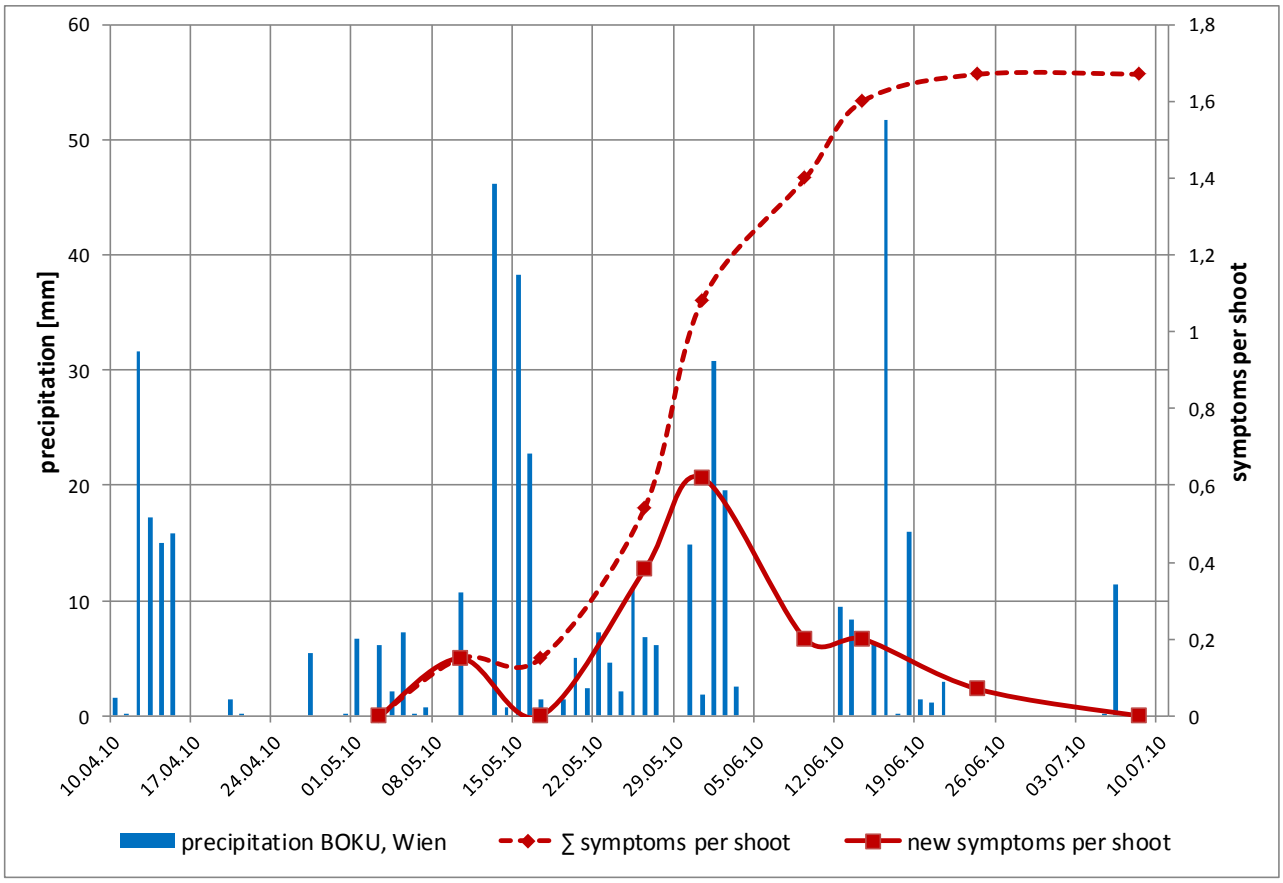


Figure 1: Precipitation amount and infestation development of *G. sabinae* on long shoots on two pear trees (*Pyrus communis, P. pyraster*) in 2010 – Boku, Vienna

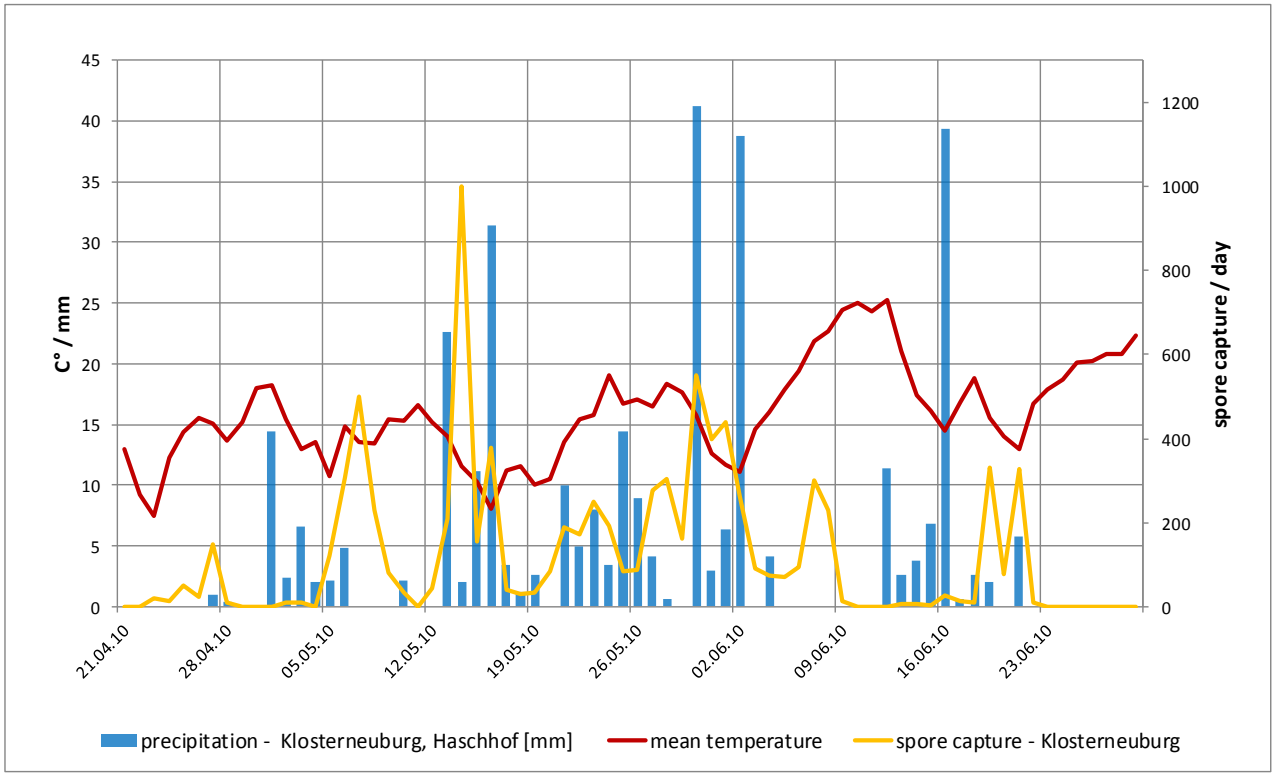


Figure 2: Precipitation amount and discharge of spores of *G. sabinae* on a spore trap in 2010 – Haschhof, Klosterneuburg

In 2011, rainfall events between the 12th and 15th of April didn't led to spore discharge. Expectedly no symptoms could be seen 4 weeks later (Figure 4, 5). For the first time a few spores were observed at the 19th of April. Later rainfall events that occurred at the end of April led to a massive spore discharge (2593 spores – 26.4.2011) and in consequence a large number of new infection symptoms could be monitored between mid and end of May. Also on the potted seedlings an enormous amount of pear rust spots was developed in this week of precipitation (data not shown). These rainfall events also caused an increase of growing and many new susceptible leaves were built. A second rain period in mid of May can be related to new infection symptoms about four weeks later, however not the infection is not accounted as very serious. As in the orchard also the potted trees showed only few symptoms after this event (see Figure 3).

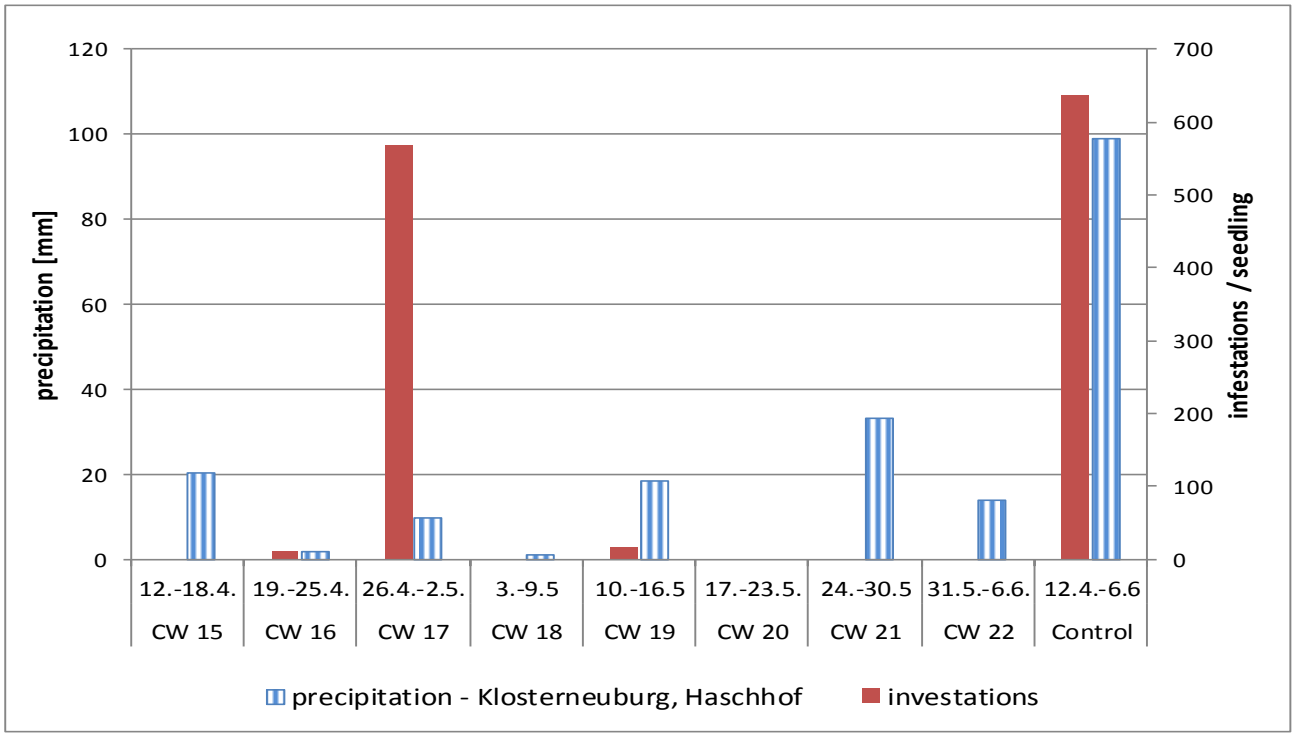


Figure 3: Precipitation amount and infestations on pear seedling in 2011 – Haschhof, Klosterneuburg

Unfortunately due to technical problems with the spore trap details on the sporulation in this time period is not available for the analysis. Later this year a small number of spores were discharged in the first days of June. From the 17th of June onwards no new spots appeared on the monitored shoots.

In this year most symptoms were formed on the 2nd, 4th and 5th leaves (data not shown).

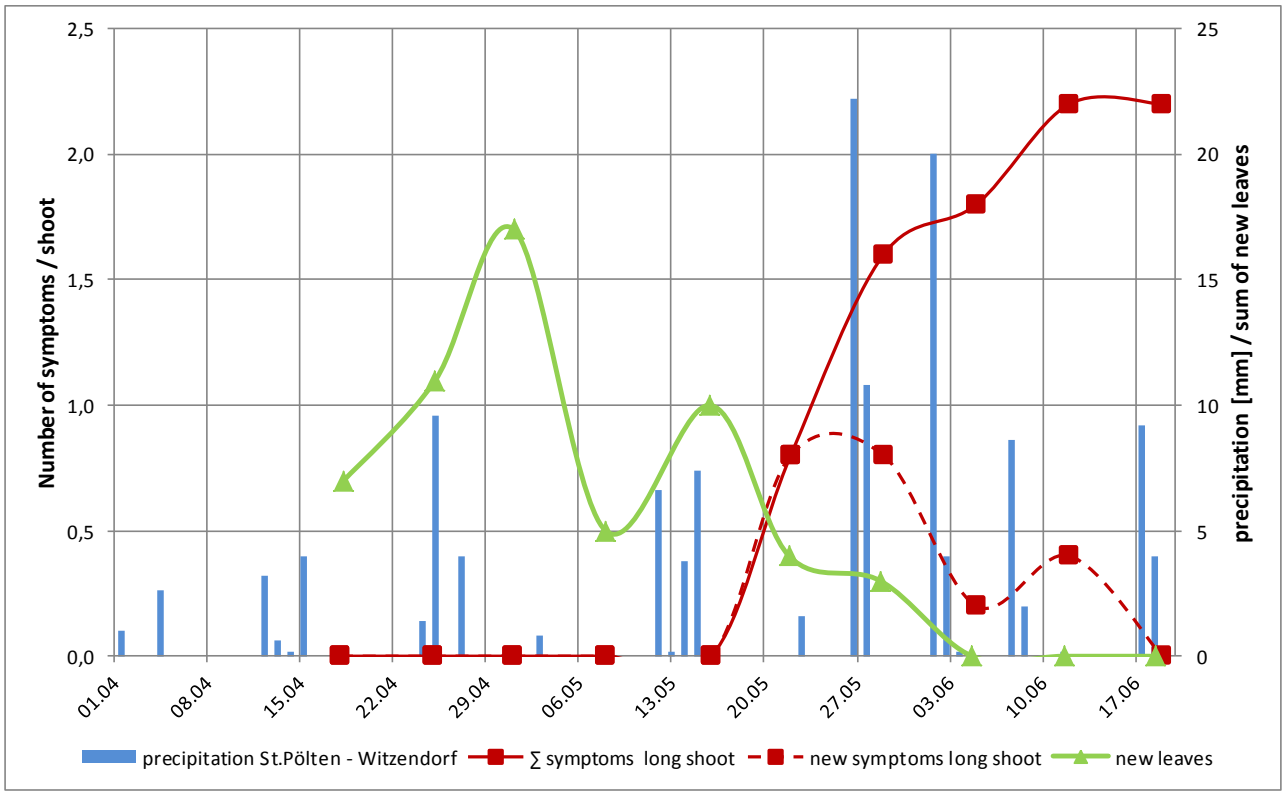


Figure 4: Precipitation amount and infestation development of *G. sabinae* on long shoots on pear trees (cv. 'Uta') in 2011 – Seeben

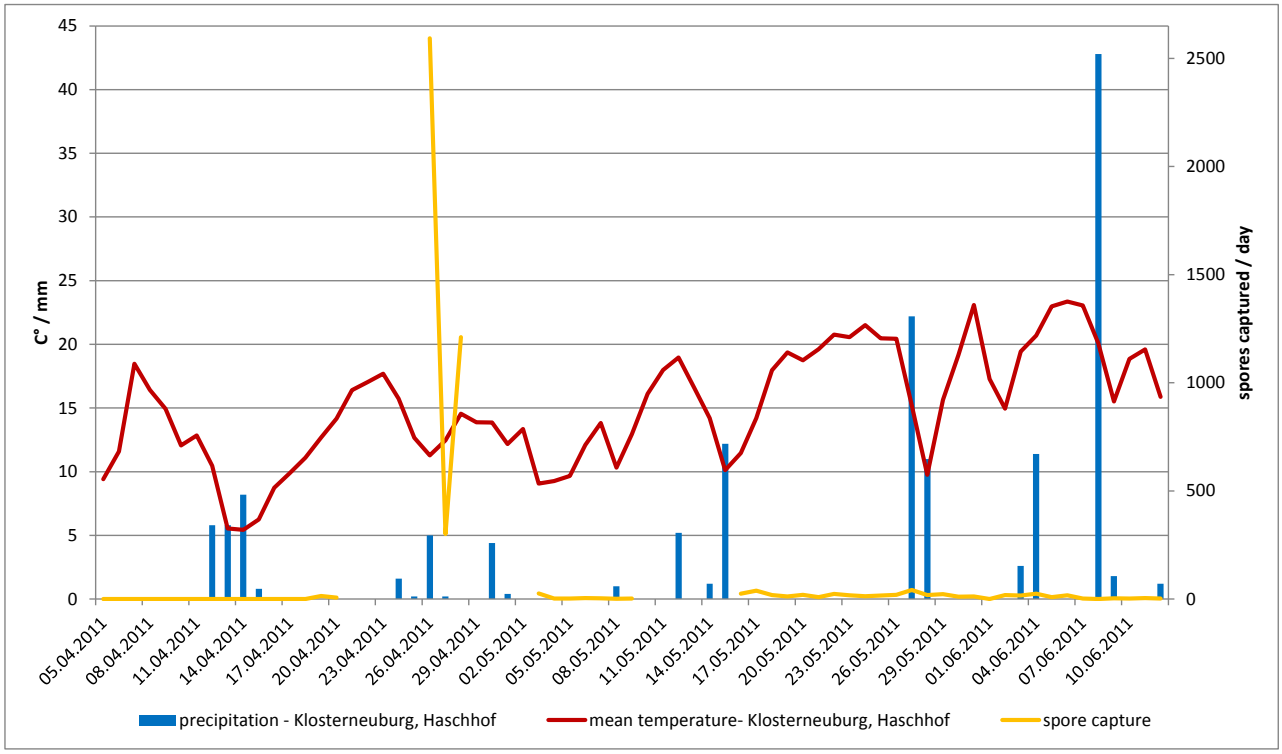


Figure 5: Precipitation amount and discharge of spores of *G. sabinae* on a spore trap in 2011 – Haschhof, Klosterneuburg

Effects of fungicide treatments and host distance on the infestation level in the monitored pear orchard

The first rain period of 2011 (12th to 15th April) with probable infestations in the organic managed pear orchard was protectively treated with 0.5l/ha copper oxychloride (Figure 6), however no infection period was estimated, as the results from the spore trap showed. The main infection period from 24th to 27th of April 2011 was treated as well as a small precipitation event at 3rd of May with 9.86 kg/ha of lime sulphur. The second and less serious period of infection was treated with 0.4 l/ha copper oxychloride (see Figure 6).

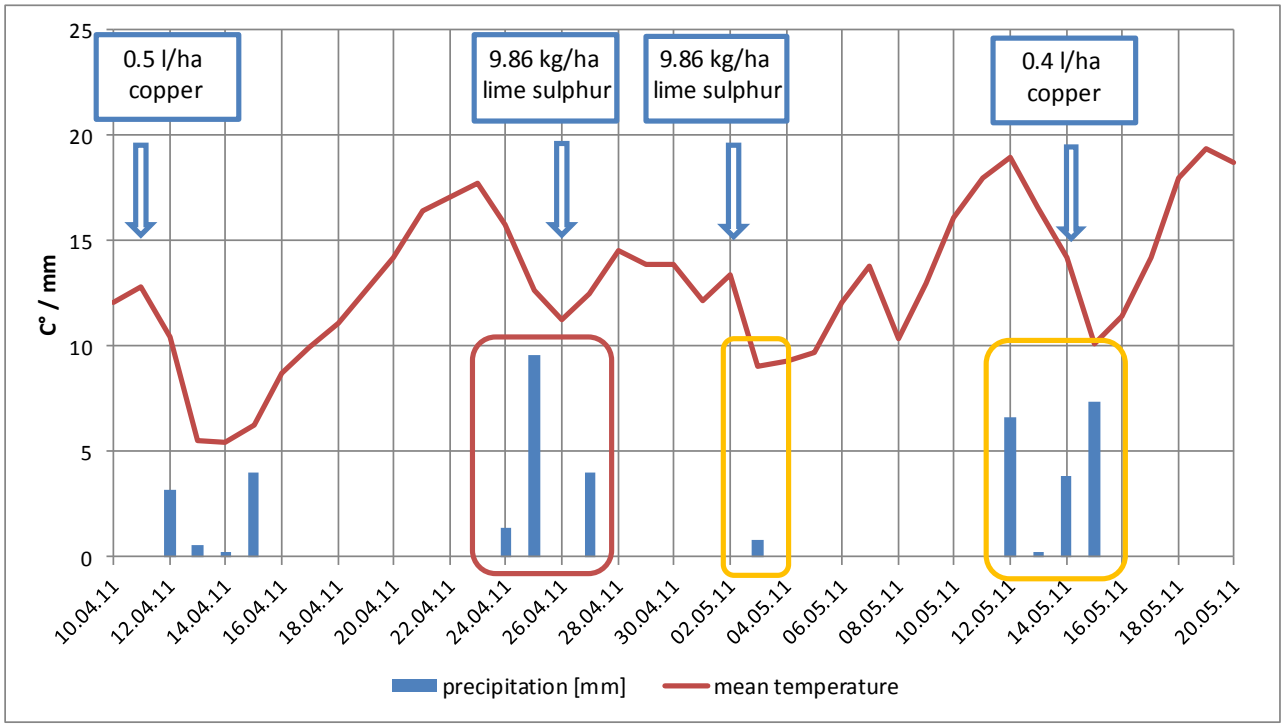


Figure 6: Fungicide treatments and precipitation in the pear orchard (cv. 'Uta') in St. Georgen, Austria in 2011.

The assessment of August 26th showed that the heaviest infections with *G. sabinae* could be found on the untreated control variant in 50 meter distance from the host plant with 33.4 spots per 100 leaves (Figure 7). At the same distance the variant treated with fungicides showed only 9.7 symptoms per 100 leaves. Also the treated trees in further distance showed fewer infestations than the trees in 50 m distance, even though there was no significant difference between all three treated variants (see Figure 7).

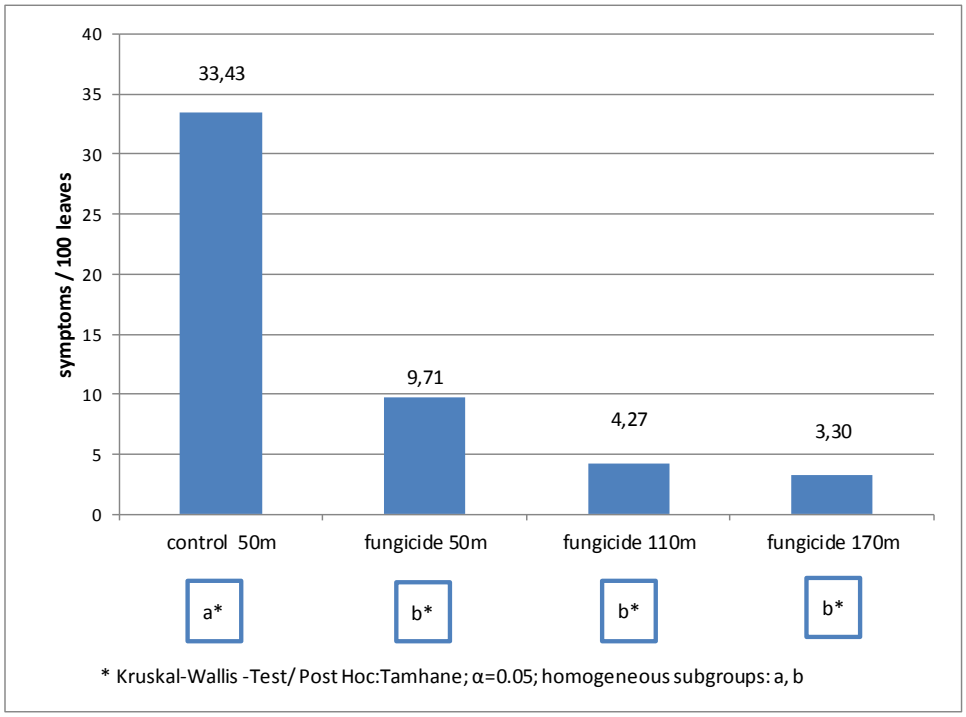


Figure 7: Infestation in depend on host distance and plant treatments

## Discussion

The investigations in pear orchards in the surroundings of St. Pölten revealed a much lower infestation level in the evaluation years 2009 to 2011 than in the year 2008, which was designated as a year of heaviest infection on pear leaves, fruits and twigs by the farmers participating in the survey.

Over the three years the main infection period occurred between the end of April and the beginning of May. Light infections also occurred from mid of April till the end of May. The results show that the precipitation event observed at the end of April 2011 caused most infections on early build leaves, whereas infections in the beginning of Mai in 2009 and 2010 led to more infections on later build leaves.

The spore trap showed a spore flying period till beginning or even mid of June, even though no later infections could be monitored on pear leaves. The alternately outdoor placed pear seedlings in 2010 seemed to be only infected during shoot growing. No new infections could be found on the seedlings after terminating growth, even though spores were discharged at this time. Earlier work on pear rust by Hilber *et al*. (1990) showed that artificial infections were only effective on young leaves. In their investigations about the closely related cedar apple rust (*G. juniperi-virginianae*) Aldwinckle et al. (1980) found, that 4 to 8 day old leaves get more lesions than 10 or 12 days old ones. Our results are in good agreement with these studies as after the end of the shoot growing period no more infections were observed at our monitored sites.

When an orchard was treated with fungicides commonly used in organic farming during the estimated main infection periods, the infestation level dropped remarkably by 70 percent. As spore discharge was largest at the end of April the main protection effect could be achieved by lime sulphur application on the 26th of April. These results show the high potential of organic fungicides in the protection of orchards from pear rust and suggest further studies on the effect of other fungicides for organic farming. The direct relationship

of infection frequency and distance to the host plant couldn't be proved statistically significant within this analysis, however a decrease of infestations with distance to the host was found. Ormrod et al. (1984) conducted a field study were pear trees in 300 meters distance to a cedar shrub were not infected by *G. sabinae.* They monitored infections in distances up to 150 meters and no effect of the direction of the pear trees from the source. The monitoring at the farms in the surroundings of St. Pölten showed a high risk of serious infections in orchards nearby an infected cedar. In addition almost every orchard monitored showed pear rust symptoms even if no infected cedar could be found in the direct neighbourhood. This provides some evidence that in windy regions like St. Pölten the distance of spore threating could be much higher than 300 meters.

For improving the control of *G. sabinae* a next step is creating a forecast model.

## Acknowledgements

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# Advantages from Canopy Related Spray Application in Organic Top Fruit Production

P. Triloff[1], G. Bäcker[2], S. Kleisinger[3]

***Summary:***

*To reduce the negative environmental impact of copper as an important fungicidal substance, especially for the control of apple scab, new molecules and formulations with lower dose rates of copper have been developed in the past years. Canopy related dosing may contribute to this desired reduction by adapting the dose rate to canopy characteristics. Since spray application produces losses to non target areas and the atmosphere and may influence quantitative and qualitative distribution of pesticides on the target, it was interesting if there are possibilities for improvements of the application process that may be utilized to once more reduce dose rates in relation to canopy characteristics or increase efficacy at dose rates that may no more be increased.*

*Based on the MABO-dosing model, relating water volume, dose rate, forward speed and fan power to the canopy, the effects on spray deposits have been compared with common dosing and application rules using fixed water volume, preset forward speeds and nominal fan power. Spray cover from three canopy systems has been analysed on deposit on the entire leaf and on coverage and droplet deposit density on both upper and lower leaf surface.*

*The canopy related application could for the very most parameters compensate a reduction of water volume per ha as canopy width decreased, leading on the upper leaf surface to very similar coverage and droplet deposit density and improved spray cover in the centre of broad canopies. On the lower leaf surface both methods resulted in a strong overdeposition increasing as canopy width increased. The results clearly showed that canopy adapted spray application improves efficacy of deposition, and thus may be utilized to further reduce dose rates in combination with canopy related dosing models.*

**Key words:** copper, fan power, spray deposit, coverage, droplet deposit density

## Introduction:

A better understanding of apple scab (*Venturia inaequalis, Cke., Wint.*) and its host in the past two decades has yielded several tools like sophisticated simulations to predict the behaviour of the fungus, sanitation means to reduce the primary inoculum to levels the fungicides can handle with acceptable success, and specific spray strategies increasing the efficacy of control of individual infections. Altogether these tools allow this important disease of apple to be controlled in organic farms at least as effectively as in integrated production in the primary season. To obtain this result in organic apple production, copper is probably the strongest fungicidal substance available and is required to control the most severe primary infections. Since copper is a heavy metal accumulating in the soil and exhibiting some negative impacts, e.g. on earth worms and aquatics, its further use in Germany has been endangered. With the development of new formulations and new copper containing molecules the amount of copper required per unit area of a crop has been reduced enormously and resulted in the registration of new copper products in

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Germany recently. However it has not yet been assessed if spray application may offer some potential to either directly reduce the farm specific total consumption of copper by canopy related dosing and more efficient spray application or indirectly by an increase of an insufficient efficacy from dose rates that may no more be increased.

Results from a project to develop a method to reduce spray drift with small droplet nozzles (Triloff, 2011) may provide another means for a further reduction of total copper consumption used for e.g. apple scab control in organic fruit production. Besides an almost horizontal air stream the main key for the reduction of spray drift from small droplets is the adaptation of fan power to the canopy by forward speed and fan speed, transporting the spray mist just into the canopy but not out again into the next alley way. Since this adaptation is contrary to the widespread opinion that only a strong air stream produces a good cover it was also interesting to assess the effect of the adaptation of fan power to the canopy on spray deposition. Therefore both methods - dosing and application after a model that adapts water volume, dose rate, forward speed and fan power to the canopy and the classical method with fixed dose rates per ha, low forward speed and full fan power - have been compared in three canopy systems; a broad three row bed, a regular slender spindle and a super spindle.

From only little research on the effect of reduced fan power on spray deposit, it is known that in trials with big canopies, already Randall (1971) reports most uniform spray deposits from highest air volumes and lowest air speed inside the canopy. In citrus Whitney & Salyani (1991) find lower spray deposits at the canopy surface than 0,6 m inside the canopy and attribute this effect to a high air volume at high speed. In fruit trees with a large canopy also Derksen & Gray (1995) did not find an increase of spray deposit after raising fan speed. An improvement of spray deposit has been achieved by Landers & Farooq (2004), who did not reduce fan speed of axial fans in top fruit but reduced air intake by wooden “donuts” with various size and so reduced air volume. An indirect prove of a positive effect of a reduced fan power on spray deposit in apple is reported from Richardson *et al*. (2000), who at crosswind found higher deposits on the upwind tree row than on the downwind *row. But also headwind not only leads to an improvement of spray deposit as* Cross et al. (2003) state, but also decrease spray drift, indicating the positive effect of a reduced reach of the air stream. Also in grape vine a reduction of fan power improved spray cover even at early developmental stages with little leaf area as Pergher & Gubiani (1995) report. Later in the season with a high leaf area the authors even found a reduced spray deposit from increased fan power and increased water volumes, indicating a poor penetration of the spray mist into the canopy at high air speed because of a shielding effect of the large leaves and a loss of spray liquid through run off at higher water volumes. The influence of fan power on spray deposit is rather high as Pergher (2005) notes which before bloom decreased spray deposit by 23% and after bloom by 21% when increasing fan power from 6,3 $m^3 \cdot s^{-1}$ to 10,6 $m^3 \cdot s^{-1}$. This improvement of the spray cover in grape vine by reduced fan power was also confirmed by Pezzi & Rondelli (2000) and Pergher & Lacovic (2005).

To evaluate combinations of a canopy adapted air stream and varying forward speed on the spray deposit, a trial series was carried out which combined a three dimensional dosing model adapting spray volume per ha, forward speed and fan power to the canopy (Triloff, 2005). While spray volume per ha and forward speed are calculated by the model and decrease resp. increase as canopy width decreases, fan power has to be adjusted visually for each canopy to a value where at the forward speed calculated by the model only very little spray mist is transported through the canopy into the next alley way.

**Material and methods:**

**Orchards:** Three apple orchards with differing dimensions have been selected from commercial farms to cover the range of planting systems at Lake Constance area:

1) „3-row bed“, variety Jonagold M9, planted in 1982, canopy width 3,20 m
2) „Slender spindle“, variety Jonagold M9, planted in 1986, canopy width 1,35 m
3) „Super spindle“, variety Jonagold M9, planted in 2004, canopy width 1,00 m

**Orchard Sprayer:** For all three orchards a tower sprayer „Wanner SZA32/1500“ with a nominal fan power of 34.000 $m^3 \cdot h^{-1}$ at the small fan gear was used, fitted with 2 x 8 hydraulic hollow cone nozzles „Albuz ATR purple“ which were used in all treatments.

**Adaptation of fan power:** To adjust fan power at each forward speed the model calculated to a value where only little spray mist was transported through the canopy into the next alley way, a few metres where sprayed in each planting system, with a second person visually monitoring the reach of the air stream in the alley way next to the sprayed tree row.

**Dosing and application treatments:** In each of the three orchards three plots were sprayed according to the owners setting of forward speed at a constant water volume of 200 $l \cdot ha^{-1}$ and nominal fan power. According to the dosing model three other plots were sprayed at differing settings of water volume$\cdot ha^{-1}$, forward speed and fan power (table 2).

Table 1: Trial treatments to evaluate spray cover

| Treatment | Training system | Application method | Forward speed $km \cdot h^{-1}$ | Fan power PTO* $min^{-1}$ | Spray liquid pressure bar | Water volume $l \cdot ha^{-1}$ |
|---|---|---|---|---|---|---|
| I | 3 row **Be**d | „**g**rower“ | **6,7** | **540** | **16,5** | **200** |
| II | 3 row **Be**d | „**m**odel“ | **3,8** | **460** | **7,5** | **237** |
| III | slender **Sp**indle | „**g**rower“ | **8,0** | **540** | **9,0** | **200** |
| IV | slender **Sp**indle | „**m**odel“ | **9,0** | **330** | **7,5** | **153** |
| V | **Su**per spindle | „**g**rower“ | **9,0** | **540** | **11,0** | **200** |
| VI | **Su**per spindle | „**m**odel“ | **12,1** | **290** | **7,5** | **114** |

* = The fan of the sprayer was operated only in the low gear

Bold letters and numbers indicate treatments in the graphs: e.g. treatment I: Be g 6,7 540 16,5 200

**Leaf sampling:** From each trial plot in the 3rd of five beds/rows sprayed, 10 leaves where picked in each of 4 canopy sectors from the top to the bottom of the canopy in each of three trees according to the protocol for spray cover trials (Schmidt & Koch, 1995; Ganzelmeier & Schmidt, 2003) shown in figure 2. Leaf samples were picked immediately after the spray cover had dried off.

**Fluorescent tracer:** As fluorescent tracer Tinopal® NFW, a 20% water soluble formulation of disodium-2,2'-([1,1'-biphenyle]-4,4'-diyldivinylene)bis(benzolsulfonate) was used. The dose rate of the tracer was set to 1,0 l$\cdot$100 $l^{-1}$ to achieve sufficient fluorescence for the

image analysis. For thoroughly mixing the tracer in the spray tank a 15 minute agitating period was required.

**Leaf sample analysis:** Leaf samples were analysed on coverage and droplet deposit density on each upper and lower leaf surface by image analysis (Media Cybernetics “Image-Pro 5.0“) followed by an analysis on spray deposit of the total leaf area by fluorometry (auto-sampler “Perkin Elmer AS 91”, luminescence-spectrometer “Perkin Elmer LS 30”).

**Results:**

**Spray deposit:** With both methods of dosing and application a decrease in spray cover is observed as canopy width decreases, even at the constant water volumes·ha$^{-1}$ in the “grower” scheme, but with a higher gradient in the “model” scheme. Adapting water volume, forward speed and fan power to the canopy, “model” improved average spray deposit in the bed system by 36% compared to “grower” although water volume was increased by only 18,5%. In the slender spindle “model” gave the same average spray deposit as “grower” despite water volume·ha$^{-1}$ was reduced by 24%. In the super spindle “model” resulted in a 23% reduction of spray deposit despite a 43% reduction in water volume per ha, compared to “grower”. Comparing “model” to “grower” in terms of overall efficiency of spray deposition, “model” resulted in a 28% more efficient use of pesticides, comprising a 16% reduction in pesticide consumption and an average increase of spray deposit by 7,7%.

Plotting spray deposit over canopy height, increasing canopy width resulted in a strong increase of spray deposit as the sampling position increased. The highest increase was observed in the bed system at the “grower” scheme with a factor of 2,4 between the

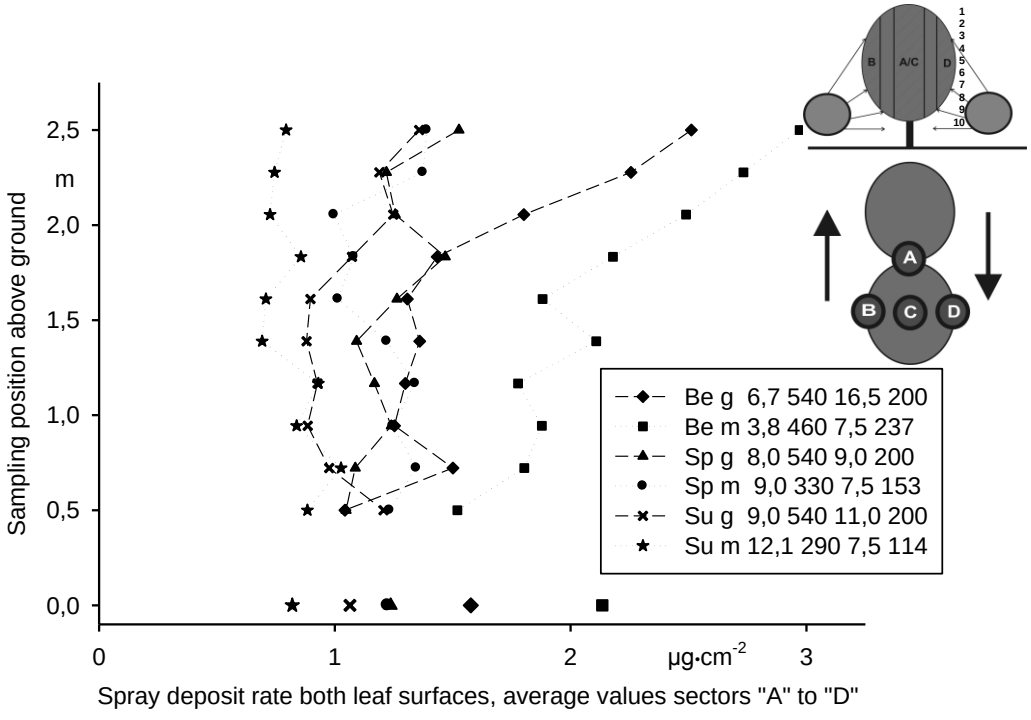


Figure 1: Spray deposits over canopy height calculated as average values of the 4 sampling sectors and average values of all individual data (n = 120) plotted at sampling position 0,0 m. Includes the scheme for leaf sampling at 4 canopy sectors (according to Schmidt & Koch, (1995), Ganzelmeier & Schmidt; (2003), modified)

highest and lowest sampling position. This increase is generally slightly stronger within the “grower” plots and is still visible in the super spindle, while “model” gave an almost uniform spray deposit over canopy height in this system (figure 1).

**Relative Coverage:** On the upper leaf surface, coverage ranged between 10% and 17% over all planting systems and spray schemes and decreased as canopy width became less. Maximum average values reached 17% and 14% in the bed system, 14,3% and 13,8% in the slender spindle and 10,5% and 10% in the super spindle for “grower” and “model” schemes. Plotting coverage data over canopy height showed a slight decrease as sampling position increased for all treatments (figure 2). Comparing “model” to “grower”, the efficiency of application decreased by 29% in the bed system but with still the same absolute values as in the slender spindle trees. In the slender spindle and the super spindle, “model” appeared to be 27% and 67% more efficient as the “grower” scheme.

**Droplet deposit density:** As observed with the spray cover on the upper leaf surface, also this parameter for both methods showed a decrease as canopy width decreased. Average values for “grower” and “model” in the bed system were 62·cm$^{-2}$ and 70·cm$^{-2}$, in the slender spindle 61·cm$^{-2}$ and 59·cm$^{-2}$, and 51·cm$^{-2}$ and 45·cm$^{-2}$ in the super spindle. When plotted over canopy height, the average values of the four sampling sectors in the bed system showed an almost vertical alignment, while in the slender spindle trees a slight decrease with increasing canopy height was observed which appeared to be more pronounced for “model” than for “grower”. In the super spindle, the values of “model” decreased with increasing canopy height while for “grower” an increase was recorded (figure 3). Separating the average values over canopy height into the 4 canopy sectors, very clearly a higher droplet deposit density has been measured in the upper part of the centre of the bed system for “model” which was not detected in the “grower” scheme. The droplet deposit densities measured on the upper leaf surface also indicate an increase of the efficiency of deposition of “model” compared to “grower” of 27% in the slender spindle and 55% in super spindle orchard, while in the bed system “model” appeared to be 5% less efficient than the application according to the “grower” scheme.

On the lower leaf surface image analysis for both relative coverage and droplet deposit density revealed a strong oversupply of spray liquid of about 2,5 x for „grower“ and 2,0 x for „model“, compared to the upper leaf surface, even without taking into account overlaying deposition on the lower leaf surface (figure 4). From this reason detailed data of the lower leaf surface are not presented.

**Discussion:**

The spray trials clearly showed that even with a fixed water volume per ha in the „grower“ plots the average spray deposit is decreasing as canopy width decreases. This decrease may be caused by the slight increase of forward speed in the “grower” plots because the relative reduction of the spray deposit complied with the relative increase of forward speed from the bed system to the super spindle. Since this reduction of the average spray cover per cm$^2$ is stronger in the “model” plots compared to “grower”, canopy related dosing models may not produce a constant average spray deposit per cm$^2$.

Plotting spray deposits over canopy height discloses a very uneven distribution of the spray deposits with a strong increase as sampling height and canopy width increase. As the results show, a great percentage of the spray mist has been deposited at the upper part of the canopy while values at the bottom of the canopy did not vary much between the

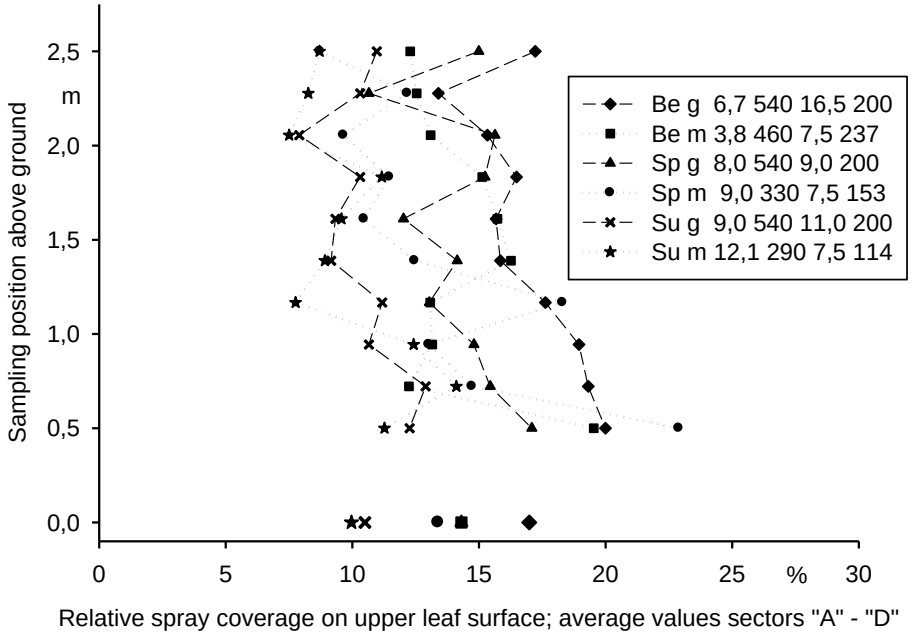


Figure 2: Relative coverage over canopy height calculated as average values of the 4 sampling sectors and average values of all individual data (n = 120) plotted at sampling position 0,0 m

three canopy structures and both dosing and application methods. Consequently a judgement of the spray cover by average spray deposits appears to be very questionable since it does not take into account an uneven distribution over canopy height and width.

However the analysis of the spray deposits of the canopy surface and the centre showed gradients from the surface to the centre which were lower for “model” than for “grower” in the spindle and super spindle trees, indicating a better deposition of spray mist inside the canopy with a canopy adapted fan power even at a high forward speed. In the bed system with a high canopy width, “model” resulted in a higher gradient but produced a higher absolute spray deposit in the canopy centre than did “grower”, indicating an improvement of spray deposition also in broad canopies. In this respect a slower forward speed at canopy adapted fan power seems to be more suitable than a high fan power at a higher forward speed.

But even a more detailed analysis of spray deposits appears to be inappropriate for judging the application of pesticides in fruit trees since it ignores an uneven distribution on the leaf surface over the canopy structure. This becomes very obvious when analysing spray coverage separately on the upper and lower leaf surface. These data clearly proved that even with the almost horizontal air stream of the tower sprayer the average coverage on the lower leaf surface over all canopy systems and methods was 2,3 x higher than on the upper surface. Since coverage mirrors mass distribution to a certain extent, it may be concluded, that also spray deposit is much higher on the lower leaf surface than on the upper one, but also much higher at the top of the canopy than at the bottom, decreasing as canopy width decreases.

Since coverage on the upper leaf surface in the three canopy structures did not vary remarkably between “grower” and “model”, an improved deposition from adapting forward speed and fan power to canopy width by the dosing model compensated a 24% reduction in spray volume·ha$^{-1}$ in the slender spindle and a 43% reduction in the super spindle,

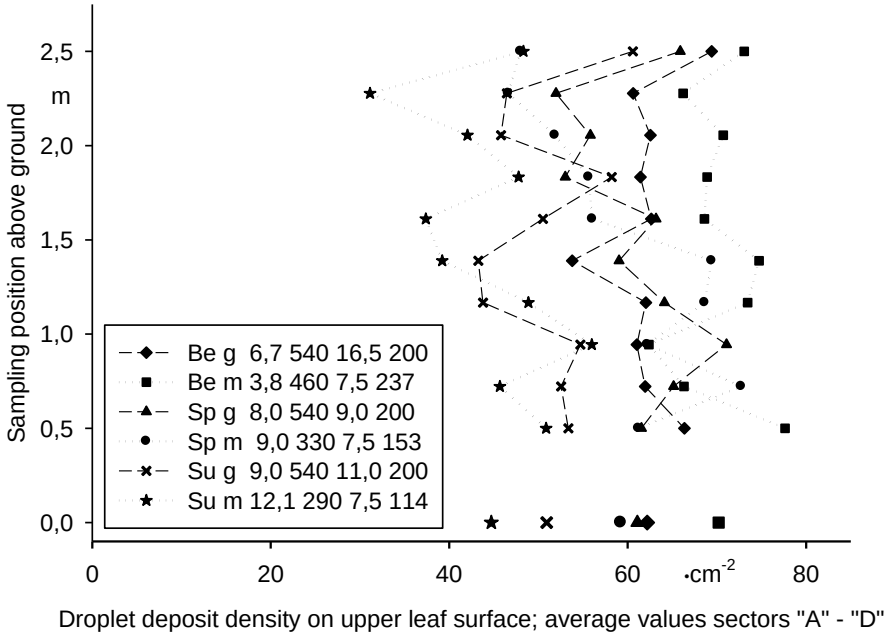


Figure 3: Droplet deposit density over canopy height calculated as average values of the 4 sampling sectors and average values of all individual data (n = 120) plotted at sampling position 0,0 m

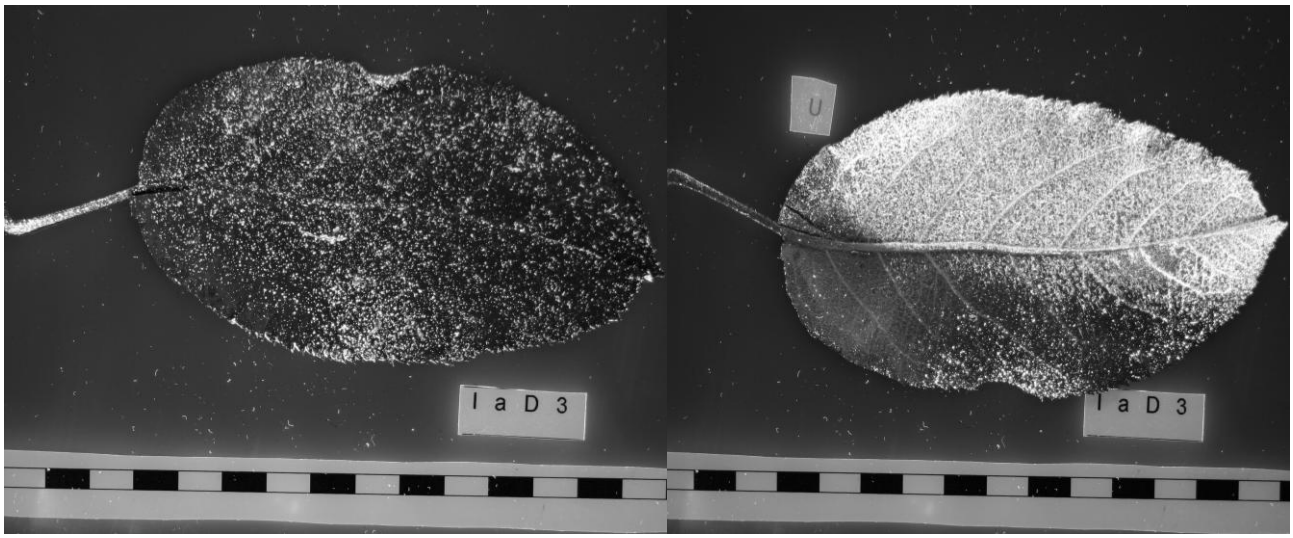


Figure 4: Differently covered leaf surfaces of the same leaf (left = upper surface; right = lower surface). On the lower leaf surface an uncovered spot (risk area) is visible (Photos: Triloff, 2007).

compared to “grower”. These results clearly show that the deposition efficiency increased as canopy width decreased and forward speed and fan power were adapted to the canopy. In the bed system “model” resulted in a 16% reduction of the coverage compared to “grower” on the upper leaf surface, but absolute values were still as high as recorded in the slender spindle from both methods.

Comparing coverage on the upper leaf surface of the canopy surface with the canopy centre, the average ratios over canopy height generally have been rather low with slightly

lower values for “model” in the bed system and the slender spindle and a slightly higher value in the super spindle, indicating that canopy related dosing and spray application does not lead to a reduction of the coverage in the canopy centre.

But also coverage on its own does not allow a sufficient judgement of spray deposits since it does not supply any information about the distribution of the spray droplets over the individual leaf surface. This aspect is especially important, since the coverage measured is far below covering the entire leaf surface and therefore at least protective fungicides require a redistribution by natural rain to cover the primarily uncovered leaf area by this secondary fungicide liquid. This is required to kill e.g. spores of apple scab that accidentally may have landed in the gaps between spray deposits, germinate and infect if the distance between individual droplets is too large in order to redistribution cover these gaps. Therefore it is necessary to introduce droplet deposit density as a further means in order to judge the quality of a spray deposit.

Not only the constant water volumes per ha as applied by the “grower“ scheme, but also the canopy related dosing and application model led to a decrease of average droplet deposit density on both upper and lower leaf surfaces as canopy width decreased. However on the upper leaf surface this decrease was more pronounced for “model” being caused by a higher value in the bed system and a lower value in the super spindle compared to the “grower” scheme. Resolved over canopy height, also droplet deposit density showed a slight decrease with increasing sampling position and decreasing canopy width, which was observed in both methods of dosing and application. Focussing on the values in the centre of the bed system, “model” yielded a clearly higher droplet deposit density on the upper leaf surface in the upper part of this canopy - even despite a reduced fan power - than did “grower”. This indicates that a low forward speed leads to an improved air stream into the centre of broad canopies carrying more droplets to this canopy sector than higher forward speed can do, even at nominal fan power. These observations are confirmed by data presented by (Walklate *et al.,* 1996; van de Zande *et al.,* 2002). Such an improved spray deposition in the canopy centre through canopy adapted forward speed and fan power has also been observed in the slender spindle and the super spindle.

From these trials may be concluded, that, when using tower sprayers for spray application with an almost horizontal air stream, the adaptation of forward speed and fan power to the

Table 2: Average changes of efficiency of spray deposition of „model“ compared to „grower“ for three canopy structures

| | 3-row Bed | Spindle | Superspindle |
|---|---|---|---|
| Spray deposit (entire leaf) | 14% | 29% | 35% |
| Relative coverage (upper leaf surface) | -29% | 26% | 67% |
| Relative coverage (lower leaf surface) | -27% | -3% | 7% |
| Droplet deposit density (upper leaf surface) | -5% | 27% | 55% |
| Droplet deposit density (lower leaf surface) | 17% | 28% | 27% |

canopy width improves deposition efficiency (table 3) which compensates the reduction of water volume and pesticide dose rate from the canopy related dosing model completely at a forward speed of 9 km·h$^{-1}$ and to a very high extent at 12 km·h$^{-1}$.

Therefore a canopy related dosing and spray application may significantly reduce time consumption for pesticide application through less fillings and related travelling, and less time consumption for spraying through higher forward speeds in slim canopies. Both savings allow better usage of meteorological conditions favourable for spray application. Additionally the reduction of fan power significantly reduces noise, fuel consumption and spray drift thus also resulting in environmental benefits, but is applicable just with small droplets since they do not require much energy to keep them in the air and control direction and reach. Apart from these reductions the canopy related dosing model in practice leads to product savings ranging from 10 - 40% on a farm level.

The remarkable increase of deposition efficiency obtained by adapting the application process to the canopy may be specially utilized for a further reduction of the general consumption of copper based fungicides, reducing their negative impact additionally to the reductions obtained from the new formulations. Generally the method is applicable for any pesticide without reducing deposition on the upper leaf surface over a wide range of forward speeds and canopy structures. The reduction of deposition efficiency obtained on the lower leaf surface is welcome since it outlines a reduction of the overdeposition caused by excessive fan power. Since this disproportion between upper an lower leaf surface is even worse with plain axial fans because of the steep angle of their air stream, fan types with cross flow characteristics should be preferred whenever possible. To guarantee a uniform air distribution over canopy height at reduced fan speeds, it is strongly advised to test the fans on air distribution test benches before applying canopy adapted spray application to avoid problems caused by a potentially poor reach of the air stream at certain sections of the air outlets.

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# Droplet size spectrum of overhead irrigation sprinklers used for targeted apple scab control

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**Abstract**

*The Research Centre Laimburg (South Tyrol, Italy) has been conducting trials on the use of plant protection products, applied via overhead irrigation, for apple scab control for more than 10 years. High levels of efficacy were achieved in several trials carried out in South Tyrol and in other fruit growing areas. However, in most countries it is still not clear whether a multi-functional overhead irrigation, developed for frost protection and irrigation, can also be used for plant protection. Different technical details such as pump pressure, sprinkler type, nozzle size, irrigation pipeline design and finally droplet size are under discussion.*

*It has not yet been established whether and to which extent (as during the application with conventional spray equipment) small driftable droplets are produced by using the overhead irrigation system. This study aimed at determining the droplet size spectrum of different circle sprinklers.*

**Keywords:** overhead irrigation, plant protection, droplet spectrum, sprinklers, droplet size, drift

## Introduction

In the orchards in South Tyrol, overhead irrigation systems have been used for decades not only for irrigation, but also for frost protection (Mantinger & Tinkhauser, 1978). Already in the 1950-ies and 1960-ies, overhead irrigation has been used in South Tyrolian orchards also for the application of plant protection products (Ramoser, 1966; 1971). Trials on the use of overhead irrigation systems for plant protection in fruit and especially grapevine production have been conducted also in Switzerland (Peyer, 1964) and Germany (Kümmerer, 1969; Goedecke, 1971; Müller, 1979). Depending on the target, results varied considerably, and for several agronomic and economic reasons the use of conventional foliar broadcast sprayers finally prevailed. In the 1990-ies the application of plant protection products via overhead irrigation was taken up again by the Research Centre Laimburg within the field of organic farming, in particular to improve scab control in organic orchards.

For scab control, many organic growers in South Tyrol rely on foliar applications directed onto the wet vegetation. Since these treatments must be carried out within a short period of time (Zemmer, 2001), their application via overhead irrigation is especially suitable, as already evidenced in several studies (Kelderer *et al*., 2000). In addition, the application via overhead irrigation proved to be effective in preventing phytotoxicity symptoms of lime sulphur (leaf burn) on the crop, which commonly occur when the product is applied as a conventional foliar broadcast spray (Kelderer *et al*., 2006).

According to the new Italian Regulation on Plant Protection, all systems that are used for the application of plant protection products, must be inspected, and information on technical details such as pump pressure, sprinkler type, nozzle size, irrigation pipeline design and finally droplet size must be provided.

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In this study we tried to overcome the lack of information concerning droplet size spectrum of overhead irrigation systems. „Pesticide drift is the movement of airborne spray droplets, vapors, or dust particles away from a target area" (Baker & Cordell, 1998). By using conventional foliar spray equipment, the spray drift of plant protection products from the target area onto adjoining crops, areas, and/or water is a major problem. Spray drift also results in the loss of part of the spray solution during application. Furthermore, spray drift can result in water contamination, health risk for animals and humans, and under- and/or over-application in small areas. Several factors, especially those of technical and meteorological origin, may result in drift of a spray solution, and droplet size is the major cause (Nuyttens *et al*., 2011). "When a liquid solution is sprayed under pressure it is atomized into droplets of varying sizes: the smaller the nozzle size and the greater the spray pressure, the smaller the droplets and therefore the greater the proportion of driftable droplets. Other causes of spray drift are spray heights, operating speed during application, wind speed, temperature, humidity, spray volume and spray product (http://www.teejet.com, 07.07.2011). Concern exists that spray drift may occur also by using overhead irrigation for plant protection. The risk of drift is considered to exist for droplets below 250 µm in size (www.wysspumpen.ch, 2010). In this study, the droplet size spectrum of different overhead sprinklers was determined.

## Material and Methods

The trials have been conducted in a sprinkler test station of the Research Centre Laimburg (South Tyrol, Italy). A digital pressure regulator was used to adjust pressure. The sprinkler types equipped with different nozzles and pressure setups and listed in Table 1 were tested.

Table 1: Tested sprinkler types, nozzle sizes, pressure setups, spray radius and flow rates (data provided by manufacturers and/or obtained from separate measurements).

| Sprinkler type | Nozzle size (mm) | Pressure (bar) | Spray radius (m) | Flow rate (l/min) |
|---|---|---|---|---|
| Kofler K 10 | 3.0 | 3.0 | 11.3 | 10.3 |
| | 3.0 | 4.0 | 12.2 | 11.9 |
| | 4.0 | 3.0 | 11.3 | 18.3 |
| | 4.0 | 4.0 | 12.1 | 21.1 |
| Kofler K 16 | 3.0 | 3.0 | 10.9 | 10.3 |
| | 3.0 | 4.0 | 11.6 | 11.9 |
| | 4.0 | 3.0 | 12.6 | 18.3 |
| | 4.0 | 4.0 | 13.5 | 21.1 |
| Perrot ZS30 | 3.5 | 3.0 | 13.5 | 12.5 |
| | 3.5 | 4.0 | 14.8 | 14.5 |
| | 4.0 | 3.0 | 14.5 | 16.5 |
| | 4.0 | 4.0 | 15.4 | 19.0 |
| NetafimMeganet* | Blau 450 | 2.5 | ca. 8 | 8.0 |
| | Blau 450 | 3,0 | ca. 8 | 8,5 l/min |

* Data obtained from own separate measurements.

*Droplet size spectrum*

Strips of water sensitive paper (Syngenta water sensitive paper 52×76) were fixed onto wooden slats and deployed along a row at 1 meter distance one from the other starting from the sprinkler. The sprinkler was then turned on for one full rotation. As soon as the water droplets had dried, the paper strips were scanned and analysed on the computer.

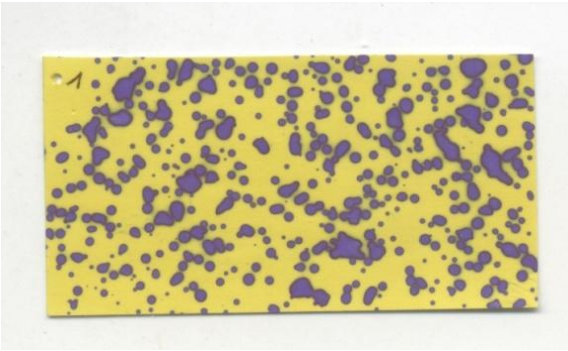

Fig. 1: water sensitive paper after one full rotation of a sprinkler.

*Water distribution*

Graduated jugs were placed along a row at 1 meter distance one from the other starting from the sprinkler. The sprinkler was then turned on for one hour and the water volume collected in each of the graduated jugs was then measured.

The weather station of the Research Centre Laimburg was used to determine wind speed and thus to decide whether to accept or repeat the measurement.

*Computations*

By using pipettes, samples of droplets of the following sizes on water sensitive paper were prepared: 100, 50, 10, 5, 2.5, 2, 1.5, 1, 0.9, 0.8, 0.7, 0.6, 0.5, and 0.4 µl. The paper strips were then scanned and the area covered by the droplets on the paper was determined by using the image processing and analysis program „Image Tool“ (http://ddsdx.uthscsa.edu/dig/itdesc.html, 25.10.2011). The curves and functions relating the covered area to droplet size were obtained by using Excel software. To find the best data fit, two distinct functions, each plotting different droplet sizes, were compiled, one for droplets ranging from 2 to 100 µl in size ($y = -4e^{-24}x^3 + 3e^{-16}x^2 + 7e^{-7}x - 3{,}5589$), and one for droplets ranging from 0.4 to 2.0 µl in size ($y = 1e^{-28}x^4 + 4e^{-21}x^3 - 4e^{-14}x^2 + 2e^{-7}x$).

The following formula (source: Wikipedia „Droplet“, 01.06.2010) was used to convert droplet volume (pl) in droplet diameter (µm):

Droplet diameter = $\sqrt[3]{\frac{Dropletvolume \times 6000}{3.14}}$

Droplet sizes obtained were then classified by using a scale such as the following (source: http://www.wysspumpen.ch/duesenrechner_feldbau.html, 01.06.2010):

>550 µm, especially big

400 – 550 µm, very big

350 – 400 µm, big

250 – 350 µm, medium

150 - 250 µm, small, risk of spray drift

< 150 µm, very small, not recommendable

By using the functions and formulas described above, the droplet size classes could finally be converted first in droplet volume, and then in droplet area.

Each area covered by the droplets on the paper strips used in the trials (which had been previously scanned and analysed by using the program „Image Tool“) could thus be assigned to one of the different classes.

Since neither visually nor with image analysis an exact distinction between blurry, overlapping droplets could be made, these were considered as one single droplet in all calculations.

## Results

For each combination sprinkler type-nozzle size-pressure setup, a table reporting water distribution (in mm/h and %), and the percentage of water per droplet size class at different distances from the sprinkler was prepared (see Table 2 as example). The total percentage of water within each droplet size class for all tested sprinkler type-nozzle size-pressure setup combinations is reported in Table 3.

Table 2: Droplet size spectrum of sprinkler type Kofler K10 with nozzle of 3 mm in size at a pressure setup of 3 bar.

| Distance (m) | Water distribution (mm/h) | Water distribution (%) | Percentag (%) of water within each droplet size class (µm Ø) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | <150 (%) | 150-250 (%) | 250-350 (%) | 350-400 (%) | 400-550 (%) | >550 (%) |
| 1 | 2.99 | 15.44 | 0.04 | 0.50 | 4.17 | 5.02 | 16,41 | 73,86 |
| 2 | 2.26 | 11.66 | 0.01 | 0.06 | 1.11 | 2.03 | 12,19 | 84,60 |
| 3 | 1.69 | 8.72 | 0.03 | 0.09 | 0.49 | 1.36 | 3,96 | 94,08 |
| 4 | 1.87 | 9.66 | 0.05 | 0.09 | 0.49 | 0.31 | 9,32 | 89,74 |
| 5 | 1.71 | 8.82 | 0.04 | 0.28 | 0.45 | 0.10 | 0,75 | 98,38 |
| 6 | 1.95 | 10.08 | 0.04 | 0.35 | 0.54 | 0.20 | 1,08 | 97,79 |
| 7 | 1.93 | 9.98 | 0.10 | 1.60 | 4.93 | 4.79 | 6,00 | 82,58 |
| 8 | 1.32 | 6.83 | 0.04 | 0.32 | 0.72 | 0.83 | 1,53 | 96,56 |
| 9 | 1.14 | 5.88 | 0.03 | 0.16 | 0.23 | 0.00 | 0,28 | 99,30 |
| 10 | 0.91 | 4.73 | 1.85 | 4.82 | 6.82 | 11.12 | 32,97 | 42,42 |
| 11 | 0.81 | 4.20 | 0.08 | 0.34 | 0.46 | 0.56 | 1,53 | 97,03 |
| 12 | 0.53 | 2.73 | 0.02 | 0.11 | 0.13 | 0.13 | 0,65 | 98,95 |
| 13 | 0.24 | 1.26 | 1.28 | 0.45 | 0.00 | 6.94 | 17,35 | 73,97 |
| **Mean percentage of water in each droplet size class** | | | **0.14** | **0.60** | **1.86** | **2.36** | **7.96** | **87.08** |

For the combination sprinkler type-nozzle size-pressure setup described in Table 2, the highest amount of small droplets (< 250 µm) was found at a distance of 10 (1.85% + 4.82%) and 13 m (1.28% + 0.45%).

Table 3: Percentage of water within each droplet size class for the tested sprinkler type-nozzle size-pressure setup combinations

| Sprinkler-nozzle-pressure | Percentage (%) of water in droplet size class | | | | | |
|---|---|---|---|---|---|---|
| | <150 µm | 150-250 µm | 250-350 µm | 350-400 µm | 400-550 µm | >550 µm |
| K10 3mm 3bar | 0.14 | 0.60 | 1.86 | 2.36 | 7.96 | 87.08 |
| K10 3mm 4bar | 0.04 | 0.27 | 0.73 | 0.66 | 2.46 | 79.06 |
| K10 4mm 3bar | 0.06 | 0.56 | 1.09 | 0.83 | 3.28 | 94.18 |
| K10 4mm 4bar | 0.03 | 0.23 | 0.62 | 0.50 | 1.39 | 97.23 |
| K16 3mm 3bar | 0.05 | 0.46 | 1.74 | 1.78 | 4.96 | 91.06 |
| K16 3mm 4bar | 0.06 | 0.22 | 0.60 | 0.55 | 1.68 | 96.90 |
| K16 4mm 3bar | 0.04 | 0.44 | 0.85 | 0.78 | 3.29 | 94.61 |
| K16 4mm 4bar | 0.04 | 0.20 | 0.44 | 0.64 | 2.22 | 96.46 |
| Perrot ZS30 3.5mm 3bar | 0.02 | 0.11 | 0.17 | 0.12 | 0.28 | 99.31 |
| Perrot ZS30 3.5mm 4bar | 0.03 | 0.12 | 0.18 | 0.14 | 0.82 | 98.70 |
| Perrot ZS30 4mm 3bar | 0.09 | 0.55 | 1.87 | 0.81 | 3.64 | 93.04 |
| Perrot ZS30 4mm 4bar | 0.04 | 0.20 | 0.69 | 0.70 | 1.89 | 96.47 |
| Netafim 2.5bar | 0.21 | 0.84 | 1.15 | 0.72 | 1.31 | 95.76 |
| Netafim 3bar | 0.02 | 0.16 | 1.15 | 1.78 | 10.63 | 86.26 |

Since droplets of both 150-250 µm and <150 µm in size are considered driftable. These two classes were combined in one single droplet size class of <250 µm (Table 3). The percentage of water within this class was very low for all sprinkler types. In fact, it exceeded 1% only for the sprinkler type Netafim at a pressure setup of 2.5 bar, while lowest values (0.13%) were recorded for the sprinkler type-nozzle size-pressure setup combination Perrot ZS30-3.5 mm-3 bar. Contrary to what assumed, the percentage of driftable droplets was always higher at low pressure than at high pressure, except for the combination sprinkler type Regner Perrot ZS30 - nozzle size 3.5 mm. In this case, the percentage of driftable droplets was comparable at both tested pressure setups, and amounted to 0.13% at 3 bar and 0.15% at 4 bar. For the sprinklers of the company Kofler an increased percentage of small, driftable droplets was recorded when these were used in combination with small nozzles, but no similar trend was observed for the sprinkler type Perrot ZS30.

## Discussion

The use of overhead irrigation systems for the application of plant protection products, especially for products against apple scab, seems to be a valuable alternative to the use of conventional foliar broadcast sprayers, because it enables the grower to safe time and money. In addition, the applications can be conducted within a very limited period of time, which positively affects targeted apple scab control. In South Tyrol, growers have been using overhead irrigation systems for decades, primarily for frost protection. The entire area cultivated with apple is therefore equipped with overhead irrigation, thus favouring its use also for plant protection.

The Department for Organic Production of the Research Centre Laimburg has been evaluating the application of plant protection products, and especially of lime sulphur for targeted scab control, via overhead irrigation for 10 years (Kelderer. *et al*., 2000). Within these studies, also the efficacy of different lime sulphur-based products was tested (Kelderer. *et al*., 2006).

Overhead irrigation systems are supposed to have an average water consumption of 4 mm/h/m2. However, the actual amount of water may vary considerably depending on the structure of the entire irrigation system. In order to establish the water use per sprinkler and thus mean water consumption, pressure setup, friction loss in relation to irrigation pipeline length and diameter, and sprinkler height must be taken into consideration. The manufacturers of the sprinklers tested in our studies declare flow rates ranging from 10.3 l/min (Kofler K10. nozzle size 3 mm. pressure setup 3 bar) to 21.1 l/min (Kofler K10 and K16. nozzle size 4 mm. pressure setup 4 bar).

Since almost no information concerning the droplet size spectrum of overhead irrigation systems exists, we decided to determine the droplet size spectrum and the proportion of small driftable droplets of different sprinkler types. Except for sprinkler type Netafim at a pressure setup of 2.5 bar, for all the other tested combinations sprinkler type-nozzle size-pressure setup, the percentage of driftable droplets was below 1%. The time period required for the application of plant protection products via overhead irrigation amounts to 5 minutes, and 5 additional minutes are necessary for rinsing (Kelderer. *et al*., 2000). The amount of water applied by each sprinkler in 5 minutes ranges from 52 l (Kofler K10, nozzle size 3 mm, pressure setup 3 bar) to 106 l (Kofler K10 and K16, nozzle size 4 mm, pressure setup 4 bar). Considering a percentage of driftable droplets of 1%, 0.5 to 1 l water are at risk of drift at each treatment application. An average irrigation system consists of approximately 50 sprinklers per hectare. Thus the amount of driftable water per hectare corresponds to 25-50 l per treatment application. According to the statement of a nozzle producing company, nozzles for a five times concentrated spray volume (300 l per meter crown height), 90% of the droplets have a diameter smaller than 250 µm and are so classified as driftable droplets. As visible in the experiment on hand, the overhead irrigation systems produce less than 1% of droplets in the driftable size range and can so be considered clearly less at risk of drift.

To clearly prove the drift potential of overhead irrigation, in addition to this experiment, spray drift trials should be carried out. It has to be considered that the proportion of small droplets prone to drift is relatively low, but all the irrigation heads are positioned above the canopies, where natural wind may easily pick up the smaller droplets below 250 µm, possibly depositing a significant fraction of those outside the orchard on non-target areas.

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# Residue decline behaviour of the natural insecticide spinosad on apples

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**Abstract**
*Spinosad is a common insecticide not only in organic but also conventional farming. It is a natural derived product from the fermentation of bacteria with a broad ranging control spectrum. The present study, carried out in the orchards of Research Centre Laimburg (Italy), aims to determine the residue decline behaviour of spinosad on apples and to determine different application strategies that results in a residue level not exceeding 0.01 mg/kg. This limit is often requested by European retailers and organic associations. The results show that 1 and 2 treatments, at application rates of 216 g/ha and the last treatment at least applied 20 days before harvest, ensures residue quantities below 0.01 mg/kg.*

**Keywords**: spinosad, insecticide, apples, residue level

## Introduction

Spinosad is a naturally derived insecticide produced through the fermentation of the bacteria *Saccharopolyspora spinosa* which occurs in nature and is not genetically engineered.
It is comprised of two complex multi-ring molecules each with a different sugar attached to the central ring structure. These two molecules are very similar in composition and are referred to as spinosyn A and spinosyn D. Commercial formulations typically consist of a mixture of spinosyn A (*CAS Registry No. 131929-60-7*) and spinosyn D (*CAS Registry No 131929-63-0*) in a ratio of approximately 85:15 (*Mertz, F. P. and Yao, R. C , 1990; Thompson, G. D. et al., 1997, 2002; Crouse, G. D. and Sparks, T. C., 1998; Dow, 2001*)
Spinosad control spectrum is quite broad ranging from Lepidoptera, Diptera, Thysanoptera, Hymenoptera, Siphonaptera, and Coleoptera (*Thompson, G.D., et al.. 2000*; *Dow, 2001*). Formulated products containing spinosad are registered worldwide in many countries on a wide range of crops and on farm animals for the control of external parasites.

Spinosad is listed in Annex I of Directive 91/414/EEC (replaced by Reg. (EC) No 1107/2009) since 2007 (*Official Journal of the European Union. 15th February 2007*).

Spinosad has been admitted since many years in the organic standards in different countries (e.g. USA, Switzerland). Due the unsatisfactory situation of insect regulation in organic farming in Southern EU Countries, in 2005 Italy proposed the introduction of spinosad in Annex 2B of the former European regulation for organic agriculture 2092/91/EC. In 2008 spinosad was admitted with the restriction of certification body in a new product category called microbial derivate products (*Official Journal of the European Union. 7th May 2008*).

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The EU- MRL (Maximum Residue Level) according to Regulation (EC) No. 396/2005 *(Official Journal of the European Union, 16 March 2005)* for spinosad (sum of spinosyn A and spinosyn D expressed as spinosad) on apple is currently set at 1 mg/kg. The EU-MRL must be respected both in conventional and organic production. For the spinosad uses approved in the EU the label PHI (minimal Pre Harvest Interval) is 7 days.
The Dow AgroSciences residue data supporting the use of spinosad on apples in the EU demonstrate that applying on apples the maximum label rate (216 g a.i/ha) four time in the season, the spinosad residue level with 7 days of PHI is lower than 30% of EU MRL, as requested by several EU Supermarkets and retailers.
Nevertheless spinosad is not very popular among different organic grower associations in the northern Europe and it is not accepted by some food retailer selling organic products which identify it as a conventional agrochemical because it is part of the screening programme in multimethod analyses for pestizide residues. For this reason some Supermarkets request to achieve 0.01 mg/kg (limit of quantification) in organic fruits.

The aim of the paper was to calculate the probability to achieve residue levels on apples below 0.01 mg/kg as a function of different application factors that correlate with residues (number of treatments, dates between application and harvest, etc). Such information would be useful to provide clear indications to the growers who wish to comply with this more restrictive requirement.

## Material and Methods

In 2009, the variety Golden Delicious, cultivated in an apple orchard of the Research Centre Laimburg (Vadena BZ), was treated three times at a distance of three days with the product Laser[5] (Active ingredient spinosad 480 g/L) at a dosage of 20 ml/hl (144 g a.i./ha). The sample taking for the analysis of residues has been carried out 6, 26 and 29 days after the last treatment, the dates are shown in table 1.

Table1: Dates of treatment and sample taking for the analysis of spinosad on fruits in 2009 (Harvest date: 15.09.09)

| **Dates of treatment** | | | **Dates of sampling** | | |
|---|---|---|---|---|---|
| 13.08.09 | 17.08.09 | 20.08.09 | 26.08.09 | 15.09.09 | 18.09.09 |

As in 2009, also in 2010 the trial was carried out on the variety Golden Delicious, cultivated in an apple orchard of the Research Centre Laimburg (Vadena BZ). One single treatment with the product Laser at a dosage of 30 ml/hl (216 g a.i./ha) was applied. This single treatment has been replicated in 4 different parcels.
Thereupon 4 dates for analysis (indicated in table 2) were fixed, in order to elevate a curve of degradation of the residues, both on foliage and on fruits.

Table 2: Dates of sample taking for the analysis of spinosad on fruits in 2010 (Harvest date: 15.09.09)

| **Date of treatment** | **Dates of sampling** | | | |
|---|---|---|---|---|
| 03.08.2010 | 10.08.10 | 18.08.10 | 24.08.10 | 17.09.10 |

In 2011 the trial was carried out on 7 different varieties. For each variety, one parcel with about 70 trees was treated one time; a second parcel was treated two times with Laser at

a dosage of 30 ml/hl (216 g a.i./ha). The varieties observed, the dates of treatment and sample taking for the analysis of residues are shown in table 3.
The sample taking has been carried out 10 / 16 / 21 / 31 / 41 / and 46 days after the last treatment.

Table 3: Variety, date of treatment and date of sampling taking for the experiment in 2011 (Harvest date: 3rd sampling)

| Variety | Dates of treatment* | | Dates of sampling | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1st treatment | 2nd treatment after 5 days | 1st | 2nd | 3rd | 4th | 5th | 6th |
| Red del. | 10.08.11 | 15.08.11 | 26.08 | 31.08 | 05.09 | 15.09 | 25.09 | 30.09 |
| Golden Del. | 10.08.11 | 15.08.11 | 26.08 | 31.08 | 05.09 | 15.09 | 25.09 | 30.09 |
| Braeburn | 16.08.11 | 21.08.11 | 01.09. | 06.09 | 11.09. | 21.09 | 30.09 | 05.10 |
| Kanzi | 20.08.11 | 25.08.11 | 05.09 | 10.09. | 15.09 | 25.09 | 05.10 | 15.10 |
| Granny Smith | 2.09.11 | 7.09.11 | 17.09 | 23.09 | 28.09 | 08.10 | 18.10 | 23.10 |
| Fuji | 2.09.11 | 7.09.11 | 17.09 | 23.09 | 28.09 | 08.10 | 18.10 | 23.10 |
| Pink lady | 23.09.11 | 28.09.11 | 08.10 | 13.10 | 18.10 | 28.10 | 08.11 | 13.11 |

*the second treatment has been applied only to the second parcel of every variety, while the first treatment was applied to all parcels

Both years, the experimental parcels have been delimitated by trees and rows, in order to avoid drift. The application was carried out with a radial parcel sprayer from Waibl (nozzles Teejet blue – pressure 7 bar) and a water volume of 500 l per meter crown height (equivalent to 1500 L/ha).

The sampling was carried as follows: 20 fruits were harvested randomly from different trees in each plot. In 2010 and 2011 samples were conserved at 1°C until the extraction and analysis. In 2009 the samples were immediately extracted and the extraction samples were kept at -20°C until the analysis

Sampling preparation, extraction, purification and spinosad residue level determination by LC-MS-MS were carried out following the UNI EN 15662:2009 method

## Results

The residue data of spinosad recorded (expressed in mg/kg) have been split in different classes on the basis of residue level, setting the value "*true*" = 1 when the residue value fall in a specific class and the value "*false*" = 0 when the value doesn't fall in the same class.
In this way the residue data have been transformed in *qualitative* or *categorical* data

*Classes:*

1. Residue ≥ EU MRL
2. Residue ≥ 50% EU MRL
3. Residue ≥ 30% EU MRL
4. 30% EU MRL < Residue ≥ 0.01 mg/kg (= LoQ: limit of quantification)
5. Residue < 0.01 (= LoQ: limit of quantification)

The categorical data can be analyzed by *Binary Logistic Multiple Regression* (Logistic Regression is also called logistic model or logit model) which is used for prediction of the probability of occurrence of an event by fitting data to a logistic function. It is a generalized linear model used for binomial regression. Like other forms of regression analysis, it makes use of one or more predictor variables that may be either numerical or categorical
In this case numerical predictor variable are active substance rate, number of treatments, days between application and harvest and categorical predictor variables are varieties.

***Equation of Probability***

$$P = 1/ 1 + e^{-z}$$

$$z = a + b_1x_1 + b_2x_2 + b_3x_3 + \ldots\ldots\ldots\ldots\ldots + b_nx_n$$

*Where:*
***a*** *= constant*
$x_1, x_2, x_3, \ldots\ldots x_n$ *= numerical or categorical factors resulted significant*

In figure 1 are showed the 112 spinosad residue data recorded in 2009, 2010 and 2011 experimental trials

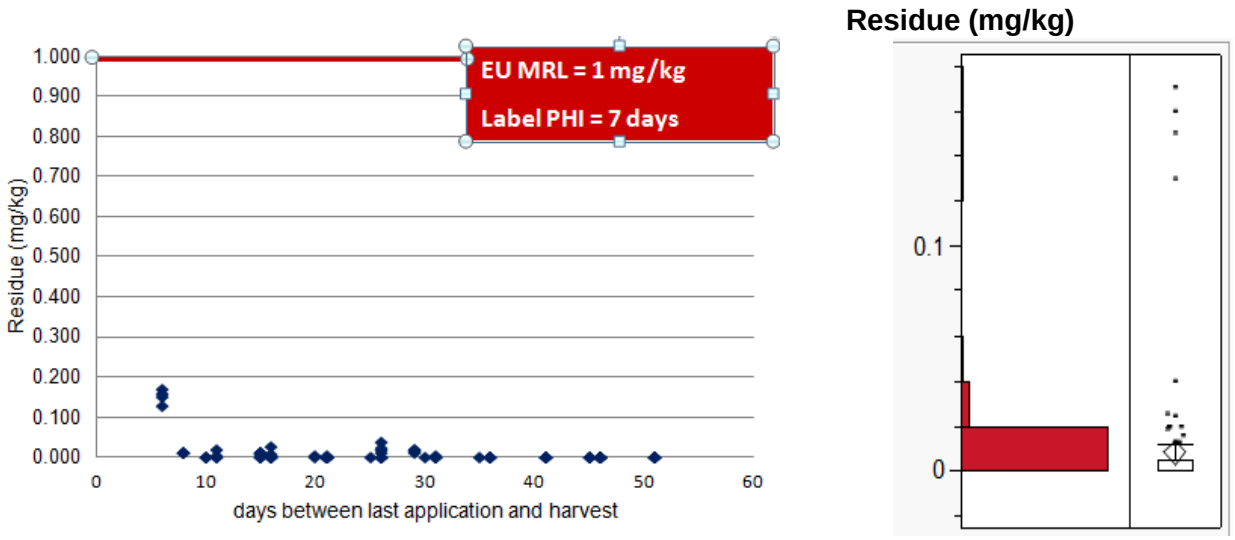


Figure 1: Spinosad residue level (mg/kg) at different days between last application and harvest

All the residue data recorded are lower than 30% MRL (0.3 mg/kg) and most of them (80%) are lower than 0.01 mg/kg with an interval of days between last application and harvest starting from 10 days (figure 2)

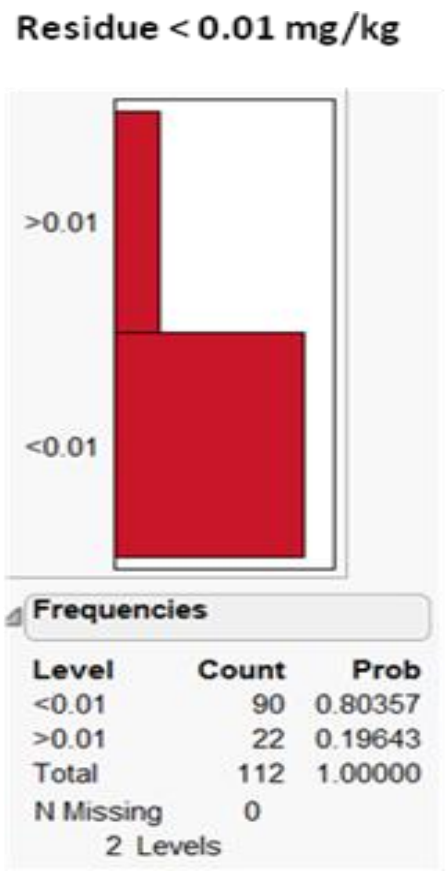


Figure 2: Spinosad residue distribution into the category "Residue lower than 0.01 mg/kg*"*

Carrying out the Binary Logistic Multiple Regression with the 112 spinosad residue data the model obtained considering the factors correlated with residue level, number of treatments (*Prob (ChiSq) = 0.0001*) and days between last application and harvest (*Prob (ChiSq) = 0.0001*), resulted significant: *Prob (ChiSq) = 0.0001 (Prob (ChiSq) < 0.05 is significant)* and $R^2$*=0.81 (*$R^2$*>0.2 is good in logistic Regression)*.
The model has also an outstanding predictive capacity (=accuracy): AUC=0.98. (*Accuracy can be expressed as the model's ability to correctly classify 0, or the ability to correctly classify 1 in the holdout dataset. AUC= 0.5 model has no predictive ability; 0.7≤AUC≤0.8 acceptable; 0.8≤AUC≤0.9 excellent; AUC ≥ outstanding*).

The equation of probability is

$P (<0.01) = 1/ 1 + e^{-z}$

*Where*
***z** = -22.66 +8.56 x*
*n. treatment (1) + 14.69 x*
*n. treatment (2) + 0.93 x days between last application and harvest.*

The probability to achieve a residue level of spinosad higher or lower than 0.01 mg/kg are represented in figure 3.

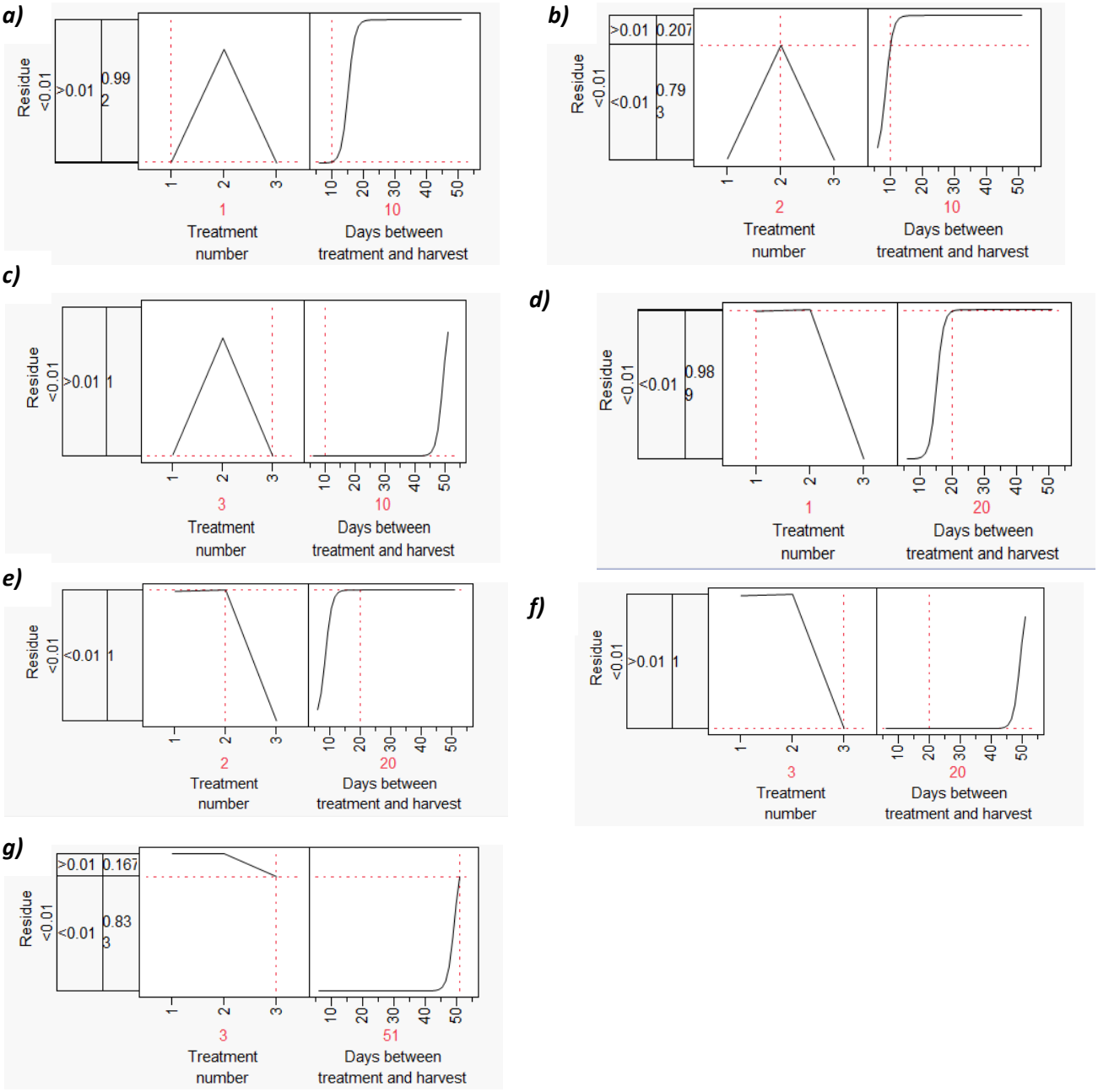


Figure 3: Probability to achieve a residue level of spinosad higher or lower than 0.01 mg/kg calculated by Binary Logistic Multiple Regression Model

In apples harvested 10 days after the last application the probability to achieve a spinosad residue level higher than 0.01 mg/kg is between 80 and 100% independently by the number of treatments (figure 3*a*, 3*b* and 3*c*).
When the days between last application and harvest increase to 20, the probability to find a spinosad residue level in apples lower than 0.01 mg/kg is around 100% with 1 or 2 spinosad treatments (figure 3*d* and 3*e*), whereas with 3 spinosad treatments there is 100% probability to have a residue level higher than 0.01mg/kg (figure 3*f*).
With 3 spinosad applications it is necessary to increase the number of days between last application and harvest to 51 days to achieve the probability of 83% to obtain a residue level lower than 0.01 mg/kg (figure 3*g*).

## Conclusions

The experimental trials carried out in the apple orchard of Research Centre Laimburg (Vadena, BZ) to evaluate the residue level of spinosad after the application of 1, 2 or 3 treatments at maximum label rate of 216 g/ha (30 ml/ha of commercial product Laser) on different apple varieties showed that 80% of the total residue data (112 data) are lower than limit of quantification of 0.01 mg/kg.

The statistical analysis (Binary Logistic Multiple Regression) applied to spinosad residue data allow to built a model with an outstanding predictive capacity (AUC=0.98) , which show a very high probability (98-100%) to find out residue of spinosad lower than 0.01 mg/kg (limit of quantification) in apple when 1 or 2 treatments of spinosad are applied and the time interval between last application and harvest is 20 days.

The growers who produce organic apples can take in account this indication on spinosad application in order to be able to supply those EU retailers who may have very restrictive requirements in term of residue in organic fruits.

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# Limitation of Codling moth (*Cydia pomonella*) with different paraffin and plant oils

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**Abstract**

*Codling moth*, Cydia pomonella, *is the major pest of pome fruit, and especially difficult to control in organic farming in Southern fruit growing areas, where this pest has two and more generations per year. Mating disruption,* Cydia pomonella *GranuloVirus (*Cp*GV) and entomopathogenic nematodes do not always provide adequate pest control. The active substance Spinosad shows high efficacy, but has also negative side effects on beneficial organisms. Furthermore residues of Spinosad remain detectable for a long time in fruits. From 2007 to 2011, several field trials with paraffin and plant oils have been conducted on apple and pear in the major Italian pome fruit growing areas, South Tyrol and Emilia Romagna. Results varied considerably: while only low efficacy levels were recorded in South Tyrol on apple, interesting and extremely promising results were obtained in Emilia Romagna on pear. Assumptions can be made to explain these differences, but none of them can be considered completely satisfactory.*

**Keywords:** Apple, codling moth, paraffin oil, plant oil

## Introduction

Codling moth, *Cydia pomonella*, is one of the major orchard pests. Apple and pear are its major hosts, but in warmer climates it may attack also quince, apricot, peach, plum, cherry, hawthorn, chest- and walnut. The pest probably originally occurred only in the Mediterranean Countries of Europe, but is now distributed almost worldwide (Pollini *et al*., 1993). In conventional and integrated production, codling moth can be adequately controlled with the chemical synthetic plant protection products currently available on the market, but in organic farming its control is still challenging due to the sometimes limited efficacy of the available insecticides. Mating disruption of codling moth with sex pheromones is one of the most important control tools also in organic farming (Kelderer, 2007). Good efficacy levels can be achieved under conditions of low pest pressure and by applying mating disruption over large areas. To increase efficacy, these systems for population reduction are usually used in combination with insecticide sprays based on *Cydia pomonella* GranuloVirus (*Cp*GV). However, in recent years, codling moth populations resistant to *Cp*GV have been detected (Fritsch *et al.,* 2005; Jehle *et al*., 2010). Furthermore, several studies show that the insecticide can provide efficient population control, but damage reduction may not always be satisfactory. The organic plant protection product Spinosad, a mixture of metabolites of the soil-dwelling bacterium *Saccharopolyspora spinosa*, shows high efficacy against insects, but its activity is not selective and residues of the substance are detected in the production. Most of the German organic fruit grower associations therefore allow the use of Spinosad only under special conditions. Also entomopathogenic nematodes, such as *Steinernema feltiae* and *Steinernema carpocapsae*, are applied for population control against overwintering codling

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moth larvae, but in the open field their efficacy is limited (Kienzle *et al.,* 2008; Peters *et al.,* 2008).

For organic farming, finding valuable alternatives for the control of this key pest is therefore of sound importance. Single-row netting structures provided good levels of control, but they are very expensive (Kelderer *et al.,* 2010).

From 2007 to 2011, several trials with special focus on the use of oily substances for codling moth control, have been carried out in the major Italian pome fruit growing regions Emilia-Romagna and South Tyrol. In South Tyrol trials were conducted on apple, while in Emilia Romagna they were performed on pear.

## Material and methods

Trials on apple in South Tyrol

In South Tyrol, trials on the efficacy of plant protection products against codling moth have been conducted from 2009 to 2011. All trials were performed in an apple cultivar Braeburn orchard (rootstock: M9), located in Auer (South Tyrol, Italy). The orchard was planted in 1997.

Details on the products tested in the trials are reported in Table 1, 2 and 3.

Table 1: Description of the products tested in 2009

| Active ingredient | Product name | Distributor | Applied rate (/100 l) |
|---|---|---|---|
| CpGV | Madex Plus | Intrachem Bio Italia | 6.7 ml |
| CpGV + Paraffin oil | Madex Plus + UFO | Intrachem Bio Italia+ Intrachem Bio Italia | 6.7 ml + 1 l |
| Spinosad | Spinosad | Dow Agroscience | 30 ml |
| Untreated control | - | - | - |

Table 2: Description of the products tested in 2010

| Active ingredient | Product name | Distributor | Applied rate (/100 l) |
|---|---|---|---|
| Chlorpyrifos* | Pyrinex ME | Makhteshim | 210 ml |
| Soy oil | Greenline | Organics | 500 ml |
| Mustard oil + Cruciferous plant meal | Duofruit | Cerealtoscana | 1 l + 300 g |
| Untreated control | - | - | - |

*applied at 14-day time intervals

Table 3: Description of the products tested in 2011

| Active ingredient | Product name | Distributor | Applied rate (/100 l) |
|---|---|---|---|
| Chlorpyrifos* | Dursban | Dow AgroSciences | 70 g |
| CpGV | Madex Plus | Biofa | 7 ml |
| Spinosad | Laser | Dow AgroSciences | 30 ml |
| Plant oil | A1 | Icas | 350 ml |
| Soy oil | Greenline | Organics | 500 ml |
| Mustard oil + Cruciferous plant meal | Duofruit | Cerealtoscana | 500 ml + 150 g |
| Untreated control | - | - | - |

*applied at 14-day time intervals

In each study year, to compare the different treatments, a randomized block design with 4 replicates of 10 trees each per treatment was used. To avoid biasing of data due to spray drift among plots, each study plot was isolated from the other plots by additional trees and rows of trees. All treatments were applied using a motorized sprayer (sprayer for experimental trials Waibl, Teejet blu), a spray volume of 1500l/ha, and a spray pressure of

7.0 bar. Except for chlorpyrifos, which was used at 14-day time intervals, all other treatments were applied at weekly time intervals from beginning of egg-hatching up to harvest.

Trials on pear in Emilia-Romagna

In the Emilia-Romagna region, trials on the efficacy of oily substances for codling moth control in organic farming have been conducted from 2007 to 2011. The trials were carried out in pear orchards (cultivars “Abate Fetel” and “William”), located in the surroundings of Modena, Ferrara, and Bologna. A randomized block design with 4 replicates per treatment was used.

Details on the products tested in the different study years and trials are reported in Table 4, 5, 6, 7, and 8.

Table 4: Description of the products tested in 2007

| Active ingredient | Product name | Distributor | Applied rate (/100 l) |
|---|---|---|---|
| Untreated control | - | - | - |
| CpGV + Paraffin oil | Carpovirusine + Biolid | Scam + Sipcam | 70 g + 1 kg |
| CpGV | Carpovirusine | Scam | 70 g |

Table 5: Description of the products tested in 2008

| Active ingredient | Product name | Distributor | Applied rate (/100 l) |
|---|---|---|---|
| Untreated control | - | - | - |
| CpGV | Carpostop | Serbios | 34 g |
| CpGV + Paraffin oil | Carpostop + Agrol | Serbios + Agrol | 34 g + 1 kg |
| Paraffin oil | Agrol | Agrol | 1 kg |
| Untreated control | - | - | - |
| CpGV | Carpovirusine | Scam | 70 g |
| Paraffin oil | Agrol | Agrol | 1 kg |
| CpGV + Paraffin oil (full rate) | Carpovirusine + Agrol | Scam + Agrol | 70 g + 1 kg |
| CpGV + Paraffin oil (half rate) | Carpovirusine + Agrol | Scam + Agrol | 70 g + 0.5 kg |

Table 6: Description of the products tested in 2009

| Active ingredient | Product name | Distributor | Applied rate (/100 l) |
|---|---|---|---|
| Untreated control | - | - | - |
| CpGV + Paraffin oil | Madex 100 + Agrol | Intrachem Bio Italia + Agrol | 21 g |
| CpGV | Madex 100 | Intrachem Bio Italia | 21 g |
| Paraffin oil (full rate) | Agrol | Agrol | 500 g |
| Paraffin oil (half rate) | Agrol | Agrol | 250 g |
| Untreated control | - | - | - |
| Emamectin benzoate+ adjuvant | Affirm + Break Thru | Syngenta | 300 g + 25 g |
| Paraffin oil | Agrol | Agrol | 250 g |
| Soy oil | Greenline 88 | Organics | 250 g |

Table 7: Description of the products tested in 2010

| Active ingredient | Product name | Distributor | Applied rate (/100 l) |
|---|---|---|---|
| Untreated control | - | - | - |
| Rape oil | Edible oil | - | 250 g |
| Soy oil | Greenline 88 | Organics | 250 g |
| Mustard oil + Cruciferous plant meal | Duofruit | Cerealtoscana | 1 kg + 300 g |
| CpGV | Carpovirusine plus | Scam | 70 g |

Table 8: Description of the products tested in 2011

| Active ingredient | Product name | Distributor | Applied rate (/100 l) |
|---|---|---|---|
| Untreated control | - | - | - |
| Paraffin oil (full rate) | Oleoter | Scam | 250 g |
| Paraffin oil (half rate) | Oleoter | Scam | 125 g |
| Rape oil | Edible oil | - | 250 g |
| Mustard oil + Cruciferous plant meal (full rate) | Duofruit | Cerealtoscana | 1 kg + 300 g |
| Mustard oil + Cruciferous plant meal (half rate) | Duofruit | Cerealtoscana | 500 g + 150 g |
| Soy oil | Edible oil | - | 250 g |
| Soy oil | Greenline 88 | Organics | 250 g |
| Corn oil | Edible oil | - | 250 g |
| CpGV | Carpostop | Serbios | 34 g |

For each study year and trial, data were compared across treatments using 1-way ANOVA followed by Tukey's HSD test for *posthoc* comparisons of means ($P<0.05$). To improve homoschedasticity, data expressed in percentages were lg10-transformed. All analyses were performed with the statistics programme PASW 17.

## Results

Trials on apple in South Tyrol

Table 9: Percentage of fruits damaged by *C. pomonella* in the different treatments in 2009

| | 06/07/2009 | | | 24/09/2009 | | |
|---|---|---|---|---|---|---|
| **Treatment** | **% damaged fruits** | **Std. Error of Mean** | **Tukey HSD** | **% damaged fruits** | **Std. Error of Mean** | **Tukey HSD** |
| Madex Plus | 11.3 | 1.8 | a | 46.4 | 1.4 | b |
| Madex Plus + UFO | 10.5 | 1.9 | a | 41.8 | 1.8 | ab |
| Spinosad | 7.8 | 0.5 | a | 38.0 | 1.9 | a |
| Untreated control | 11.8 | 1.7 | a | 49.0 | 3.3 | b |

No significant increase in the efficacy of *Cp*GV was achieved by adding paraffin oil to the tank mixture.

Table 10: Percentage of fruits damaged by *C. pomonella* in the different treatments in 2010

| | 12/07/2010 | | | 07/10/2010 | | |
|---|---|---|---|---|---|---|
| **Treatment** | **% damaged fruits** | **Std. Error of Mean** | **Tukey HSD** | **% damaged fruits** | **Std. Error of Mean** | **Tukey HSD** |
| Pyrinex ME | 2.2 | 0.4 | a | 13.1 | 1.4 | a |
| Greenline | 3.6 | 0.7 | a | 17.8 | 2.0 | a |
| Duofruit* | 3.7 | 1.4 | a | 15.9 | 1.9 | a |
| Untreated control | 5.0 | 1.4 | a | 29.0 | 2.2 | b |

*slight leaf burn was observed

The products Greenline and Duofruit showed a certain level of efficacy against both 1$^{st}$ and 2$^{nd}$ generation larvae, statistically comparable to that of the conventional standard insecticide Pyrinex ME (chlorpyrifos), applied at 14-day time intervals.

Table 11: Percentage of fruits damaged by *C. pomonella* in the different treatments in 2011

| | 01/07/2011 | | | 19/09/2011 | | |
|---|---|---|---|---|---|---|
| **Treatment** | **% damaged fruits** | **Std. Error of Mean** | **Tukey HSD** | **% damaged fruits** | **Std. Error of Mean** | **Tukey HSD** |
| Pyrinex ME | 6.4 | 1.6 | ab | 32.3 | 2.8 | ab |
| Madex Plus | 9.8 | 2.4 | b | 44.4 | 2.8 | c |
| Spinosad | 3.9 | 1.6 | a | 27.5 | 1.9 | a |
| A1 | 10.5 | 3.2 | b | 47.3 | 2.1 | c |
| Greenline | 9.9 | 0.8 | b | 36.3 | 2.4 | b |
| Duofruit* | 8.3 | 1.1 | b | 34.9 | 2.2 | b |
| Untreated control | 14.3 | 2.4 | b | 45.1 | 2.8 | c |

*slight leaf burn was observed

Even though at the 2nd assessment fruit damage in some of the oil-based treatments was significantly lower than in the untreated control, the oil-based products showed generally low efficacy in reducing fruit damage caused by both 1st and 2nd generation larvae.

Trials on pear in Emilia-Romagna

Table 12: Percentage of fruits damaged by *C. pomonella* in the different treatments in 2007

| **Treatment** | **% damaged fruits** | **Std. Error of Mean** | **Tukey HSD** |
|---|---|---|---|
| Untreated control | 12.0 | 2.0 | b |
| Carpovirusine + Biolid | 4.0 | 1.8 | a |
| Carpovirusine | 8.0 | 0.4 | ab |

A considerable, although not statistically significant increase in the efficacy of the CpGV-based product in reducing codling moth fruit damage was obtained by adding paraffin oil to the tank mixture.

Table 13: percentage of fruits damaged by *C. pomonella* in the different treatments in 2008

| **Treatment** | **% damaged fruits** | **Std. Error of Mean** | **Tukey HSD** |
|---|---|---|---|
| Untreated control | 23.5 | 5.1 | a |
| Carpostop | 13.5 | 4.7 | a |
| Carpostop + Agrol | 11.8 | 2.6 | a |
| Agrol | 8.5 | 2.5 | a |
| Untreated control | 62.0 | 2.4 | a |
| Carpovirusine | 43.8 | 3.4 | ab |
| Agrol | 29.8 | 7.0 | b |
| Carpovirusine + Agrol (full rate) | 26.0 | 5.9 | b |
| Carpovirusine + Agrol (half rate) | 26.3 | 2.4 | b |

In both trials, an increased efficacy of CpGV in reducing fruit damage was observed, when paraffin oil was added to the tank mixture, but differences among treatments failed significance. In one out of the two trials, even paraffin oil applied alone significantly reduced codling moth fruit damage in comparison to the untreated control.

Table 14: Percentage of fruits damaged by *C. pomonella* in the different treatments in 2009

| Treatment | % damaged fruits | Std. Error of Mean | Tukey HSD |
|---|---|---|---|
| Untreated control | 17.8 | 3.2 | c |
| Madex 100 + Agrol | 0.5 | 0.5 | a |
| Madex 100 | 3.0 | 0.7 | b |
| Agrol (full rate) | 6.3 | 1.5 | b |
| Agrol (half rate) | 5.3 | 1.3 | b |
| Untreated control | 8.3 | 2.1 | a |
| Affirm + Break Thru | 1.5 | 0.7 | a |
| Agrol | 4.3 | 2.0 | a |
| Greenline 88 | 2.5 | 1.3 | a |

Also in 2009, the addition of paraffin oil increased the efficacy of CpGV against codling moth, and paraffin oil applied alone again resulted in a significant fruit damage reduction in comparison to the untreated control in one out of the two trials.

Table 15: Percentage of fruits damaged by *C. pomonella* in the different treatments in 2010

| Treatment | % damaged fruits | Std. Error of Mean | Tukey HSD |
|---|---|---|---|
| Untreated control | 18.5 | 3.1 | c |
| Edible rape oil | 10.3 | 2.3 | b |
| Greenline 88 | 5.5 | 0.9 | ab |
| Duofruit | 6.5 | 0.9 | ab |
| Carpovirusine plus | 1.3 | 0.6 | a |

All tested plant oils significantly reduced fruit damage in comparison to the untreated control, with differences among the different formulated and not formulated oils not being significant. Slightly, though not always significantly lower fruit damage levels were recorded for the *Cp*GV-based product than for the plant oils.

Table 16: Percentage of fruits damaged by *C. pomonella* in the different treatments in 2011

| Treatment | % damaged fruits | Std. Error of Mean | Tukey HSD |
|---|---|---|---|
| Untreated control | 16.5 | 1.9 | b |
| Oleoter (full rate) | 5.0 | 1.1 | a |
| Oleoter (half rate) | 5.5 | 3.0 | a |
| Edible rape oil | 4.0 | 1.4 | a |
| Duofruit (full rate) | 3.8 | 2.1 | a |
| Duofruit (half rate) | 4.5 | 1.2 | a |
| Edible soy oil | 6.3 | 3.6 | a |
| Greenline | 4.8 | 1.7 | a |
| Edible corn oil | 2.5 | 0.9 | a |
| Carpostop | 0.5 | 0.3 | a |

Codling moth fruit damage was always significantly lower in treated than in untreated control plots, with differences among treated plots not being significant. The *Cp*GV-based product showed slightly, though not significantly higher efficacy than the oil-based products in controlling the target pest.

## Discussion

In Southern areas, codling moth, *Cydia pomonella*, is the major pest in organic pome fruit production. Mating disruption, entomopathogenic nematodes and *Cydia pomonella* GranuloVirus (*Cp*GV) frequently do not provide satisfactory levels of control of this pest. Even though Spinosad has recently been included into Annex II of EU Regulation 889/2008, many grower associations are disinclined to recommend the use of Spinosad because of its negative side effects on beneficials and its long-lasting detectable residue levels. From 2007 to 2011, several field trials aiming at evaluating the efficacy of oily substances against codling moth, have been conducted in South Tyrol on apple and in Emilia Romagna on pear. Several paraffin oil-based products, differing in their origin and manufacturing, and various plant oils, obtained from different plant species, have been tested.

No satisfactory results were obtained in the trials carried out on apple in South Tyrol. Efficacy levels against codling moth of the oil-based products were low, and almost no significant damage reduction in comparison to the untreated control was recorded. In addition, the plant oil-based product Duofruit, which showed highest efficacy, caused considerable leaf and fruit burn.

In the trials conducted on pear in Emilia Romagna, instead, the same active substances showed efficacy values ranging from 50 to 80%. These substances thus seem to be promising tools to be included into currently commonly used control strategies of codling moth.

Doubts on the mode of action of oily substances may arise, and an acceptable explanation for the observed differences in efficacy is warranted. In the past it was assumed that oily substances may act as ovicides, and contradictory studies, reporting from low up to excellent efficacy levels, can be found also in literature. Riedl *et al*. (2002) investigated the mode of action of oily substances in laboratory studies, and concluded that their efficacy depends on the likelihood of interrupting egg respiration. Egg respiration, and thus the efficacy of oils, seems to be strongly influenced by the location of the oviposition site: eggs deposited on the lower leaf surface are hardly affected by the oil, while eggs deposited on the upper leaf surface and on fruits may be killed. Literature does not provide a definite answer whether differences exist in the oviposition sites of codling moth between apple and pear.

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# POSEIDON - a tool to support the orientation towards a sustainable improvement of organic fruit growing in participative working groups

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**Abstract**

*In the frame of the project "network for improvement of the production system in organic fruit growing" (BOELN-project Nr. 04OE178/06OE100) a group of fruitgrowers, consultants and researchers from all German regions started to discuss approaches to improve the orientation of their production system towards the principles of organic farming (POA). Several working groups on different topics have been generated in this network. In a participative process involving fruit growers, researchers and consultants, POSEIDON, a first indicator system has been developed to support the decision making process of these working groups by presenting relevant data from practice and analysis. In the BOELN-project 2810OE024, a field record system and a benchmarking system to collect and present the parameters based on these indicators necessary for the working group "reduction of copper and of general input of plant protection products" are prepared and evaluated.*

*POSEIDON aims to support the discussions about different strategies with data presentation. POSEIDON does not aim to replace the discussion about the best strategies to improve the orientation towards the POA by a rating system for "best practice" referring to the actual state of scientific knowledge. The nucleus for the improvement of the production system is not the model but the working groups consisting of growers, consultants and scientists.*

**Keywords:** system approach, sustainability, indicator system, organic fruit growing

## Introduction

Since 2004, in the frame of the project "network for improvement of the production system in organic fruit growing" (BOELN-project Nr. 04OE178/06OE100) a group of fruitgrowers, consultants and researchers from all German regions discuss possibilities to improve their production system (Kienzle *et al*., 2008, 2010). In the last years a necessity was felt to proceed in a system approach and in a concerted action to ensure a sustainable further development.

Furthermore, discussions with authorities required to picture the production system of organic fruitgrowing, especially regarding aspects of plant protection. In consequence, a working system that allows to improve organic fruit growing supported by on farm data should be developed.

## Material and Methods

The system was developed in a participative process involving fruit growers, researchers and consultants. At the end of 2007, in the frame of the BOELN-project-Nr. OE06100 "network for improvement of the production system in organic fruit growing", a working group "POSEIDON" was founded. This group had the task, to work out first drafts for a system that should allow to structure an aim-oriented purposeful sustainable further development of the production methods. The system should also be suitable to explain the

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production system and the trends set in further development, especially in the plant protection system, to persons and authorities outside the organic sector.

The practicability of this system was evaluated in discussions about several topics, mainly regarding the areas plant protection and biodiversity:

- Reduction of the amount of copper used
- Strategies for resistance management, especially for codling moth
- Pro and Contra of the use of Spinosad in codling moth control
- Integration of aims of nature conservation in the cultivation methods

In the last years the subject of copper reduction was discussed very intensively in Germany in the organic sector and also with authorities. Thus, this topic was chosen for the first evaluation of the system in several working groups consisting of fruit growers, consultants, researchers and representatives of organic associations.

Since most topics regarded plant protection, in a first step the presentation of data about the plant protection practice in common models for sustainable agriculture was examined.

In most farm management systems as e.g. REPRO, an 'application-index' is used to summarize the data of the plant protection practice (Heyer *et al.,* 2005; von Haaren *et al*., 2008). The working group decided, that this kind of presentation was not suitable as a data basis for a sustainable aim-oriented development of organic fruitgrowing since most relevant data (kind of products used and their side effects, toxicity and persistence) about the products applied were not considered and other measurements than the application of plant protection products were not included at all.

Thus, a specific concept for presentation of data relevant for the plant protection system suitable for organic farming was developed. The first concept was presented in a first workshop in June 2009. After the discussion it was revised and finally discussed in the annual meeting of the "network for improvement of the production system in organic fruit growing" in December 2009. The data requirements and the calculation and presentation of the single parameters were worked out in smaller working groups consisting of 4-6 fruitgrowers and one or two consultants and/or researchers. During this activity the idea arose to use this system also as an instrument for the extension service. A draft for a benchmarking system related to the system used by the Bioland Beratung GmbH in quality management systems (Boehm *et al*., 2011) was evaluated in small working groups of 4-5 fruitgrowers and one consultant in several German fruit growing regions (Lake Constance, Neckar valley and Baden, Rhineland Palatinate, Northern Germany, Saxony).

The issue of these working groups was that there was great interest to have a benchmarking system based on this first draft. In consequence of this discussion, a project (2810OE024) was started at the end of 2010. In this project, software to record and evaluation of the data and for benchmarking the parameters is developed. Working groups consisting of 4-6 growers and one consultant in each of the four regions involved actually are evaluating and improving the software. Furthermore, in 2011, methods for a fast and practicable estimation of the success of the strategies (level of infestation with several pests and diseases) are elaborated and evaluated by the group.

## Results

### *1. Targeting and sustainability concept*

In the participative workshops with fruit growers, a "theoretical" discussion about the different concepts of sustainability used in Germany (Brand & Jochum, 2000) was not practicable. The first definition of sustainability was based on Meadows (Meadows *et al*., 2006) "a development is not sustainable if it erodes its own basis and for this reason will

not endure". In the model of the "three pillars" used in Germany (Brand & Jochum, 2000) this is a classical base of economic sustainability. In the discussion it became obvious that the basis of organic farming are the principles of organic farming (POA) as formulated by ifoam (www.ifoam.org). Since the POA are the basis for the "society pact" for higher price for products from organic farming, for organic farmers there is a crucial difference to conventional farming regarding economic sustainability: For organic farming, which aims explicitly in a holistic approach to enhance the stability and biodiversity of the agroecosystems as well as the fertility of the soil, it is economically crucial to consider this in the further development of the production methods.

Thus, since the POA cover the important fields of sustainability, the POA were just adapted as "sustainability concept" for the orientation of the further development of the production method. A better orientation to the POA was set as the "major aim" of all further development. If particular targets are followed, as for instance the reduction of the amount of copper used, care must be taken to consider all aspects of the development and to evaluate also an eventual cost of the success in other areas by reaching the particular target. Reaching a particular target must never lead to a decline of the whole production process in its orientations towards the POA. To picture this concept, the terms "aims", "bricks" (to achieve the aim) and "guardrails" (to point out the cost of success") were introduced.

### *2. Indicators*

Indicators are used for the measurement of certain parameters. In most systems, indicators derive from several parameters that are summarized and weighted following specific criteria. Qualitative parameters are transformed into numeric parameters to make possible a general summarizing of all parameters. The issues of some analysis are "sustainability rates" derived from weighted and summarized parameters (Kuestermann *et al*., 2002). The growers refused forcefully this kind of presentation and rating. During the discussion about the parameters and the data to collect they realized that with a justifiable effort it seemed not realistic to determine the whole production system in a way that a really holistic rating considering all aspects would be possible.

Their arguments reflected also the principle of care which advises to consider that there is an incomplete understanding of ecosystems and agriculture and that "scientific knowledge alone is not sufficient but must be backed by practical experience as well as by traditional and indigenous knowledge". Furthermore, in organic fruit growing there is still a rather fast development of knowledge and the "state of scientific knowledge" in organic fruitgrowing is a rather variable factor during time. Thus, the weighting and summarizing of the parameters into indicators for a evaluation of "good practice" and "bad practice" seemed not reasonable as base for the orientation of further development. Instead of using the rating by a model for orientation, the growers demanded a discussion process according to the common practice in the "Working net" but based on real data from practice. The single parameters should be presented individually. The process of rating and weighting should be part of the discussion where growers, consultants, scientists and members of associations should be involved. The competence for orientation of the further development was clearly seen in this discussion process. What was required was a presentation of real data as support for the decision in a discussion process. Since it is impossible to present really all data for the whole production system, the level of detail and the number of parameters for the data presentation is the key factor that has to be decided. Actually, several particular targets for the development have been roughly defined by the group just discussing about a better orientation towards the POA. Thus, it is possible to decide according to the particular target about the level of detail and the kind of parameters to

present as a base for the discussion process for further development. The group demanded to have a detail level in presentation of the main parameters regarding the relative strategies that allows a structured discussion. The main parameters regarding a possible success, a possible cost of success and the “bricks” for possible strategies must be presented individually. Thus, the level of detail of the presentation depends also on the strategies discussed. If, for instance, it is discussed to replace a treatment with a high amount of copper by a treatment with potassiumhydrogencarbonate, there is no need to specify the risk for aquatic organisms with any model since the situation is evident. However, if very low amounts of copper are replaced by high amounts of sulphur, it is maybe difficult to estimate just by evidence the impact of the two different strategies on aquatic organisms. In such a case, if available, a simulation model like SYNOPS (Gutsche & Strassemeyer, 2007) is needed to calculate the theorical impact of the two strategies on certain key species. If simulation models are used, the growers demanded that the path of calculation must be revealed. If different risks must be considered and appropriate models are not available, the risk must be presented in an acceptable mode in spite of the lack of a model. For the possible long term effects of low amounts of copper on soil organisms, for instance, it is actually difficult to find an appropriate model. Certainly, it is not possible just to neglect a preventive minimization of this possible risk. The parameter for presentation used in this case is simply the amount of copper in kg used per unit of area (ha) and year – just according to common practice in the guidelines for organic production for many years. Generally, the fruit growers exercised the system approach not only in the evaluation of their production methods but also in the evaluation of potential risks of plant protection products. The first criterion to evaluate the potential risk of a product was the occurrence in the system. The risk of a product was the more questioned the more external of the production system it occurred in nature. Ultimately, the restriction to natural substances in organic farming can be explained by this system approach in risk reduction.

In Table 1, as an example, the set of parameters for the target “reduction of the amount of copper” is described. For targets regarding the plant protection system, parameters are grouped in three pillars:

- Input of products and energy
- Sustainable farm management
- Functional biodiversity

For the presentation of several parameters, the data from the farm units can be computed in models that can provide further informations. For the estimation of the potential risk for aquatic organisms, for instance, an interface to SYNOPS designed by the institute for risk assessment of the JKI (Gutsche & Strassemeyer, 2007) is projected.

The particular *potential of a single active ingredient for resistance building* of the substances, if relevant, is expressed in a first approach based on the existing experience sorting the substances in 5 classes:

- Class 1: Applied since more than 50 years on large areas, no resistance observed. Unspecific mode of action (e.g. sulphur, copper)
- Class 2: Applied since more than 20 years on large areas, no resistance observed. Unspecific mode of action or more than one active ingredient
- Class 3: Applied since less than 20 years on large areas, no resistance observed. Unspecific mode of action and/or more than one active ingredient. The risk of resistance building in literature is estimated low but not excluded.

- Class 4: Applied since less than 20 years on large areas, no resistance observed. Only one or two rather purified active ingredients. The risk of resistance building in literature is estimated higher or similar products have shown resistance or for this product resistance building was observed on pests or diseases on other crops.
- Class 5: Cases of resistance observed with active ingredients with similar mode of action.

Table 1: Parameters for the presentation regarding the target “reduction of the amount of copper applied” grouped in the three pillars of the plant protection system. For parameters with *) details are explained in the text

| **Input of plant protection products and energy** | **Sustainable farm management** | **Functional biodiversity** |
|---|---|---|
| **Active ingredients and quantity applied.**<br>*If this is not sufficient for decision-making:*<br>- Parameters for the potential risk for human health and environment *)<br>- Parameters for the risk for resistance building against the active ingredient *)<br>- Parameters regarding a life cicle analysis of the active ingredients | **Sustainable application**<br>- Use of forecasting models<br>- Application technique<br>- Formulation | **Genetic biodiversity**<br>- Genetic biodiversity of the varieties *)<br>- Genetic diversity of variety resistance |
| | **Cultivation methods**<br>- Reduction of the inoculum<br>- Enhancement of the fertility of the soil<br>- Balanced fertilization<br>- Balanced tree growth and crop load regulation measures | |
| | **Choice of Variety**<br>- Percentage of scab-resistant varieties<br>- Percentage of varieties with low susceptibility to scab or (in special regions) canker | **Species diversity**<br>- Protection*) and enhancement of beneficial organisms |
| **Number of applications necessary**<br>*If this is not sufficient for decision-making*<br>Energy consumption for the applications | **Alternate application** of active ingredients if necessary regarding the risk of resistance building | |
| | **Organization of the farm unit**<br>- Number of application equipments in correlation to the area of scab susceptible varieties | |
| | **Economic success of the farm unit**<br>- Yield and quality *)<br>- Cost of the strategy<br>- Risk of losses *)<br>- Marketing success | |
| | **Local and seasonal factors**<br>- Seasonal infection pressure<br>- Local attitude for infections | |

The general *genetic biodiversity* is expressed using the data based partly on the data collected by Bannier (2011). An index based on the conformity of the genetic heritage based on the genealogy of the varieties is actually tested. For the strategy of copper reduction this index must be completed by guardrails regarding the sensitivity of the different varieties against different races of scab as the disease of main importance. These data are not yet available but should be collected.

The *side effects of plant protection products on beneficials* will be shown using a decision-making support model in preparation by Kienzle & Zebitz. At first, beneficials are classified as system relevant (efficacy to suppress the relative pest without other control mechanisms is documented), relevant (important effect on pest is documented) and less relevant. As data base for the side effects of the products mainly classifications related to the IOBC classification and available on the registration sheet of the products and other publications are used. However, in the interpretation of the data it must be taken into account that the basic data are not always comparable (laboratory and field data). The presence of stages of the insects sensitive to the products is indicated using data sheets where their occurrence is listed in relation to the BBCH-Code. The mobility of the insects is taken into account using a class system regarding their mobility over longer distances and their possibility to immigrate from landscape elements as hedges in the orchard.

In the context of plant protection strategies the most important parameter for the *quality of the fruits* is the percentage of infested fruit by a certain pest or disease. It is not practicable for the fruit growers to assess the infestation in their orchards with methods similar to researchers. However, it seems practicable for them to monitor the infestation levels of their fruits using a class-divided system. In 2011, a first schedule for 5 classes for scab and sooty blotch infestation was tested in the working groups. The survey of the infestation level is also used for the discussion about regional thresholds for the application of different strategies, e.g. for scab control.

It is important to remember that especially for the evaluation of plant protection strategies it is important to have long term data from different farm units in different regions. Failing this, a reliable estimation of the *risk of losses* and, thereby, of the real economic efficiency of the strategy is impossible. The risk of losses is expressed by the probability of losses (number of years with losses assessing a certain number of years and a certain number of farm units).

## *3. Data collection*

For the collection of the data in the field a field record system is used. Basic unit is always the farm unit. For the fruit growers, it is very important, that data has to be recorded only one time. Thus, the field record system must be suitable also for the recording of the data relevant for audits, e.g. Global GAP or the controls of the associations and for the requirements of the agricultural administration. Actually, one field record system is adapted to these general requirements of the organic fruitgrowers and simultaneously for those of the data collection for the benchmarking system of POSEIDON. The parameters are preset so that benchmarking is possible. Generally, the data collected should not exceed substantially the data that the fruitgrowers collect for their other necessities.

## *4. Benchmarking system*

The different strategies are presented by a list of the parameters relevant for the strategy. The data regard the level of farm unit, plot and single variety. New strategies are defined looking at the differences to a “standard strategy”. This standard strategy is defined usually as the mean values for the relevant parameters of the participating farm units from a

defined area (e.g. one region). If necessary, the pool of farm units can be specified even more about several selection criteria. The data of the single farm unit remain anonymous. Each fruit grower is able to view his own data and the mean of the group. Thus, a kind of self evaluation is possible.

Since plant protection strategies usually are described using a large amount of parameters, it could be difficult to have a clearly arranged presentation of the data.

Thus, parameters with values different to the standard strategy (the criteria for discrimination can be defined by the user) are marked and listed first. Even if the list of parameters to describe the strategies is long, in fact the user is working on the few parameters that show relevant differences. However, if a new strategy shows a difference to the standard strategy in some new parameter, this is not neglected since all relevant parameters are always listed and can be marked.

### *5. Presentation of the working groups*

In the German “Working net” there are different working groups with main focus on variety and rootstock testing, reduction of the input (especially copper) or the enhancement of biodiversity in the orchard. These groups will present their work on the Foeko homepage. The presentation of the activities to improve the orientation towards the POA was discussed also intensively in the working net. It was considered very important for this aim that the presentation of the working groups is transparent and faithful, scientifically sound and fair. The presentation should not downgrade other farmers or farming systems and should contain in the medium term concrete targets the working group wants to achieve. It should show the progress but also eventual measures to evitate a cost of this progress. In this context, even if special targets are followed, always the whole production system must be considered.

Furthermore, working groups should share their experience with the other organic farmers, and, in this way, contribute to a sustainable advancement of the whole sector.

## Discussion

The participative development in an interactive discussion process produced a working system very near to the origins of organic fruitgrowing. At the beginning, the production method was developed in small regional working groups of fruitgrowers. Base of the development were the principles of organic farming. In a second step, consultants and researchers joined the group and integrated scientific knowledge.

POSEIDON gives the possibility to reproduce this kind of work on a larger scale. The existing data from many farm units can be presented anonymously and structured. If necessary, smaller calculations or models can be integrated to evaluate certain parameters. The development of the system can follow the needs of the main topic and of the working group. In this way, POSEIDON can support the discussions about different strategies with data presentation. POSEIDON does not aim to replace the discussion about the best strategies to improve the orientation towards the POA by a rating system for “best practice” referring to the actual state of scientific knowledge. Since it is never possible to consider really all parameters of a system a really holistic picture of the production system seems not realistic. Thus, a rating based on a certain indicator set could easily set trends that do not really lead to an improvement of the orientation of the production system towards the POA but, in contrary, can compromise the holistic system approach of organic farming.

The nucleus for the improvement of the production system is not the rating by the indicator system but the working groups consisting of growers, consultants and scientists. The

benchmarking system does not aim to increase the competition between the fruit growers. It aims to enhance their collaboration in a collective improvement of their production system – always learning from each other.

In the next years, POSEIDON will be evaluated as a tool for the presentation of real data and as a support for decision making with the aim to improve the efficiency of these working groups. It will be also tested by extension service as a tool to record and to structure the experience of a large number of fruit growers over the years.

Several years experience will show how the system can be used best and several years will be needed to adapt the first version to the needs of different working groups and regions.

Actually, a first software based version is evaluated in the working group “copper reduction” by 20 fruit growers in four regions in collaboration with the respective extension service.

If POSEIDON succeeds to connect data regarding the state of scientific knowledge and the practical long term experience and, thus, to combine knowledge with experience, it will be a valuable instrument for participative working groups to collaborate successfully in improving the orientation of organic fruit growing towards the POA.

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# Successful blossom thinning and crop regulation for organic apple growing with potassium-bi-carbonate (Armicarb®): Results of field experiments over 3 years with 11 cultivars

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**Abstract**

*With field trials over 3 years in a commercial organic orchard in Switzerland we have tested the efficacy of Armicarb® (potassium-bi-carbonate) for flower thinning in organic apple production. Over time, Armicarb was tested on 11 cultivars, at different application periods, in different concentrations, and always in comparison to other agents that are already allowed for thinning in organic fruit production in the European Union as e.g. lime sulphur, molasses, mechanical rope-thinner or combinations of methods. Armicarb proved to be an efficient and reliable thinning agent with an efficacy similar to the now recommended methods with rope device, molasses or lime sulphur but has the advantage to be an environmentally very friendly product. On the other hand, the risk for fruit russeting is comparably elevated especially with cultivars Elstar, Golden Del. and Gala. Finally, we have elaborated cultivar-specific recommendations for the use of Armicarb for thinning purposes, which were the basis for the Swiss Federal approval to use Armicarb for thinning in conventional apple production in 2011. Its approval for thinning purposes for Swiss organic apple production is expected for the season 2012.*

**Keywords:** apple, organic, thinning, crop regulation, potassium-bi-carbonate

## Introduction

One of the main challenges in organic apple growing is the regulation of the crop load to, i) prevent bi-annual bearing, ii) improve fruit quality, and iii) save labour costs for manual thinning. Up to now, there are only few methods and agents allowed for certified organic agriculture: e.g. the mechanical rope thinner device (Bertschinger *et al.,* 1998). After Weibel *et al.* (2008), however, 1-2 treatments with the rope thinner alone seldom provide a satisfying result and should be combined with a desiccant agent such as e.g. molasses. Also with 2-3 molasses treatments during flowering period, for rewarding results a combination with the rope device is recommended by the latter authors. In most EU countries lime sulphur is the standard thinning agent. At dosages of 2-2.5 vol.% and 2-3 treatments over flowering period it provides a fairly good efficacy, and induces no risk for fruit russeting. In Switzerland, however, lime sulphur is not registered by the Federal authorities because of its potential human toxicity.

For the use as a contact fungicide Armicarb (potassium bi-carbonate; $KHCO_3$) is already licensed for organic apple production. The active component is 85% potassium-bi-carbonate which acts on fungi by changing the pH and the osmotic pressure plus the direct ionic effect of potassium-bi-carbonate on the cell walls (Stähler Suisse SA, Zofingen, CH). After promising pre-trials in 2006 and 2007 to apply Armicarb also for crop regulation, we conducted from 2009-2010 replicated thinning trials with several cultivars under very close to praxis conditions (e.g. using a commercial orchard sprayer). The main questions to answer were: i) thinning effect of Armicarb in comparison to other methods; ii) thinning

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effect and negative side effects (e.g. phytotoxicity) of Armicarb with different cultivars; iii) optimal concentration for different cultivars; iv) optimal application period and frequency. For this publication, we display and discuss mainly the results related to Armicarb.

## Material and Methods

The field trials were conducted on the commercial organic fruit farm of family Ch. Vogt at Remigen. Situated at the edge of the eastern Swiss Jura mountains, 450 m above sea level; av. temp. 9.1 °C/y; av. rainfall 900 mm/y; soil is a pseudo-gleyic, medium-deep brown soil of 18.9% clay, 45.8% silt and 35.1% sand, pH($H_2O$) is 5.5-5.9.

The experiments were performed in 2008, 2009 and 2010; the last return-to-bloom assessment was in April 2011. Per cultivar, there were usually 3, minimum 2 replicated plots randomly distributed. A plot consisted of usually 18-27 trees (minimum 9). Most trees were on rootstock M9 (except Maigold M27, and Topaz M27 in 2009); in full production age between 7 and 18 years old; spaced 1 x 3 m and under a hail protection net. Usually 10, minimum 5 representative trees per plot were selected as measuring and counting trees. Flowering intensity of the trees used for the experiment was at least 75% but mainly 90-100%. Usually the products were applied with a commercial orchard sprayer (Lochmann RPS) using 1000 L water per ha; case-wise a motor backpack sprayer (Birchmeier M155) was used. Usually the test agents were applied twice during flowering stage F and $F_2$ (BBHC 61-65); 3 applications were occasionally necessary when flowering period was long. The rope device was applied at pre-bloom at stage red tip (E, BBCH 57) at high driving speed (9-11 km/h) in order to keep physical damages to leaves and branches as low as possible. Because the agents tested (Tab. 1 Armicarb treatments, Tab. 2 other agents and methods) are all desiccants, we applied them at warm days (around 20 °C at midday) with no rain announced for the following 24 h, and when a maximum of un-pollinated flowers were open, thus, spraying time began from 9-10 a.m. on. For the rope device, however, we aimed for colder, cloudy weather to enhance the physiological shock of the treatment (according to Weibel *et al.,* 2008).

Fruit set was counted before and after June drop: from each measuring tree 4-6 representative branches in the centre zone of the canopy were chosen Over the entire length of each branch the amount of fruit clusters and the number of fruits per cluster (0,1, 2, 3+) was counted using a multiple hand counter tally. Russeting was assessed as % incidence; when treatment-induced russeting seemed to be more intensive, we also assessed severity as percentage of affected fruit skin. Few days before harvest final fruit set was estimated as percentage from an optimal crop load (set as 100%); and 25 representative fruits per replicated plot were collected to assess fruit diameter and wight of the fruits. To assess the treatments' influence on bi-annual bearing, return to bloom was counted in mid April of the following year as percentage of flower buds of total buds.

For statistical analysis we used ANOVA models (treatment, cultivar, replicated block (nested with cultivar) and interaction cultivar*treatment). For multipe treatment comparison a post ANOVA Tukey test was performed ($p<0.05$; JMP V. 8.0.1, SAS Inc.).

Table 1: Variants tested with Armicarb from 2008-2010 (beside untreated control and hand thinning of 2/3 of the flower clusters).

| Year | Application frequency and timing | Concentrations (kg/ha) | Cultivars |
|---|---|---|---|
| **2008** | 3 x during flowering period (F-$F_2$, BBHC 61-65) | 5, 10, 15, 20 | Golden Del., Idared, Elstar, Maigold, |
| **2009** | 1 x at F(61) or $F_2$(63) or $F_2$(64) or $F_2$(65) or at T-stage | 20, 15 at T-stage | Topaz, Otava (both scab resistant) |
| | 2 x at F(61) and $F_2$(65) | 10, 15, 20 | Golden Del., Gala Elstar, Maigold, Topaz (M9), Topaz (M27), Otava |
| **2010** | 2 x at F(61) and $F_2$(65) | 15 | Golden Del., Braeburn, Pinova, Gravensteiner, Topaz, Otava, Ariane (scab resistant) |
| | 2 x at F(61) and $F_2$(65) | 20 | Maigold |
| | 2 x at F(61) and $F_2$(65) combined with rope thinner | 15 | Elstar, Topaz |

Table 2: Treatments tested in comparison to Armicarb from 2008-2010 (beside untreated control and hand thinning of 2/3 of the flower clusters).

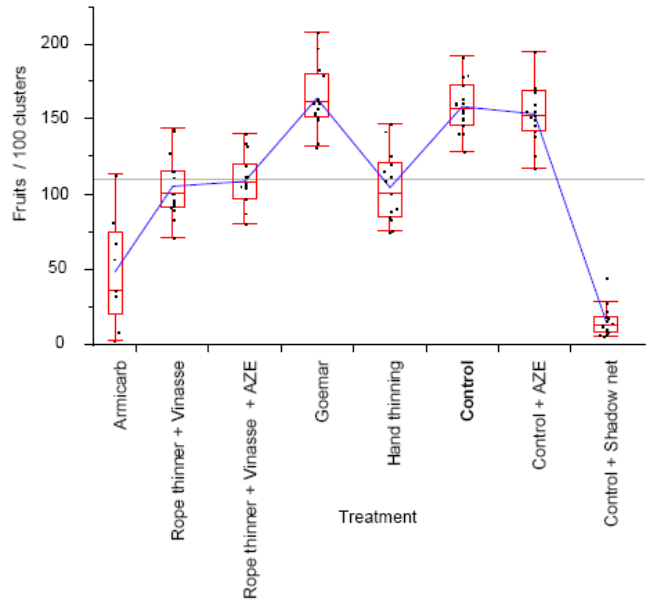
| Product | Description | Application |
|---|---|---|
| **Rope device** | "Gessler" (Friedrichshafen, DE), 286 Nylon ropes of 50 cm length on a 2 m vertical axis with 300 rev./min. | 1-2 at stage E(BBHC 57) at 9-11 km/h driving speed |
| **Shadow net** | "AGROFLOR" (Nendeln, FL) with 74% light reduction | for 3-5 days 20 days after full bloom |
| **Lime sulphur** | Ca-Polysulphid 381g/L "Polisenio", IT | 2.5 vol%; 2-3 x during flowering period (F-$F_2$, BBHC 61-65) |
| **Vinasse** Also in combination with rope device application (see above) | Molasses from sugar beet "Bioorga-NK-flüssig" (60 g N/L, 70 g K/L); Hauert HGB Dünger AG, Switzerland | 5-7 vol.%; 2-3 x during flowering period (F-$F_2$, BBHC 61-65) |
| **Acetic Acid** | "Apfelessig" for cooking purposes with 5 g AA/L (Bio Farm, Switzerland) | 3 vol %; 2-3 x during flowering period (F-$F_2$, BBHC 61-65) |
| **"Black oil"** | Self made mixture of pine oil (NuFilm 1ml/L) and dust of active carbon (25 g/L) to induce a micro-shading of the flower clusters by the black colour | 2-3 x during flowering period (F-$F_2$, BBHC 61-65) |
| **Goemar®** | An algae substrate containing natural GA 14 and micro-nutirents, Stähler Suisse SA, Zofingen, CH | 0.3 vol.% 2-3 times during flowering period (F-$F_2$, BBHC 61-65) |

## Results

*Experiments in 2008*

Figure 1 shows example-wise the thinning effects counted before June drop in 2008 on cv. Elstar. The treatments effects were similar but less expressed in the parallel trials on cv. Idared. Armicarb (in that year at a dosage of 20 kg/ha) had a strong, in this case almost too radical thinning effect by decreasing the fruit set from 159 fruits per 100 flower clusters (FlCl) to 49. This corresponds to a thinning effect of 69.4 %. Like this, the Armicarb treated trees had only half of the fruit set compared to hand thinning (removal of 2/3 of the flower

clusters) and the organic standard treatment rope-device plus vinasse, both showing thinning effects of 33 %. Net-shadowing caused a far too intensive fruit drop down to only 16 fruits/100 FlCl remaining.



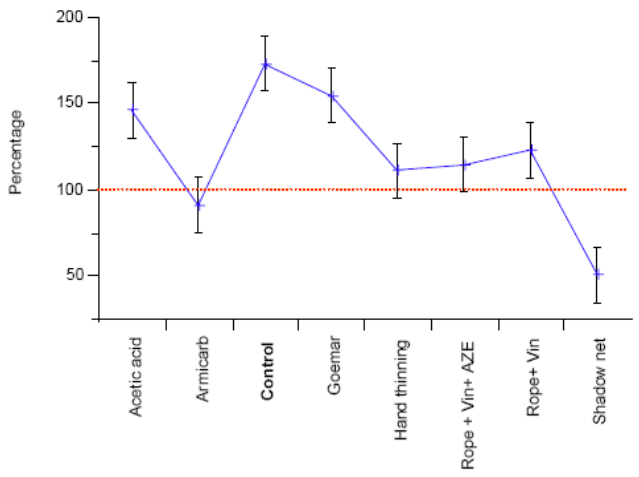

*Tukey HSD Test*

| *Treatment* | | *Mean* |
|---|---|---|
| Goemar | A | 164.0 |
| **Control** | A | 159.1 |
| Control + AZE | A | 154.0 |
| Rope thinner + Vin + AZE | B | 109.3 |
| Rope thinner + Vin | B | 105.7 |
| Hand thinning | B | 104.7 |
| Armicarb | C | 48.7 |
| Control + Shadow net | C | 16.0 |

*Levels not connected by same letter are significantly different. LSD = 43.37*

Figure 1: Number of fruits/100 clusters with cv. Elstar under 8 different thinning treatments counted before June drop in 2008. Represented are Box Plots with the great mean line (horizontal line). AZE = acetic acid. Vin = vinasse, LSD = least significant difference

The effect of the treatments on crop load before harvest in 2008 can be seen in Figure 2: the untreated control was clearly over loaded with 173.3 % of an optimal crop load; Armicarb treated trees were slightly over-thinned showing 91.7 % of an optimal crop load, shadow nets clearly over-thinned to only 51.7 %, meanwhile the positive control treatments like hand thinning and rope device plus vinasse were between 112 and 123 %; Goemar and Acetic Acid had no effect.




*Tukey HSD-Test ($p < 0.05$)*

| *Treatment* | | | | | *Mean %* | *SD* |
|---|---|---|---|---|---|---|
| Control | A | | | | 173.3 | 5.8 |
| Hand thinning | | | C | D | 111.7 | 7.6 |
| Goemar | A | B | | | 155.0 | 13.2 |
| Acetic acid (AZE) | A | B | C | | 146.7 | 25.2 |
| Rope thinner + Vin | | B | C | D | 123.3 | 5.8 |
| Rope thinner + Vin+ AZE | | | C | D | 115.0 | 13.2 |
| Armicarb | | | | D | 91.7 | 10.4 |
| Shadow net | | | | E | 51.7 | 12.6 |

*Levels not connected by same letter are significantly different. LSD=37.02*

Figure 2: Percentage of optimal fruit load (visually estimated) before harvest with cv. Elstar. Dotted line = optimal fruit load (100%). Vin = vinasse, AZE = acetic acid. LSD = least significant difference.

As a consequence, fruit weight of Armicarb treated trees increased by 25. 2 % from 130 g/fruit in the untreated control to 174.3 g/fruit. Return to bloom in the Armicarb treated Elstar plots in the following year with 89.6 % flower buds was clearly higher, almost too high, compared to hand thinning and rope device plus vinasse with only 7-23 % flowering buds whereas untreated control developed only 0.9 % of flower buds (data per treatment not shown in detail).

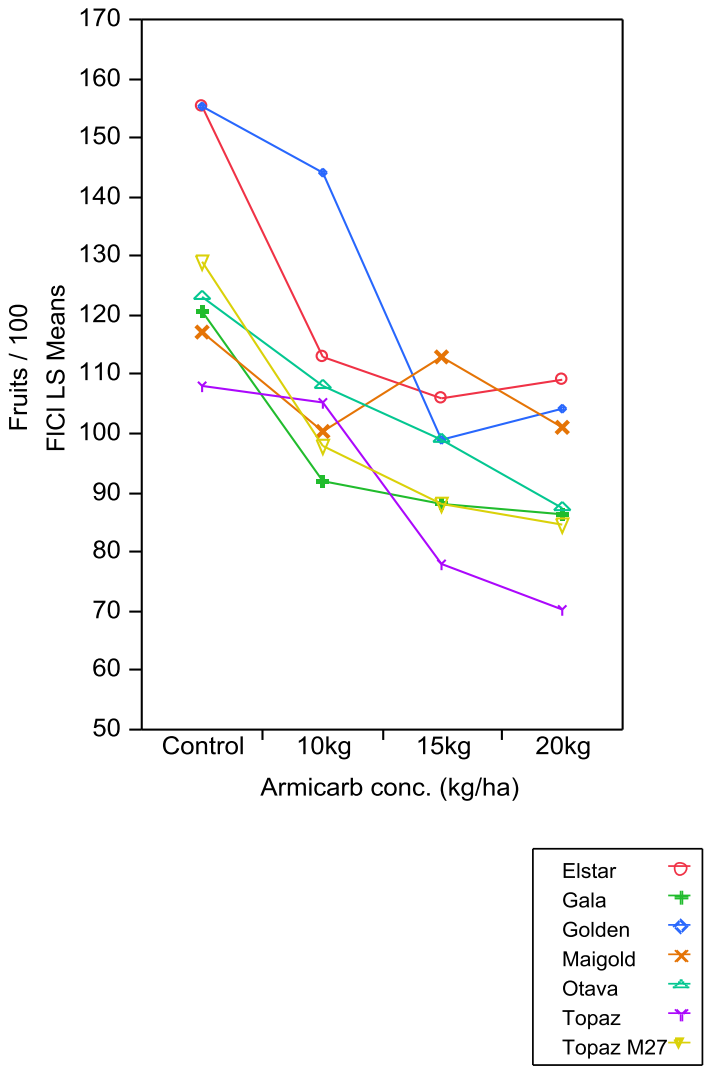
The trials with different Armicarb concentrations in 2008 with cv. Golden Delicious, Idared, Elstar and Maigold revealed that concentrations must be between 10-20 kg/ha. The results showed a tendency that with most cultivars 5 kg have almost no thinning effect, 15 kg/ha are significantly more effective than 10 kg/ha, but 20 kg/ha do not further improve the thinning effect compared to the 15 kg/ha concentration. Furthermore, the incidence of fruit russeting - mainly with Elstar - increased at 15 and 20 kg/ha (data not shown in detail, see results 2009).

*Experiments in 2009*

In 2009 the experiments on different Armicarb concentrations were repeated with 10, 15 and 20 kg/ha on cv. Gala, Golden Delicious, Elstar, Maigold, Topaz (M9, scab resistant), Topaz (M27) and Otava (scab resistant). The results confirmed that a relevant thinning effect can be achieved only from 15 kg/ha on. This trend was obvious for all cultivars tested. In that year, the thinning effect by 15 kg/ha Armicarb as it was assed before June drop was around 40 % with Topaz on M9 and M27 and Elstar, around 30 % with Maigold and Otava but only 2 % with Golden D (data not shown). After June drop (Fig. 3), Golden D. 'catched up' to a thinning effect of 30%, similar to Elstar, Gala and Topaz (M9 and M27); with Maigold, due to a high natural June drop, only a 9.4 % thinning effect resulted at this date. With most cultivars the 15 kg/ha Armicarb concentration led to a close to optimal crop load before harvest. Exceptions were Maigold, where a concentration of 20 kg/ha gave a better final result without a concerning increase of russeting. In this year, especially with Elstar, Gala and Golden Del. the 15 kg/ha dosage of Armicarb increased the incidence of fruits affected with russeting in a magnitude of 10-17 %.

The data on return to bloom as percentage of flower buds in the following year (2010) did not reveal significant treatment effects except for Otava with an increase of 61% flower set. Nevertheless, in the plots treated with 15 kg/ha Armicarb, crop load before harvest in 2010 was improved towards optimal fruit set in the magnitude of 9 % (Elstar and Otava) to 34 % (Topaz) (data not shown in detail).

In the separate trial to test different timing of Armicarb with cv. Topaz and Otava with only a single application, we could see that the thinning effect of later Armicarb applications at stage $F_2$ 65 is superior (22 %) than with earlier applications at stage $F_2$ 61 or 62 or 63 (8.2 %). The reason for this pattern is that the later the more flowers are open and affected by the agent. Also the incidence of russeting increased with later applications from 4 % at $F_2$ 62 up to 10% at $F_2$ 65. Russeting damages were particularly severe - reaching 22 % incidence - in the case where 20 kg/ha Armicarb were applied on Topaz at late flowering stage $F_2$ 65 shortly before it began to dizzle with rain. We assume that under these circumstances Armicarb got entirely in solution and too intensively into contact with the fruit epidermis. The data of the timing trial are not shown in detail.




| Cultivar and Armicarb conc. kg/ha | Fr/100 FrCl | Diff. to Control % | % of opt. crop load | Russet. incid. % | Fruit weight g |
|---|---|---|---|---|---|
| Elstar, 10kg | 112.8 | 24.0 | 125 | 15 | 102 |
| Elstar, 15kg | 105.8 | 28.7 | 108 | 17 | 120 |
| Elstar, 20kg | 109.1 | 26.5 | 110 | 20 | 128 |
| Elstar, Contr. | 148.4 | 0 | 135 | 0 | 99 |
| Gala, 10kg | 92.0 | 20.3 | 110 | 5 | 117 |
| Gala, 15kg | 88.2 | 23.6 | 100 | 10 | 121 |
| Gala, 20kg | 86.2 | 25.5 | 100 | 13 | 124 |
| Gala, Contr. | 115.4 | 0 | 115 | 0 | 116 |
| Golden, 10kg | 143.9 | -1.4 | 105 | 5 | - |
| Golden, 15kg | 99.0 | 30.2 | 105 | 10 | - |
| Golden, 20kg | 104.3 | 26.5 | 105 | 15 | - |
| Golden, Contr. | 141.9 | 0 | 100 | 0 | - |
| Maigold, 10kg | 100.3 | 14.3 | 120 | 3 | - |
| Maigold, 15kg | 106.1 | 9.4 | 120 | 5 | - |
| Maigold, 20kg | 101.2 | 12.6 | 107 | 7 | - |
| Maigold, Contr. | 117.0 | 0 | 130 | 0 | - |
| Otava, 10kg | 108.0 | 12.3 | 115 | 2 | 106 |
| Otava, 15kg | 99.0 | 19.6 | 100 | 8 | 112 |
| Otava, 20kg | 87.3 | 29.2 | 105 | 10 | 118 |
| Otava, Contr. | 123.2 | 0 | 110 | 0 | 94 |
| TopazM9,10kg | 105.1 | 2.6 | 103 | 3 | 99 |
| TopazM9,15kg | 78.0 | 27.7 | 95 | 6 | 113 |
| TopazM9,20kg | 70.3 | 34.8 | 88 | 12 | 116 |
| TopazM9,Contr | 107.90 | 0 | 108 | 0 | 104 |
| TopazM27,10kg | 98.0 | 24.0 | 108 | 5 | 106 |
| TopazM27,15kg | 88.1 | 31.7 | 103 | 8 | 107 |
| TopazM27,20kg | 84.7 | 34.3 | 103 | 10 | 108 |
| TopazM27,Contr | 128.9 | 0 | 112 | 0 | 91 |

Figure 3: Armicarb concentration trials in 2009 with cv. Elstar, Gala, Golden D., Maigold, Otava, Topaz (on rootstocks M9 and M27) with 0, 10, 15 and 20 kg/ha Armicarb in 1000L water 2 x during bloom: Number of fruits per 100 flower clusters (FlCl) after June drop; thinning effect compared to control (%); crop load before harvest (% of optimum); incidence of fruit russeting (% incidence); fruit weight (g). Interaction treatment * cultivar is significant; in all cases differences between control and 15 kg/ha were significant, except for Maigold where this is the case with 20 kg/ha.

In 2009 also different alternative methods were tested on cv. Topaz and Otava. Natural fruit fall, however, was high this year due to a relatively cold climate causing sub-optimal conditions for assimilation for the trees during Mai. For this reason, even in the untreated control variant and with both cultivars, crop load at harvest was only 10 % too high in the untreated control plots. At the fruit counting date before June drop, the treatments 2 x 20 kg/ha Armicarb and rope device plus 3 x 7% vinasse showed a significant but low thinning effect of 12.8 and 18.9 %. 3 x 2.5% lime sulphur with 30.4 % thinning effect was significantly more effective than the latter treatments. As in 2008, net shadowing reduced fruit set too radically by 67.1 %. The interaction treatment * cultivar was not significant. The results of this trial are not shown in detail.

*Experiments 2010*

In 2010 Armicarb concentration trials were performed with 15 kg/ha on cv. Golden Del., Braeburn, Pinova, Gravensteiner, Topaz, Otava, Ariane (scab resistant), and with 20 kg/ha on Maigold. The good thinning effect of Armicarb at these concentrations could be confirmed, though, as a consequence of the varying intensity of the natural June drop in the control plots, the thinning effect (expressed as % difference to the untreated control) could vary considerably between the fruit counting before and after June drop (Tab. 3). Nevertheless, with all cultivars, either before or after June drop significant and for practical fruit growing relevant thinning effects in the magnitude of 13 % (Otava) to 52 % (Gravensteiner) could be achieved.

Table 3: Thinning effect of Armicarb ( 2 x 15 kg/ha) in the 2010 trials with different cultivars as assessed before and after June drop. Asterisks indicate statistically significant effects (post ANOVA Tukey HSD tests at $p < 0.05$).

| **Cultivar** | **Thinning effect before June drop (%)** | **Thinning effect after June drop (%)** |
|---|---|---|
| Braeburn | 22.4* | 21.8* |
| Golden | 30.0* | 44.5* |
| Gravensteiner | 29.0* | 52.3* |
| Otava | 11.9* | 13.2* |
| Pinova | 24.6* | 15.6 |
| Topaz | 9.8 | 37.1* |
| Maigold (20 kg/ha) | 45.8* | 16.7 |

Figure 4 shows that beside the total thinning effect, with all cultivars, the 15 kg/ha Armicarb treatment also reduced the proportion of flower clusters with 3 or more and 2 fruitlets in favour of clusters with 1 or 2 fruitlets, respectively; from the perspective of the fruit grower a most desirable pattern.

The 2010 trials to compare different alternative methods carried out on cv. Elstar and Topaz included applications of lime sulphur, Armicarb standard (15 kg/ha), Armicarb 15 kg/ha in combination with a rope device application at stage E (59) and “Black Oil”. In 2010 the weather conditions right after blooming period were unusually cold and rainy during 24 days. The conditions for assimilation and fruit set were therefore sub-obtimal. For these reasons, the results of these method comparison trials are to some extent difficult to interpret and not shown in detail: The counting before June drop revealed a generally very high fruit set with 230 to 245 fruits per 100 flower clusters over all treatments including the by 2/3 hand thinned trees (as a compensation reaction, these latter trees kept most of the remaining fruits and thus had a high number of > 2 and > 3 fruits per fruit cluster). At this date, only the lime sulphur treatment (2 x 2.5 vol.%) revealed a moderate thinning effect of 22.3%. After June drop, however, fruit set dropped drastically to 85-111 fruits/100 FlCl in all treatments (including untreated control) with the exception of the lime sulphur treatment which decreased to 58 fruits/100 FlCl, and therefore was even over-thinned. Consequently at harvest 2010, the remaining treatments appeared with a near optimal crop load, again without significant treatment differences.

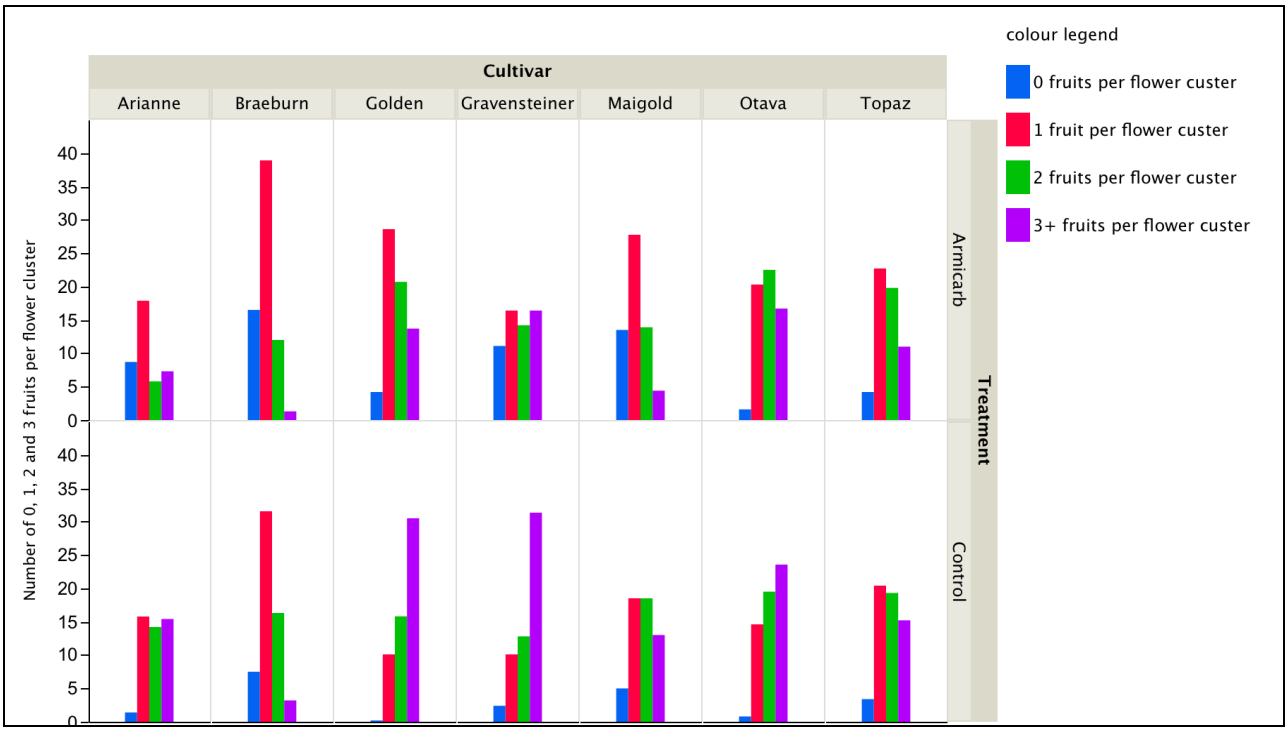



Figure 4: Mean numbers of fruits per flower cluster per cultivar and treatment, counted before June drop 2010 in the treatment with 2 x 15 kg/ha Armicarb; Maigold with 20kg Armicarb.

In the trials of 2010 no variant showed signs of treatment-induced russeting. For this reason, the assessments of russeting incidence and severity were not carried out that year.

## Discussion

Armicarb is a well known product against scab and sooty blotch on apple (Tamm *et al.,* 2006). Since presently no active compound is allowed for fruit thinning in organic apple production in Switzerland, Armicarb is an interesting candidate for organic apple thinning. Pfeiffer (2008) studied the thinning effect of Armicarb where it was applied as a fungicide at a rate of 5 kg/ha: Very much in line with our study, there was too little thinning effect of Armicarb at that dosage. To our knowledge, so far there are no other longer-term and multi-cultivar studies published (including return to bloom data) where Armicarb was tested at higher dosages as a thinning agent. In our experiments, we could show that Armicarb has a high thinning potential and that its effect is cultivar and concentration dependent. The concentration experiments over 3 years showed that Armicarb has a significant thinning effect with a concentration of 15 kg/ha for 10 of the cultivars tested and with 20 kg/ha for cv. Maigold. Interestingly, a further concentration increase did not induce a stronger thinning effect but increased the incidence of russeting.

Our results are in line with previous findings (Weibel *et al.,* 2008) confirming that the rope device combined with vinasse is a fairly efficient thinning method with a comparable efficacy as hand thinning and lime sulphur. Furthermore, with a vinasse concentration of 5-7.5% and 2-3 applications during blooming phase, we did not observed phytotoxic effects. To avoid damages on wood, spurs and leaves (Baab & Lafer, 2005) it is important to use the rope device as soft as possible by driving at high tractor speed (9-11 km/h) and at moderate rev./min. of the rope spindle (Damerow 2007 & Weibel *et al.,* 2003).

In our experiment, hand thinning by 2/3 was consequently included as ‘positive’ control treatment (Dennis, 2000 and 2002). However, beside unaffordable costs for labour these

trees tend to compensate the removed flowers by keeping a high proportion of fruits on the remaining flower clusters and show a high proportion of flower clusters with 2 and 3 and more fruits (Kloss & Weibel, 2009).

Goemar® GA14, vinegar (5 g/L acetic acid) and “Black Oil“ did not show significant thinning effects in these trials and are therefore not profoundly discussed.

Our results on shadowing are in line with previous studies which showed that tree shading can be an efficient thinning method (Stadler *et al.,* 2005, Kockerols *et al.,* 2008). According to Stadler *et al.* (2005) we mounted the nets 22-23 days after full bloom and we based our shading duration on the recommendation of A. Widmer (ACW, Wädenswil) who suggested 5-6 days for Elstar and 4 days for Idared but got over-thinning with both cultivars in both years. We suppose that in our experiment, this shading duration was too long under the circumstances given with low natural radiation and an additional hail net.

## Conclusions

From our experiments, and for the cultivars and conditions tested, we draw the following conclusions for the practical application for organic thinning measures during bloom:

1. With the majority of cultivars, 2 applications during bloom of 15 kg/ha potassium-bi-carbonate (Armicarb) gave a satisfying result. Only with cultivar Maigold 2 x 20 kg gave a better result.
2. When applying Armicarb on not yet tested cultivars, 15 kg/ha is a recommendable starting concentration for tests. The optimum, however, can range between 12-20 kg/ha.
3. The application of Armicarb should take place at warm, sunny days without rain in the following 12 h and at a time with a maximum of still un-pollinated flowers wide open. Thus, depending on the duration and intensity of the blooming phase, 2-3 applications are necessary.
4. With some cultivars like Elstar, Gala, Golden Delicious and climatic conditions that favour russeting, the use of Armicarb for thinning can increase the incidence and severity of fruit russeting. In particular it has to be avoided that it starts drizzling shortly after the application of Armicarb.
5. When Armicarb is also intensively used for scab and sooty blotch control, the potassium levels in the fruit flesh can increase to a magnitude where inner fruit quality can be negatively affected (K:Ca-ratio > 35) (Weibel 2010, unpublished data).
6. We achieved good thinning results also with 2-3 applications of lime sulphur at 2.5%, and vinasse at 7% concentration at the same conditions as mentioned above for Armicarb. Vinasse is particularly efficient with e.g. cultivar Topaz.
7. The efficacy of vinasse can or even should be improved with a soft application of the rope thinning device at stage red tips (E 59), followed by two or three applications of the desiccant agent during flowering period.
8. The thinning effect of the rope device is due to a physiological shock followed by a lack of assimilates for the development of the fruitlets (Greene 2002, Wünsche and Ferguson 2005). Thus, this method should be applied at colder, cloudy days to increase the effect of the photosynthesis decreasing effect. The Rope device should not be used during full bloom because then too many primary leaves will be destroyed and the physiological shock will cause long-lasting negative effects (e.g. compensatory shoot growth in summer etc.).

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# Comparison of different crop regulation methods for organically grown apples (cultivar 'Pinova' and 'Elstar')

S. Sinatsch[1], B. Pfeiffer[1], T. Schult[2] , J. Zimmer[2], L. Brockamp[3], B. Benduhn[3]

**Abstract**

*Over a period of three years (2009-2011) different thinning methods and compounds were tested at three different sites. At two sites the same trees were observed over three seasons, strength of thinning was adapted to the intensity of flowering depending on the reaction of the trees. At different field trials with the cultivar 'Pinova' and 'Elstar' thinning by hand was compared to thinning with the Darwin rope thinner and to lime sulphur. Using the Darwin rope thinner showed the best results for 'Pinova'. Lime sulphur also reduced the remaining time necessary for thinning by hand, but not as efficient as the rope thinner.*

**Keywords:** thinning, apples, organic, rope thinner, lime sulphur

## Introduction

In organically grown apple orchards thinning is an important tool to help the tree to regulate its yields, to achieve a good fruit quality and to avoid alternating bearing. In recent years different methods of thinning were tested (Eis *et al*. 2008, Weibel *et al*. 2008). In spring 2009 a research project „Increasing of crop safety and optimizing of crop loading of organic grown pome fruit" (FuE 2806OE197) started, founded by the „Bundesprogramm Ökologischer Landbau und andere Formen nachhaltiger Landwirtschaft". The aim is to examine different possibilities for securing yield and optimizing crop load in organically grown apples and pears. The project is a cooperation between the research facilities LVWO Weinsberg, DLR Rheinpfalz and ÖON Jork.

## Material and Methods

Trial 1 was carried out on an organic apple orchard at the fruit experimental station of LVWO Weinsberg. In all three years (2009-2011) the same trees of the variety 'Pinova' planted in spring 2003 at a spacing of 1.2 x 3.5 m (crown height 2.3 m) were observed (10 trees per treatment, each tree was counted as replication). In spring the number of blossom clusters per tree was counted. In May/June all trees were thinned by hand (except of untreated control) and adjusted to an average amount of 110-130 apples per tree. Time for thinning was stopped. Number of apples removed by hand was counted and time saving for thinning by hand was computed. At harvest number, weight and quality of fruits were determined for each tree. In 2009 8 different treatments were carried out, in 2010 three new and in 2011 two further treatments were added (table 1). The Tree-Darwin rope thinner (Fruit-Tec) was used at three different BBCH flower stages: pink bud (57), flowers forming a hollow ball (59) and 40-50 % of flowers open (64-65). Lime sulphur and sunflower oil were applied with a tunnel sprayer using a spay volume of 800 l water per ha. Additional pruning was done in 2009 and 2011 to reduce the number of blossom clusters.

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Table 1: Thinning treatments at Weinsberg, ‘Pinova’ 2009 - 2011.

| Treatment | Application / Dosage 2009 | Application / Dosage 2010 | Application / Dosage 2011 |
|---|---|---|---|
| untreated control | | | |
| thinning by hand 1 | | | |
| rope thinner 1 BBCH 57 | 200 U/min, 8 km/h | - | 220 U/min, 6 km/h |
| rope thinner 1 BBCH 59 | 220 U/min, 8 km/h | - | 220 U/min, 6 km/h |
| rope thinner 1 BBCH 64-65 | 220 U/min, 8 km/h | 180 U/min, 8 km/h | 220 U/min, 6 km/h |
| lime sulphur | 3 x 30 l/ha + 1,5 l/ha Bioblattmehltaumittel | 1 x 30 l/ha | 3 x 30 l/ha |
| additional pruning | ✓ | not necessary | ✓ |
| foliar fertilizer 1 (Wuxal Aminoplant) | 1 x 15 l/ha | 1 x 20 l/ha | 1 x 20 l/ha |
| thinning by hand 2 | | | |
| rope thinner 2 BBCH 64-65 | | 180 U/min, 8 km/h | 220 U/min, 6 km/h |
| rope thinner 2 + lime sulphur | | 180 U/min, 8 km/h + 1 x 30 l/ha | 220 U/min, 6 km/h + 1 x 25 l/ha |
| Sunflower oil + TS-forte | | | 3 x 25 l/ha + 2 l TS-forte |
| Sunflower oil + Rimulgan | | | 3 x 25 l/ha + 2,4 l Rimulgan |

Trial 2 at Klein-Altendorf was on-farm placed in an organic orchard within two rows of ‘Pinova’ (1.0 x 3.0 m). Thinning by hand was compared to thinning with lime sulphur (600 l water/ha), as well as to mechanical thinning by the Darwin rope thinner (table 2). The tested treatments have been replicated four times with seven to nine trees per testing plot. Five trees per plot have been evaluated (20 trees per variant). In April blossom clusters per tree were counted. After treatment the trees have been adjusted to an average amount of 100-110 apples/tree. Number of apples removed by hand was counted and time saving for thinning by hand was computed under presumption that removing one apple per tree needs one hour per ha (2500 trees/ha). At harvest yield (kg) and amount of fruits/tree were recorded.

Table 2: Thinning treatments at Klein-Altendorf, ‘Pinova’ 2009 - 2011.

| Treatment | Application / Dosage |
|---|---|
| thinning by hand | |
| rope thinner BBCH 59 | 200 U/min, 8 km/h |
| rope thinner BBCH 63-64 | 200 U/min, 8 km/h |
| lime sulphur | 3 x 30 l/ha |

Trial 3 was carried out in an organic ‘Elstar’-orchard (planted 1998, 1.0 x 3.5 m) at the fruit growing station ESTEBURG (Jork). Thinning by hand, lime sulphur (1000 l water/ha) and mechanical thinning by Darwin rope thinner with different rotations were compared (table 3). Due to a large variety of flowering intensity the trial was repeated every year with new sample trees. Only trees with high flowering rate were chosen to assess the fundamental effect of each thinning method. In spring the intensity of flowering per tree was rated on a scale from one to nine (1=no flowers, 6=optimal). The treatments were replicated four times with ten trees per replication. Three trees per replication have been evaluated. In opposite to Weinsberg and Klein-Altendorf trees weren’t thinned by hand after treatment

(except for 2009). Only the trees of the treatment thinning by hand have been adjusted to an average amount of 110 apples per tree after June drop.

Table 3: Thinning treatments at Jork, 'Elstar' 2009 - 2011.

| Treatment | Application / Dosage 2009 | Application / Dosage 2010 | Application / Dosage 2011 |
|---|---|---|---|
| untreated control | | | |
| thinning by hand | after june drop | after june drop | after june drop |
| rope thinner BBCH 58-59 | 240 U/min, 8 km/h | 230 U/min, 8 km/h (BBCH 61) | 250 U/min, 8 km/h |
| rope thinner BBCH 65 (A) | 240 U/min, 8 km/h BBCH 63-64 (A) | 250 U/min, 8 km/h | 250 U/min, 8 km/h |
| rope thinner BBCH 65 (B) | 210 U/min, 8 km/h BBCH 63-64 (B) | 230 U/min, 8 km/h | 230 U/min, 8 km/h |
| rope thinner BBCH 65 (C) | 270 U/min, 8 km/h BBCH 63-64 (C) | 270 U/min, 8 km/h | 270 U/min, 8 km/h |
| rope thinner BBCH 67 | 240 U/min, 8 km/h | 230 U/min, 8 km/h | 250 U/min, 8 km/h |
| lime sulphur (BBCH 65-66) | 3 x 30 l/ha (BBCH 61, 65-66) | 3 x 30 l/ha | 3 x 30 l/ha |

## Results

Trial 1

Regarding the quality (figure 1) and yield (table 4) within the tested treatments over all three years, rope thinner 1 at BBCH 59 (64 kg/tree) and BBCH 64-65 (61 kg) had the highest yield, especially the rope thinner 1 BBCH 57 had a high amount of fruits with an excellent colour, while unthinned control had lots of green and small fruits. All treatments with the rope thinner (1) had a higher accumulated yield than the treatment thinning by hand (1). Looking at the time needed for thinning by hand after treatments, using the rope thinner (1) saved between 60-110 h/ha compared to the treatment thinning by hand (1).

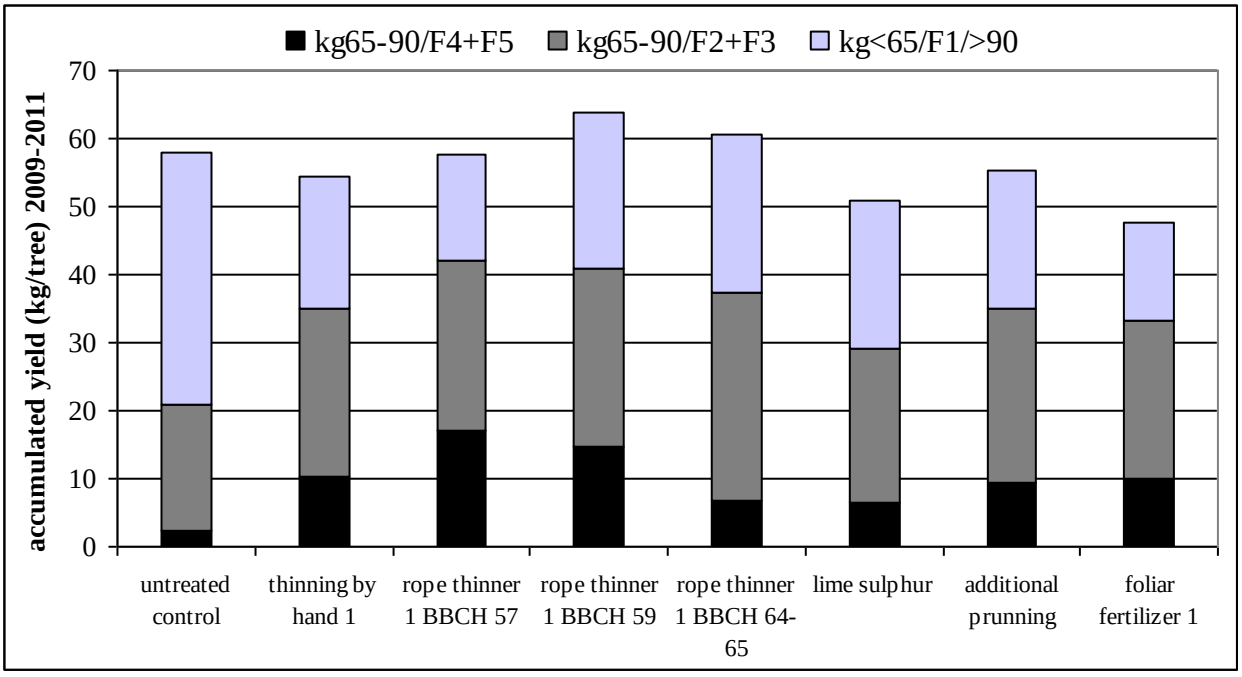


Figure 1: Accumulated yield (kg/tree) 2009-2011 for three quality levels (size and colour): 65-90mm/F4+F5 (good size + excellent coloured), 65-90/F2+F3 (good size + well coloured), <65/F1/>90 small (or to large) and green fruits).

Using 3 x lime sulphur also reduced the time for thinning by hand. An overthinning in 2009 with lime sulphur because of an application mistake lead to a lower accumulated yield (sum 2009-11) than thinning by hand (1).

The treatment additional pruning lead to a good yield with good quality, but about 43 % more time than the treatment thinning by hand (1) was needed to remove the apples by hand. Treatments with the rope thinner (2) had a yield of 32-34 kg/tree (175-184 g/fruit) combined with a good fruit quality. Yield of the treatment thinning by hand (2) was higher, but more green and small fruits (155 g/fruit) were harvested. The combination of rope thinner plus a late lime sulphur application on open flowers of one-year-old branches had the lowest effort for thinning by hand in this part of the trial and the highest setting of blossoms in 2011, as well as a favourable effect on the fruit quality. Comparing the two sunflower oil treatments in 2011, the formulation with TS-forte lead to a higher yield and better fruit quality; time reduction for thinning by hand was about 13-17 h/ha (comparable to lime sulphur).

In general, the pollination is very well in this organic orchard because there are 15 bee colonies nearby. In 2009 good weather conditions lead to a good pollination, but thinning by hand after treatment was not strong enough. Spring 2010 started late, around pink bud stage minimum temperature of -0,6 °C was observed. Because of alternation, in 2010 lower yield was observed. In 2011 a high number of flower buds on annual shoots occurred. Late frost on Mai 4$^{th}$ 2011, 2 ½ weeks after full blossom, lead to a severe June drop. Most varieties were strongly affected, but 'Pinova' with the tremendous high number of blossom clusters not as much.

Table 4: Blossom clusters/tree, accumulated yield (kg/ha) and accumulated amount of apples removed by hand (2009-2011), as well as time saving for thinning by hand for different treatments at Weinsberg, 'Pinova' (2400 trees/ha), (Tukey-test α=0.05).

| Treatment | Blossom clusters/tree | | | Accumulated yield (kg/tree) 2009-2011 | Accumulated amount of apples removed by hand 2009-2011 | Time saving (h/ha) in comparison to thinning by hand*** (%) |
|---|---|---|---|---|---|---|
| | 2009 | 2010 | 2011 | | | |
| untreated control | 274 | 51 a | 552 cde | 58.53 ab | | |
| thinning by hand (1) | 236 | 117 abcd | 520 cde | 54.42 ab | 349 ab | 100 % |
| rope thinner (1) BBCH 57 | 233 | 86 ab | 595 de | 57.67 ab | 236 a | 32 |
| rope thinner (1) BBCH 59 | 267 | 110 abc | 644 de | 63.85 b | 264 ab | 24 |
| rope thinner (1) BBCH 64-65 | 223 | 172 cd | 381 ab | 60.61 ab | 290 ab | 17 |
| lime sulphur | 249 | 178 d | 506 bcd | 50.84 a | 336 ab | 4 |
| additional pruning | 220 | 80 ab | 359 a | 55.38 ab | 499 c | -43 |
| foliar fertilizer (1) | 227 | 72 a | 486 bcd | 47.70 a | 379 bc | -9 |
| thinning by hand (2)* | | 191 | 405 a | 40.00 b | 366 b | 100 % |
| rope thinner (2) BBCH 64-65* | | 200 | 417 a | 31.57 a | 196 a | 47 |
| rope thinner (2) + lime sulphur* | | 189 | 468 a | 33.92 ab | 133 a | 64 |
| thinning by hand (2)** | | | 405 | 21.52 ab | 199 a | 100 % |
| sunfloweroil + TS-forte** | | | 405 | 22.68 b | 182 a | 9 |
| sunfloweroil + Rimulgan** | | | 417 | 19.37 a | 186 a | 7 |

*2010-2011, **2011, *** Presumption: thinning one apple per tree needs one hour per ha (2500 trees/ha)

Trial 2

In 2010 and 2011 the control, which was only thinned by hand, showed the lowest number of blossom clusters per tree. In spring 2010 in most of the treatments including the control the highest number of blossom clusters was counted. The treatment with Darwin rope thinner at the BBCH stage 59 led to a homogeneous number of blossom clusters over all three years (table 5).

From 2009 to 2011 all treatments reduced the remaining time for thinning by hand compared to the control (thinning by hand). Using Darwin rope thinner at BBCH 59 and BBCH 63-64 the number of apples removed by hand was reduced by half, which means a time saving of about 300 h/ha. In the lime sulphur treated plots 502 apples still had to be removed. This corresponds to a time saving of only 90 h/ha in comparison with the control. The accumulated yield of the years 2009-2011 with about 59.0 kg per tree was nearly the same in the control and the mechanically treated plots. The lime sulphur treatment had with 55.8 kg the lowest yield.

Table 5: Blossom clusters/tree, accumulated yield (kg/tree), accumulated amount of apples removed by hand and time saving for thinning by hand for the different treatments at Klein-Altendorf, 'Pinova' 2009 - 2011, (Tukey-test α=0.05).

| Treatment | Blossom clusters/tree | | | Accumulated yield [kg/tree] 2009-2011 | Accumulated amount of apples removed by hand 2009-2011* | Time saving (h/ha) in comparison to thinning by hand*** (%) |
|---|---|---|---|---|---|---|
| | 2009 | 2010 | 2011 | | | |
| Thinning by hand | 227 | 242 | 225 | 58.5 a | 596 b | 100 % |
| Rope thinner BBCH 59 | 265 | 268 | 246 | 58.7 a | 287 a | 52 |
| Rope thinner BBCH 63-64 | 265 | 278 | 229 | 59.0 a | 309 a | 48 |
| Lime sulphur | 263 | 261 | 229 | 55.8 a | 502 b | 16 |

* Presumption: thinning one apple per tree needs one hour per ha (2500 trees/ha)

Trial 3

At Jork the influence of every thinning method was assessed at 'Elstar' from 2009 to 2011 by means of flowering intensity in the following year, yield and fruit quality. The mechanical thinning caused an average yield reduction of about 30 % over the years compared to the untreated control (table 6). In 2009 it was given with 50 % towards untreated control, except for treatment at BBCH 63-64 (B) and 210 U/min with 30 %. In 2010 and 2011, using the rope thinner at BBCH 58-59, yields about 18 kg/tree could be reached – only 15 % reduction towards untreated control. The lowest yield (12 kg/tree) showed the mechanical thinning at BBCH 64-65 (C) with a high rotation of 270 U/min. From 2009 to 2011 a yield reduction of 15-20 % (except for 2010) was observed when thinning with lime sulphur. This treatment achieved yields about 16-22 kg/tree. The lower yield resulted in better fruit qualities. An increase of size, weight and better colour of the fruits could be observed. Most fruits of untreated control were graded at size 70-75 mm with a weight of 140 g, but trees thinned with rope thinner showed the highest peak at size 75-80 with 170 g/fruit.

Over the years a reduction of biennial bearing was shown after mechanical thinning (BBCH 64-65, A, C) and treatment with lime sulphur (table 6). In 2010 and 2011 the intensity of flowering was given with constant notes of five at both variants. Especially the upcoming biennial bearing in 2010 has been stopped. The trees of untreated control and thinning by hand showed a low and alternating intensity of flowering (2010).

Mechanical thinning may stimulate vegetative tree growth. This effect was assessed by counting and measuring new shoots in summer (pruning) and January/February the following year. The variants “untreated control”, “thinning by hand” and “lime sulphur” had the lowest vegetative growth (19 m/tree) in 2009 and 2010. But the trees of mechanical thinning showed an increasing growth (trend). Using the rope thinner on BBCH 64-65 with 240-250 U/min achieved the highest value (27 m/tree) in both years.

Table 6: Intensity of flowering (1-9) and total yield at Jork, ‘Elstar’ 2009 - 2011, (Tukey-test =0.05).

| Treatment | 2009 | | | 2010 | | | 2011 | |
|---|---|---|---|---|---|---|---|---|
| | Bloom 2009 | Yield (kg/tree) | Bloom 2010 | Bloom 2010 | Yield (kg/tree) | Bloom 2011 | Bloom 2011 | Yield (kg/tree) |
| untreated control | 5.6 | 23.0 a | 3.8 | 4.5 | 20.7 a | 4.2 | 4.7 | 21.2 a |
| thinning by hand | 6.0 | 19.2 a | 3.7 | 4.1 | 17.7 ab | 4.3 | 4.4 | 19.8 a |
| rope thinner BBCH 58-59 | 5.8 | 9.4 b | 4.6 | 4.6* | 18.6* ab | 4.2* | 4.8 | 18.0 a |
| rope thinner BBCH 64-65 (A) | 6.5 | 11.0 b | 4.7 | 4.8 | 14.3 ab | 4.5 | 4.8 | 15.3 a |
| rope thinner BBCH 64-65 (B) | 6.1 | 15.3 ab | 3.7 | 4.7 | 15.0 ab | 4.5 | 4.7 | 12.6 a |
| rope thinner BBCH 64-65 (C) | 6.1 | 9.7 b | 4.6 | 4.9 | 11.7 b | 4.6 | 5.1 | 15.1 a |
| rope thinner BBCH 67 | 6.3 | 8.9 b | 3.7 | 5.0 | 18.3 ab | 3.8 | 4.9 | 15.5 a |
| lime sulphur | 6.4 | 20.2 a | 4.8 | 5.0 | 22.1 a | 4.6 | 5.4 | 16.1 a |

*BBCH 61

## Discussion

At **Weinsberg** using the Darwin rope thinner at the cultivar ‘Pinova’ saved between 60-110 working hours per hectare for thinning by hand over a period of three years. ‘Pinova’ trees at Weinsberg are much more affected by alternation than in Klein-Altendorf. Therefore at Weinsberg about 200 hours less were needed for thinning by hand compared to Klein-Altendorf (where about 300 hours were saved). In general, growth of ‘Pinova’ reacted not as strongly as 'Elstar' to the use of rope thinner. The best results for breaking alternation showed the rope thinner at BBCH 64-65. Also the combination of rope thinner and lime sulphur worked well. The most important difference between the treatments (except for saving time at thinning by hand) was the better fruit quality at the treatments with the rope thinner (less green and small fruits). Using three times lime sulphur was not as efficient as the rope thinner. Lime sulphur supported a high flower setting in the following year. Both sunflower oil treatments saved some time at thinning by hand, similar to the results described by EIS ET AL. (2008), but there the breaking of alternate bearing was not successful. At **Klein-Altendorf** in all three trial years the treatments with the Darwin rope thinner in the variety ‘Pinova’ showed the best results. Besides that the amount of larger fruits (75+) was generally higher. Applications with lime sulphur also reduced the time for thinning, but they were not efficient enough. These and others here not described results of field trials in the variety ‘Elstar’ and ‘Braeburn’ proved a positive effect of the treatments on biannual bearing (SINATSCH ET AL. 2011). At **Jork** mechanical thinning (BBCH 59-63, 250 U/min) and lime sulphur showed the best results over the years, regarding the yield, fruit size, weight and colour. Also a first reduction of biennial bearing was shown after both treatments. The stimulation of vegetative tree growth by mechanical thinning has to be examined in the following years.

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# Crop regulation on different apple cultivars with transpiration inhibitors

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**Abstract**

*In organic apple growing in South Tyrol, yield control is commonly achieved by removing buds and flowers with mechanical thinning machines and/or lime sulphur sprays. To allow for thinning also later in the season, trials with shading nets have been carried out over several years. By shading trees with close-meshed nets before June fruit drop, photosynthesis in leaves can be drastically reduced. Notwithstanding the good trial results, the method is not used in the field, because shading trees with nets is labour-intensive and expensive. During the last years we therefore tested different substances as alternatives to shading nets. First promising results were obtained with different oily substances. However, based on our current knowledge, negative side effects such as leaf burn and fruit russeting, can not be excluded. In this experiment, the paraffin oil-based product UFO (Ultra Fine Oil) was applied on different apple cultivars and its thinning efficacy and the side effects were recorded. There have been promising results in thinning, but the differences between the varieties were large. It is therefore clear, that the treatments have to be decided individually for each apple cultivar.*

**Keywords** apple, June drop, thinning, transpiration inhibitors

## Introduction

Yield control is an essential practice in apple growing to obtain consistent and high-quality yields. In integrated farming systems, growers rely primarily on synthetic plant growth regulators. Depending on their active substance and application rate, these products may be applied also very late in the season (Südtiroler Beratungsring für Obst- und Weinbau, 2011). As a consequence, fruit set can be estimated accurately and unnecessary manual thinning can be avoided. These products are not allowed in organic farming. Thinning in organic orchards in South Tyrol is done at flowering by using mechanical thinning machines (Strimmer *et al*., 1997; Kelderer *et al*., 2009; Weibel & Walther, 2003) and/or by applying lime sulphur sprays (Kelderer *et al*., 2006). Methods, which allow for thinning later in the season, have been tested for several years. Promising results, that is a drastical reduction of the net photosynthesis of apple trees, were obtained by using close-meshed shading nets (75 – 90% sunlight reduction), and highest efficacy was achieved by shading trees at fruit size up to 10 – 15 mm (Byers *et al.*, 1985; Kelderer *et al.,* 2008; McArtney *et al.,* 2004; Musacchi & Corelli Grappadelli, 1994; Stadler *et al.*, 2005; Widmer *et al.*, 2008). However, the method is not used in the field, because shading nets are very expensive and opening and closing nets for short periods of time is labour-intensive.

Different substances have already been tested as alternatives to shading nets. A partial success was achieved with applications of bentonite sprays, but at harvest visible remains of the substance were still present around the calyx and stalk end of fruits, which thus became unmarketable (Prantl *et al.*, 2004). It is known from literature that oily substances can inhibit transpiration in leaves, close stomata, and thus affect photosynthesis. In 2008 and 2009 different oily substances such as pine oil-, paraffin oil-, soybean oil- and canola oil-based products have been applied and compared (Kelderer, 2010). Our recent trials aimed at evaluating the thinning efficacy and possible negative side effects of the paraffin

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oil-based product UFO (Ultra Fine Oil; distributor: Intrachem Bio Italia S.p.A., Grassobbio, Italy) on the main apple varieties cultivated in South Tyrol.

## Material and Methods

Trial design:

The trials were conducted in 2010 and 2011 in different apple orchards under integrated management, at the Research Centre Laimburg (Pfatten, South Tyrol, Italy) and in Latsch

(Venosta Valley, South Tyrol, Italy). Both study orchards are located in the valley floor. Laimburg is situated at 220 m, and Latsch at 780 m above sea level. A randomised block design with 4 replications per treatment was used, and assessments were made on 5 trees per plot, uniform in growth, size, and number of flowers. All paraffin oil-based treatments were applied with a motorized sprayer for experimental trials from WAIBL (transverse current blower). A detailed description of the study orchards, the tested treatments, and the timing of the applications is provided in Table 1 and 2.

In 2011, all experimental plots were treated also with lime sulphur before and after bloom in order to assess for potential negative side effects (phytotoxicity) caused by the application of lime sulphur and paraffin oil in combination. In organic agriculture, lime sulphur is the most applied plant protection product after flowering. It must therefore be considered, that part of the thinning effect observed in this experiment might have been due to the application of lime sulphur.

Table 1: Description of the 2010- and 2011-study orchards (cultivars, rootstock, year of planting and planting density).

| Year | Cultivar/Clone | Rootstock | Planting Year | Planting density |
|---|---|---|---|---|
| 2010 | Pinova | M9 | 2000 | 0,8 × 3,2 m |
| | Golden Delicious/Klon B | M9 | 1993 | 0,7 × 3,5 m |
| | Braeburn | M9 | 1998 | 0,9 × 3,2 m |
| | Red Delicious/Red Chief | M9 | 2000 | 0,7 × 3 m |
| | Pink Lady | M9 | 2001 | 0,8 × 3,15 m |
| | Gala/Royal Gala | M9 | 1997 | 0,8 × 3,5 m |
| | Fuji/Kiku | M9 | 2004 | 1 × 3,25 m |
| 2011 | Pinova | M9 | 2000 | 0,8 × 3,2 m |
| | Gala/Royal Gala | M9 | 1997 | 3,5 x 0,8 |
| | Golden Delicious/Klon B | M9 | 1993 | 3,5 x0,7 |
| | Fuji/Kiku | M9 | 2004 | 3,25 x 1 |
| | Pink Lady/Crips Pink | M9 | 2001 | 3,15 x 0,8 |
| | Red Delicious/Red Chief | M9 | 1997 | 3,2 x 0,9 |
| | Braeburn | M9 | 1998 | 3,2 x 0,9 |
| | Kanzi | M9 | 2008 | 3,0 x 0,8 |
| | Granny Smith | M9 | 2009 | 3,2 x 0,8 |

Table 2: Tested treatments

| Year | Cultivar | Treatment | Trade name | Producer/ distributor | Applied rate | No. Applications | Phenological stage/Fruit size (mm) |
|---|---|---|---|---|---|---|---|
| 2010 | Pinova | Paraffin oil | UFO | Intrachem Bio Italia | 1,5 l/hl | 2× | 15 mm |
| | | Untreated control | - | - | - | - | - |
| | Golden Del. | Metamitron | | Experimental product | 350 ml/hl | 1× | 15 mm |
| | all other cultivars + Golden Del. | Paraffin oil | UFO | Intrachem Bio Italia | 1,5 l/hl | 2× | 15 mm |
| | | Paraffin oil | UFO | Intrachem Bio Italia | 1 l/hl | 3× | 15 mm |
| | | Untreated control | - | - | - | - | - |
| 2011 | Pinova | Paraffin oil | UFO | Intrachem Bio Italia | 1,5 l/hl | 2× | 15 mm |
| | | Untreated control | | | | | |
| | all other cultivars | Paraffin oil | UFO | Intrachem Bio Italia | 1,5 l/hl | 2× | 15 mm |
| | | 6-Benzyladenine+Naphthaleneacetic acid+Surfactant | Brancher Dirado+ Dirager | Agrimport, Gobbi, Sipcam | 100 ml + 10 ml + 100 ml | 1× | 15 mm |
| | | Untreated control | - | - | - | - | - |

Assessments:

Thinning: to assess for the thinning efficacy of the different treatments, in each plot, after June fruit drop, the number of fruits was counted on 100 randomly selected flower clusters (henceforth FC) per tree. To take into consideration also the position of the flowers on the tree, 40 FC were selected in the upper third of the tree, and 60 in the lower part of the tree, uniformly distributed within the outer and inner part of the tree canopy. Counts were made using Fankhauser's method (Fankhauser *et al.*, 1979): after June fruit drop, the number of fruits was counted on all FC present on entire branch sections. The number of fruits per 100 FC was then inferred by calculating the mean value of the assessed data.

Fruit russeting: to assess for fruit russeting, in each plot, at harvest, fruits were checked for symptoms of fruit russeting and classified according to a scale ranging from 0 to 10, with 0 = fruit with no russeting symptoms, 1 = fruit with russeting symptoms at stalk cavity, 2 = fruit with 10-20% fruit area affected by fruit russeting, and so on. Based on this the percentage of russeted fruit surface was calculated.

Flower formation: in 2010, to assess for possible side effects of the different treatments on flower formation the next season, the percentage of flowers on the sprouted buds was determined the following year in spring.

Leaf drop: we also made visual assessments on leaf drop. To establish leaf drop incidence, a scale ranging from 0 to 5 such as the following was used: 0 = no leaf drop, 1 = light drop of rosette leaves, 2 = medium drop of rosette leaves, 3 = medium drop of rosette leaves and first symptoms of leaf drop on shoots, 4 = high drop of rosette leaves and light-medium leaf drop on shoots, and 5 = high drop of rosette leaves and on shoots.

Yield and fruit weight: at harvest, all fruits present on the 5 sample trees within each plot were harvested, and fruit yield (kg/tree) and fruit weight (g) were assessed, using a sorting machine from AWETA.

The number of fruits/100 FC, fruit weight (g), yield (kg/tree), percentage of russeted fruit surface and percentage of flower buds in the following season were compared across treatments using 1-way ANOVAs followed by Student-Newman-Keuls' test for posthoc comparisons of means ($P<0.05$). To improve homoschedasticity, data expressed in percentages were arcsin(radq(x/100))-transformed. All analyses were performed with the statistics programme PASW 17.

## Results

Table 3: Trial results 2010. Assessments after June fruit drop for no. fruits/100 FC, on 17th of May for leaf drop, and before harvest for fruit russeting.

| Treatment | No. Fruits/100 FC | | Leaf drop | % Fruit russeting | |
|---|---|---|---|---|---|
| Gala 1.5l×2 | 132.5 | a | 1 | 10.6 | a |
| Gala 1l×3 | 126.0 | a | 2 | 11.1 | a |
| Gala Control | 169.7 | b | 0 | 10.9 | a |
| Fuji 1.5l×2 | 100.0 | a | 1 | 14,1 | b |
| Fuji 1l×3 | 106.0 | ab | 1 | 9.0 | a |
| Fuji Control | 117.4 | b | 0 | 6.4 | a |
| Pink 1.5l×2 | 129.1 | a | 0 | 1.2 | a |
| Pink 1l×3 | 128.4 | a | 1 | 2.0 | a |
| Pink Control | 139.7 | a | 0 | 2.1 | a |
| Red Del. 1.5l×2 | 101.3 | a | 1 | 0.2 | a |
| Red Del. 1l×3 | 107.1 | a | 2 | 0.4 | a |
| Red Del. Control | 148.0 | b | 0 | 0.2 | a |
| Braeburn 1.5l×2 | 72.3 | a | 2 | 3.3 | a |
| Braeburn 1l×3 | 69.9 | a | 2 | 3.6 | a |
| Braeburn Control | 92.1 | b | 0 | 2.8 | a |
| Golden 1.5l×2 | 101.3 | ab | 4 | 34.0 | b |
| Golden 1l×3 | 107.7 | b | 3 | 30.3 | b |
| Golden Metamitron | 91.0 | a | 0 | 24.6 | a |
| Golden Control | 150.6 | c | 0 | 23.8 | a |
| Pinova 1.5l×2 | 66.8 | a | - | 0.1 | |
| Pinova Control | 122.9 | b | - | 1.4 | |

In the first trial year (2010), the paraffin oil-based product UFO was tested on different apple cultivars at two different application rates and timing: in one treatment UFO was sprayed twice at 1.5 l/100 l, while in the other treatment UFO was applied three times at 1.0 l/100 l. On the cultivar Golden Delicious, also the conventional thinning product Metamitron was tested (one application at 350 ml/100 l).

A statistically significant thinning effect was achieved with UFO on all cultivars except Pink Lady (Table 3). On Gala, Red Delicious, Golden Delicious and Braeburn, the number of fruits/100 flower clusters was significantly lower in both UFO-based treatments than in the untreated control. On the cultivar Fuji, instead, only 2 applications of UFO at 1.5 l/100 l achieved a significant thinning effect in comparison to the untreated control, while 3 applications of UFO at 1.0 l/100 l differed significantly neither from the other UFO-based treatment nor from the untreated control. On the cultivar Golden Delicious, also the chemical reference treatment Metamitron was tested: the standard showed highest efficacy in thinning, but a slightly lower, though statistically comparable thinning effect was recorded for UFO applied twice at 1.5 l/100 l. On the cultivar Pinova, only one UFO-based treatment was tested (2 applications at 1.5 l/100 l), and also in this case a significant thinning effect in comparison to the untreated control was obtained.

Leaf drop was estimated visually according to a scale ranging from 0 (= no leaf drop) to 5 (=high leaf drop of rosette leaves and light-medium leaf drop on shoots). In the UFO-treated plots, leaf drop was recorded on all cultivars, while no leaf drop was observed in untreated control plots (Table 3). Leaf drop values ranged between 1 and 2, and were thus relatively low on all cultivars except Golden Delicious, where high leaf drop was observed (3 and 4).

Table 4: Trial results 2010. Assessments at harvest for fruit weight (g) and yield (kg/tree), the following year in April for the percentage of flower buds.

| Treatment | Yield (kg/tree) | | Fruit weight (g) | | % Flower buds | |
|---|---|---|---|---|---|---|
| Gala 1.5l×2 | 15.6 | b | 120.8 | b | 2.9 | a |
| Gala 1l×3 | 12.3 | a | 124.9 | b | 7.3 | b |
| Gala Control | 17.6 | b | 108.1 | a | 2.8 | a |
| Fuji 1.5l×2 | 30.0 | a | 135.5 | a | 0.0 | a |
| Fuji 1l×3 | 29.9 | a | 135.5 | a | 0.0 | a |
| Fuji Control | 30.8 | a | 128.6 | a | 0.0 | a |
| Pink 1.5l×2 | 30.1 | a | 143.9 | b | 27.5 | b |
| Pink 1l×3 | 30.5 | a | 140.6 | ab | 27.0 | b |
| Pink Control | 32.9 | a | 135.5 | a | 16.0 | a |
| Red Del. 1.5l×2 | 16.8 | a | 136.1 | b | 2.9 | a |
| Red Del. 1l×3 | 17.6 | a | 145.7 | c | 8.7 | a |
| Red Del. Control | 18.1 | a | 121.6 | a | 5.8 | a |
| Braeburn 1.5l×2 | 27.5 | a | 150.3 | b | 37.8 | b |
| Braeburn 1l×3 | 26.0 | a | 158.4 | c | 51.5 | c |
| Braeburn Control | 37.9 | b | 123.7 | a | 10.0 | a |
| Golden 1.5l×2 | 21.2 | a | 159.2 | b | 13.5 | b |
| Golden 1l×3 | 22.2 | a | 171.7 | c | 8.9 | b |
| Golden Metamitron | 22.4 | a | 185.0 | d | 15.1 | b |
| Golden Control | 30.6 | b | 128.6 | a | 1.5 | a |
| Pinova 1.5l×2 | - | | - | | 75.5 | b |
| Pinova Control | - | | - | | 68.0 | a |

Increased fruit russeting in the UFO-based treatments was registered only on the cultivars Fuji and Golden Delicious: on Fuji, the fruit surface affected by russeting was significantly higher for UFO applied twice at 1.5 l/100 l UFO (14.1%) than for UFO applied 3 times at

1.0 l/100 l (9.0%) and the untreated control (6.4%). On Golden Delicious, both UFO-based treatments showed significantly more russeted fruit surface (34.0 and 30.3%) than the Metamitron-based treatment (24.6%) and the untreated control (23.8%) (Table 3).

Statistically significant differences among treatments in yield emerged only on three cultivars (Table 4). On the cultivar Gala, yield was significantly lower in the plots treated with 3×1.0 l/100 l UFO (12.3 kg/tree) than in those treated with 2×1.5 l/100 l UFO (15.6 kg/tree) and in untreated control plots (17.6 kg/tree). On the cultivars Braeburn and Golden Delicious, instead, yield was significantly lower in both UFO-based treatments (respectively 27.5 and 26.0, and 21.2 and 22.2 kg/tree) than in the untreated control (respectively 37.9 and 30.6 kg/tree). Furthermore, on Golden Delicious, yield in the UFO-based treatments was statistically comparable to that in the reference treatment Metamitron (22.4 kg/tree).

A significant effect on fruit weight was recorded on almost all cultivars (Table 4). On the cultivar Gala, mean fruit weight was significantly higher in UFO-treated plots (120.8 and 124.9 g) than in untreated control plots (108.1 g). On the cultivar Pink Lady, fruit weight was highest for 2×1.5 l/100 ml UFO (143.9 g), intermediate for 3×1.0 l/100 l UFO (140.6 g), and lowest for the untreated control (135.5 g). On the cultivars Red Delicious and Braeburn, instead, fruit weight was significantly higher for 3×1.0 l/100 l UFO (145.7 and 158.4 g) than for 2×1.5 l/100 l UFO (136.1 and 150.3 g) and finally for the untreated control (121.6 and 123.7 g). On the cultivar Golden Delicious, the plots treated with Metamitron showed highest fruit weight (185.0 g), followed by those treated with 3×1.0 l/100 l UFO (171.7 g), those treated with 2x1.5 l/100 l UFO (159.2 g), and by untreated control plots (128.6 g).

For flower formation (% flower buds on sprouted buds) the following spring, significant differences among treatments emerged on all cultivars except Fuji and Red Delicious (Table 4). On the cultivar Gala, the percentage of flower buds was significantly higher for 3 applications of UFO at 1.0 l/100 l UFO (7.3%) than for 2 applications of UFO at 1.5 l/100 l (2.9%) and for the untreated control (2.8%), with the latter two treatments not differing significantly one from the other. On the cultivar Pink Lady, the percentage of flower buds was significantly higher for both UFO-based treatments (27.5 and 27.0%) than for the untreated control (16.0%). On the cultivar Braeburn, the percentage of flower buds was highest for the treatment 3x1.0 l/100 l UFO (51.5%), intermediate for the treatment 2x1.5 l/100 l UFO (37.8%), and lowest for the untreated control (10.0%). On the cultivar Golden Delicious, the Metamitron- and UFO-based treatments (15.1, 13.5 and 8.9%) showed significantly more flower buds than the untreated control (1.5%). On the cultivar Pinova, significantly more flower buds were formed in the UFO-based treatment (75.5%) than in the untreated control (68.0%).

In the second trial year (2011), the thinning efficacy of the paraffin oil-based product UFO was tested in comparison to the reference product Brancher Dirado (Benzyladenin) applied in tank mixture with the adjuvant Dirager (henceforth B+D treatment; distributors in Italy: Agrimport, Gobbi, and Sipcam).

On the cultivar Golden Delicious, both the UFO and the B+D treatment (73.0 and 78.4 fruits/100FC) showed a significant and comparable thinning effect in comparison to the untreated control (129.1 fruits/100 FC) (Table 5).

Table 5: Trial results 2011. Assessments after June fruit drop for no. fruits/100 FC, on 13th of May for leaf drop, and on 5th of August for fruit russeting.

| Treatment | No. Fruits/100 FC | | Leaf drop | % Fruit russeting | |
|---|---|---|---|---|---|
| Golden Del. UFO | 73.0 | a | 3 | 13.3 | a |
| Golden Del. B + D | 78.4 | a | 0 | 10.6 | a |
| Golden Del. Control | 129.1 | b | 0 | 9.5 | a |
| Pink Lady UFO | 33.6 | a | 1 | no fruit russeting has been recorded | |
| Pink Lady B + D | 72.5 | b | 0 | | |
| Pink Lady Control | 158.6 | c | 0 | | |
| Braeburn UFO | 23.3 | a | 3 | | |
| Braeburn B + D | 53.3 | b | 0 | | |
| Braeburn Control | 96.7 | c | 0 | | |
| Fuji UFO | 47.7 | a | 2 | 5.7 | a |
| Fuji B + D | 60.9 | b | 0 | 3.3 | a |
| Fuji Control | 103.2 | c | 0 | 4.3 | a |
| Gala UFO | 57.9 | a | 2 | no fruit russeting has been recorded | |
| Gala B + D | 74.7 | b | 0 | | |
| Gala Del. Control | 98.7 | c | 0 | | |
| Granny Smith UFO | 21.4 | a | 1 | 4.7 | b |
| Granny Smith B + D | 27.8 | b | 0 | 1.4 | a |
| Granny Smith Control | 29.4 | b | 0 | 1.0 | a |
| Red Del. UFO | 16.3 | a | 4 | no fruit russeting has been recorded | |
| Red Del. B + D | 47.3 | b | 0 | | |
| Red Del. Control | 69.0 | c | 0 | | |
| Kanzi UFO | 2.5 | a | 5 | | |
| Kanzi B + D | 23.1 | b | 0 | | |
| Kanzi Control | 34.0 | c | 0 | | |
| Pinova UFO | 61.8 | a | - | | |
| Pinova Control | 73.3 | b | - | | |

On the cultivars Pink Lady, Braeburn, Fuji, Gala, Red Delicious, and Kanzi, instead, the thinning effect was significantly higher for the UFO treatment than for the B+D treatment, which still significantly reduced the number of fruits/100 FC in comparison to the untreated control. On the cultivar Granny Smith, only the UFO treatment resulted in a significant reduction of fruit set (21.4 versus 27.8 fruits/100 FC for the B+D treatment and 29.4 fruits/100 FC for the untreated control). Also on the variety Pinova, the UFO treatment resulted in a significant thinning effect (61.8 fruits/100 FC) in comparison to the control (73.3 fruits/100 FC).

Leaf drop was recorded only for the UFO-based treatment, and this on all tested varieties. Highest leaf drop was observed on the cultivars Kanzi (5 = high drop of rosette leaves and on shoots) and Red Delicious (4 = high drop of rosette leaves and light-medium leaf drop on shoots), while the cultivar Pink Lady was the least affected variety (1 = light drop of rosette leaves).

Fruit russeting could be recorded only on the cultivars Golden Delicious, Fuji, and Granny Smith (Table 5). On the cultivars Golden Delicious and Fuji, the percentage of fruit surface affected by russeting did not differ significantly among treatments, while on the cultivar

Granny Smith, the UFO treatment showed a higher percentage of fruit russeting (4.7%) than the B+D treatment (1.4%) and the untreated control (1.0%).

## Discussion

In organic apple growing in South Tyrol, yield control is achieved by using mechanical thinning machines and/or lime sulphur sprays during flowering (Südtiroler Beratungsring für Obst- und Weinbau, 2011b). These tools allow for flower thinning. At this crop stage it is already visible how many flowers each tree will bear, but actual fruit set depends also on several additional factors, which can not be predicted at the moment of flower thinning. Frequently, successive steps must be undertaken to assure yield of good quality. In integrated farming systems this is achieved by applying phytohormones. In organic farming, instead, at the moment, the only available tool is manual thinning, which is labour-intensive and expensive.

With the aim of reducing transpiration and net photosynthesis of leaves on treated trees, and thus increasing June fruit drop, the paraffin oil-based product UFO (Ultra Fine Oil) was tested at the research centre Laimburg (South Tyrol, Italy) on different apple varieties in 2010 and 2011. The trials were conducted in different integrated apple orchards (training system: spindle). In 2010, UFO was tested at two different application rates and timing: 2 applications at 1.5 l/100 l and 3 applications at 1.0 l/100 l, respectively. The trials have been conduced in integrated management orchards and it is known, that trees have a different behaviour under integrated and organic conditions. Therefore it would be interesting, to test this experiment also under organic conditions, but it has to be clear, that thinning effects vary significantly between different cultivars, orchards and areas and so there will never be one single thinning application for all apple growing.

In 2010, on all cultivars except Pink Lady, a significant thinning effect of the UFO-based treatments emerged. On the cultivar Golden Delicious, also the synthetic reference product Metamitron was tested. This product showed the highest thinning effect, followed by 2x1.5 l/100 l UFO, 3x1.0 l/100 l UFO, and the untreated control, each treatment differing significantly one from the other. Leaf drop on all cultivars occurred in the UFO-treated plots, but not in the untreated control. However, leaf drop values ranged between 1 and 2, and were thus relatively low. Statistically significant differences among treatments in fruit russeting emerged only on the cultivars Gala and Golden Delicious, with a high percentage of russeted fruit surface in the treatment 2x1.5 l/100l UFO. Significantly lower yield in the UFO-based treatments than in the untreated control was recorded on the cultivars Gala, Braeburn, and Golden Delicious, which is due to the thinning effect of the paraffin oil. Significant differences in fruit weight could be recorded on almost all cultivars. In general, fruit weight was higher in UFO-treated than in untreated control plots, and on the varieties Red Delicious, Braeburn, and Golden Delicious mean fruit weight was highest for 3x1.0 l/100 l UFO, intermediate for 2x1.5 l/100 l UFO, and lowest for the untreated control. Flower bud formation the following spring was generally higher in UFO-treated than in untreated control plots.

In 2011, the paraffin oil-based product UFO was tested in comparison to the integrated phytohormone Brancher Dirado applied in tank mixture with the adjuvant Dirager (B+D treatment) on different apple cultivars. The highest thinning effect was achieved with the UFO-based treatments, followed by the B+D treatment. Leaf drop on all varieties was observed only for the UFO-based treatments. Fruit russeting could be recorded only on the cultivars Golden Delicious, Fuji and Granny Smith, but remained below 15% in all treatments.

It can be concluded that the tested paraffin oil-based product UFO showed a promising thinning potential on all apple cultivars, but, at the moment, the risk of leaf drop and slight fruit russeting can not be excluded.

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# Photo-optical, non-invasive detection of the fire blight pathogen *E. amylovora*

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**Abstract**

Erwinia amylovora*, the causal agent of fire blight, causes enormous losses in pome fruit production, especially in apples and pears. The limited options for disease control make early detection of fire blight infections important in order to start specific and efficient counter measures in due time. Spectroscopic methods take advantage of non-invasiveness and speed of measurements. Measurements are repeatable, and a quick survey of large areas and remote sensing is possible. The method uses reflectance characteristics of plant tissue, which provides information about the health status of the plant. In this project, a fluorescence spectrometer was used to perform ratings on inoculated and healthy trees of different apple cultivars. First results indicate the possibility of differentiation between healthy and infected trees due to spectrometric data.*

**Keywords**: *E. amylovora*, remote sensing, fluorescence spectroscopy

## Introduction

Fire blight is a major threat for pome fruit production, causing economical losses worldwide especially in apples and pears (Bonn and Van der Zwet 2000). Specific symptoms are the emergence of polysaccharide containing exudates, the burnt appearance of leaves and flowers, and the typical shepherd's crook, a bending of the shoot apex due to decreasing turgor pressure. Once the disease has occurred, there are very few countermeasures like the excision of diseased tissues or the removal of infected trees, even whole orchards. Besides these curative measures stands the protective use of antibiotics e.g. streptomycin or biological control using antagonists (Kunz *et al.* 2011). An early detection of fire blight infections, amongst other parameters, may be helpful for decision making in order to start specific and efficient countermeasures in due time. The advantages of spectroscopic methods are obvious: the measurements are non-invasive and therefore repeatable; a quick survey of large areas and remote sensing is possible. The method uses the reflectance characteristics of plant tissue, which provides information about the health status of the plant. Changes in plant reflectance spectra can be used to make statements about the presence of pathogens. In 1996, Luedeker *et al.* could use chlorophyll fluorescence to identify powdery mildew on apple leaves earlier than a visual rating. Experiments on scab-infected and healthy apple trees were successful putting into practice an infrared spectroscopy-based differentiation (Delalieux *et al.* 2007). In addition, attempts were undertaken using near infrared spectroscopy to detect fire blight in asymptomatic pear plants (Spinelli *et al.* 2006).

## Material and methods

Apple plants from six different susceptible cultivars were used: *Adams Parmäne, Danziger Kantapfel, Gala, Öhringer Blutstreifling, Rewena,* and *Schneiderapfel*.

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For each cultivar, twelve apple shoots were used. One half of the shoots were artificially inoculated with a bacterial suspension of *E. amylovora* strain *Ea*385. Inoculation was performed by injection with a needle and syringe just beneath the youngest fully developed leaf. The other half was left untreated as control. The experiment was conducted in the greenhouse, with 12 h light and 27°C/15°C day/night temperature. Measurements were taken immediately before inoculation as well as 1, 2, 4, 7, 11, 16, 22 and 29 dpi. Leaves at five different positions on the shoot were measured three times at each point; additionally photographs were taken to document symptom development. The sensor used was the fluorescence spectrometer Multiplex® (Force A, Orsay, France). As an active sensor it is independent from ambient light because of its internal light source, allowing measurements regardless from external light conditions. The sensor registers twelve signals resulting from four excitations (UV, blue, green and red light) and three emissions (blue, red and far-red). Out of this signals several ratios are calculated, which give information about different plant constituents/components (Multiplex® User's guide 2008). The main two categories are fluorescence excitation ratios of far-red chlorophyll emission (FRF) and fluorescence emission ratios (e.g. FRF/RF and BGF/FRF).

**Results**

Data were tested for normality of distribution to decide which classification method should be used. Testing was done using the Shapiro-Wilk test in R (R Development Core Team 2011) for each of the datasets (324 sets for each cultivar: two treatments – inoculated and control, nine dates - 0 to 29 dpi, and 18 variables tested individually). Out of these datasets, only 21% (*Adams Parmäne*, Fig. 1) respectively 25% (*Rewena*, Fig. 3) were normally distributed with a p-value greater than 0,05 in Shapiro-Wilk test. The four other cultivars showed similar results (data not shown). Since most of the data were not normally distributed, a tree based modeling was done to decide, which of the variables were most useful in differentiating between healthy and infected plants. Decision trees consist of nodes, connected by branches and ending in leaf nodes. At each decision node the measured attributes are tested, resulting in a branch that leads either to another node or to a terminating leaf node (Larose 2005). From these trees it is possible to generate decision rules to decide between healthy and infected populations based upon new measurements. The technique used in this work could accurately classify the measured attributes based on recursive partitioning (Breiman *et al.* 1984). The decision trees were created using "rpart" in R (R Development Core Team 2011).

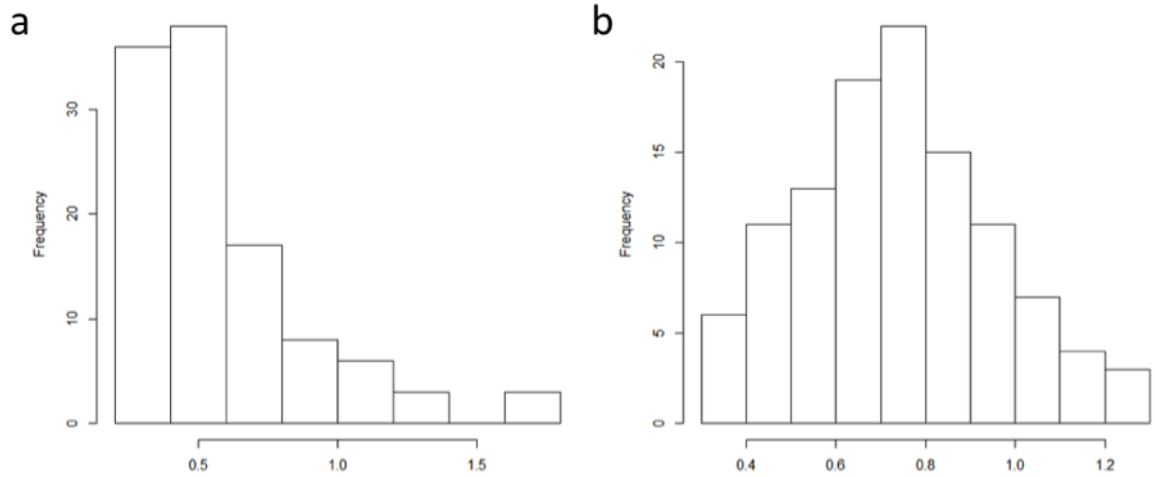


Fig. 1: Histograms representing a non normal (a) and a normal distribution (b) of the variables BRR_FRF (p-value=8,355E-7) and NBI_R (p-value=0,1119) on infected *Adams Parmäne* plants at 4 dpi. BRR_FRF: Blue-to-Red Fluorescence Ratio under UV excitation; NBI_R: Nitrogen Balance Index, red and UV excitation.

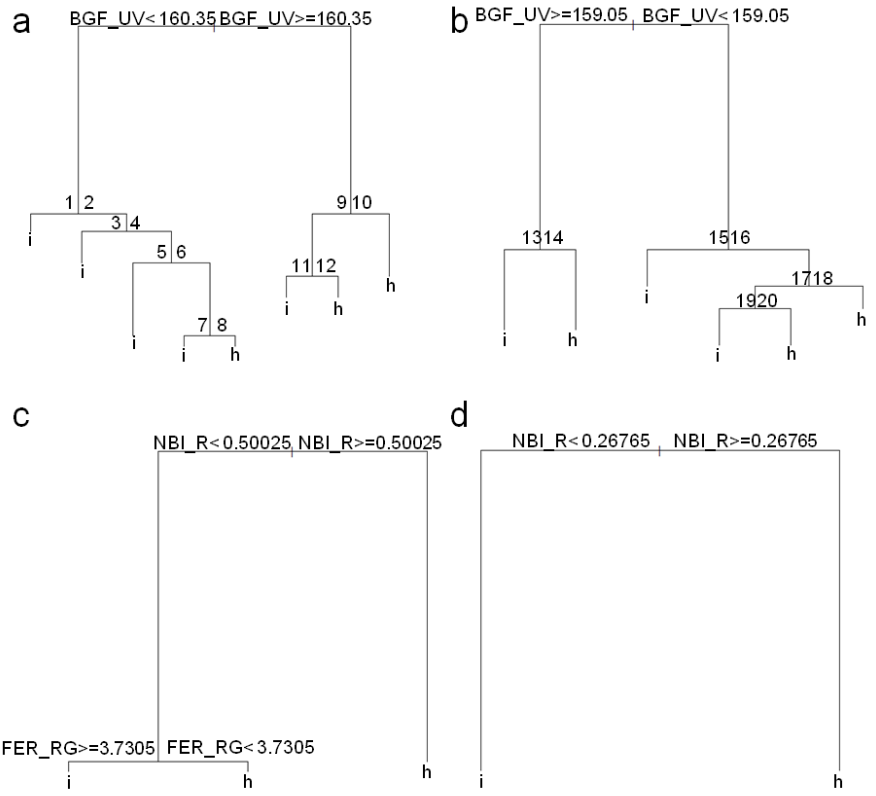


Fig. 2: Decision trees derived from measurements on healthy and infected *Adams Parmäne* plants. a: 1 dpi, b: 7 dpi, c: 16 dpi, d: 29 dpi; i: infected leaves, h: healthy leaves; 1: RF_UV>=120.4; 2: RF_UV< 120.4; 3: BGF_UV< 155.9; 4: BGF_UV>=155.9; 5: BGF_UV>=159.15; 6: BGF_UV< 159.15; 7: NBI_G>=2.7775; 8: NBI_G< 2.7775; 9: BGF_UV>=165.05; 11: BGF_UV< 167.75; 12: BGF_UV>=167.75; 10: BGF_UV< 165.05; 13: NBI_G< 4.229; 14: NBI_G>=4.229; 15: RF_UV< 51.55; 16: RF_UV>=51.55; 17: BGF_UV>=157.75; 19: BGF_G>=412.5; 20: BGF_G< 412.5; 18: BGF_UV< 157.75. Abbreviations for the attributes are explained in the Multiplex® User's guide (2008).

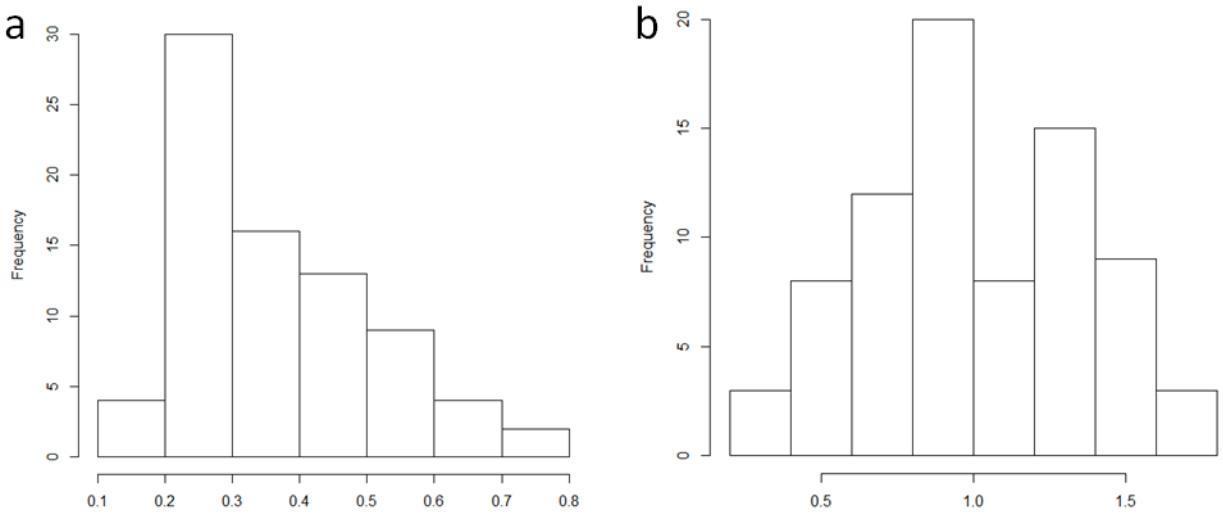


Fig. 3: Histograms representing a non normal (a) and a normal distribution (b) of the variables BRR_FRF (p-value=1,99E-4) and NBI_R (p-value=0,1269) on infected *Rewena* plants at 4 dpi. BRR_FRF: Blue-to-Red Fluorescence Ratio under UV excitation; NBI_R: Nitrogen Balance Index, red and UV excitation.

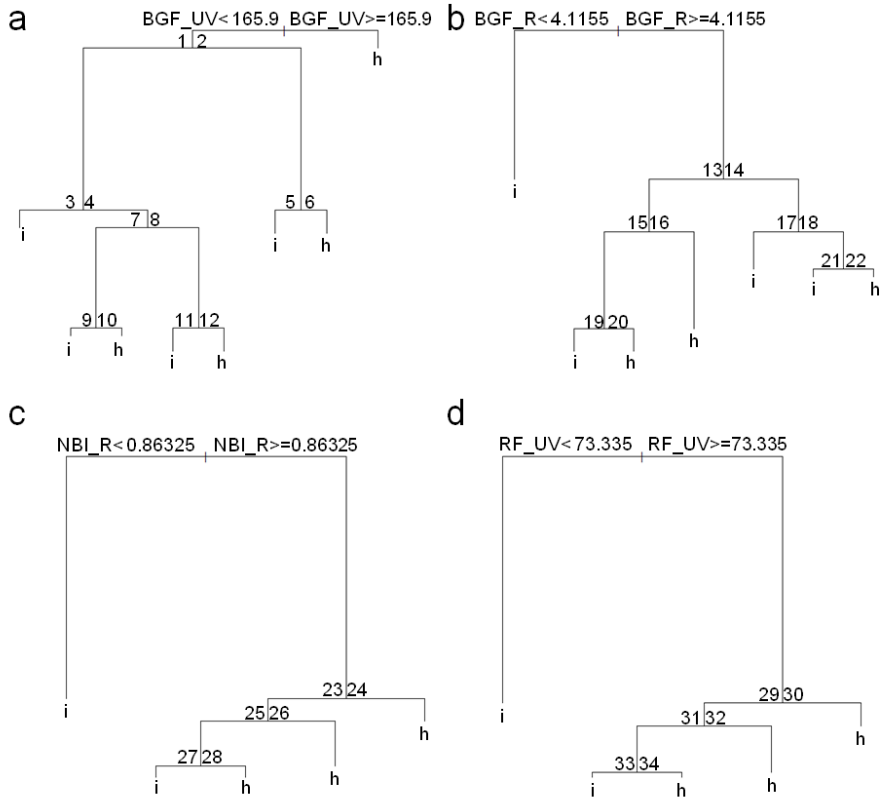


Fig. 4: Decision trees derived from measurements on healthy and infected *Rewena* plants. a: 1 dpi, b: 7 dpi, c: 16 dpi, d: 29 dpi; i: infected leaves, h: healthy leaves; 1: BGF_G>=383.65; 2: BGF_G< 383.65 51; 3: RF_R< 448.3; 4: RF_R>=448.3; 5: NBI_G< 4.145; 6: NBI_G>=4.145; 7: SFR_G< 6.1815; 8: SFR_G>=6.1815; 9: SFR_R>=4.4185; 10: SFR_R< 4.4185; 11: BGF_UV>=159.55; 12: BGF_UV< 159.55; 13: NBI_R< 1.0555; 14: NBI_R>=1.0555; 15: SFR_R>=4.9815; 16: SFR_R< 4.9815; 17: BGF_UV>=169.15; 18: BGF_UV< 169.15; 19: FRF_G< 808.2; 20: FRF_G>=808.2; 21: RF_UV>=210; 22: RF_UV< 210; 23: BGF_UV>=164.05; 24: BGF_UV< 164.05; 25 FRF_UV>=488.3; 26: FRF_UV< 488.3; 27: RF_UV< 150.7; 28: RF_UV>=150.7; 29: BGF_UV>=161.45; 30: BGF_UV< 161.45; 31: SFR_G< 8.744; 32: SFR_G>=8.744; 33: BGF_G< 405.2; 34: BGF_G>=405.2. Abbreviations for the attributes are explained in the Multiplex® User's guide (2008).

Decision trees were used to derive a set of conditions that permit accurate classification of infected and healthy trees based on the measured attributes. Fig. 2 and 4 show the results from the measurements at 1, 7, 16 and 29 dpi. At 7 dpi all *Adams Parmäne* trees showed visible symptoms, *Rewena* trees at 16 dpi. Taking Fig. 2d as example, the tree can be interpreted as follows: if the value of NBI_R is smaller than 0,26765 one would take the left branch and the corresponding measurements would be classified as infected. In more complex trees (Fig. 2a, b and c) one has to decide on more nodes which direction to take until the leaf node is reached. While at 1 dpi – regarding the measurements on *Adams Parmäne* - many nodes are needed to separate the populations, the number of nodes gets smaller until at 29 dpi only one attribute is needed to completely separate infected from healthy plants. With the less susceptible cultivar *Rewena*, the number of nodes at 1 and 7 dpi (Fig. 4a and b) is comparable to the ones of *Adams Parmäne* but at 16 and 29 dpi (Fig. 4c and d) the decision trees don't enable a proper differentiation like the ones derived from the susceptible cultivar. Similar results were obtained from the other cultivars: The classification of susceptible cultivars is more distinct than of less susceptible cultivars. Additionally, error rates of the classification accuracy for each tree were obtained in calculating the prediction column and confusion matrix. The corresponding error rates in table 1 indicate a better distinction for infected and healthy *Adams Parmäne* plants regarding further developed infections, too. Error rates are higher at the beginning and decrease with progression of infection; the distinction between healthy and infected populations gets clearer. The error rates for *Rewena* don't show this decline up to 29 dpi.

Table 1: Error rates of the classification accuracy.

| dpi | 1 | 2 | 4 | 7 | 11 | 16 | 22 | 29 |
|---|---|---|---|---|---|---|---|---|
| error rate [%] | | | | | | | | |
| *Adams P.* | 15,97 | 13,89 | 9,14 | 12,78 | 7,22 | 5,00 | 4,39 | 0 |
| *Rewena* | 12,5 | 8,33 | 10,93 | 14,44 | 8,33 | 12,22 | 3,95 | 6,78 |

## Discussion

This experiment gives insight into the potential of fluorescence spectroscopy to differentiate between fire blight infected and healthy apple trees. Even though a secure classification is only possible after visible symptoms are present, the photo-optical detection of fire blight infections could be useful in reducing the effort of manual ratings of orchards. Results suggest that decision trees are able to distinguish apple plants infected with fire blight from healthy plants of the susceptible cultivar *Adams Parmäne*. These results are of considerable interest for pome fruit production, where an early detection based on photo-optical input could lead to earlier and better counter measures and thus to secure and increased production. For the less susceptible cultivar *Rewena* this distinction is not that well-defined. Other statistical methods should be taken into account. In further studies, other parameters like secondary plant metabolites such as flavonoids that might be generating the changes in reflectance characteristics of infected plant tissue and the quantitative detection of *E. amylovora* using Real Time PCR could be combined with the results of spectroscopic measurements. Furthermore, the effect of other biotic stresses for apple plants like scab or powdery mildew on the reflectance characteristics should be investigated.

**Acknowledgements**

We want to thank Roland Gerhards and Martin Weis as well as Ulrich Mayr and Hans-Thomas Bosch. This work was supported by a grant provided by the European Union within the Interreg IV program „Gemeinsam gegen Feuerbrand“ to RTV.

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# Summary of an eight year research project on fire blight control

S. Kunz[1], A. Schmitt[2], P. Haug[3]

**Abstract**

*In organic fruit growing effective control strategies are needed to prevent blossom infections by the fire blight pathogen* Erwinia amylovora*. In a research project, funded by the "BundesprogrammÖkologischerLandbau" from 2004 to 2011, 64 different preparations have been tested in laboratory trials for efficiency against the fire blight pathogen. 26 of them were further tested in field trials according to EPPO guideline PP1/166(3).From the products, which are commercialised in Germany, BlossomProtect had the highest efficiency, followed by Myco-Sin, FolanxCa 29, Funguran and Serenade.*

*Blossom Protect contains blastospores of the yeast-like fungus* Aureobasidium pullulans*. Therefore strategies were developed to integrate Blossom Protect applications in commercial spray schedules for apple scab control. In additionthe influence of the selected products on fruit russet was evaluated. The increase in fruit russetting depended on the number of applications of Blossom Protect and on the variety treated. Combined control of fire blight and apple scab control could be achieved using tank mixtures of Blossom Protect and wettablesulphur. With a strategy of alternating Blossom Protect applications with sprays of a mixture of wettablesulphur and Myco-Sin, fire blight and apple scab were both controlled significantly. In addition, this strategy reduced the risk of fruit russetting.*

*In field trials, carried out since 2006, the abundance of* E. amylovora *was measured in blossoms with qPCR and was correlated with the disease incidence in untreated plots. These data indicate that control measures can be limited to orchards infested with the fire blight pathogen.*

**Keywords:** Fire blight control strategy, *Erwinia amylovora*, Blossom Protect, Myco-Sin

## Introduction:

Fire blight caused by *Erwinia amylovora* is the most serious bacterial disease in apple and pear. During the last four decades it has spread throughout Europe. Sanitation methods like pruning of infected shoots and uprooting of infected trees are necessary to reduce infection pressure in the orchards. However, it is not possible to eliminate all fire blight bacteria due to their epiphytic and endophytic abundance on and in trees free of symptoms(Voegele *et al*., 2010). Under favourable weather conditions *E. amylovora* multiplies on blossom surfaces (e.g. stigma) and invades the plant tissue by the nectarthodes in the hypanthium (Pusey and Smith, 2008). Each blossom is a potential infection site and therefore efficient control agents are needed to prevent blossom infections. In addition information on the risk for infections is needed to successfully schedule the applications.

A three-step evaluation procedure was established consisting of laboratory tests *in vitro* and *in vivo* as well as field trials. The laboratory tests in shaken cultures and on detached blossoms gave information on the mode of action of the control agents (Kunz *et al*., 2009) and was used to select control agents to be tested under field conditions.

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Products used for fire blight control have to be integrated into spray schedules of organic pome fruit producers. Moreover, strategies are needed for the control of both, fire blight and apple scab, and strategy components should not have any negative side effects.Therefore, control agents showing high efficacy against fire blight were also tested for phytotoxicity (fruit russetting) and combatibility with fungicides.

Besides the weather conditions, which are considered in fire blight prediction models, presence of the pathogen in the orchard is a prerequisite for infections. Real Time PCR is a fast and sensitive method that allows quantification of *E. amylovora* in blossoms (Voegele *et al*., 2010). Since 2007 the abundance of *E. amylovora* in blossoms of untreated trees was measured and correlated with the disease incidence. Implementation of measurement of the abundance of *E. amylovora* in the orchard in decision making for control measures is discussed.

**Material and Methods:**

Measurements of growth rates of *E. amylovora* in liquid cultures and the reduction of fire blight symptoms on detached blossoms was measured as described in Kunz *et al*. 2009.

**Field trials to test the efficiency of products** and strategies against fire blightwere carried out in accordance with the EPPO guideline PP1/166(3). One to four trees per orchard plot were inoculated with the pathogen. From the inoculated trees *E. amylovora* was spread over the entire orchard by natural vectors. Only the symptoms from trees, which had not been inoculated, were taken into account. Results from field trials conducted in the year 2004 in Groß-Umstadt, 2006-2008 in Karsee and Darmstadt (Kunz *et al*., 2009 and literature cited therein), 2009-2010 in Darmstadt(Kunz *et al*., 2010; Kunz *et al.*, 2011) and from 2012 in Mühlingen and Darmstadt (Kunz *et al*., 2012) have already been published.

**Field trials on the influence of treatments on fruit russet:** Experiments were conducted in organic apple orchards in a randomised block design with four replications per treatment. All of the fruit from 4-6 trees per plot were classified into 4 classes (cl) according to the russeted area per apple (cl1= 0% of the surface russeted; cl2=0-10%; cl3=10-30%; cl4= >30%). For each plot the russet index (RI) was calculated (RI = (Ncl1 x 1 + Ncl 2 x 2 + Ncl x 3 + Ncl 4 x 4)/ (Ncl1+Ncl2+Ncl3+Ncl4). (Kunz *et al.,* 2009).

**Real Time PCR analyses** were performed as described by Vögele *et al.* 2010. Samples consisted of washing fluids from blossoms containing intact bacteria. No DNAextraction was performed prior to PCR analysis. Absolute quantification of bacteria insamples was done by standardization with respect to serial dilutions of purecultures of *E. amylovora*.

Twenty blossoms were collected in Whirl-Pak bags (Carl RothGmbH, Karlsruhe), and incubated with 2 ml $H_2O$ per blossom for 15 min. A 1 ml aliquotwas removed and centrifuged for 1 min at 15,000 g. The supernatant was discarded and pellets resuspended in an equal volume of $H_2O$. Samples were either analysed directly orstored at -20°C.

**Results**

In a systematic evaluation 64 control agents have been tested in different test systems during the last eight years. From these, 38 control agents suppressed *E. amylovora* in vitro, illustrating their bacteriostatic behaviour. However, on detached apple blossoms only 19 preparations were able to reduce symptom development by more than 60%. Six of them were copper products, five products contained *Aureobasidium pullulans*, two were from other fungal origin and three products contained*Bacillus*sp.. The group of efficient products furthermore included Myco-Sin, Chitoplant and LX4630.

In 13 field trials since 2004, 26 different preparations have been tested for efficacy against fire blight, from which 16 products were commercially available in Germany as plant protection agents, plant strengtheners or fertilizers. In the following we focus on the results with the commercialised products. Blossom Protect on average reduced fire blight incidence by 78% and Myco-Sin by 61%, when sprayed three to five times per season according to the phenological development of the blossoms (fig. 1).FolanxCa29 (calciumformate) reduced blossom blight by 59% in our trials. The copper fungicide Funguran applied with 135g metallic copper per ha, showed 58% efficiency (fig. 1). Other copper formulations (Protex-Cu and Cueva) used with 100g metallic copper/ha showed lower effects against fire blight in field trials. Serenade (*Bacillus subtilis*) reduced fire blight symptoms by 56%. All the other products, which had efficiencies lower than 50% (fig. 1)cannot be recommended for use in fire blight control.

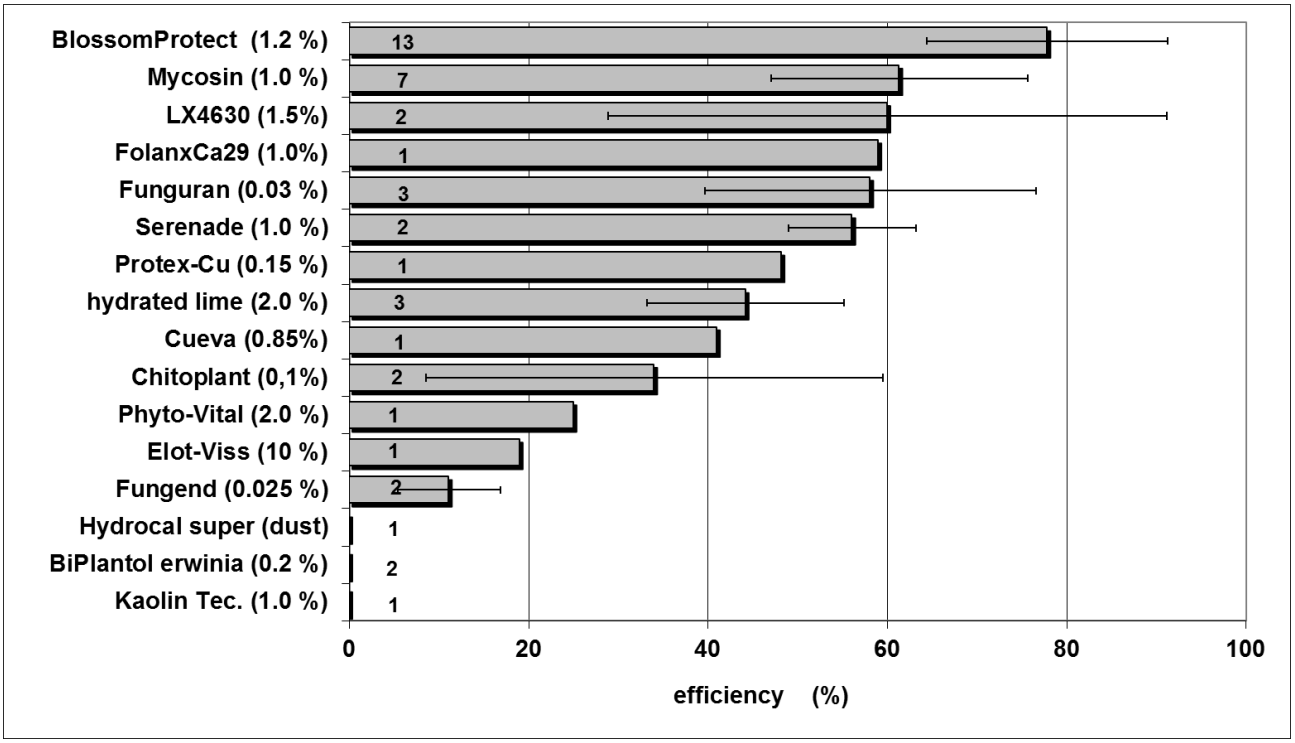


Figure 1: Efficiency against fire blight blossom infections in field trials 2004 to 2011of products commercially available in Germany. Efficiencies were calculated from evaluations of disease incidence of trees with secondary infections. The products were applied with the given concentration 3 to 5 times according to the phenological stage of the blossoms. The number of trials for each product is shown in the column.

In practice, spray strategies have to control both, fire blight and apple scab.In general, sulphur did not hamper the efficiency of Blossom Protect against fire blight, when applied in alternation with Blossom Potect. However, the use of lime sulphur tended to be more critical than the use of NetzschwefelStulln and the total number of applications during bloom was too high (tab. 1). The use of tank mixtures of Blossom Protect with NetzschwefelStulln gave good control of both, fire blight and apple scab, in three field trials and reduced the number of applications (tab. 1). As Myco-Sin is known to enhance the efficacy of sulphur against apple scab and since it has a proven effect against fire blight, a strategy to alternate Blossom Protect applications with sprays of a mixture from NetzschwefelStulln and MycoSin was tested. In four field trials this strategy was almost as effective against fire blight as Blossom Protect as stand alone treatment. The use of this

strategy allowed the reduction of total applications during bloom from 6-8 to 4 and a reduction of the number of Blossom Protect treatments per year from 4 to 2, which reduces costs and the risk of fruit russet.

Table 1: Efficiency (%) of BlossomProtect and spray strategies in field trials 2004-2011 in Karsee (KA), Darmstadt (DA) and Mühlingen (MÜ). Only results on disease incidence from trees not artificially inoculated were considered. The numbers in brackets indicate the number of applications of the agents used in the described strategies.

| | **KA 04** | **KA 06** | **DA 06** | **KA 07** | **KA 08** | **DA 09** | **DA 10** | **MÜ 11** |
|---|---|---|---|---|---|---|---|---|
| BlossomProtect (12 g/l) | 85 (4) | 86 (4) | 85 (4) | 89 (4)<br>83 (3) | 80 (4) | 81 (4) | 82 (4) | 91 (4)<br>84 (3) |
| BlossomProtect (12 g/l) altern. lime sulphur (15 ml/l) | 68 (4)<br>(4) | | 87 (3)<br>(1) | 77 (3)<br>(3) | | | | |
| BlossomProtect (12 g/l) altern. wettable sulphur (3 g/l) | | 88 (4)<br>(3) | 85 (4)<br>(1) | 84 (3)<br>(3) | | | | |
| BlossomProtect (12g/l) altern. wettable sulphur (3g/l) + MycoSin (10g/l) | | | | 87 (3)<br>(3) | 70 (3)<br>(2) | 74 (2)<br>(2) | | 76 (2)<br>(2) |
| tank mixture: BlossomProtect (12 g/l)+ wettablesulphur (3 g/l) | | | | | 77 (4)<br>(4) | | 74 (4)<br>(4) | 89 (4)<br>(4) |
| Vacciplant (0.375 ml/l) before Blossom Protect (12 g/l) | | | | | | | | 90 (2)<br>(3) |

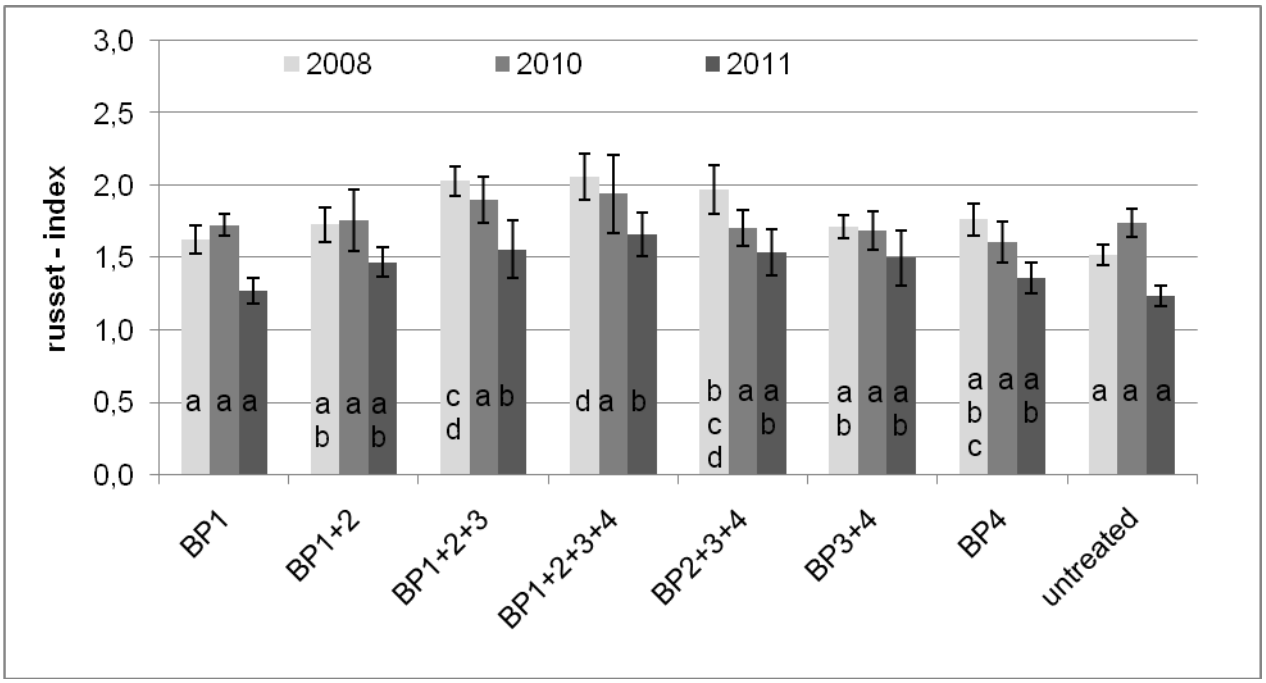


Figure 2: Russet index on fruits of the variety ´Santana´ in Mainau 2008, 2010 and 2011 after varying numbers of treatments with 12 g/l Blossom Protect (BP) in comparison to an untreated control**.** The numbers 1-4 represent the dates of the treatment.Different letters indicate a significant difference in Tukey´s Multiple Comparison test ($p \leq 0.05$) in one year

The influence of products and strategies on fruit russet was tested in organic apple orchards. Copper or Blossom Protect increased fruit russet significantly on some varieties (‘Jonagored’, ‘Jonagold’, ‘Golden Delicious’, ‘Idared’, ‘Sansa’, ‘Santana’) whereas in ‘Gala’, ‘Goldrush’, ‘Summerred’, ‘Pinova’, ‘Braeburn’ and ‘Topaz’ no effect was visible (Kunz *et al*., 2010; Kunz *et al*., 2009). In a three years trial in MainauBlossom Protect (12g/l) was applied 1 to 4 times during bloom at different application dates on the variety `Santana`. One or two applications had no significant influence on fruit russet, independent of the date of application. Three or four treatments increased fruit russet significantly in 2008 and 2011 (fig. 2).

The addition of wettable sulphur to Blossom Protect or strategies alternating Blossom Protect with a mixture of Myco-Sin and wettable sulphur did not further increase the risk for russeting on ‘Jonagold’. Tank mixtures of copper and Blossom Protect or a mixture of Vacciplant with Blossom Protect increased fruit russet compared to Blossom Protect applied as a stand alone treatment on the variety ‘Jonagold’, but not on ‘Topaz’ (data not shown).

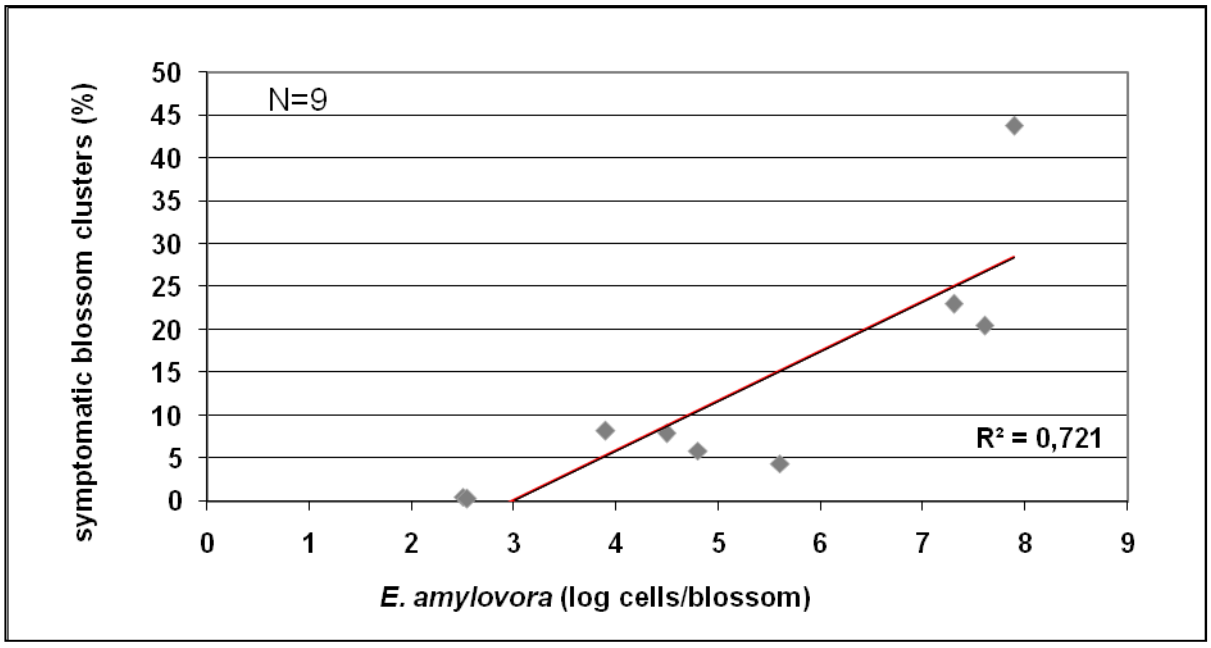


Figure 3: Correlation of fire blight incidence (% symptomatic blossom clusters) andmaximum abundance of *E. amylovora*(log cells/blossom) in blossoms during the flowering period in 9 field trials from 2006 to 2011.

Since 2006 in 9 field trials the abundance of *E. amylovora* was measured in blossoms of untreated plots of not inoculated trees. *E. amylovora* showed a typical growth curve starting below the detection level at beginning of bloom and reaching numbers of nearly $10^8$ cells/blossom at the end of the blossoming period (Kunz *et al*., 2009), in most of the trials. In two trials cell counts below 1’000 per blossom were measured and no increase in abundance of *E. amylovora* over the blossoming period was detected. In both trials the fire blight incidence was below 1% infected blossom clusters (fig. 3). In all of the 9 field trials the maximum number of pathogens measured in blossoms correlated with the disease incidence in untreatedplots. More than 5’000 *E. amylovora* bacteria per blossom were necessary to result in a measurable disease incidence.

**Discussion**

Investigation of products for efficacy against fire blight in field trials is expensive and time consuming. Trials under natural infection conditions are seldom successful and trials with artificial inoculation are restricted to certain trial sites as *E. amylovora* is a quarantine organism. Only a small number of products or strategies can be tested in the field each year. Reliable methods to screen products for efficacy are needed to avoid a waste of resources in field trials.Therefore, in this project a three step evaluation procedure was established composed of tests in shaken cultures, tests on detached blossoms and field trials (Kunz *et al*., 2009). The efficacy of a product on detached blossoms corresponded well with its efficacy in field trials. We suggestto first test all control agents in the detached blossom system and then to evaluate only products with proven efficacy under field conditions.

The aim of the project was to investigate products for fire blight control, which are available for organic growers. Nevertheless test preparations not registered have been included in the study. Although having good efficiencies in some cases(Kunz *et al*., 2009), these test preparations were not developed further by the companies. Therefore, during the last year, we focused on products commercially available in Germany.

Based on the results of this project, BlossomProtect is recommended for fire blight control in organic orchards in Germany and other European countries. Due to high efficiencies in field trials in the USA, which were comparable to that of antibiotics, the use of BlossomProtect will also be recommended in pome fruit production in the USA after finalization of registration (Kunz *et al.*, 2012). BlossomProtect contains blastosporesof the fungus*Aureobasidium pullulans.* Reports on *A. pullulans* causing fruit russeting in apple and pear (Spotts and Cervantes, 2002) have been addressed in several field trials during our project. The results indicate that the enhancement of fruit russeting caused by Blossom Protect depends on the variety and on the number of treatments. On susceptible varieties the number of applications should be reduced to two. This can be achievedby using a strategy applying Blossom Protect twice in periods with high risk for fire blight infections and additional applications of a tank mixture of Myco-Sin + wettable sulphur, when additional risk days occur or applications for apple scab control are necessary.

Applications against fire blight were done according to the phenological stage of the blossoms in our field trials in order to achieve the coverage of all blossoms. However, blossoms can only be infected when certain weather conditions are fulfilled during their life span.Under conditions unfavourable for fire blight, applications of control agents are unnecessary. In two field trials we proved that the timing of Blossom Protect applications according to the forecast modelMaryblyt (Lightner and Steiner, 1992; Moltmann, 1996) reduced the number of applications in comparison to the timing according to the phenological stage without reducing efficacy. Therefore the timing of applications according to forecast models is recommended. Blossom Protect or Myco-Sin should be applied the day before predicted infection conditions.

All the forecast systemsonly take physical factors like temperature or moisture/wetness into account, but not theactual presence of the pathogen.WithqPCRa fast, specific and quantitative detection of the pathogen in blossoms is possible (Voegele *et al.*, 2010).In our filed trials, the dissemination of *E. amylovora* from artificially inoculated trees to not inoculated trees as well as the multiplication of the pathogen in not inoculated trees was measured. The amount of *E. amylovora* bacteria detected in the blossoms correlated with the disease incidence in nine field trials. In trials without measurable dissemination of bacteria the abundance in blossoms was below 5’000 cells/blossom resulting in no or only sporadic symptom development. In our trials 20 blossoms were taken per sample and four samples per day. In a monitoring established in commercial orchards during the last years

100 blossoms per sample and two samples per day were taken. The correlation between abundance of *E. amylovora* in blossoms and disease incidence was comparable in both studies (Voegele and Kunz, unpublished). Monitoring of *E. amylovora* in the orchards during bloom can give additional input to the decision process on when and how often control agents should be applied to control fire blight. As long as the pathogen is not detectable by qPCR, the application of control agents can be postponed.

**Acknowledgement**

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# Cultivar testing, pathogenesis and quantitative distinction of live and dead cells of *E. amylovora*

M. Joos[1,*], A. Rehfus[1], A. Rex[1], S. Buck[1], R.T. Voegele[1]

**Abstract**

*Fire blight, caused by the Gram-negative bacterium* Erwinia amylovora, *is the most devastating bacterial disease of pome fruit. In order to identify standard cultivars less susceptible to fire blight, we tested 39 old standard apple cultivars from the Lake Constance region using artificial shoot inoculation and subsequent visual rating over a period of four weeks. Since it was previously shown that there is a built up of large bacterial populations in asymptomatic tissue, we also quantified the pathogen load within shoots of Regalis® treated and untreated trees of the apple variety Jonagold. A possible explanation for the detected high bacterial load might be a high number of dead* E. amylovora *cells in the infected asymptomatic tissues. Thus, we developed methods for a Live/Dead-distinction of* E. amylovora*. The most promising method is based on the combination of Real Time PCR and FACS-analyses.*

**Keywords:** *E. amylovora,* live and dead cells, pathogenesis, Real Time PCR

## Introduction

The most harmful bacterial disease throughout the world in economical important rosaceous plants (e.g. apple) is fire blight (Stöger *et al.* 2006). The causative agent of this disease is the Gram-negative bacterium *Erwinia amylovora* which is peritrichous flagellated and surrounded by a mucilaginous capsule. Symptoms of fire blight are the discoloration of infected twigs, leafs or blossoms, the appearance of exudate and the typical deformation of infected shoots, formally known as “sheperd’s crook”. Until now no resistance against fire blight is reported. Therefore, the search for at least less susceptible cultivars is a very important part of the fight against fire blight. To investigate the susceptibility of cultivars, Real Time PCR is a very powerful method to get an overview of the degree of infection and the quantity of the pathogen (Higuchi *et al.* 1993), and to investigate the susceptibility of cultivars. In reference to Salm and Geider (2004), we used whole bacteria extracted from shoot samples from pathogenesis-experiments. During Real Time PCR analysis of field samples we detected very high cell numbers in asymptomatic tissue. These findings suggest that there are large numbers of living and dead cells accumulated in the samples tested. This phenomenon is called latent infestation. The fact that Real Time PCR is not capable to discriminate between live and dead bacteria might lead to a significant overestimation of the effective pathogen load (Weißhaupt 2008). Based on a modified Real Time PCR and FACS-analysis we are able to clearly discriminate between live und dead cells and therefore exclude „false positive“ estimates of pathogen load.

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## Material and Methods

### Cultivar testing and pathogenesis:

For the testing of different cultivars and pathogenesis experiments we used freshly grafted apple trees provided by the Kompetenzzentrum Obstbau Bodensee (KOB) within the Interreg IV project „Gemeinsam gegen Feuerbrand“. All experiments were done in the greenhouse using the following parameters: 12 h light and 27 °C/15 °C day/night temperature. At the beginning of the experiments apple trees were needle-inoculated with an *E. amylovora* suspension containing $10^9$ cells per ml. The experimental setup for cultivar testing was composed of 29 different apple cultivars, each represented by 10 plants. Determination of different symptoms caused by *E. amylovora* was done by visual rating four times in weekly intervals starting one week after inoculation. Absolute shoot length and length of lesion were measured.

Within the pathogenesis experiments an untreated apple cultivar was tested in comparison to a cultivar treated with the growth regulator Prohexadion-Ca (BASF). This regulator is supposed to have an influence on the course of infection (Bubán *et al.* 2004). Treatment was done 14 days and 1 day before inoculation, according to the recommendation of BASF (2009). Each time 1.6 g Regalis®-granulate were dissolved in 1000 ml water. Shoots were cut 2, 3, 4, 6 and 12 days post inoculation. Cut shoots were processed as described by Voegele *et al*. (2010). After processing the samples were analysed by Real Time PCR.

### Quantification:

Real Time PCR analyses were performed as described by Voegele *et al*. (2010).

### Live/Dead-Distinction:

For Live/Dead-distinction the Real Time PCR method was modified by the addition of Propidium Monoazide (PMA). Since PMA binds covalently to DNA of dead cells, the amplification and quantification during Real Time PCR is restricted to DNA of living cells (Nocker *et al.* 2006; 2007). Unbound PMA was photo-inactivated with a 500 W spotlight positioned in a distance of 20 cm and 5 min exposure-time (Nocker *et al.* 2006). The modified Real Time PCR was performed with whole *E. amylovora* cells and purified plasmid DNA to determine if there are significant differences. Plasmid DNA was purified after 10 min PMA-treatment of the bacterial culture.

Another method based on intercalating dyes and labelled antibodies was also used: FACS-analyses. In this method dead bacteria were stained with Propidium Iodide (Boulos *et al.* 1999). *E. amylovora* was detected using a specific primary and a Fluorescein Isothio-cyanate-labeled secondary antibodies. This allowed the differentiation of living (green) and dead (red and green) *E. amylovora* cells.

## Results

Our work on *E. amylovora* principally focussed on three main aspects: a) susceptibility of different apple standard cultivars (Cultivar Testing) b) description of the quantitative distribution of *E. amylovora* within tissue with or without symptoms (Pathogenesis) c) distinction of live and dead cells in environmental samples (Live/Dead-Distinction).

Cultivar Testing. Against the background of field experience about the susceptibility of different apple cultivars, we started to screen several old apple standard cultivars from the Lake Constance region.

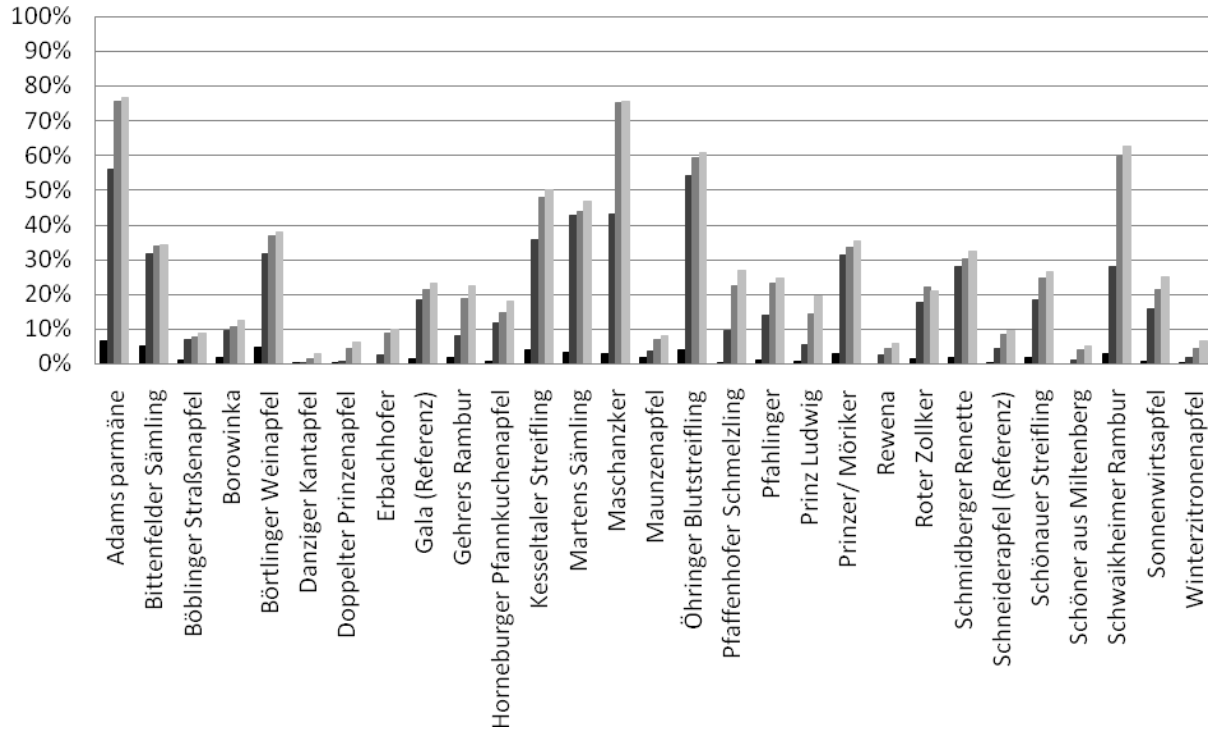


Figure 1**:** averaged relative length of lesion (%) of apple cultivars (2010)
x-axis: standard cultivars; ■ 1 week post inoculation (wpi), ■ 2 wpi, ■ 3 wpi, □ 4 wpi

During visual rating in 2010 we observed the highest increase in lesion-length during the second week post inoculation for most of the cultivars (Figure 1). Within the third and fourth rating only slight increases were recognised except for Adamsparmäne, Börtlinger Weinapfel, Maschanzker and Schwaikheimer Rambur.

In 2010 the cultivars tested most susceptible were: Adamsparmäne, Bittenfelder Sämling; Börtlinger Weinapfel, Kesseltaler Streifling, Martens Sämling, Maschanzker, Öhringer Blutstreifling, Prinzer/Möriker, Schmidberger Renette and Schwaikheimer Rambur.

In 2011 (Figure 2) we retested 19 cultivars, including the references Gala and Schneiderapfel, to validate our results from 2010. Additionally, we tested ten other standard cultivars. The higher increase in lesion-length during the first week post inoculation, in comparison to 2010, is due to an upgrade of the greenhouse lightning.

As shown for 2010 we also observed the highest increase in lesion-length in 2011 during the second week post inoculation.

The cultivars tested most susceptible in 2011 are: Aargauer Jubiläumsapfel, Fießers Erstling, Grüner Stettiner, Kronprinz Rudolf, Oberländer Himbeerapfel, Öhringer Blutstreifling, Pfaffenhofer Schmelzling, Prinz Ludwig and Sonnenwirtsapfel.

The cultivars tested least susceptible over two years are: Böblinger Straßenapfel, Doppelter Prinzenapfel, Glockenapfel, Rewena and Schöner aus Miltenberg.

Thus, we have successfully verified large parts of the results of 2010. We retested some cultivars, e.g. Doppelter Prinzenapfel or Winterzitronenapfel, less or least susceptible. We also retested some cultivars most susceptible in similar ranges, e.g. Öhringer Blutstreifling. However, for some cultivars conflicting results were obtained in 2011. The cultivar

Danziger Kantapfel for example was tested least susceptible in 2010 and intermediate susceptible in 2011.

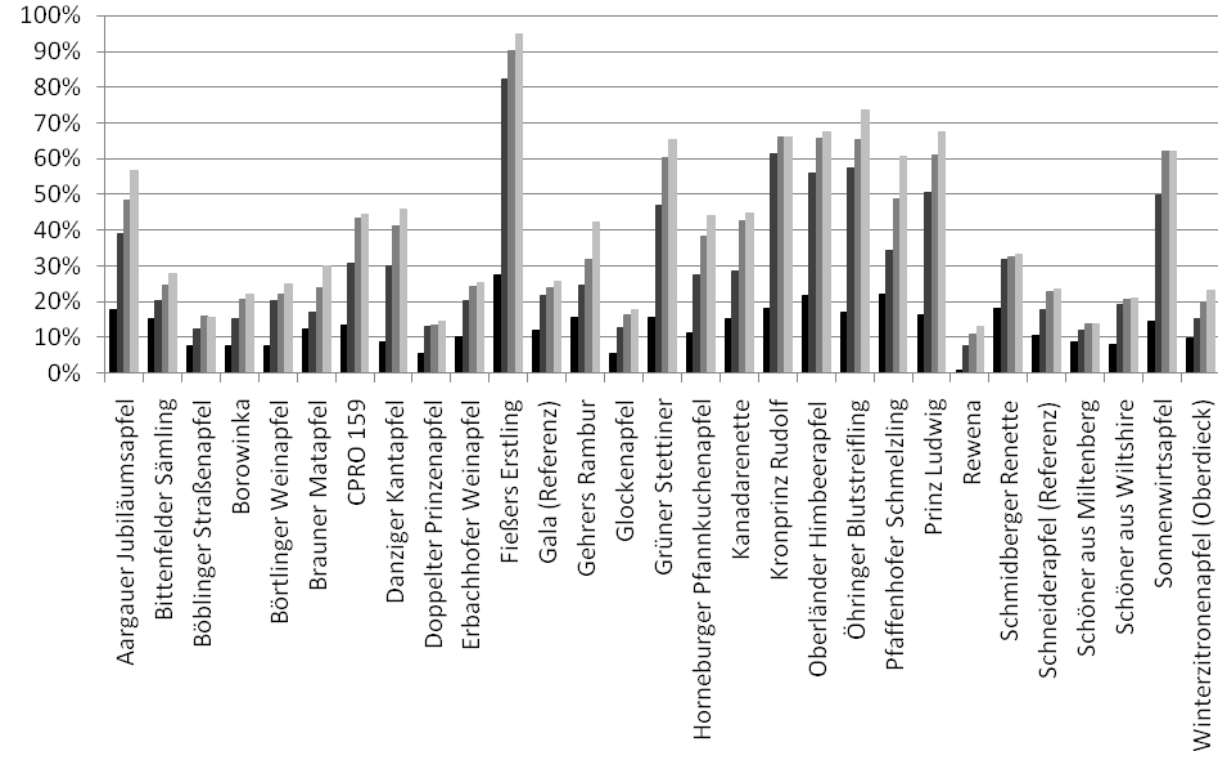


Figure 2**:** averaged relative length of lesion (%) of apple cultivars (2011)
x-axis: standard cultivars; ■ 1 week post inoculation (wpi), ■ 2 wpi, ■ 3 wpi, □ 4 wpi

Pathogenesis. As expected there was an observable difference between untreated and treated trees. Trees treated with Prohexadion-Ca showed reduced shoot growth and thickened shoots in comparison to the untreated trees.

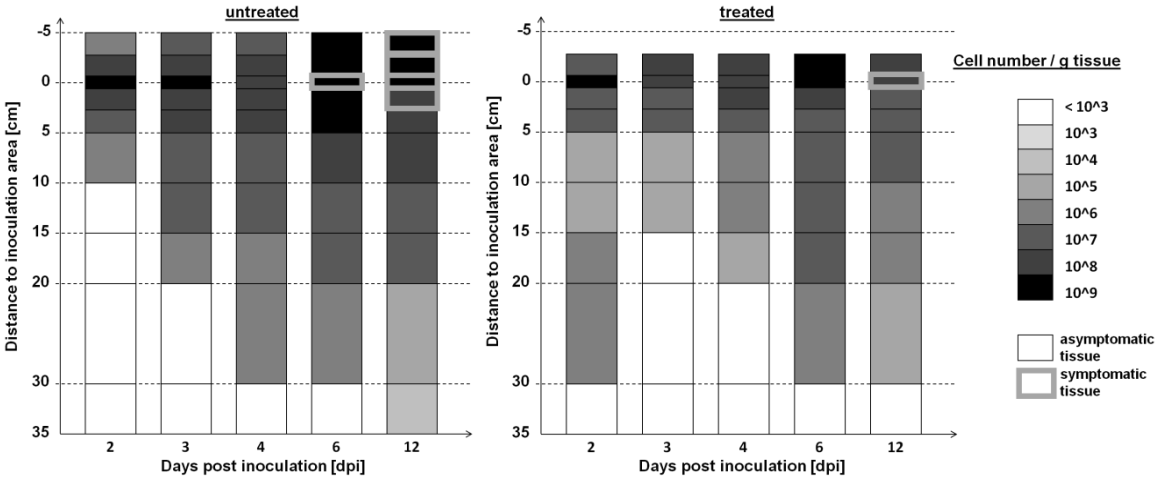


Figure 3: Spread of *E. amylovora* in young apple trees of the cultivar Jonagold after artificial shoot inoculation. 25 trees untreated/treated, 10 trees control; each time point shows the average of 5 shoots
Left: untreated apple trees, Right: Regalis® treated apple trees

With respect to symptoms, there is a remarkable influence of the treatment. In untreated trees symptoms appeared after 6 days post inoculation whereas symptoms only appeared after 12 days post inoculation in treated trees. However, cell numbers were very similar in both setups.

Live/Dead-Distinction. During our efforts to distinguish between live and dead cells we were able to inhibit the amplification of dead cell DNA using PMA in Real Time PCR.

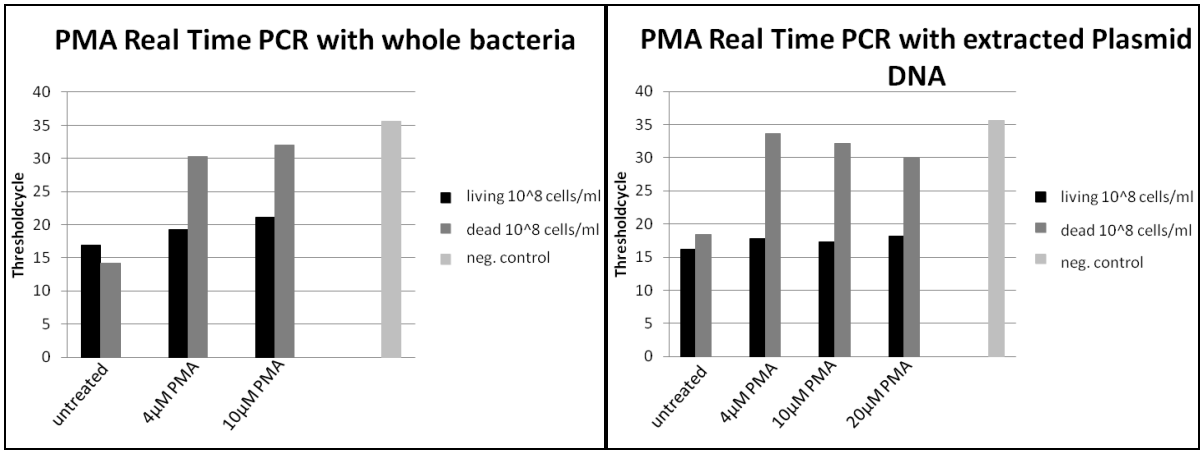


Figure 4: PMA Real Time PCR with whole bacteria and extracted Plasmid DNA
Left: living and dead whole bacteria tested untreated and treated with 4 µM and 10 µM PMA, Right: extracted Plasmid DNA of living and dead bacteria, which were untreated or treated with 4 µM, 10 µM and 20 µM PMA prior to DNA extraction

As shown in Figure 4 there is only a small difference between thresholdcycles (cts) of untreated samples. However in the treated samples there is a remarkable shift of about 12 ct values for whole bacteria and 16 to 12 ct values for extracted DNA with increasing PMA concentration. This means that the amplification of DNA arising from dead cells was inhibited by the PMA treatment. There is no difference between the 4 µM and the 10 µM PMA treatment for reactions with whole bacteria. For purified DNA best effects were achieved using a PMA concentration of 4 µM.

During the development of the FACS-analyses method for Live/Dead-Distinction we succeeded to calibrate the Flow-cytometer (FACSCalibur, BD Biosciences) for Live/Dead-staining. The calibration was done using pure cultures stained with Propidium Iodide (Boulos *et al.* 1999) and a specific primary and a Fluorescein Isothio-cyanate-labeled secondary antibody. After the calibration was done field samples were tested (Figure 5). These included a visually symptomatic Sonnenwirtsapfel (Erwinia.008) and a visually asymptomatic one (Erwinia.008.2).

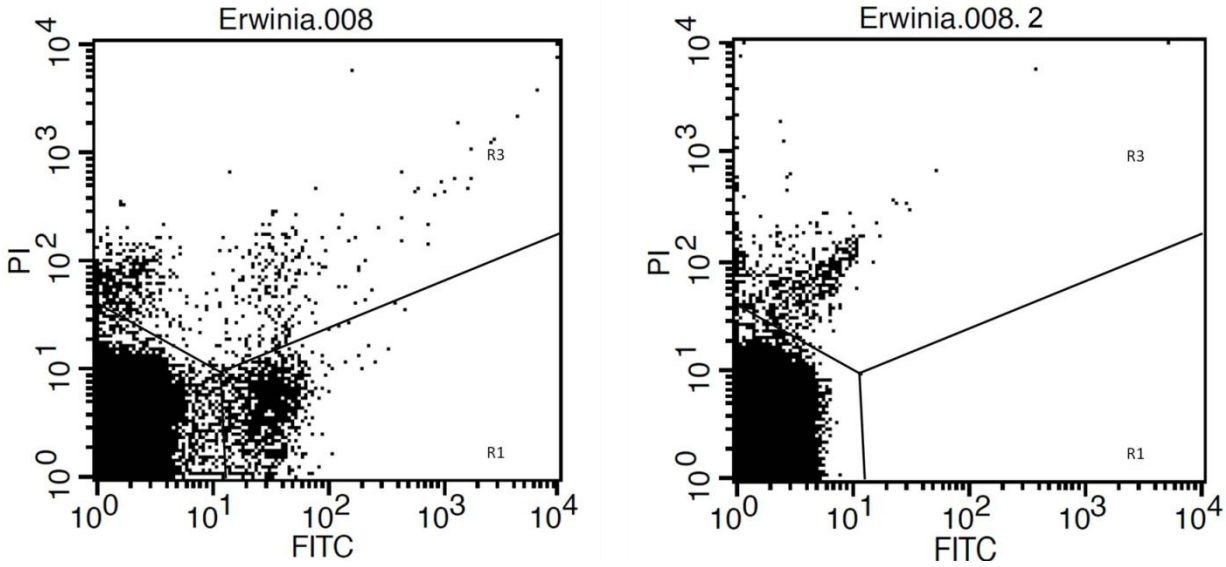


Figure 5: Environmental samples measured by flow cytometry.
Left: symptomatic apple tree, the number of detected bacteria (5510) is composed of 89 % foreign bacteria, 9 % live (R1) and 2 % dead (R3) *E. amylovora*.
Right: asymptomatic apple tree, *E. amylovora* not detectable.

Comparing both diagrams in Figure 5 one notices that the most hits/bacteria are positioned in the lower left corner. This is caused by the fact that these bacteria are unstained and therefore represent “other living bacteria” (not *E. amylovora)*. Green stained and therefore living *E. amylovora* would be positioned in the lower right corner (R1). In the symptomatic sample (Erwinia.008) 9 % bacteria stained green, no living *E. amylovora* cells were found in asymptomatic sample (Erwinia.008.2). Dead and therefore red stained bacteria would be positioned in the upper left corner and green-red stained (dead *E.amylovora*) in the upper right corner (R3). Thus, in the symptomatic sample there were all together 89 % foreign bacteria detected and 2 % dead *E. amylovora*. Contrary to this there were no *E. amylovora* detected at all in the asymptomatic sample.

These results show that we are able to detect *E. amylovora* by FACS-analyses and additionally to distinguish between different bacteria as well as living and dead cells.

## Discussion

The cultivars tested less and least susceptible during our cultivar testing will be analysed by Real Time PCR to get an idea of the pathogen spread inside shoots. Probably the trees appear only less susceptible while being highly infested. In parallel, an analysis of physiological and metabolic differences between less and highly susceptible apple cultivars will be forced initiated to determine the reason for less susceptibility.

Comparing the results obtained in 2010 and 2011, several re-tested cultivars showed different behaviour. Some tested less susceptible in 2010 but intermediate or highly susceptible when re-tested in 2011 and vice versa.

In order to get a stable reproducible test-setup, the reason for these differences has to be identified. Probably the differences observed, are due to the upgrade of the greenhouse lightning.

In the pathogenesis experiments we could show, that there is no crucial difference between cell numbers in Regalis® treated trees compared to untreated. We will proceed with analysing trees from cultivar testing. Physiological and metabolic analyses of cultivars treated with Regalis® in comparison to untreated ones will also be performed.

As shown in the results of Live/Dead-Distinction we are able to nearly suppress the complete signal of dead cell DNA. This method in combination with Flow-cytometry gives us a tool at hand to analyse latent infections on their pathogenic potential because Flow-cytometry was found to provide highly reproducible, statistically safeguard data. With respect to this fact and a possible automation of the Flow-cytometry protocol this method can also be used for high throughput analyses of environmental samples.

**Acknowledgements**

I want to thank Dr. Ulrich Mayr and Hans Thomas Bosch.

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# Field trials on adulticide bait (spintor-fly®) to control the cherry fruit fly in Emilia-Romagna (North Italy) in 2010 and 2011

MG. Tommasini[1] and S. Caruso [2]

**Abstract**

*In Emilia-Romagna Region (North Italy) trials to identify new strategies for the control of cherry fruit fly have been carried out. The aim was to find an alternative to the active substances commonly used until last years (e.g., dimethoate, phosmet) in Integrated Pest Management but at risk to be excluded from the market due to E.U. PPP revision, and to identify an effective way to control cherry fruit fly in organic farming. For this reasons field trials have been performed in 2010 and 2011 to evaluate the efficacy of a Spinosad-based bait adulticide (Spintor-Fly®) against* Rhagoletis cerasi *L. in cherry orchards. The results obtained were positive in both years. Spintor-Fly ® proved to be very effective to control the pest. An extension of its use in open field is awaited. However, its use on a large scale could be limited by its low persistence (it has to be applied at least weekly), poor rainfastness and phytotoxicity on the treated areas although widely tolerated. For these reasons new formulations of this experimental product developed to mitigate the negative aspects as mentioned above are awaited to be evaluated.*

**Keywords:** *Rhagoletis cerasi*, cherry fruit fly, control, Spintor-Fly.

## Introduction

In the last years in Italy the control of cherry fruit fly (*Rhagoletis cerasi* L.) has become more complex due to the low availability of pesticides.

As it is known, following the revision of European products (reduction RMA), dimethoate is no longer usable and phosmet showed problems of phytotoxicity on different cultivars in different contexts (Caruso & Boselli, 2011). Consequently, in Integrated Pest Management (IPM), there is a shift towards the use of neonicotinoids (acetamiprid, thiacloprid, and thiamethoxam). These products while showing a decent effect from the first experiences in Italy (Caruso & Boselli, 2011), present some problems. For example not all neonicotinoids are authorized for the control of cherry fruit fly. They are already used against the black aphid and their further repeated use may increase the risk of resistance development. For these reasons a broader range of formulations to be included in ordinary control strategies is needed. Furthermore, apart anti-insect nets (Grassi *et al.* 2010) which have not had wide application for the rather high costs and limits of practicality in their use, no means of control against *R. cerasi* in organic production, are available.

Among new products being evaluated, the fruit fly bait Spintor-Fly® is promising. Besides the bait ingredients it contains spinosad as adulticide. (It is authorized for cherry fruit fly control in USA, Canada and for olive fruit fly (*Bactrocera (Dacus) oleae* Gmelin) and Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) control in Europe. In the United States (Yee and Alston, 2006; Alston, 2009) and Canada (Edwards, 2004; Thistlewood, 2010) the system has been used for several years with good results. Its potential for cherry fruit fly control has been ascertained in Germany by Köppler *et al*. (2008).

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In Italy, it showed promising results in trials carried out in 2010 in cherry orchards in the Emilia-Romagna (Caruso & Tommasini, 2011) and Sardinia (Marras *et al*., 2011) as well as against the olive fruit fly and the Mediterranean fruit fly. In this paper a summary of results from trials carried out in Emilia-Romagna in 2010-2011 are reported.

**Material and Methods**

The trial was carried out in 2010 in three farms, one located at Linaro of Cesena (Province of Forlì-Cesena, FC) and the other two in Vignola (Province of Modena, MO) both placed in North Italy. In 2011 the trial was undertaken in six farms, three in Vignola and three in Forlì-Cesena. In both years the selected farms were characterized by high infestation of cherry fruit fly in previous years (more than 50% damage recorded). In general the farms were located in the foothills (150-300 m a.s.l.), and in 2011 the farm six was located in a hill country (700 m a.s.l.). In each farm the experimental design provided a comparison between a plot treated with Spintor-Fly® and an untreated plot as control (Tab. 1 and 3). The untreated plots were located at a distance within 500-600 meters from treated plots. This trial scheme was necessary due to the mode of action of Spintor-Fly® (bait adulticide) which results in higher efficacy when applied on surface areas of a quite large size (greater than 1000 square meters). This new formulationhighly accepted as food by the cherry fruit flies, contains a small amount of the active ingredient Spinosad (0.24 g/l). Adults feeding on the bait ingest the active substance, too. Adults die within a few hours after feeding making it impossible for them to mate. To be effective Spintor-fly® must be homogeneously distributed in the orchard, spraying a small portion (approx. 50 $cm^2$) of the upper part of the vegetation of each plant and leaving coarse droplets of the product on leaves. Sprays were performed with a manual sprayer applying 5 liters of bait solution per hectare (consisting of 1 liter of product and 4 liters of water).

The sprays were performed on a weekly basis except for reaplication, at shorter intervals, required in case of rain. The product is in fact easily washed off. In order to detect the onset of flight activity of cherry fruit fly in due time and to start immediately the applications of Spintor-Fly®, 2 sticky yellow traps (Rebell type) were placed in each field on the $8^{th}$ of May 2010 and on $26^{th}$ of April 2011. The traps should also serve as an indicator of the efficacy of sprays (an increase of the catch would correspond to a reduction of the attractiveness of the bait). Traps were checked with short intervals (1-2 days) before the first detection of adults, and later, weekly. Within 24 hours from the beginning of pest flight sprays with Spintor-Fly® started (Tab. 1 and 3). To assess the efficacy of Spintor-Fly® a survey was carried out in each farm at harvest by sampling 100 fruits for cultivar / treatment. When less than 5 cultivars were present in the plot more fruits were sampled for cultivar reaching always 500 fruits / treatment. The percentage of damaged fruits by *R. cerasi* of the selected cultivars was assessed by opening each cherry fruit and checking visually the presence of cherry fruit fly larvae or the damage caused by them into the fruits. The cultivars to check the infestation included those at medium and late harvest which are the most susceptible to cherry fruit fly (Tab. 2 and 4).

Table 1 – Trial set-up and treatments carried out with Spintor-Fly® in 2010.

| Farm N. | Place (Province) | Treatment | Surface (ha) | Sprays (No.) | Date of sprays |
|---|---|---|---|---|---|
| 1 | Linaro (FC) | Spintor-Fly<br>Control | 0.25<br>0.5 | 5<br>- | May (24,30);<br>June (03,09,16) |
| 2 | Vignola (MO) | Spintor-Fly<br>Control | 1<br>0.1 | 6<br>- | May (17,20,25);<br>June (04,09) |
| 3 | Vignola (MO) | Spintor-Fly<br>Control | 0.3<br>0.8 | 6<br>- | May (17,20,25);<br>Jun (04,11) |

Table. 2 - Cultivars on which trials were carried out in 2010.

| Farm | Cultivar |
|---|---|
| 1 – Linaro (FC) | Ferrovia, Sweet Heart, Cornina, Maraschina |
| 2 – Vignola (Mo) | Mora di Vignola, Nero I, Durone dell'Anella, Ferrovia, Durone della Marca |
| 3 – Vignola (Mo) | Mora di Vignola, Nero I, Ferrovia, Durone della Marca |

Table 3 – Trials set up and treatments carried out with Spintor-Fly® in 2011.

| Farm no. | Place | Treatment | Surface (ha) | Sprays (no.) | Date of sprays |
|---|---|---|---|---|---|
| 1 | Vignola (Mo) | Spintorfly<br>Control nt | 1,0<br>0,1 | 6<br>- | **May**<br>(6,12,17,21,28)<br>**Jun**<br>(13) |
| 2 | Vignola (MO) | Spintor-Fly<br>Control | 1,0<br>0.1 | 6<br>- | **May**<br>(8, 16,19,25)<br>**Jun**<br>(1,13) |
| 3 | Vignola (MO) | Spintor-Fly<br>Control | 1,0<br>0.8 | 6<br>- | **May**<br>(7, 13,17, 21, 28)<br>**Jun**<br>(13) |
| 4 | S. Romano (FC) | Spintor-Fly<br>Control | 0,8<br>0,1 | 7<br>- | **May**<br>(8, 16,22, 26)<br>**Jun**<br>(05, 14) |
| 5 | Dovadola (FC) | Spintor-Fly<br>Control | 1,0<br>0,1 | 5<br>- | **May**<br>(8,15,21,27)<br>**Jun**<br>(03) |
| 6 | Cusercoli (FC) | Spintor-Fly<br>Control | 1,0<br>0,1 | 8<br>- | **May**<br>(10,17,21, 28)<br>**Jun**<br>(02, 09,15,22) |

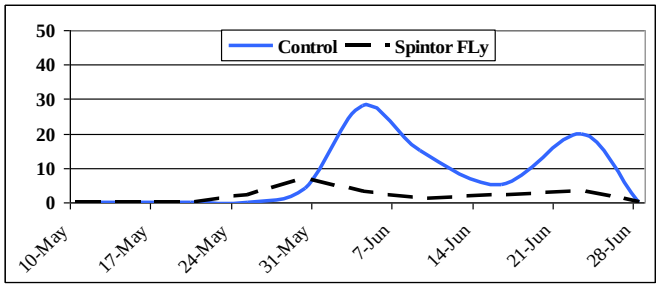
Table 4: Cultivars on which trials have been carried out in 2011.

| **Farm no.** | **Cultivar** |
|---|---|
| 1 – Vignola | Nero I, Durone dell'Anella (DA), Ferrovia, Nero II, Ciliegione |
| 2 – Vignola | Durone Anella, Nero I, Nero II |
| 3 – Vignola | DA, Nero I, Giorgia, Nero II, Ferrovia, Lapins, Durone del Cortile, Sweet heart |
| 4 –S. Romano | Sunburst, Ferrovia, Nero III, Morandina, Cornina |
| 5 – Dovadola | Mora di Vignola, Sunburst, New Star, Lapins |
| 6 – Cusercoli | Mora di Vignola, Cornina |

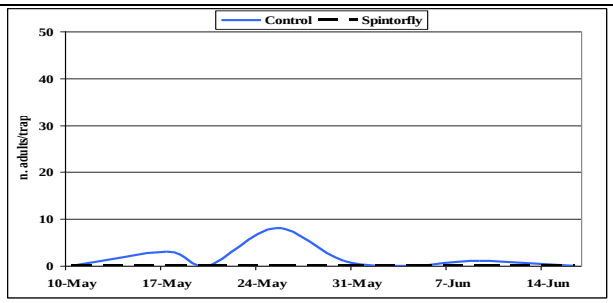
## Results and Discussion

The average adult catches recorded on traps during 2010 in the treated plots with Spintor-Fly® were basically always very low (Fig. 1). This result provided a preliminary indication of the positive activity of the bait spray.

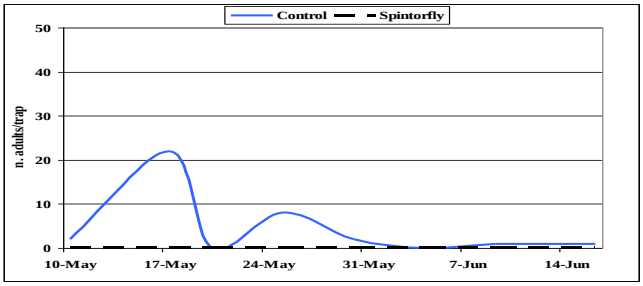
In table 5 the results of the field trial in 2010 are shown. The infestation rate was clearly reduced in all plots treated with Spintor-Fly® compared to the untreated control (not always of the same cultivar for lack of availability). In the treated plots 0,0 to 5.5% of the fruits were infested, whereas in the untreated plot infestation was much higher, ranging from 33% to 90%.




Farm no.1 – Linaro (FC)




Farm no. 2 – Vignola (Mo)




Farm no. 3 – Vignola (MO)

Figure 1 - *R. cerasi flight* 2010

Table 5 - Results 2010: Effect of bait treatments on larval infestation of cherries by *R. cerasi* (average of the different cultivars).

| Farm No. | Thesis | Check at harvest (dd/mm) | Sample size (N. cherries) | Damaged fruits (%) |
|---|---|---|---|---|
| 1 – Linaro (FC) | Spintor Fly® | 9/6; 23/6 | 500 | 1,8 |
| | Control | | 500 | 53,8 |
| 2 – Vignola (Mo) | Spintor Fly® | 8/6; 15/6 | 500 | 2,2 |
| | Control | | 500 | 33,0 |
| 3 - Vignola (Mo) | Spintor Fly® | 8/6 | 500 | 0,0 |
| | Control | | 500 | 90,2 |

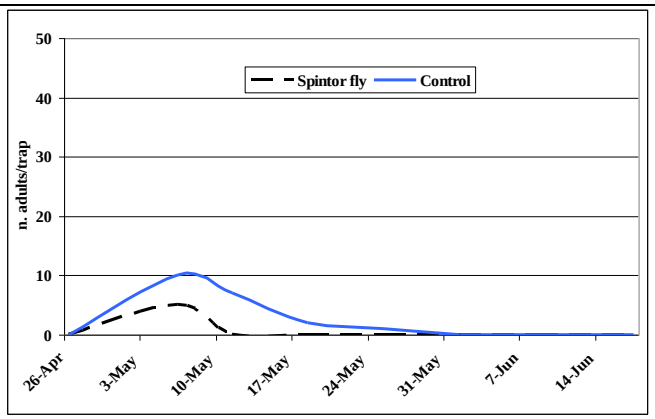
The average adult catches on traps during the trial in 2011 (Fig. 2) in the treated plots with Spintor-Fly® in most of the farms were lower than the catches recorded in the untreated plot, also in the farm 2 (Fig 2) where the pest pressure was very high, confirming the results of 2010. In table 6 the results on the percentage of fruits damaged by cherry fruit fly at harvest in 2011 are shown. In the treated plots the damage by *R. cerasi* on fruits fluctuated between 0.0 to 3.2% compared to an infestation level in the untreated control always higher than 15% and in some cases close to 40%.

Although a lower presence of *R. cerasi* was observed in all farms in 2011 compared to 2010, the results confirm those of 2010. A clear reduction of cherry fruit fly infestation was achieved in the plots treated with Spintor-Fly® compared to the untreated control plots.

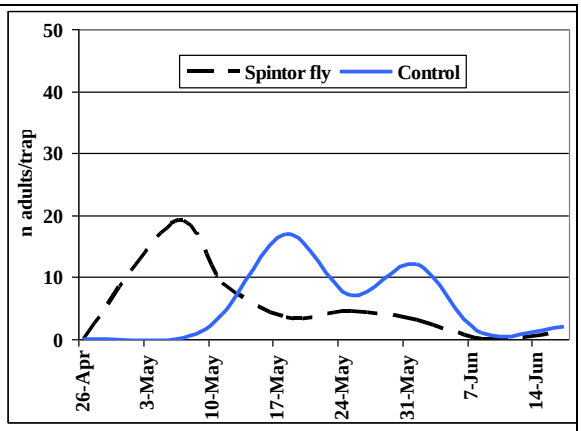
On the other hand symptoms of phytotoxicity in the areas of the treated vegetation were observed both in 2010 and 2011. These symptoms are considered tolerable by the farmers and they are not significantly damaging the cherry trees.

Table 6 – Results 2011: Effect of bait spray treatments on larval infestation of cherry by *R. cerasi* (average of the different cultivars).

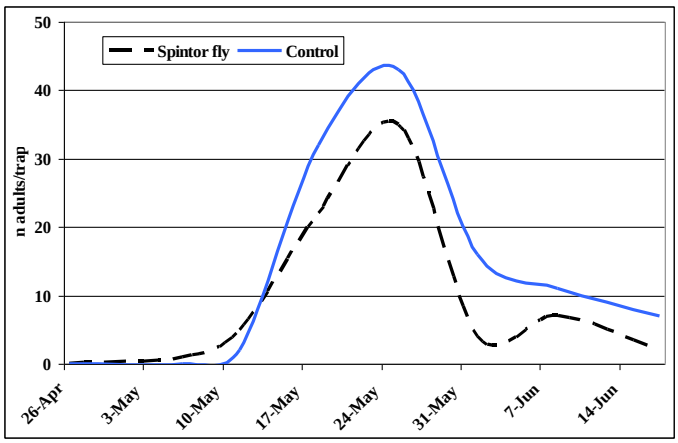
| Farm No. | Thesis | Check at harvest (dd/mm) | Sample size (N. cherries) | Damaged fruits (%) |
|---|---|---|---|---|
| 1 – Vignola (Mo) | Spintor Fly® | 26/5; 7/6; 12/6 | 500 | 0,1 |
| | Control | | 500 | 15,0 |
| 2 – Vignola (Mo) | Spintor Fly® | 1/6; 8/6 | 500 | 0,3 |
| | Control | | 500 | 39,0 |
| 3 - Vignola (Mo) | Spintor Fly® | 31/5; 10/6; 17/6 | 500 | 0,7 |
| | Control | | 500 | 17,2 |
| 4 – S. Romano (FC) | Spintor Fly® | 28/5; 10/6; 24/6 | 500 | 3,2 |
| | Control | | 500 | 20,4 |
| 5 – Dovadola (FC) | Spintor Fly® | 28/5; 3/6 | 500 | 0,0 |
| | Control | | 500 | 28,0 |
| 6 – Cusercoli (FC) | Spintor Fly® | 9/6; 22/6 | 500 | 2,7 |
| | Control | | 500 | 34,3 |



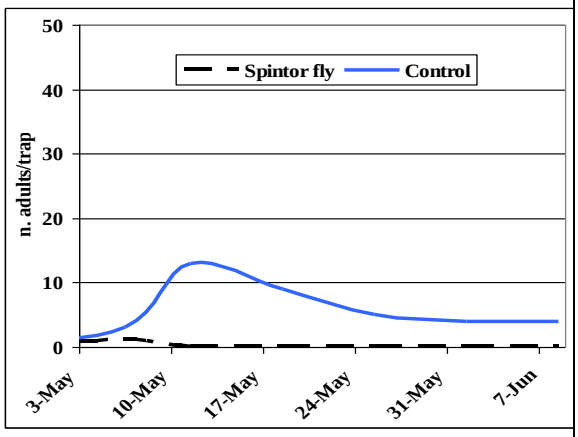

Farm no. 1.Vignola (Mo):



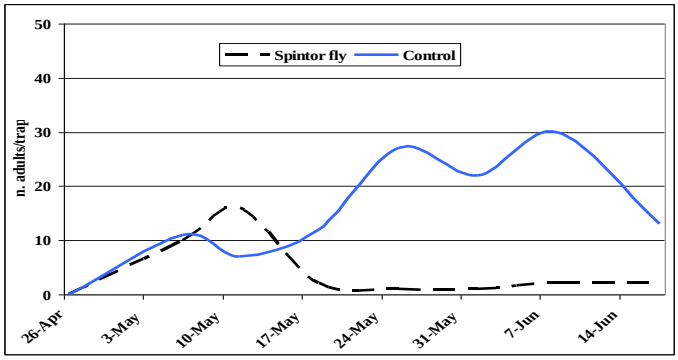

Farm no. 4 – S. Romano (FC)



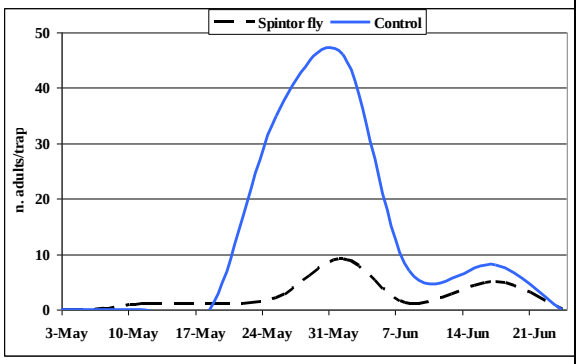

Farm no. 2.Vignola (Mo):




Farm no. 5 – Dovadola (FC):




Farm no. 3 – Vignola (Mo):




Farm no. 6 – Cusercoli (FC):

Figure 2 - *R. cerasi* flight 2011

## Conclusion

The trials carried out in 2010 and 2011 gave positive results highlighting the efficacy of Spintor-Fly® to control cherry fruit fly on cherry. Nevertheless there are a few drawbacks which can prevent its extensive use in open field. In particular the short persistence, which requires repeated applications (weekly) during the period of adult flight and fruits ripening. This is aggravated by its poor rainfastness even with few millimiters of rain. Phytotoxicity on treated areas seems to be negligible and largely tolerable.

It would be useful to evaluate new experimental formulations of this product which limit the negative aspects mentioned above to provide an improvement in their use in both organic and IPM production.

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# Promising field and semi field results forcherry fruit fly control using Neem

E. Böckmann[1], E. Hummel[2], H. Vogt[1]

## Abstract

*The European cherry fruit fly,* Rhagoletis cerasi *(Diptera: Tephritidae), is the major pest species in cherry orchards throughout Europe. Adequate control measures are lacking to date, especially in organic fruit growing. In our prior studies the proof has been given for neem (a.i. azadirachtins) to interfere with ovary development and impede successful reproduction in* R. cerasi*. In fruit fly control under field conditions, however, it usually failed. To our belief the latter is mainly founded in the unsuitable layout of these field trials. Three main conditions have to be fulfilled to apply neem successfully under field conditions: First, females have to be detained from immigrating from the control to the treated area. Second, the application of neem products has to be started together with the first detections of flies on Yellow Traps. Third, the active ingredient has to be present in the field constantly. Taking these precautions into account, we here present semi field and field trials we carried out with neem in bait spray and semi field trials with cover spray. The tested baits and the cover spray contained NeemAzal-T which is an oil-free formulation. Beforehand, we compared acceptances of bait containing either the latter formulation or, the already licenced oily formulation NeemAzal-T/S under laboratory conditions.*

*In laboratory studies* R. cerasi *preferred the oil-free formulation in bait. In semi field and field trials infestation rates were significantly reduced by all bait treatments as well as the cover spray. Infestation in the treated trees was below the economic threshold. The outcome of the study reveals the high efficacy of neem under exclusion of immigration of mature females and is essential in decision making regarding preconditions of orchards necessary for a successful application of neem products for cherry fruit fly control.*

## Keywords

GF 120, *Rhagoletis indifferens*, *Rhagoletis cingulata*, Azadirachtin, field trial design

## Introduction

Cherry growers in Europe have one main problem in order to produce marketable fruits: The infestation with larvae of the cherry fruit fly, *R. cerasi*. Its control still relies on the use of the systemic insecticide Dimethoate, but its use becomes increasingly restricted. A withdrawal from the market due to the EU wide reduction program for broad spectrum insecticides can be suspected. Furthermore Dimethoate is not an option for organically managed orchards. Against this background there is a high need for new environment-friendly control strategies. In this study we focused on the use of bait sprays. Bait has to be ingested by the target insect and therefore contains phagostimulants like proteins and sugar (Yee & Chapman 2005, Mangan *et al*. 2006). Together with the spatially restricted application, bait sprays reduce application rates of insecticides and are more selective as compared to cover sprays.

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A good example for the successful use of bait sprays against tephritids is the use of the spinosad containing GF-120™ Naturalyte Fruit Fly Bait (Dow Agrosciences, Indianapolis, Indiana, USA) (Burns *et al*. 2001, Yee 2007, Thistlewood *et al*. 2010). In our study, two different neem formulations were incorporated into bait. Whilst we have proven in prior studies the principal effectiveness of both formulations, namely NeemAzal-T and NeemAzal-T/S, in laboratory and semi-field they usually failed in our field experiments (Vogt 2009, Kleeberg & Vogt, 2010). We suspect that immigration of mature females is the main reason for the gap between efficacy in the lab or semi field and under field conditions.

In the present study we first tested the influence of the insecticide formulation on bait acceptance. Afterwards we carried out semi field trials to prove the principal efficacy of neem under environmental influences like precipitation and UV-light. Furthermore we developed and tested a field trial setup suitable to evaluate the efficacy of neem bait under exclusion of immigration of mature females.

## Material and Methods

### Study insects and baits

The insects derived from collections of the previous year, where pupae were first stored under room conditions (20 to 25°C) and subsequently transferred to the cold room (3 to 5°C) for at least 5 months. Post-diapause development was allowed in a climate chamber (25 ± 0.5°C / 18 ± 0.5°C, RH 65 ± 5 %, photo period light : dark16:8 h, 4 to 6 klux) (Köppler *et al.* 2009). .

The baits used in experiments contained the oily insecticide formulation NeemAzal-T/S® or the oil free formulation NeemAzal-T® (products of the Trifolio-M GmbH, Lahnau, Germany. Note that NeemAzal-T is not yet marketed). Furthermore baits contained sugar and yeast in the relation 400:1, the bioemulgator Ledophil® (Handelsvertretung Ledophil, Traude Klose, 99439 Büttelsted) to enhance rainfastness, and water. The abbreviations used in this paper are summarized in Table 1. Note that the baits NATS 1% and NAT 0.2% are comparable concerning their insecticide content.

Table 1: Component abbreviations used in the paper.

| Component | Abbreviation |
|---|---|
| 1% NeemAzal-T/S in bait (resultant ai = 0.0001%) | NATS 1% |
| 0.2% NeemAzal-T in bait (resultant ai = 0.0001%) | NAT 0.2% |
| 1% NeemAzal-T in bait (resultant ai = 0.0005%) | NAT 1% |
| 0.2% NeemAzal-T + 98% water (resultant ai = 0.0001%) | NATcover |

### Experimental setups

### Laboratory

No choice and choice experiments were carried out in the climate chamber (conditions as described above) in order to investigate the acceptance of different baits by the study insects. For both treatments we used white plastic boxes (6 x 11 x 11 cm) with transparent lid and a water reservoir in the center. In case of no choice tests, one hole of approximately 1.5 x 2.5 cm was cut in the center of the lid and three drops (each 10 µl) of respective bait were presented on a rectangular cover slip placed on the hole. Droplets were dried for 24h in the climate chamber and afterwards 5 female flies, starved for about 17h were introduced into the box. Flies were up to 7 days of age. The cover slip with the dried out bait was weighed before and after the experiment and the difference in weight

was calculated. For all baits, a control of the same setup without flies was run and weight differences in the treatment were corrected for the mean weight difference in the control. If only one bait was offered at a time (no choice design), experiments were carried out from approximately 10 am of day 1 to 10 am at day 2, i.e. 24h. In choice tests the same boxes were used, now with two holes cut centrically at opposite sides of the lid, where two different baits were presented at a time. This experiment was carried out with pre-fed flies for three days from 10 am at day 1 to 10 am at day 4, i.e. 62h. As in treatment 1, weight differences were calculated and corrected for mean values of a control, carried out simultaneously. In each experiment at least 4 replicates of the control were carried out per bait. In the choice experiment comparing NAT 0.2% vs. NAT 1% one replicate was excluded because flies escaped.

**Semi field and field trials**

Both were carried out in Dossenheim in the experimental orchards of the institute (Baden-Württemberg, north of Heidelberg) (latitude: 49°27.02'; longitude: 8°40.48').

**Semi field**

Single caged trees (cages approximately 9 $m^3$; cherry varieties 'Hedelfinger' and 'Kordia') were treated with 30 ml of a respective bait spray once a week for four weeks. Bait was sprayed on three dispersed spots of the treetop. Within the first 3.5 weeks, young flies (<4 days) were introduced into the cages twice a week, totaling 33 males and 30 females per cage. Starting with the first introduction of flies one application of bait or cover spray (cf. Table 1) was carried out once a week for 5 weeks with 40 ml of bait or 1.5 l of cover spray applied each time. Bait was applied using an air pressure driven color sprayer, for cover spray a knapsack sprayer was used. Two controls were carried out, one matching the conditions in bait sprays with weekly blank bait applications (blank bait control), one without treatment (untreated control). All fruits were collected at the 15.06.2011 and a random sample of 400 fruits per tree was examined.

**Field trial**

The sweet cherry orchard used for the trial was, planted in 2007 with the cherry variety 'Regina'. The orchard is 0.52 ha in size and has 9 rows with 33 trees with a planting distance of 5 x 3.5 m. The control and the treated area consisted each in 165 cherry trees. Between both areas, we installed a chemical barrier consistent in three cherry tree rows, that were treated weekly with 0.1% Pirimor and a not yet marketed pyrethroid bait. In the treated area we applied 40 ml NAT1% per tree using a knapsack sprayer. The application dates were 10.05, 17.05, 25.05, 03.06, 09.06.2011. Because Yellow Trap captures indicated a very low cherry fruit fly population that year, young flies (<4 days) were released centrally in both areas. In total about 160 males and 180 females were released in the control and the treated area, respectively. Infestation rates were calculated by defining 10 blocks à 4 trees centrally in both areas. From every block a sample of 200 fruits was examined, with 50 fruits randomly collected per tree. Samples were taken at the 24.06.2011.

**Statistical analyses**

Because the assumptions for parametric tests were not always met we used non-parametric tests in all experiments. In all statistical analyses the significance level was $\alpha=0.05$.

In laboratory experiments the Kruskal-Wallis test was applied. In case more than two baits were compared, approximate p-levels were estimated using the Kruskal-Wallis test and subsequently the Nemenyi-Damico-Wolfe-Dunn test (Hollander & Wolfe 1999) was carried out to ascertain multiple significance levels.

In semi field trials we tested if infestation rates of NATcover < untreated control and if NAT 1% and/or NAT 0.2% < blank bait control and if untreated control < blank bait control. These comparisons were chosen, because it was expected that all treatments reduce infestation rate and that the application of blank bait may increase infestation rates. The analyses were carried out using the Wilcoxon Exact Test with subsequent Holm correction for the familywise error rate.

In the field trial the Kruskal-Wallis-Test was carried out to compare infestation rates.

**Results**

Under laboratory conditions, significantly more NAT 0.2% than NAT 1% was fed in no choice and choice experiments (Table 2). No significant difference was found between NAT 0.2% and NATS 1% when comparing feeding amounts under no choice conditions, but when having a choice, females fed significantly higher amounts of NAT 0.2% (Table 2). In semi field trials, fruit infestation was significantly reduced in all treatments against the respective control, but there was no significant difference between both controls (Figure 1). Under field conditions application of NAT 1% significantly reduced infestation from about 9% in the control to 0.5% in the treated area.

Table 2: Comparison of the amount of bait fed by females (n=5) in one day supplied with only one bait (No choice design) and in three days supplied with two baits simultaneously (Choice design), respectively. Mean values, given with SD, show differences of bait weight after the experiment as compared to before, corrected for the weight loss of the respective bait in a control (n≥4) without insects. In all cases the Kruskal-Wallis-Test was carried out. Significant p-values are given in bold.

| Experimental design | Bait | Mean ± SD (mg) | N | P |
|---|---|---|---|---|
| **No choice** | NAT 0.2% | 2.6 ± 0.4 | 8 | **< 0.001** |
| | NAT 1% | 1.1 ± 0.3 | 8 | |
| **Choice** | NAT 0.2% | 4.8 ± 0.7 | 15 | **< 0.001** |
| | NAT 1% | 0.2 ± 0.4 | 15 | |
| **No choice** | NAT 0.2% | 2.8 ± 0.8 | 9 | 0.389 |
| | NATS 1% | 2.1 ± 0.8 | 9 | |
| **Choice** | NAT 0.2% | 3.5 ± 1.8 | 16 | **0.001** |
| | NATS 1% | 1.3 ± 0.1 | 16 | |

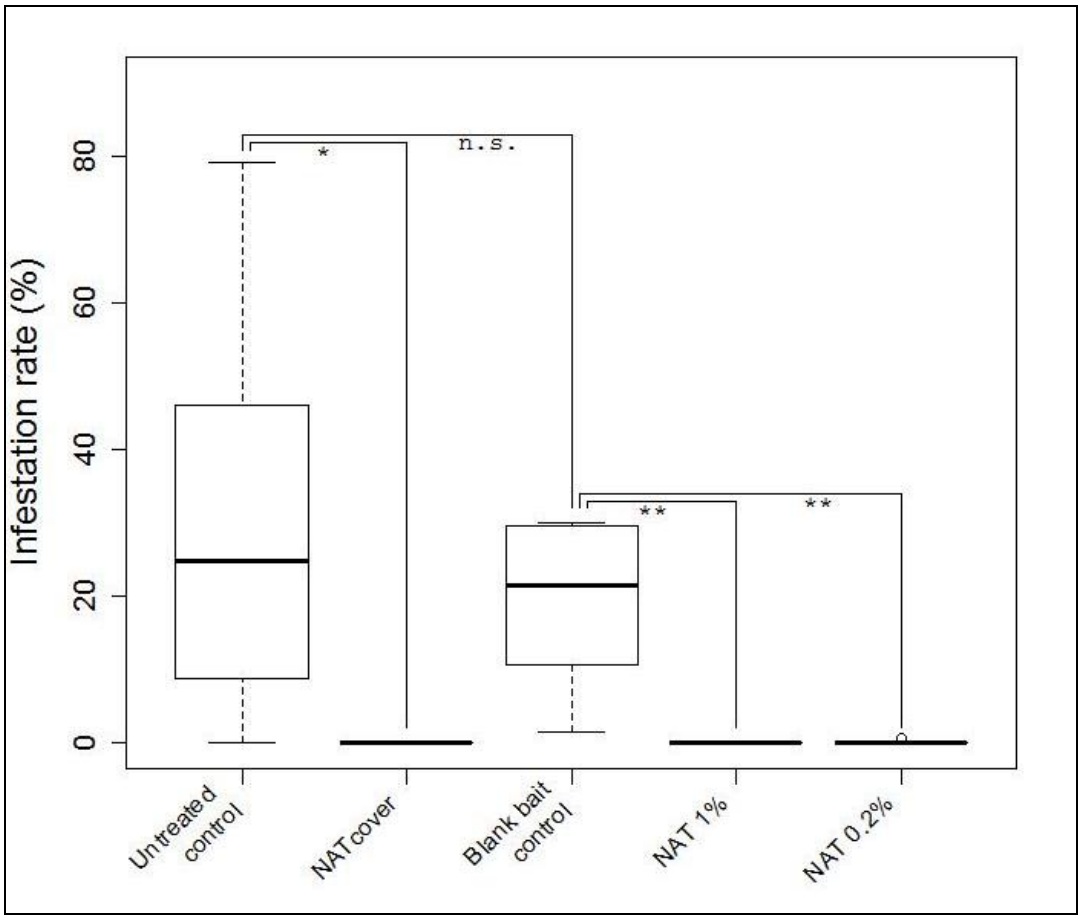


Figure 1: Boxplots of infestation rates in semi field trials with 5 replicates per treatment and control. Connecting lines show which treatments and controls were compared statistically (cf. methods) and if the effect was significant (** = $p < 0.01$, * = $p < 0.1$, n.s. = not significant).

## Discussion

Bait mixtures containing neem in different formulations and concentrations were tested for their efficacy in cherry fruit fly control in laboratory, semi-field and field assays. Based on preliminary work (Vogt 2009, Kleeberg & Vogt, 2010) and especially due to our results presented here from 2011, we can state that neem bait has high potential for control of *R. cerasi.* Oil-free formulations are most promising because they are less repellent. From an efficacy point of view there is no difference in semi field trials if bait or a cover spray is applied. However the cover spray experiment has to be seen as a first trial to estimate its principal efficacy. The application rate with 3 ml insecticide formulation per tree is 7.5 times higher than with bait spray. Before initiating field trials using cover spray it remains to be investigated if reduced rates of such a spray remain effective. An additional finding of the study is that *R. cerasi* is able to survive and to cause infestations on caged trees with or without blank bait application. In fact we even found the highest infestation rate at an untreated tree with about 80% infested cherries. Hence flies encounter all resources for surviving and reproduction on cherry trees, presumably for the most part by grazing on leaves (Yee 2008).

For successful control, females should be provided with bait consistently after emergence (i.e. treatments should start with the first sight of flies on Yellow Traps), because neem affects ripening of ovaries and consequently egg production as well as fertility of eggs. An alternative tactic may be the pretreatment with an effective contact insecticide such as SpruzitNeu® (Neudorff GmbH KG, Emmerthal, Germany) within an adequate time span

after first sight of flies, in order to reduce initial population pressure in the orchard, followed by neem treatments. Up to date, weekly applications of neem are recommended with additional treatments after high precipitation events. Furthermore immigration of females has to be obviated. Our field results show that if the mentioned prerequisites are complied, neem products can effectively control *R. cerasi* in cherry orchards. Generally their use is most promising in isolated orchards or if collectively coordinated treatments of adjacent cherry orchards are carried out.

Due to the similar foraging and as neem seems to have similar impact on other tephritids (De Ilio, 1999) it is well possible that our results can be carried over to other pests such as the olive fruit fly (*Bactrocera olea*) and the Mediterranean fruit fly (*Ceratitis capitata*). Mediterranean areas with low summer precipitations are advantageous for bait application whereas long growing periods of fruits and occurrence of multivoltine pest species will increase seasonal work load and costs. Corresponding field trials would be of high interest in order to assess the possibilities of an elevated use of this environment friendly control method.

## Acknowledgement

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# Controlling cherry fruit fly under organic farming in France: Hopes and despairs

F. Warlop[1], E. Filleron[2]

**Abstract**

*Cherry Fruit Fly is still a very damaging pest in Europe, and control measures so far are not fully convincing. Alternatives to chemicals show intermediate levels of efficacy, and growers can not rely on them yet. Although nets offered a very sufficient protection to trees, they do not appear like a easy-to-adapt solution, in traditional orchards. Efforts must be continued to assess new active matters and new strategies, especially while an important new threat occurs,* Drosophila suzukii.

**Keywords:** organic farming, cherry fruit fly, insect-proof net, *Beauveria bassiana*

## Introduction

As mean temperatures increased since 2003, pests generally, and fruit flies in particular have been more and more problematic, in organic farming as well as in conventional. Early cherry cultivars may be a part of the solution, but can not be the only ones to be planted. Many strategies have been identified and tested, but so far none could give satisfying level of protection with affordable cost. Natural parasitism is occurring, but at a very low level. Summer or spring soil tillage is supposed to exert an effect on pupae survival rate, but it has never been possible to prove in our conditions. Mass trapping shall be limited to parcels above one hectare, or very isolated from other cherry trees, and its efficacy remains rather irregular with respect to its cost.

New strategies had to be experienced, in order to propose affordable means to cherry growers. Therefore, a task group investigated the relevance of nets for cherry orchards from 2008 to 2010, whereas other natural products were also tested in the field (Severac *et al.,* 2010).

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## Results

*1) Field results with plastic nets*

In 2008 and 2009, several orchards have been protected with two kinds of nets, with two different mesh sizes:

Figure 1: Overview of two different mesh sizes: medium 4x5 (left) / small 6x6 (right)

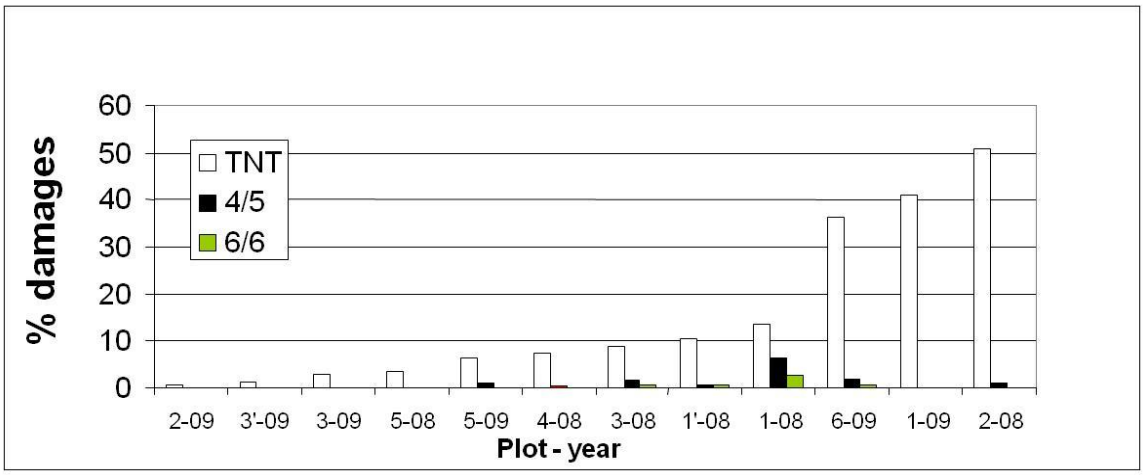


Figure 2: % of damages obtained in 2008 and 2009 on experimental plots, with both kinds of nets (4x5, 6x6) compared with control (TNT) trees.

Table 1: Efficacy of the protection with both kinds of nets

| *Efficacy measured* | *Mesh 4x5* | *Mesh 6x6* |
|---|---|---|
| ***Average*** | **91,3%** | **96,4%** |
| *Minimum* | 52,6% | 81,5% |
| *Maximum* | 100% | 100% |

Figure 3: Outlook of nets on cherry trees in Provence

*Conclusions*

Nets are the most effective solution so far, but cherry orchards need to be redesigned and intensified to be adapted to nets, and in order to limit investment costs. Therefore, growers are not willing to use this technique yet.

*2) Field results with integral plastic mulch*

Plastic mulch has been tested in Switzerland (by FiBL) with interesting results, and trial has been repeated in 2009 and 2010, as the barrier may prevent adults from soil emergence, as well as larvae to penetrate soil to pupate.

As picture enclosed shows, a 3000m² organic and isolated cherry orchard, newly abandoned, has been fully covered with a plastic cover (permeable to water and gases). No control plot could be maintained on the trial, in order to avoid neighbouring flies.

Figure 4: Installation of an integral plastic mulch under cherry trees

The effect of the mulch on emerging flies has been assessed on both cultivars concerned (Burlat & Napoleon).

In 2009, pest pressure was very high since temperatures were very favourable. Accordingly, the early cultivar Burlat could be harvested in end of may, and damages could be prevented. On late Napoleon, damages reached 45% which could be considered as lower than expected : this would lead to the conclusion that plastic cover may play a role in limiting the fly damages, though it was not important enough in our conditions. In 2010, the overall population level was too low to evaluate the effect of the mulch.

Considering the time requested to fix the device, the condition of an isolated orchard, an efficacy very limited and environment-dependant, this technique may not be currently recommended to organic growers.

*3) Field results with natural products*

Natural products remain among the most promising and relevant techniques to control *R. cerasi* (Daniel, 2009). Dimethoate could shortly be withdrawn for ecotoxicity and residues problems, therefore also conventional growers are expecting new field results from alternative compounds.

Various trials have been carried out from 2008 to 2011 by a task group including Ctifl, DGAL, Ministry and experimental regional centres (Warlop *et al.,* 2012), mostly with the new formulation of *Beauveria bassiana* called Naturalis®, but also with kaolin.

*Naturalis*

As shown on the figure below, seven different partners carried out 12 field trials to assess the efficacy of Naturalis, either on experimental plots or by organic growers, mostly in south of France.

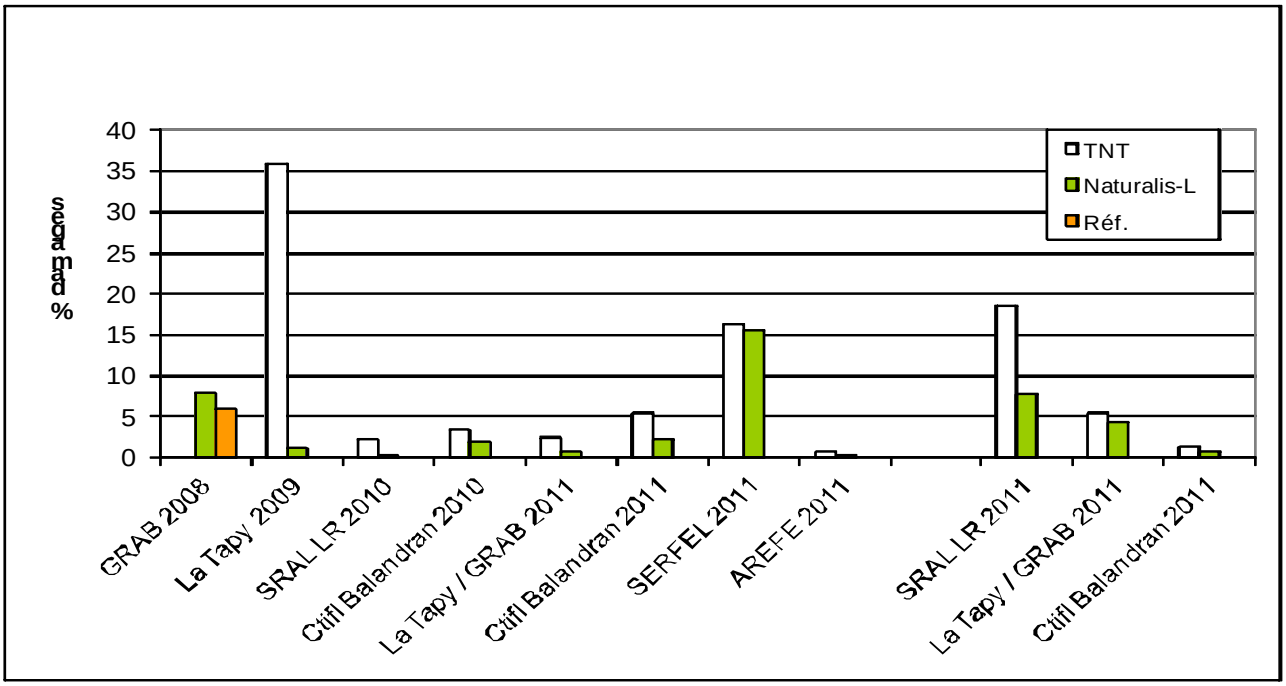


Figure 5: Compared % of damages in 11 field trials done from 2008 to 2011, between control (TNT), Naturalis-L treatments and growers reference. (8 first results on *Rhagoletis cerasi*; 3 last trials on *Drosophila suzukii*).

Results show different levels of pressure, ranging from 0 to 35% of damages at harvest. Efficacy of *B. bassiana* was also very fluctuating, from 5 to 96%, with most of trials giving a mean efficacy of 50-60%, under a low pressure. Efficacy was especially low this year, but this may be due to a formulation problem from company's side.

This active substance however looks irregular in efficacy so far, and dependant of humidity at the time of application. Weather conditions during treatments (may or june) can be rather unfavourable to the germination and contamination of the fungus. It is also essential to spray *Beauveria bassiana* every 7 days until harvest (Daniel & Häseli, 2010).

*Kaolin*

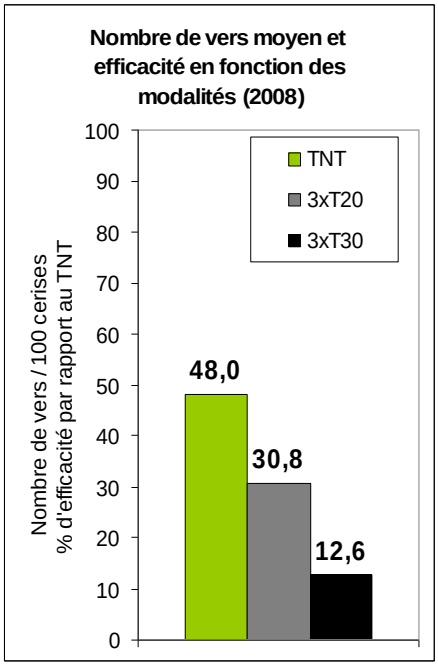


Figure 6: Mean number of larvae for 3 different strategies: control (TNT), kaolin with 2 different dosages.

Kaolin clay has shown interest, as it did on olive fruit fly. Trials led by La Tapy (Filleron, 2010) led to efficacies from 40 to 95%, according to the dosage and strategies used. The graph enclosed shows an efficacy much improved with 3 treatments at 30 kg/ha compared to 3 treatments at 20 kg/ha.

The main concern is about anaesthetic residues, although health risks should be very limited. Machines in packinghouses have been tested for their ability to wash cherries while separating stems. Most of the surface can be cleaned, only the cavity of the cherry keeps traces.

Figure 7: Appearance of cherries before (left) and after (right) washing.

## Discussion

Although advances and progress have been achieved in the past years, we are not yet able to recommend reliable strategies to control cherry fruit flies under the conditions of organic farming, except with nets for intensive orchards. This difficult situation is even worse for the last 2 years, since a new invasive pest has been recorded in France, *Drosophila suzukii*, coming from Japan through Italy and commercial exchanges.

This very prolific pest lays eggs on healthy cherries, but also on strawberries, small fruits or peaches. So far, no control strategy is available, and this new situation threatens the economical sustainability of many fruit growers.

**Acknowledgements**

We thank colleagues and students who helped us in collecting the data, and growers for confidence by putting their orchard to our disposal.

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# *Drosophila suzukii* (Matsumura), a revolution for soft fruits in Trentino

A.Grassi, M.Pallaoro [1]

**Abstract**

*In September 2009, Spotted Wing Drosophila (SWD),* Drosophila suzukii *(Matsumura), was detected for the first time in Italy and Europe on raspberry (*Rubus idaeus *L.), highbush blueberry (*Vaccinium corymbosum *L.) and strawberry (*Fragaria x ananassa *Duch.) in several cultivated fields in Trentino, north-eastern Italy. Two years after, SWD reached an extraordinary population development, causing serious damages on cherries and soft fruits: an important infestation was also observed for the first time on wine grape. Due to the very high pest pressure, conventional insecticides, even if applied many times at the harvest, were unsuccessful in reducing the fruits damage at acceptable levels. Interesting results were obtained with alternative control methods, such as the mass trapping and the anti-insect nets. A multi-method approach seems to be the best to manage* D.suzukii *infestations in a sustainable way in Trentino region.*

**Keywords**: Spotted Wing Drosophila, *Drosophila suzukii*, soft fruits, mass trapping, anti-insect nets

## Introduction

The Spotted Wing Drosophila, *Drosophila suzukii* (Matsumura) is one of the two species of the genus *Drosophila* to lay eggs in healthy (whole) fruits instead of damaged or overripe ones (Sasaki and Sato 1995, 1996). It preferentially oviposits on mature fruits but can also lay eggs on immature and spoiled fruit of suitable varieties at lower rates (Kanzawa 1939; Mitsui *et al.* 2006).

The larval feeding causes the fruit to collapse around the oviposition site. The oviposition scar exposes the fruit to secondary attack by pathogens and other insects (Hauser and Damus 2009). The damage caused by *Drosophila suzukii* larvae renders the fruit unsuitable for sale.

The pest was recorded for the first time in Trentino, north-eastern Italy, in September 2009. Soft fruits and strawberry production is an important component of agriculture in our region, where these fruits showed a high intrinsic susceptibility to *D.suzukii* infestations, probably because of their long harvest period, the thin skin and a sweet juicy pulp.

In 2010, we carried out a monitoring study, field and lab surveys to better understand the distribution of the pest on our territory, to deepen its biology and seasonal phenology, to determine the host range (cultivated and spontaneous) and to assess the efficacy of registered conventional insecticides in controlling SWD infestations.

The insecticides must be used during the ripening period of the fruits, to kill the adults and to prevent so the egg laying inside the ripe fruits, the most susceptible stage. For several aspects, this approach is very complicated on soft fruits: they are harvested over a long period and at very short intervals, so that it's very difficult to correctly time the sprays complying with the pre-harvest interval. Moreover, the repeated applications of insecticides can cause serious commercial problems due to the accumulation of residues on the product, are unsustainable over a long period for the negative impact on the environment

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and on the IPM on soft fruits, on the health of growers and consumers, and may lead to resistance problems in the SWD populations.

In 2011, we repeated the territorial monitoring and conducted some trials in order to evaluate the effectiveness of alternative control methods, like the mass-trapping and the anti-insect nets.

**Material and Methods**

Monitoring of SWD adult flight activity. To determine the distribution of the pest on the territory and the seasonal adults activity, we used 65 apple cider vinegar traps. A trap consisted of a durable plastic bottle of 500-1000 ml capacity with a screw-top. Six 0.6 mm diameter holes were drilled on the side and 200 ml of pure apple cider vinegar were added as a bait.

The traps (one per site, hung on shady side of the plant) were deployed since the beginning of April (week 14) or beginning of May (week 19) till the beginning of December (week 49) in some woody areas, fields of highbush blueberry, raspberry, blackberry and strawberry and orchards of sweet cherry, apricot, wine grape. In some cases, traps placed on site in 2010 were regularly inspected also during the winter and the 2011 season.

Traps were replaced once a week, the solution was filtered in laboratory and males and females were counted under dissecting microscope. Some of the traps are still now on place in order to document the flight of the adults during the winter time, and will remain till the next season.

Fruits infestation assessment. In most of the sites where the adults flight was monitored, the infestation on fruits was weekly estimated during the harvest time on a sample of 50-100 soft fruits/site (25-50 cherries and apricots/site), collected at the right commercial ripening stage from an area of about 15-20 metres diameter around the trap. On wine grape, 10 bunches/site were sampled at the harvest time from the edges of the vineyard close to woods or bushy borders and we inspected 10-15 berries/bunch.

A total of 739 samples were inspected. Fruits were accurately examined one by one under dissecting microscope, to search for the eggs of *D.suzukii*, typically inserted in the skin.

Mass-trapping trial. The aim of the trial was to evaluate the effectiveness of a dense barrier of traps deployed along the edges of a highbush blueberry plantation (var.Elliot – surface of about 800 square metres) in preventing the immigration of SWD adults and their damage on the fruits during a complete harvest period.

The traps, of the same type used for monitoring the SWD adults flight activity on the territory, were placed in the field prior to the reddening of the fruits (week 28 – 12 July). They were hung on the blueberry bushes on the borders at a distance of about 2 metres between them. A few traps were placed also on trees around the plantation and a control trap was deployed in the middle of the field. As a bait, we used a solution of 150 ml of pure apple cider vinegar and 50 ml of red wine per trap. The bait was weekly replaced until the end of the trial (week 34 – 22 August, end of harvest). The solution was filtered in lab and adult males and females were counted under dissecting microscope.

The damage on the fruits was weekly estimated on samples of 100 fruits (at the right commercial ripening stage) picked up both from the bushes on the edges and from the bushes in the central part of the field. The fruits were examined one by one under dissecting microscope, searching for the SWD eggs inserted in the skin.

Anti-insect nets. The aim of the trial was to assess the efficacy of nets (0.5 mm x 0.8 mm mesh size) in reducing the infestation of *D.suzukii* on the fruits of sweet cherry and highbush blueberry.

Two sweet cherry orchards (cv. Regina) were selected in the production areas of Vigolo Vattaro and Susà. The SWD adult flight was monitored using 2 apple cider vinegar traps in each orchard. Before the reddening of the fruits (week 22), five trees (about 4 metres tall) were randomly selected and singly bagged with a bag of net. The bag was closed on the base of the trunk with a rope. A control plot (5-10 trees), where insecticides were not applied for the control of *D.suzukii*, was arranged in a edge of each orchard. In addiction, some branches with fruits were protected during the insecticides sprays with a waterproof tissue, that was removed after the treatment to let the adults lay the eggs in the cherries. The infestation on fruits was assessed at the harvest time (week 27 and 28): samples of 50 cherries were collected from every bagged tree, from the control plot and branches and from the trees sprayed with the insecticides. The fruits were inspected in the lab under dissecting microscope, to search for the SWD eggs inserted in the fruits.

In a highbush blueberry plantation (cv. Brigitta) in Ronchi Valsugana site, two tunnels with nylon covering were singly completely wrapped on their sides with a band of the net, prior to the reddening of the fruits (week 24 – 15 June). An apple cider vinegar trap was placed in each tunnel and in a control plot (a tunnel where net and insecticides were not applied) to monitor the adults flight. The infestation was assessed at weekly intervals during the harvest on a sample of 100 fruits randomly collected at the right ripening stage from each of the tunnels covered with net, from the control plot and from the insecticides sprayed tunnels. The fruits were examined in lab under dissecting microscope to search for the SWD eggs. Meteo data (temperature and high humidity) were also recorded in covered and uncovered tunnels by means of dataloggers.

## Results

Monitoring of the adults flight activity

Some adults were caught in apple cider vinegar traps during the winter time in few sites at different altitudes and situations. Most of them were collected in two lowland site, in S.Michele and Mezzocorona (Table 1). This means that the pest spent the winter as an adult (mainly females) directly on our territory. It's likely that a higher number of adults survived at lower altitudes, where temperatures are milder and the refugees are probably more abundant (houses, greenhouses, composters, etc.). The catches of adults in winter are probably due to adults that during particularly sunny days may fly away from the refugees, to search for food sources to maintain themselves during the winter.

No adults were collected by beatings, aspiration (on conifers, evergreen shrubs) and from dead leaves samples collected from raspberry and bluberry fields in winter and kept in lab till the next season.

The adults catches continued in spring in the two lowland sites. No adults were detected by means of beatings on wild and cultivated flowering *Prunus* and *Rosaceae* species.

The first *D.suzukii* eggs were found the 18$^{th}$ of May by visual inspection on cherry fruits collected from a wild tree in Roncogno (430 m a.s.l), near Pergine Valsugana. This was the first sign of the beginning of adults activity on the territory: in fact, the following weeks, we recorded the first adults in traps and some other eggs in fruits, always in cultivated sweet cherry orchards. These observations confirm that cherry is an important host for this pest, the first generations develop on it. To get a good control of the infestations on cherry is likely a crucial point to try to limit the development of the populations and the damages on the crops that will ripe later in the season.

What happened in the following period, summer and autumn, is really a dramatic population outbreak (Figure 1). Compared to 2010, the flight started about 2 weeks earlier: also the ripening of the susceptible crops was earlier and this better synchronism pest/crop

gave rise to a faster and more conspicuos demographic increase, with a peak in September much higher and earlier than the previous season. The climate in winter and spring was warmer than in 2010 and in addiction June was particularly rainy and humid. This positively affected the development of the pest, since *D.suzukii* seems to prefer high humidity conditions. Moreover, the rain damaged many cherries just at harvest time and many growers didn't harvest the fruits: from these fruits a lot of adults could develop and spread the infestations on the territory.

As in 2010, the pest was recorded everywhere in Trentino. It is interesting to observe that most of the adults were caught at medium-high altitudes (Figure 2): many soft fruits in Trentino are cultivated in this range, at the feet of the mountains and in lateral valleys, where also wild soft fruits are more abundant and the forests are closer to the crops. Also the climate at these altitudes is more suitable than in the bottom of the valleys, is milder during the summer, more rainy and more wet.

Fruits infestation

Cherries and strawberries are the first fruits that ripen in Trentino (Table 2). Cherries are the first host crop for *D.suzukii*. Most of the infested fruits recorded in May, June and July were sampled from unsprayed or rain unprotected orchards: in fact, in most of the cases, the insecticides on cherry were effective against SWD, probably because they were applied in a very early and susceptible stage of its population development. The insecticides showed to be ineffective where the cherry orchards were rain unprotected, because the residues were probably washed away. It is important to observe that cherry is highly susceptible to SWD: even if the adult population is still low, its damage on cherries may be severe if no chemicals are applied.

The strawberries harvested in May and June were undamaged, probably because cherry is more attractive than strawberry in this moment of the season. Moreover, the growers use to spray a lot of insecticides on strawberry, using different compounds: the population is still low in this stage and the chemicals are probably more effective. In any case, strawberry seems to be the least susceptible crop in Trentino.

Raspberry, blueberry and blackberry are highly susceptible crops. For the first season, we recorded also a quite severe and widespread infestation on wine grape: an earlier harvest and a better synchronism with the peak of adults population, were probably the main reason for this damage in 2011.

The highest levels of damage were recorded from July to October, with a peak in August-September. At that time, most of the infested samples had more than 50% of the fruits damaged.

For soft fruits the harvest period is much longer than on sweet cherry. Due to the very high pest pressure in 2011, a lot of adults could continuously immigrate into the fields from surrounding sites and lay the eggs in ripe and unripe fruits. Even if a lot of insecticides were applied during the harvest time, they were ineffective in controlling the infestation on fruits (Figure 3 and 4). These graphs suggest that we can not rely only on insecticides to get a good control of this pest in Trentino situation. Alternative control techniques and a multiple control methods approach are necessary.

The mass trapping trial showed quite promising results (Table 3). A very high number of adults was caught by the traps along the edges of the blueberry plantation during the trial, but just a few individuals were captured by the control trap in the middle of the field. Weekly catches demonstrate that males and females can continuously immigrate into the cultivated fields from external sources and that it's necessary to stop their incoming flight. The traps were successful for a long time in stopping this flight and preventing the infestation on the fruits in the centre of the field. Their effectiveness decreased just after

the peak of adults catches, almost close to the end of the harvest, when probably a higher number of adults was able to pass the traps barrier and lay more eggs in the fruits.

The anti-insect nets gave the best results in reducing the damage on the fruits. On cherry, we obtained 100% of efficacy in the trial carried out in the orchard in Susà site (Table 4). A single cherry fruit collected from a bagged tree, probably because in contact with the net, resulted infested with one egg in the trial performed in Vigolo Vattaro (Table 5).

It's interesting to observe that the insecticides applied by the growers against the Cherry Fruit Fly (*Rhagoletis cerasi*) at the fruit reddening phase in both the sites were unable to provide a protection against SWD, that occurred later. This means that with the available insecticides, generally provided with a short persistence of activity against *D.suzukii*, additional treatments in Trentino must be planned nearness the harvest to effectively control this pest, that prefers to attack fruits completely red and ripe. We expect that the number of insecticide sprays and their side effects will increase a lot on sweet cherry and this represent an important reason to exert every effort to find alternative control methods.

Also the trial on highbush blueberry produced promising results (Table 6). A weak infestation was recorded just at the end of harvest in one of the two tunnels protected with the net. We suspect that some adults got into the tunnels during the harvest procedures, when the pickers had to lift the net at the main openings to reach the bushes. This happened many times during the trial. No significant effects on climate (temperature and humidity) due to the net covering were recorded in this trial.

Table 1: Adults catches in apple cider vinegar traps during the winter time in 2011

| **site** | S.Michele | Mezzocorona | Gaggio | V.Vattaro | V.Vattaro | Vattaro |
|---|---|---|---|---|---|---|
| **crop** | vineyard | small vineyard, garden | forest | forest | strawberry | forest |
| **altitude (m a.s.l)** | 260 | 213 | 910 | 718 | 727 | 696 |
| **week n° and date** | **SWD adults catches** | | | | | |
| 2 - 10-16 January | | | 1 ♂ | | | |
| 3 - 17-23 January | 1 ♀ | 1 ♀ | | | | |
| 5 - 31 Jan. - 6 Feb. | 1 ♀ | 1 ♀ | | | | |
| 9 - 28 Feb. - 6 Mar. | | | | 2 ♀ | | |
| 10 - 7-13 March | 4 ♀ | 4 ♀ | | | | |
| 12 - 21-27 March | 4 ♀ | 1 ♀ | | | 1 ♂ + 1 ♀ | 1 ♂ |
| 13 - 28 Mar. - 3 Apr. | 1 ♀ | 1 ♀ | | | | |

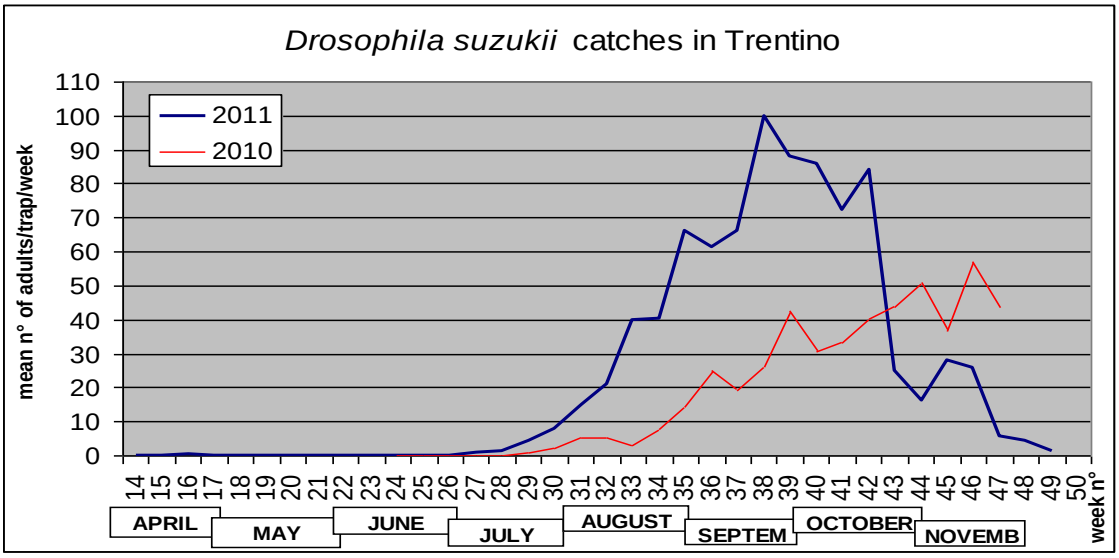


Figure 1: SWD flight recorded in Trentino in 2010 and 2011

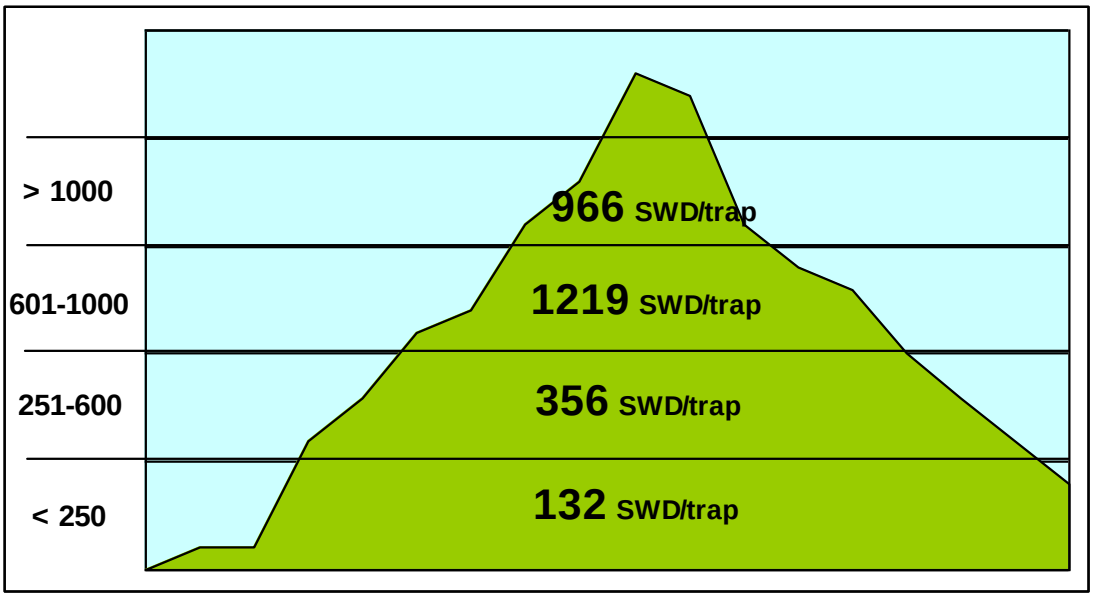


Figure 2: SWD adults catches according with the altitude of the trapping sites

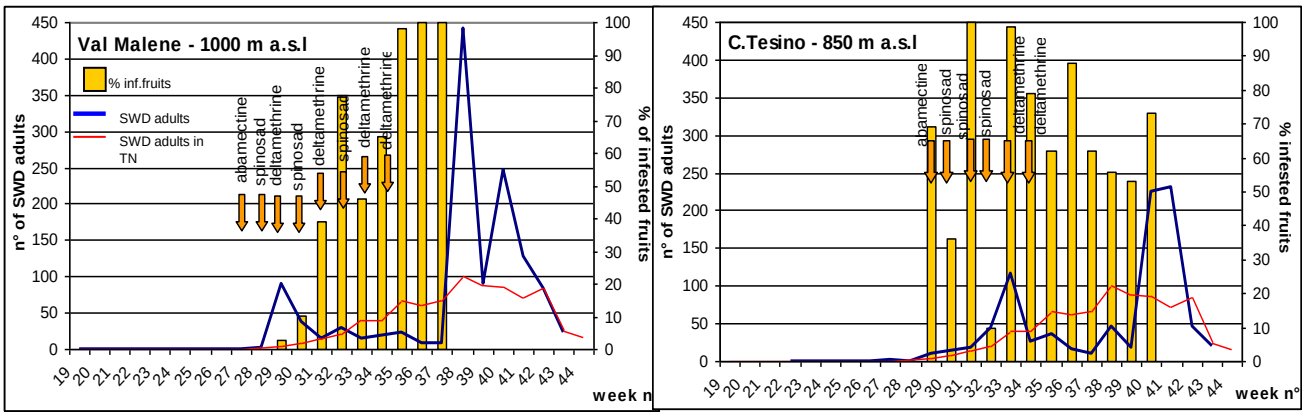


Figure 3 and 4: SWD flight and damage in a strawberry and a blackberry plantation

Table 2: infestation recorded on fruit samples in 2011

| | PERCENTAGE OF FRUIT SAMPLES INFESTED BY SWD | | | | | |
|---|---|---|---|---|---|---|
| **grape** | | | | 25 | 71 | |
| **blackberry** | | 0 | 82 | 100 | 100 | 75 |
| **blueberry** | | 0 | 51 | 95 | 100 | |
| **raspberry** | | 7 | 80 | 100 | 97 | 92 |
| **strawberry** | 0 | 0 | 50 | 70 | 71 | 53 |
| **cherry** | 3 | 11 | 46 | | | |
| | **May** | **June** | **July** | **August** | **Septem.** | **October** |

Table 3: results of mass trapping trial on highbush blueberry

| week | SWD adults | | % infested fruits | | |
|---|---|---|---|---|---|
| | central trap | border traps | from central bushes | from border bushes | |
| 21 | *posit.* | | | | |
| 22-27 | 0 | | | | |
| 28 | 0 | *posit.* | 0 | | **H** |
| 29 | 0 | 11 | 0 | | **A** |
| 30 | 0 | 84 | 0 | 7 | **R** |
| 31 | 1 | 156 | 0.8 | 5 | **V** |
| 32 | 1 | 111 | 0 | 2.2 | **E** |
| 33 | 0 | 1048 | 9 | 18 | **S** |
| 34 | 0 | 681 | 32 | 34 | **T** |
| 35 | 1 | ***end*** | 30 | | |

Table 4 and 5: results of the trial with anti-insect nets on sweet cherry in Susà and V.Vattaro sites

| week | SWD adults | % infested fruits | | | | |
|---|---|---|---|---|---|---|
| | | control plots | | sprayed trees* | bagged trees | |
| | | unsprayed branches | unsprayed trees | | | |
| 19-26 | 0 | | | | | |
| 27 | 1 | 6 | 3.3 | 8 | 0 | HARVEST |
| 28 | 0 | 100 | 90 | 94 | 0 | |

**week 21 and 23: acetamiprid+spinosad - week 24 deltamethrine*

| week | SWD adults | % infested fruits | | | | |
|---|---|---|---|---|---|---|
| | | control plots | | sprayed trees* | bagged trees | |
| | | unsprayed branches | unsprayed trees | | | |
| 19-25 | 0 | | | | | |
| 26 | 0 | nc | 2 | nc | nc | |
| 27 | 0 | 3.04 | 2 | 0 | nc | HARVEST |
| 28 | 3 | 100 | nc | 70 | 0.4 | |

** week 25: deltamethrine - week 26: spinosad*

Table 6: results of the trial with anti-insect nets on highbush blueberry in Ronchi Valsugana site

| week | SWD adults | | | % infested fruits | | | | |
|---|---|---|---|---|---|---|---|---|
| | control | covered tunnel 1 | covered tunnel 2 | control | covered tunnel 1 | covered tunnel 2 | sprayed tunnel | |
| 21-26 | 0 | 0 | 0 | | | | | |
| 27 | 1 | 0 | 0 | | | | | |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | HARVEST |
| 29 | 6 | 1 | 0 | nc | nc | nc | nc | |
| 30 | 2 | 1 | 0 | 6 | 0 | 0 | 0 | |
| 31 | 33 | 0 | 0 | 15 | 0 | 0 | 12 | |
| 32 | 30 | 0 | 0 | 27 | 0 | 0 | 1 | |
| 33 | 96 | 3 | 1 | 77 | 7.7 | 0 | 19 | |
| 34 | 46 | 19 | 16 | 88 | end of harvest; net covering removed | | nc | |
| 35 | 120 | removed | | 100 | | | nc | |

## Discussion

The complexity of the environment, of the agro-ecosystems and the high concentration of small size soft fruits plantations in this small territory, represent an important additional force for *D.suzukii* in Trentino. The control of the pest was further complicated in 2011 by an extraordinary population development: also the insecticides showed to be an unreliable method in this situation. The alternative methods we tested this season demonstrated that can effectively contribute to the control of the pest. A multi-method approach seems to be the best way to manage *D.suzukii* infestations in a sustainable way in Trentino region.

## Acknowledgements

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# Madex Twin, a new *Cydia Pomonella* Granulovirus Isolate for the Control of both Codling Moth *Cydia Pomonella* and Oriental Fruit Moth *Grapholita Molesta*

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**Abstract**

*Madex Twin, a new* Cydia pomonella *granulovirus (CpGV) isolate for the control of both codling moth (CM) and oriental fruit moth (OFM), has been developed and tested in bioassays and field trials by Andermatt Biocontrol, Switzerland. At field level, the product has been tested in a total of 44 field experiments in the Northern and Southern hemisphere. The product showed promising efficacy results against both pest species in the laboratory, as well as in the field. Applications for registration of Madex Twin in Southern Europe and in other countries all over the world have already been initiated.*

**Keywords:** CpGV, codling moth, oriental fruit moth, Madex Twin

## Introduction

OFM is a key pest in peach and nectarine production all over the world. A possibility of residue free control of OFM would be highly appreciated as residue requirements are becoming more and more important. The Swiss company Andermatt Biocontrol selected a granulovirus isolate (Madex Twin) on OFM in the laboratory. Madex Twin was then tested against CM and OFM in laboratory bioassays and in numerous field trials in the relevant pome and stone fruit growing regions. Selected trial examples as well as a review of its average performance under field conditions will be presented here.

## Material and Methods

*DNA Restriction Endonuclease Analysis (REN)*

The viral DNA of the test item (Madex Twin) and the reference item (CpGV, Neustadt Mexican isolate) was digested with the restriction enzymes *Bam*HI, *Eco*RI, *Sal*I and *Eco*RV. All digested DNAs were electrophoresed in a 0.8% agarose gel over night (25 V) using TAE as a buffer system.

*PCR Amplification and Sequencing of Marker Genes*

The partial sequences of late expression factor *lef-8* and the granulin (*polh/gran*) genes were amplified using the degenerate primer method described by Lange et al. (2004) and Jehle et al. (2006). PCR products used for direct sequencing were purified using the GFX PCR DNA and Gel Band Purification Kit (Amersham, Freiburg, Germany), and both DNA strands were sequenced using M13 universal, M13 reverse and T7 standard primers (MWG, Germany). The sequences were aligned using BioEdit with the corresponding sequences of further CpGV isolates determined previously and described by Eberle et al. (2009).

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*Phylogenetic Analysis*

Partial *polh/gran* and *lef-8* sequences determined for Madex Twin were concatenated and aligned with the corresponding sequences of Cryptophlebia leucotreta granulovirus (Cr1eGV) (Lange & Jehle, 2003) as an outgroup using Clustal W (Thompson et al., 1994) implemented in BioEdit 7.0.5.3 (Hall, 1999). A phylogenetic analysis using minimum evolution algorithms was performed using MEGA 4.1 (Kumar et al., 2004).

*Genome Sequencing*

Genome sequencing of Madex Twin was performed by a commercial supplier by 454 pyrosequencing (Genterprise Genomics, Mainz). Sequences obtained were assembled using DNAStar Lasergene Seqman NGen® Software. Analysis and joining of contigs was done with DNAStar Lasergene SeqMan Pro (Version 8.1.5). The trace files were checked by eye and minor sequencing mistakes were corrected if necessary. In case of sequence ambiguities, primers were designed using DNAStar Lasergene PrimerSelect and the sequence parts were re-sequenced by Sanger sequencing (Genterprise Genomics, Mainz). Methionine-initiated open reading frames (ORFs) encoding 40 amino acids or more were determined using GeneQuest (DNAStar Lasergene 8.1.4). ORFs were checked for homology to ORFs in other dsDNA viruses using the BLAST programs blastn and blastx; the consensus sequence of Madex Twin was aligned to CpGV-M (Eberle, 2010) on nucleotide level by bl2seq. The predicted ORFs were compared to the re-sequenced CpGV-M (Eberle, 2010) in BioEdit 7.0.5.3.

*Bioassay*

The bioactivity of Madex Twin was determined in a concentration-response bioassay on semisynthetic diet (WARD's) with CM and OFM larvae. A CpGV-M isolate of known activity was used as reference. Up to 6 different concentrations between $2x10^2$ and $2x10^6$ occlusion bodies per gram diet were tested on both insect species for each virus isolate. For each virus concentration, 50 neonate larvae were kept individually in darkness at 25°C. Mortality was assessed after 12-14 days. 200 larvae on virus-free diet were used as untreated control.

The median lethal concentration (LC50) and the relative potency (RP) were calculated by probit analysis (Finney, 1971) using Priprobit 1.63. Normal Distribution and the model "Preference (a,b,c: Natural Preference to Treatment Side), D=0" was used. Visualisation of the results was done with Microsoft Excel®.

*Field Trials*

Trial site 1, OFM-Peach: Madex Twin was applied at different rates and compared to the reference product Calypso (Thiacloprid). A total of 5 applications took place on 11th, 20th, and 28th of May (1st generation applications), 15th of July and 26th of August (2nd generation applications) in a well established peach orchard in Veselé, Slovakia in 2010.

Pest infestation damage was scored as "stopped damage" (superficial empty gallery where larvae have been killed) and "active damage" (deep damage to the core with living larva or signs that larva had emerged from the fruit and completed its development). "Stopped damage" was distinguished from "active damage" by cutting the fruit open.

Trial site 2, OFM-Pear: The pilot pear orchard was situated close to a peach field in the Bologna province of Italy, where the presence of OFM has been high throughout the summer 2011. Treatments were done during the 3rd OFM generation close to harvest. The

products were applied from beginning of August 2011 until harvest time. Madex Twin was applied at two dose rates of 50 (6 day interval) and 100 ml/ha (8 day interval) respectively.

The final assessment was carried out at harvest time by counting the number of damaged fruit on 200 randomly selected fruits per plot. The damaged fruits were sectioned to control the type of damage ("deep entries" or "stings" for *C. pomonella*, "deep entries" for *G. molesta*) and to classify the larvae inside the fruit.

Trial site 3, CM-Apple: In order to study the field performance of Madex Twin on CM in pome fruit, a trial site was selected in the Bologna region of Italy. Madex Twin was compared to the normal CpGV-M strain (Madex) and a chemical reference. The CpGV based products were applied at an 8 day interval (starting from 6$^{th}$ of May 2011) until the end of the first CM generation. Coragen (Rynaxypyr), a new larvicide highly effective against CM, was applied twice at a 15 day interval (29$^{th}$ of April and 14$^{th}$ of May 2011). The final assessment was carried out on 11$^{th}$ of June counting the number of damaged fruit on 300 randomly selected fruits per plot.

## Results and Discussion

*DNA Restriction Endonuclease Analysis*

On the basis of DNA restriction analysis using *Sal*I, *Eco*RV, *Eco*RI and *Bam*HI, it could be concluded that Madex Twin is a CpGV isolate. The predominant genotype corresponds to CpGV-M (NW) and is therefore an A type genome isolate. However, there were some submolar bands observed in the *Eco*RI and *Eco*RV profiles, indicating that there is a second genome type present at a low level.

*Phylogenetic Aalysis*

On the basis of the concatenated *polh/gran* and *lef*-8 sequences, Madex Twin did not differ in its predominant genome type from CpGV-M. Madex Twin was found to contain a predominant A type genome.

*Genome Sequencing*

Whole genome sequencing of Madex Twin confirmed that it is a CpGV isolate with a predominant type A genome. With a genome size of 123,345 bp it is slightly shorter than CpGV-M (Eberle, 2010). All 142 ORFs annotated for CpGV-M were found in Madex Twin. No additional ORF could be observed. Five ORFs differ in their predicted amino acid sequence to CpGV-M due to insertions, deletions or SNPs. Sequence identity to CpGV-M on nucleotide level is 99%.

Beyond the predominant type A genome, a second genotype is visible at a ratio of about 50% in ten ORFs. In nine ORFs, this leads to amino acid sequence differences to CpGV-M. Analysis of the *polh/gran* and *lef-8* sequences revealed that a type D genome is also present in Madex Twin at a very low level of about 2%.

*Bioassay*

Reaching a 50% lethal concentration (LC50) of 2.9x103 OB/g diet in the laboratory bioassay on OFM, Madex Twin showed a 45 times higher activity than CpGV-M (Table 1). With a LC50 of 1.1x103 OB/g diet in the bioassay on CM larvae (Table 2), Madex Twin performed equally well compared to the highly effective CpGV-M reference isolate.

Table 1: Activity of CpGV-M and Madex Twin against OFM.

| | LC50 [OB/g diet] | 95% lower limit | 95% upper limit | relative potency | 95% lower limit | 95% upper limit |
|---|---|---|---|---|---|---|
| CpGV-M | 130'190 | 81'686 | 199'530 | 1.0 | 0.7 | 1.5 |
| Madex Twin | 2'892 | 1'799 | 4'444 | 45.0 | 29.5 | 69.1 |

Table 2: Activity of CpGV-M and Madex Twin against CM.

| | LC50 [OB/g diet] | 95% lower limit | 95% upper limit | relative potency | 95% lower limit | 95% upper limit |
|---|---|---|---|---|---|---|
| CpGV-M | 1'058 | 824 | 1'322 | 1.00 | 0.73 | 1.37 |
| Madex Twin | 1'107 | 863 | 1'382 | 0.96 | 0.70 | 1.31 |

*Field Trial Results*

Trial site1: OFM-Peach: Figure 1 shows the mean reduction of active and total fruit damage. All test products significantly reduced fruit attack in comparison to the untreated plot. Treatments with Madex Twin 100 and 200 ml/ha and Calypso showed equally good results of up to 84%. An increase in the dose rate from 100 to 200 ml/ ha did not result in a significant improvement of efficacy. This data reflects the well-known flat dose-response relationship of granulovirus products under field conditions (Kienzle et al., 2001).

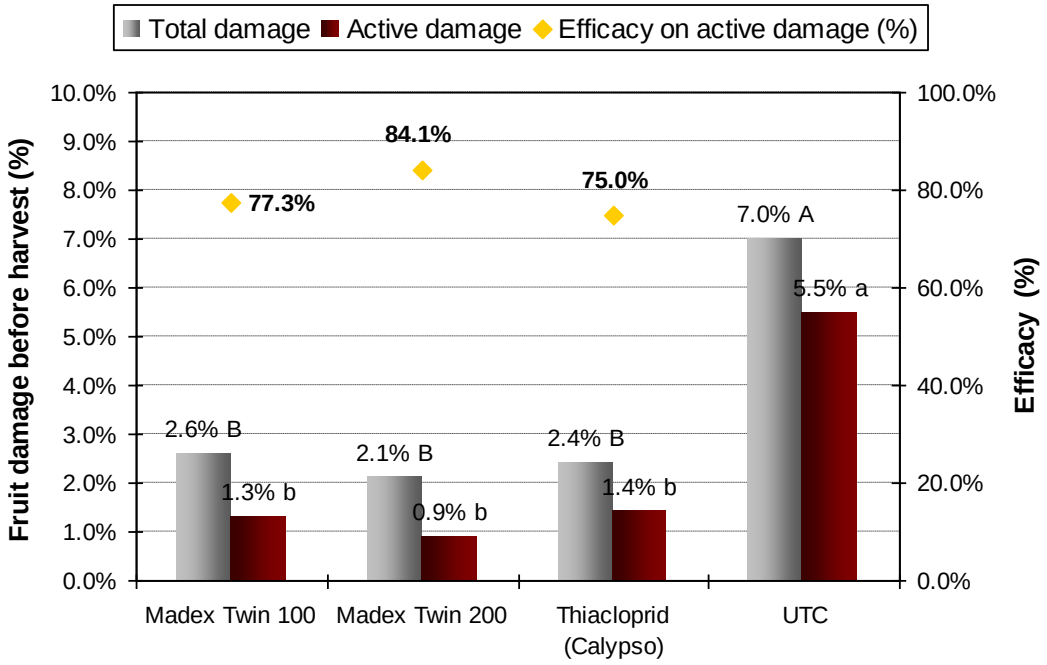


Figure 1: Evaluation of total and active damage of OFM before harvest on peach treated with Madex Twin at different dose rates and compared to a chemical reference product. (Tukey test; P<0.05)

Trial site 2, OFM-Pear: In the untreated control (UTC) the mean total fruit damage amounted to 4.4%, while in the other treatments mean total fruit damage ranged between 0.75% and 1.88% (Figure 2).

The percentage of deep entries caused by CM was lowest in plots treated with Madex Twin (100 ml/ha), intermediate in plots treated with Madex Twin (50 ml/ha), Madex (CpGV-M, 100 ml/ha) and the chemical standard, and highest in untreated control plots.

In the Madex Twin (100 ml/ha) treatment, the percentage of fruit damaged by OFM was significantly lower than in untreated control plots. In the Madex Twin (50 ml/ha) and Madex (100 ml/ha) treatments, the percentage of fruits damaged by OFM neither differed significantly from Madex Twin (100 ml/ha) nor from the untreated control, while in the chemical standard (Trebon Up) OFM damage was statistically comparable to that recorded in the untreated control. The percentage of total fruit damage (all fruits damaged by *C. pomonella* and *G. molesta*) was significantly lower in plots treated with Madex Twin (100 ml/ha) than in untreated control plots.

The efficacy of the Madex Twin treatment (100 ml/ha) was 80.9%, thus higher than the efficacy of the chemical reference treatment and the other CpGV-based treatments.

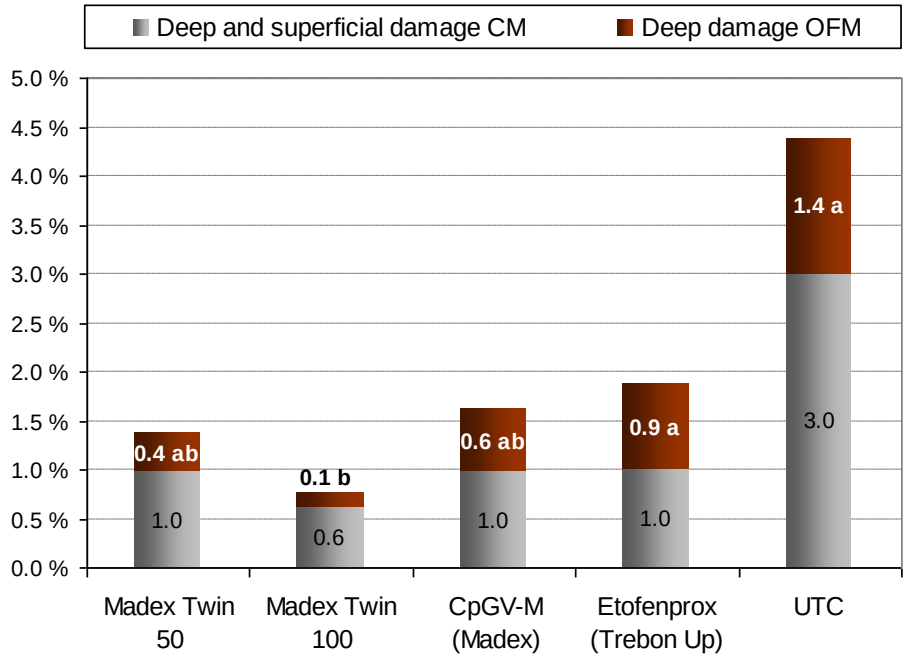


Figure 2: Mean percentage of fruits with deep entries and stings caused by CM and with deep entries caused by OFM. Italy 2011.

Trial site 3, CM-apple: In the untreated control (UTC) the mean fruit damage amounted to 8.7%, of which 7.2% were “deep entries” and 1.5% “stings” (Figure 3).

The percentage of fruits damaged with “deep entries and superficial damage” was significantly lower in treated than in untreated control plots, with differences among treated plots not being significant.

Madex Twin showed a very high efficacy level and was as effective as the normal CpGV-M isolate (Madex) against CM in apple.

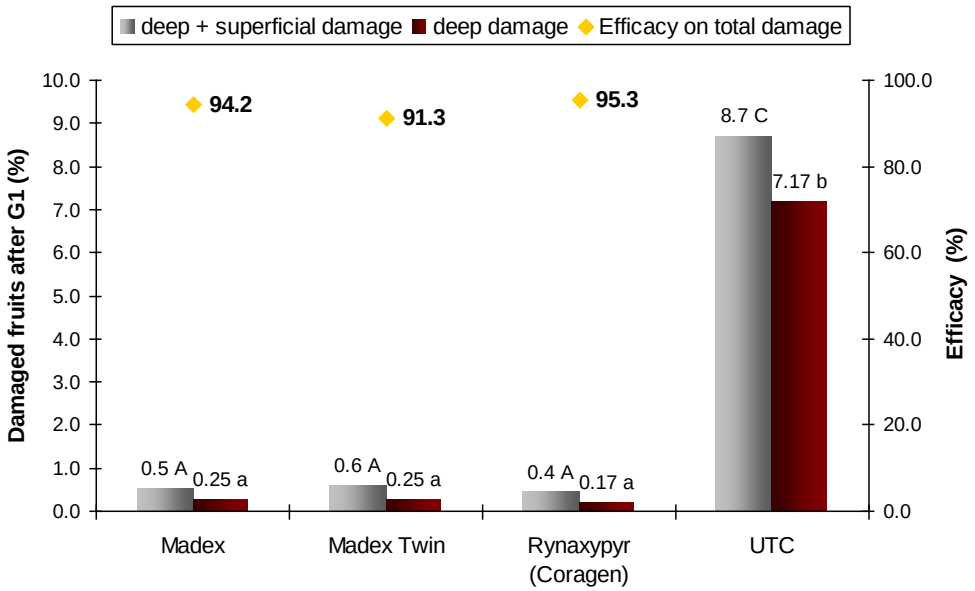


Figure 3: Mean percentage of fruits with deep damage, total fruit damage and efficacy level of Madex Twin compared to CpGV and chemical standard. Italy, 2011.

## Conclusion

Madex Twin differs only slightly from CpGV-M in REN analysis and sequencing profile. However these differences seem to be the reason for the higher potency of Madex Twin towards OFM in the bioassays. Based on the extensive testing, Madex Twin can effectively be used for the control of OFM on stone and pome fruit as well as for control of CM on pome fruit (Table 3). The registration process has already been initiated in several countries all over the world.

Table 3: Average performance of Madex Twin at standard rate of 100 ml/ha. Review of field trial results from 2010/ 2011 (Min./Max. and number of trials)

| | Apple (CM) | Peach (OFM) | Nectarine (OFM) | Apple (OFM/CM) |
|---|---|---|---|---|
| Average efficacy on shoot strike reduction | | 61.9% (50 - 83%; n=9) | 76.3% (n=2) | |
| Average efficacy on reduction of total fruit damage | 69% (45-91%; n=11) | 64.5% (52-86%; n=10) | 67.7% (64.3 - 70%; n=3) | 66.2% (39 - 81%; n=3) |
| Average efficacy on reduction of deep damage | 83% (61.5 - 95.4; n=12) | 80.8% (n=2) | | |

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# Regulation of plum moth (*Cydia funebrana*) with *Cydia pomonella* granulovirus (CpGV) in organic plum orchards - first results

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**Abstract**

*As a further regulation strategy of plum moth (*Cydia funebrana*) CpGV isolate V15 in two different formulations was tested in different organic plum orchards in Germany in 2010 and 2011. On most plots CpGV was used in combination with mating disruption with "Isomate OFM Rosso". Various efficacies were found, they were in average not as high as in lab and ranged from 80 % to negative in worst case.*

**Keywords**: Plum moth, CpGV isolates, *Cydia funebrana*, organic plum growing

## Introduction

The plum fruit moth, Cydia funebrana is regarded as one of the key pests of organic plum production in Europe. On higher infestation levels biological control is limited. To test if infection of plum moth larvae is principal possible, viral suspensions of ten different CpGV isolates were sprayed on mature plums in the laboratory of the Department of Phytomedicine at the Geisenheim Research Center in 2009 (Reineke et. al 2010, Rueß et. al. 2010). The results demonstrated, that an infection of C. funebrana with CpGV is principally possible with an efficacy of up to 63%. Therefore the CpGV isolate with the highest efficacy was field tested in different organic plum orchards in Germany in 2010 and 2011.

## Material and Methods of the field trials

In the field trial 2010, it was the main aim to test if the promising laboratory infection experiments with the isolate CpGV V15 (Andermatt Biocontrol) could be extended to the field situation in organic orchards. In 2011, an additional attempt was directed to closer application intervals and another formulation. In 2010 and 2011 CpGV was applied in seven orchards in Rhineland-Palatinate on nine field trials in block design, as well as in one (2010) and two (2011) exact field trials with four replications. In 2010 and 2011 in Baden-Wuerttemberg, CpGV was applied in six field trials in block design in five orchards.
Weekly applications of CpGV V15 with an application rate of 150 ml per meter crown height (ml/mch) ($3x10^{10}$ occlusion bodies (OB)/ml) beginning with the flight of plum moth were compared to an untreated control. In 2010 CpGV V15 was used in a concentration of 500 ml/mch as a third treatment in an exact field trial. In further trial in 2011, a new formulation of CpGV V15, called "V42" ($1,7x10^{10}$ occlusion bodies (OB)/ml), was applied in two plots with an application rate of 261 ml/mch. 2011 in Rheinland-Pfalz in one plot CpGV V15 was applied as a third variant every 5 to 6 days and the numbers of application was increased from 12 to 23. Details of the orchards are shown in Table 1.

Details of the treatments, the numbers and the dates of start and end of applications in 2010 and 2011 are shown in Table 2.

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Table 1: Details of the orchards in Rhineland-Palatinate and Baden-Wuerttemberg where on-farm trials with CpGV V15 and V45 were done in 2010 and 2011.

| Site (Province RP or BW) | Plot No. | Variety | planted | plot [ha] |
|---|---|---|---|---|
| Wackernheim (RP) | 1.1<br>1.2<br>1.3 | Top (old)<br>Top (new)<br>Presenta | | 0.5<br>0.5<br>0.9 |
| Grafschaft (RP) | 2.1<br>2.2<br>2.3 | President<br>Hauszwetschge, Ortenauer<br>Hauszwetschge | 1994 up to 1996 | 0.3<br>0.32<br>0.5 |
| Kettig (RP) | 3 | Presenta, C. Schöne | 2002 | 1.0 |
| Uhlbach (BW) | 4 | Elena, C. Schöne, Valjevka, Katinka<br>Presenta | 1993<br>2003 | 0.75 |
| Sulzburg-Laufen (BW) | 5.1 | Herman, C. Schöne, Ersinger, Valjevka | 1996 | 0.42 |
| | 5.2 | Herman, C. Frühe, C. Schöne, Elena Valjevka<br>Presenta | 1994 / '96'<br>2001 | 0.25 |
| Backnang (BW) | 6 | Felsina, Hanita, Elena | 1999 | 0.25 |

Table 2: Treatments, type of field trail, amount of water, numbers and dates of application of CpGV V15 and V42 in 2010 and 2011

| Plot | Variants | Type of trial exact / field [1)] | Water l/ha | Year | Applications | | |
|---|---|---|---|---|---|---|---|
| | | | | | numbers | start | end |
| 1.1<br>1.2 / 1.3 | 1. Control<br>2. 150 ml V15 | field | 500 | 2010 | 11 | 03.06. | 13.08. |
| 1.1<br>1.2 / 1.3 | 1. + 2. | field | 400 | 2011 | 12 | 31.05. | 17.08. |
| 2.1 | 1. + 2.<br>3. 500 ml V15 | exact | 500 | 2010 | 14 | 25.05. | 31.08. |
| 2.1 | 1. + 2.<br>3. 261 ml GV V42 | exact | 400 | 2011 | 13 | 13.05. | 05.08. |
| 2.2 / 2.3 | 1. + 2. | field | 500 | 2010 | 14 | 25.05. | 31.08. |
| 2.2 | 1. + 2.<br>3. 150 ml V15 all 5-6 days | exact | 400 | 2011 | 13/23 | 13.05. | 05.08. |
| 2.3 | 1. + 2. | field | 400 | 2011 | 13 | 13.05. | 05.08. |
| 3 | 1. + 2. | field | 500 | 2010 | 6 | 09.06. | 14.07 |
| 4 | 1. + 2. | field | 600 | 2010 | 7 | 04.06. | 24.08. |
| 5.1 | 1. + 2.<br>3. 261 ml V42 | field | 360 | 2011 | 12 | 11.05. | 26.07. |
| 5.2 | 1. + 2. | field | 360 | 2011 | 13 | 11.05 | 03.08. |
| 6 | 1. + 2. | field | 900 | 2011 | 14 | 14.05. | 08.08. |

[1)] field trail in block design or exact field trail with 4 replications (exact)

In all plots, except plot 4 in Baden-Württemberg 2010, application of CpGV was used in combination with mating disruption with "Isomate OFM Rosso".

For population monitoring pheromone traps were installed and controlled weekly (data not shown, Rueß et. al 2011, 2012).

The infestation of the fruits was assessed at two times depending on flight peaks of plum moths; the assessment was made in June or at beginning of July (1st generation), the second shortly before harvest (2nd generation) by counting the rate of infested plums in treated and untreated plots (minimum 1000 fruits per plot). For the 1st generation the

infested fruits were counted on the trees. For the infestation assessment of the 2nd generation the fruits were cut open.

CpGV infection of cadavers and living larvae from field trails were tested by PCR amplification at the Geisenheim Reasearch Center (Reineke 2010, Rueß et al 2010).

## Results

The results of the field trials with CpGV V15 in comparison to untreated control are reported here. In the trials 1.1 - 1.3 (Table 3) the infestation rate of the 2nd generation 2010 raised up to more than 10% in all three plots, in untreated and treated plot, except for treated plot 'Presenta' (plot 1.3), where the infestation was 5.2%.

First generation 2011 started with low infestation levels and did not increase in the 2nd generation. The efficacies ranged from negative to 53%, except for the plot 1.2. (2nd gen.) with 84% efficacy. The low efficacy was partly caused by very low infestation levels. An exception was the negative efficacy in plot 1.2 at 2nd generation 2010 in general very high infestation level.

Table 3: Infested plums (%) of the 1st and 2nd generation and efficacies (ABBOTT) at Wackernheim, 2010 and 2011

| **1. Wackernheim** | | | | Infestation in % | | |
|---|---|---|---|---|---|---|
| **No** | **variety** | **Year** | **Generation / Date** | **Untreated control** | **CpGV V15** | **Efficacy [%])** |
| 1.1 | Top (old). | 2010 | 1st | 5.2 | 3.5 | **32** |
| | | | 2nd | 13.6 | 12.1 | **11** |
| 1.1 | | 2011 | 1st (29.06.) | 2.9 | 2.0 | **29** |
| | | | 2nd (25.08.) | 0.9 | 0.6 | **31** |
| 1.2 | Top (new) | 2010 | 1st | 8.2 | 6.0 | **26** |
| | | | 2nd | 12.8 | 15.2 | **-19** |
| 1.2 | | 2011 | 1st (29.06.) | 3.0 | 2.3 | **24** |
| | | | 2nd (25.08.) | 0.8 | 0.1 | **84** |
| 1.3 | Presenta | 2010 | 1st | 0.5 | 0.3 | **50** |
| | | | 2nd | 11.1 | 5.2 | **53** |
| 1.3 | | 2011 | 1st (29.06.) | 0.1 | 0.1 | **1** |
| | | | 2nd (25.08.) | 0.0 | 0.1 | **-** |

Nearly the same situation was found in Grafschaft (Table 4). After moderate infestation rates in the 1st generation 2010 infestation increased to more than 10% (trial 2.3.) and up to 37% (trial 2.2) in 2nd generation. The efficacies ranged from 10 to 74%. In 2011 Grafschaft (trial 2.3) both generations caused in both treatments very low infestation levels of about 1%, resulting in low or even negative efficacies.

In Plot 3 (Table 4) in 2010, the cultivar 'Cacaks Schöne' had moderate infestation levels of 5.8% (1st gen. control) and 3.3% (1st gen. V15) with 42% efficacy, and of 6.5% (2nd gen. control) to 2.4% (2nd gen. V15) with an efficacy of 64%.

In the late variety 'Presenta' (plot 3, Table 4), the infestation levels were 4.6% (1st gen., control) and 3.9% (1st gen. V15) as well as 17.5% (2nd gen., control) and 16.4% (2nd gen. V15) with efficacies of 16 and 6%, respectively.

Table 4: Infested plums (%) of the 1st and 2nd generation and efficacies (ABBOTT) at Grafschaft and Kettig, 2010 and 2011

| | | | | Infestation [%] | | |
|---|---|---|---|---|---|---|
| **No** | **Site** | **Year** | **Generation** | **Untreated control** | **CpGV V15** | **Efficacy [%] (ABBOTT)** |
| 2.2 | Grafschaft | 2010 | 1st | 4.4 | 2.3 | **48** |
| | | | 2nd | 37.3 | 22.9 | **39** |
| 2.3 | Grafschaft | 2010 | 1st | 3.0 | 0.8 | **74** |
| | | | 2nd | 13.0 | 11.7 | **10** |
| 2.3 | Grafschaft | 2011 | 1st (04.07.) | 0.7 | 0.7 | **3** |
| | | | 2nd (30.08.) | 1.2 | 1.3 | **-4** |
| 3 | Kettig / C. Schöne | 2010 | 1st | 5.8 | 3.3 | **42** |
| | | | 2nd | 6.5 | 2.4 | **64** |
| 3 | Kettig / Presenta | 2010 | 1st | 4.6 | 3.9 | **16** |
| | | | 2nd | 17.5 | 16.4 | **6** |

In the plot 4 (Table 5) in 2010, the infestation of variety 'Katinka' was 4.8% (control) and 4.7% (treated) in 1st generation (efficacy 2%). In the 2nd generation, the infestation level was 0.6% (control) and 0.3% (treatment). The infestation of the varieties 'Elena' and 'Presenta' was low in 1st generation. It increased in the 2nd generation to moderate level (4.1%) for the variety 'Elena' and to 8.9% for 'Presenta'. Efficacies were found to be 9% to 40% (Table 5). The early ripening cultivars 'Ruth Gerstetter', 'Cacaks Schöne', 'Valjevka' and 'Cacaks Fruchtbare' were only assessed for the 1st generation. The infestation levels were moderate and the efficacies were 17%, 3% and 23%, respectively. Only for 'Cacaks Fruchtbare' a negative efficacy was assessed.

Table 5: Infested plums (%) of the 1st and 2nd generation and efficacies (ABBOTT) plot 4, Uhlbach, 2010

| **4. Uhlbach** | | **Infestation [%]** | | | |
|---|---|---|---|---|---|
| **Variety** | **Generation Date of harvest** | **Untreated control** | **CpGV V15** | **Efficacy [%]** | **Fruit load** |
| Ruth Gerstetter [1)] | 1st (08.07.) | 2.3 | 1.9 | **17** | low |
| C. Schöne [1)] | 1st | 2.1 | 2.0 | **3** | low-medium |
| Valjevka [1)] | 1st | 3.5 | 2.7 | **23** | low-medium |
| C. Fruchtbare [1)] | 1st | 2.7 | 3.2 | **-19** | low-medium |
| Katinka | 1st (08.07.) | 4.8 | 4.7 | **2** | low-medium |
| | 2nd (29.07.) | 0.6 | 0.3 | **48** | |
| Elena | 1st (08.07.) | 1.2 | 0.8 | **34** | medium-high |
| | 2nd (16.09.) | 4.1 | 2.9 | **29** | |
| Presenta | 1st (08.07.) | 1.1 | 1.0 | **9** | low |
| | 2nd (16.09.) | 8.9 | 5.3 | **40** | |

[1)] early varieties, no 2nd assessment

In the plot 5.2 (Table 6) the early variety 'Herman' (1st gen.) and the latest variety 'Presenta' (2nd gen.) caused high rates of damaged fruits with 10.4 and 12.3% in the control. The rate of damaged fruits in the treatment with V15 was 2% at the variety 'Herman' and 20.1% at the variety 'Presenta'. The efficacies were 80 and -63%. All other efficacies obtained in this plot are between these two numbers.

Table 6: Infested plums (%) of the 1st and 2nd generation and efficacies (ABBOTT) at plot 5.2, Sulzburg, 2011

| **5.2 Sulzburg** | | **Infestation** [%] | | | |
|---|---|---|---|---|---|
| **Variety** | **Generation Date of harvest** | **Untreated control** | **CpGV V15** | **Efficacy [%] (ABBOTT)** | **Fruit load** |
| Herman [1)] | 1st (16.06.) | 10.4 | 2.0 | **81** | medium |
| C. Frühe [1)] | 1st (16.06.) | 7.7 | 4.8 | **37** | medium |
| C. Schöne | 1st (16.06.) | 1.7 | 1.4 | **20** | medium |
| | 2nd (07.07.) | 1.3 | 1.8 | **-38** | |
| Valjevka | 1st (16.06.) | 3.7 | 3.4 | **7** | medium |
| | 2nd (01.08.) | 5.6 | 6.9 | **-23** | |
| Elena | 1st (16.06.) | 1.3 | 1.5 | **-9** | very high |
| | 2nd (12.08.) | 5.3 | 6.5 | **-24** | |
| Presenta | 1st (16.06.) | 0.9 | 0.5 | **46** | medium-high |
| | 2nd (12.08.) | 12.3 | 20.1 | **-63** | |

[1)] early varieties, no 2nd assessment

In 2011 trial 6 (Table 7) the 1st generation caused in both treatments very low infestation levels of about 1%, resulting in efficacies of 50 and 44%. The rate of infested plums at the variety 'Hanita' in 2nd generation was very low with 0,3% control and 0,4% V15. The efficacy was a negative one. The latest variety in this trial 'Elena' with rates of damaged fruits of 9% control and 4.8% V15 of 2nd generation had an efficacy of 47%.

Table 7: Infested plums (%) of the 1st and 2nd generation and efficacies (ABBOTT) at plot 6, Backnang, 2011

| **6 Backnang** | | | **Infestation** [%] | | | |
|---|---|---|---|---|---|---|
| **Variety** | **Year** | **Generation Date of harvest** | **Untreated control** | **CpGV V15** | **Efficacy** [%] (**ABBOTT**) | **Fruit load** |
| Hanita | 2011 | 1st (21.06.) | 2.0 | 1.0 | **50** | high |
| | | 2nd (05.08.) | 0.3 | 0.4 | **-33** | |
| Elena | 2011 | 1st (21.06.) | 1.0 | 0.6 | **44** | very high |
| | | 2nd (24.08.) | 9.0 | 4.8 | **47** | |

The application of CpGV V15 as an additional treatment (2010, plot 2.1) at a concentration of 500 ml/ha/mch did not show a higher efficacy in any case (Table 8). The numbers of damaged plums was very high in general in the 2nd generation.

Table 8: Infested plums (%) of the 1st and 2nd generation and efficacies (ABBOTT) at plot 2.1, Grafschaft, 2010 (exact)

| | | | **Infestation** [%] | | | | |
|---|---|---|---|---|---|---|---|
| **No** | **Site** | **Generation** | **Untreated control** | **V15 150 ml** | **Efficacy [%]** | **V15 500 ml** | **Efficacy [%] (ABBOTT)** |
| **2.1** | Grafschaft | 1st | 6.9 | 6.3 | **9** | 5.9 | **14** |
| | | 2nd | 29.8 | 19.5 | **35** | 22.5 | **25** |

In 2011 another formulation of CpGV (termed V42) was used in plots 2.1 and 5.1 (Tables 9 and 10). In plot 2.1 an efficacy of 47% was obtained in 1st generation, but in 2nd generation it was negative.

Table 9: Infested plums (%) of the 1st and 2nd generation. and efficacies (ABBOTT) at plot 2.1, Grafschaft, 2011 (exact)

| | | | Infestation [%] | | | | |
|---|---|---|---|---|---|---|---|
| **No** | **Site** | **Generation** | **Untreated control** | **V15** | **Efficacy [%]** | **V42** | **Efficacy [%]** |
| **2.1** | Grafschaft | 1st (04.07.) | 4.6 | 4.7 | **-3** | 2.4 | **47** |
| | | 2nd (30.08.) | 3.8 | 1.8 | **52** | 4.4 | **-14** |

On plot 5.1 (Table 10) V42 showed higher efficacies than V15 at the varieties 'Herman' with 58% and 'Cacaks Schöne' with 80% (both at 1st generation).

In the 1st generation the infestation rates of the middle-early ripening variety 'Valjevka' were 4.2% (treatment V42), 3% (untreated plot) and 2.3% (V15). The infestation rates of the 2nd generation from 'Valjevka' raised up to a very high level of 17% in untreated plot. So efficacies of 21% (V15) and 23% (V42) were realized, but the damage in both treated plots was unacceptable high considering the fact, that 12 applications were done. 'Ersinger' (2nd gen.) treated with V15 as well as 'Cacaks Schöne' (2nd gen.) and 'Ersinger' (1st and 2nd gen.) treated with V42 showed on low infestation levels negative efficacies.

Table 10: Infested plums (%) of the 1st and 2nd generation and efficacies (ABBOTT) at plot 5.1, Sulzburg, 2011

| **5.1 Sulzburg** | | **Infestation [%]** | | | | | |
|---|---|---|---|---|---|---|---|
| **variety** | **Generation** | **Untreated control** | **V15** | **Efficacy [%]** | **V42** | **Efficacy [%])** | **Fruit load** |
| Herman | 1st (16.06.) | 5.0 | 4.2 | **17** | 2.1 | **58** | Medium-high |
| | 2nd | Fruits still harvested | | | | **--** | |
| C. Schöne | 1st (16.06.) | 2.7 | 0.8 | **71** | 0.6 | **80** | High |
| | 2nd (06.07.) | 0.5 | 0.1 | **80** | 0.9 | **-80** | |
| Ersinger | 1st (16.06.) | 2.2 | 1.4 | **35** | 2.3 | **-5** | Very high |
| | 2nd (06.07.) | 0.1 | 0.5 | **-400** | 0.3 | **-200** | |
| Valjevka | 1st (16.06.) | 3.0 | 2.3 | **25** | 4.4 | **-47** | high |
| | 2nd (08.08.) | 17.0 | 13.5 | **21** | 13.1 | **23** | |

Another treatment with narrow application intervals of 5 to 6 days between the applications of V15 was tested in comparison to the normal interval of 8 to 10 days (Table 11). For the variety 'Ortenauer' in 1st generation the efficacy of V15 sprayed every 5-6 days was higher than V15 sprayed every 8-10 days. In 2nd generation the opposite results were observed: Applications every 8-10 days showed a higher efficacy. At variety 'Hauszwetschge' (2nd gen.) a similar efficacy of both treatments of 32 and 28% was observed.

Table 11: Infested plums (%) of the 1st and 2nd generation and efficacies (ABBOTT) at plot 2.2, Grafschaft, 2011 (exact)

| **2.2 Grafschaft** | | **Infestation [%]** | | | | |
|---|---|---|---|---|---|---|
| **Variety** | **Generation** | **Untreated control** | **V15 5-6 d** | **Efficacy [%]** | **V15 8-10 d** | **Efficacy [%])** |
| Ortenauer | 1st (04.07.) | 2.8 | 2.0 | **29** | 2.5 | **11** |
| | 2nd (22.08.) | 4.2 | 2.8 | **34** | 2.2 | **48** |
| Hauszwetschge | 2nd (22.08.) | 3.6 | 2.4 | **32** | 2.6 | **28** |

## Discussion and Conclusion

The field trials with CpGV V15 showed heterogeneous results with efficacies up to 50% and exemptions up to 80% on low infestation levels, mainly during 1st generation. Also on moderate to high infestation levels efficacies of 50% could be reached in 1st and 2nd generation. But there are also negative efficacies in both generations. The average efficacy over all results in both years is 10%.One reason for various efficacies could be the short time, larvae of plum moths are crawling on the surface of the plum fruit and the behaviour to penetrate the fruit through the egg, so that the uptake of CpGV maybe is not high enough for a lethal dose. In case of abundant fruiting, the wetting of the single fruit with virus maybe not sufficient. A higher concentration with 500 ml/ha/mch did increase the efficacy for 1st generation, but not for the 2nd generation. It has to be considered that the used concentration of 150 ml/mch is higher than in the regulation of Codling moth. Also the use of CpGV V42 did not show a clear better result than V15. To set the applications closer (all 5-6 days) intervals did not raise the efficacies in all cases, but raised the amounts of application from 12 to 23. As a consequence CpGV is not recommended for the use of plum moths control

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# Assessment of disease susceptibility and fruit quality of 28 peach cultivars

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## Abstract

*The susceptibility to diseases is not considered as a key-criterion in peach breeding programs. Even if the turn-over of peach cultivars is important, suitability for organic and low-input systems remains unknown for most of the cultivars. A first program has been carried out from 2001 to 2008 to assess peach leaf curl, powdery mildew and aphid susceptibilities of 18 cultivars in an experimental station. Fruit quality was also assessed. A high variability in leaf curl* (Taphrina deformans) *and powdery mildew* (Sphaerotheca pannosa *var* persicae) *susceptibilities and fruit quality was observed among the cultivars. Reine des Vergers and Belle de Montélimar were identified as relevant cultivars for specific markets, because of low susceptibilities to diseases and high fruit quality. In 2009, they have been included in a second assessment program among 10 other cultivars, which is still going on. The variability of peach leaf curl susceptibility between cultivars was strongly influenced by the disease pressure. In 2011, we identified different levels of tree recovery among the cultivars after severe damages caused by peach leaf curl and* Monilinia *spp.*

**Keywords:** cultivar susceptibility, peach, leaf curl, *Monilinia spp.*, powdery mildew

## Introduction

The choice of the peach cultivar is one of the keystones to set up less input-dependent orchards. During the last decades, breeders have mainly selected peach cultivars on yield and fruit appearance (Byrne, 2002). As a consequence, most of the commercial cultivars are highly susceptible to peach diseases which are difficult to control in organic and low-inputs orchards. Nowadays, the choice of peach cultivars is large but reliable data concerning their suitability to organic and low-inputs systems are rare.

The lack of reliable information on peach cultivars' susceptibility to diseases and pests is partially explained by several methodological bottlenecks. Indeed, the choice of the experimental design strongly affects the assessment results. Random and block tree distribution can affect the spatial dissemination of diseases in different ways. The pest and disease management is also a key-point, because the optimal date for spraying is not necessarily the same for the range of tested cultivars. Moreover, the difference in susceptibility to diseases between cultivars can be very narrow. As many factors are difficult to be controlled by the experimental design and management, misleading results could be observed.

To help the growers in their choice of peach cultivars, the susceptibility to main diseases of a wide range of peach cultivars was assessed in two field trials.

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## Material and Methods

**Trial 1.**

In 2001, 18 cultivars were planted at the INRA Gotheron experimental station in the Rhône Valley production area, in the South-East of France. Some promising old cultivars and parents coming from INRA breeding nursery with low susceptibility to diseases were selected for the experiment (Warlop *et al.*, 2006). All cultivars were own-rooted and planted at 1.5 m distance in a single row in a stony loam soil. Water was supplied with a microjet irrigation system. Each cultivar was represented by one tree. The commercial cultivar Summergrand was used as reference for high leaf curl susceptibility.

Mineral oil was applied in years when aphid (mainly *Brachycaudus persicae*) pressure was high (table 1). A sulphur-based treatment was applied in 2003 –to control powdery mildew (*Sphaerotheca pannosa* var *persicae*) and avoid a heterogeneous growth of the young trees. No other fungicides and insecticides were applied during the period 2001-2008. Leaf curl (*Taphrina deformans*) and powdery mildew (*S. pannosa* var *persicae*) symptoms were observed each year in April-May and in May-June, respectively. The severity of damages was ranked in 3 classes: high, medium, low. Organoleptic fruit quality was assessed by one score ranging from 1 (low quality) to 10 (very good quality) during tasting sessions.

**Trial 2.** In 2008 and 2009, 12 cultivars (including the two most promising cultivars selected in trial 1) were planted at the same experimental station in an adjacent plot. All cultivars were grafted on GF305 rootstock and 10 trees per cultivar were.planted at 4 x 4 m distance randomly-located in the plot. The two most promising cultivars from trial 1, Belle de Montélimar and Reine des Vergers, had to be grafted again in August 2009 and 2010 because of bud grafting failure. Water was supplied with a microjet irrigation system. Bénédicte cultivar was the reference for low leaf curl susceptibility. Mineral oil completed with localized chemical treatments was applied when aphid pressure was high (table 1). Copper and sulphur-based treatments were restricted to critical situations (1) to limit leaf curl damages on young grafted trees and (2) to limit interactions between diseases and permit the assessment of all studied diseases.

Leaf curl and powdery mildew symptoms were observed twice a month on leaves as long as they occurred (table 2). Flower necroses caused by *Monilinia spp.* symptoms (mainly *Monilinia laxa*) were observed after bloom. Each year, the results are given for the date when severity scores were at maximum. Statistical analyses were computed using Statgraphics plus 5.1 software (Statgraphics plus 5.1, Manugistics, Rockville, MD, USA). The level of significance was set at 5% for all the statistical tests performed. Anova and Bonferroni post-hoc tests were used to assess the differences between cultivars.

Table 1: Pesticide applications against aphids and diseases in trial 1 and 2. Dose of copper and sulphur per treatment is indicated in brackets. Sanitary methods (*e.g.* decayed fruit removing) were also realised.

| | **Trial 1** | **Trial 2** |
|---|---|---|
| **Plot management** | Organic farming | Conventional / very low inputs |
| **Disease treatments** | sulphur (6kg/ha): 24/04/2003 | copper (0.7kg/ha): 25/02/2010; 10/05/2010<br>sulphur (6.0kg/ha): 13/08/2010 ; 03/05/2011 ; 24/06/2011 |
| **Aphid treatments** | mineral oil : 26/02/2003 ; 04/03/2004 ; 15/03/2006 ; 01/03/2007 ; 21/02/2008 | mineral oil: 17/03/2010 ; 21/02/2011<br>localized treatments: 24/04/2009 ; 27/04/2010 ; 10/05/2010 ; 18/04/2011 ; 27/05/2011 |

Table 2: Severity classes for the assessment of leaf curl and powdery mildew symptoms observed on leaves (expressed in percentage of leaf damage in the whole crown) and *Monilinia spp*. symptoms observed on flowers (expressed in percentage of flower necrosis in the whole crown) in trial 2.

| Infection class | Leaf curl and powdery mildew damaged leaves (%) | *Monilinia spp.* damaged flowers (%) |
|---|---|---|
| 0 | 0% | 0% |
| 1 | 1 – 5% | 1 –10% |
| 2 | 6 – 30% | 11 – 25% |
| 3 | 31 – 60% | 26 – 50% |
| 4 | 60 –100% | 51 – 100% |

## Results

### Trial 1

Three different groups of cultivars could be identified from the 6-year data on leaf curl, powdery mildew and fruit quality in trial 1 (table 3). Cultivars in group A had a low to medium susceptibility to leaf curl and a high fruit quality. Reine des Vergers and Belle de Montélimar were found to be the most promising cultivars for these criterions. In group B, cultivars were highly susceptible to leaf curl, and their fruit quality was interesting. In group C, the high disease susceptibility and/or the low fruit quality made them unsuitable for commercial production.

Table 3: Leaf curl and powdery mildew susceptibility (Hi: high, Me: medium, Lo: low) and organoleptic fruit quality of 18 cultivars. Values are the mean susceptibility and fruit quality scores from 2003 to 2008 (1: low quality, 10: very good quality). *N* = 1 tree / cultivar.

| Cultivar | Group | Harvest | Leaf curl | Powdery mildew | Fruit quality |
|---|---|---|---|---|---|
| Belle de Montélimar | A | 29/8 | Lo | Me | 8 |
| Reine des Vergers | | 01/9 | Lo | Lo | 8 |
| Mme Guilloux | | 28/8 | Lo | Hi | 6 |
| GF 305 | | 25/8 | Lo | Hi | 6 |
| GF305-1 × S3928 | | 25/8 | Lo | Me | 6 |
| (S3928 × GF305-1-2)[6] | | 27/7 | Me | Me | 5 |
| 5745[2] | | 25/8 | Lo | Me | 4 |
| Surpasse Amsden | | 10/7 | Me | Hi | 5 |
| Combet | | 02/9 | Me | Me | 8 |
| Summergrand | B | 07/8 | Hi | Hi | 5 |
| Genadix 4 | | 10/7 | Hi | Me | 7 |
| Tournier | | 21/7 | Hi | Hi | 7 |
| Véraud | | 27/7 | Hi | Me | 8 |
| Précoce de Hale | | 27/7 | Hi | Me | 7 |
| 2240 : 23 : 2 × S4577 | C | 28/8 | Me | Hi | 2 |
| Génard | | 03/8 | Hi | Hi | 4 |
| Marnas | | 22/8 | Lo | Me | 1 |
| (S3747 × GF305-1-1)[2] | | 27/7 | Me | Hi | 2 |

**Trial 2**

In 2009, a high variability in leaf curl development was observed and four groups could be statistically identified (figure 1a). This variability was less important in 2010 and 2011 (figure 1b, 1c). Differences in susceptibility between cultivars across years could be related to leaf curl pressure which was moderate in 2009, high in 2010 and very high in 2011. Bénédicte remained the less susceptible cultivar during the observation period. The low susceptibility of Belle de Montélimar and Reine des Vergers observed in 2011 confirmed the previous results of trial 1 (table 3).

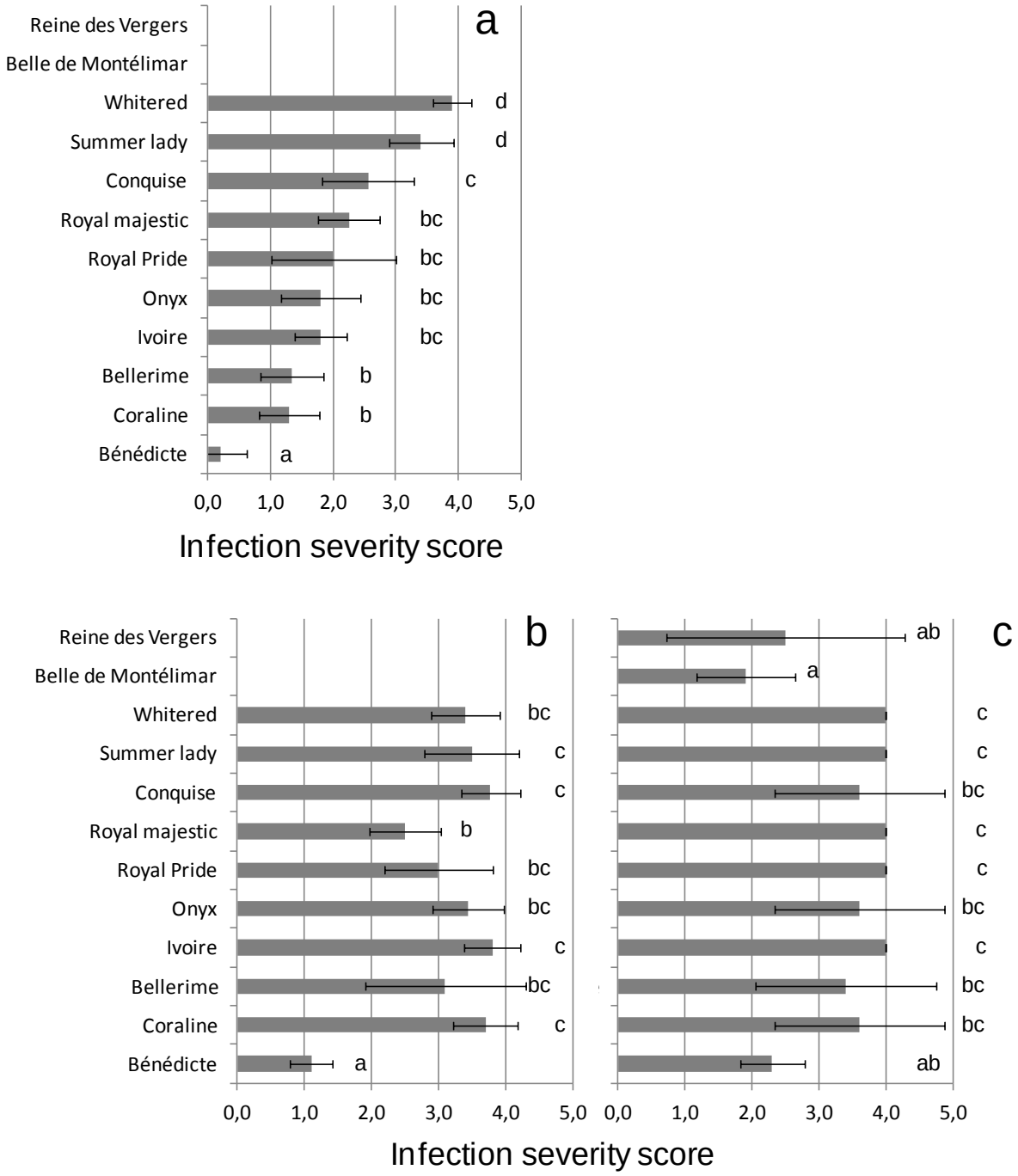


Figure 1: Mean leaf curl severity scores on 22 April 2009 (a), 6 May 2010 (b) and 21 April 2011 (c) in trial 2. Reine des Vergers and Belle de Montélimar cultivars were not observed in 2009 and 2010. Standard deviations are indicated by bars. Results of the Bonferroni test are indicated by letters.

*Monilinia spp.* damages on flower ranged from 0.4 to 2.0 in 2010 and 1.5 to 2.0 in 2011 (figure 2), no damage was observed in 2009. *Monilinia* spp. symptoms were not observed on Reine des Vergers and Belle de Montélimar cultivars in 2010 and 2011 because grafts were not developed enough. Statistical differences were displayed in 2010 only. In 2011, climatic conditions were very favourable to *Monilinia spp.* contamination at bloom for all cultivars.

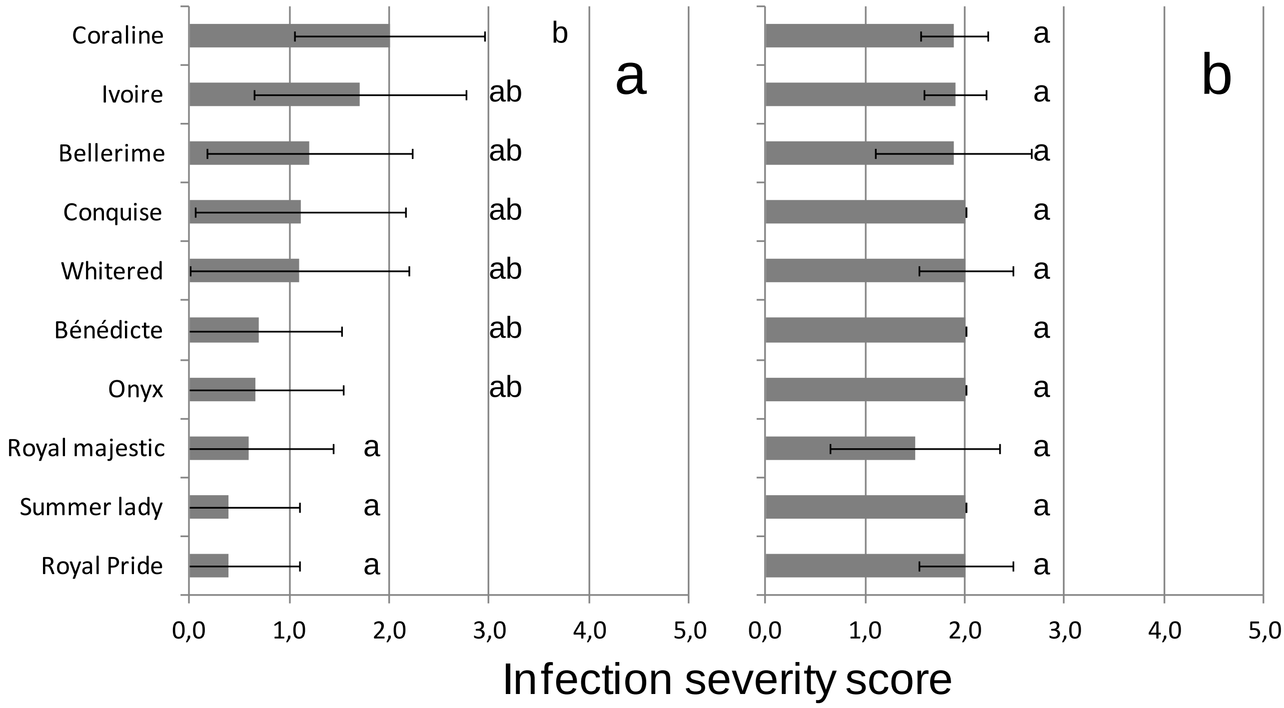


Figure 2: Mean *Monilinia spp.* severity scores on 12 May 2010 (a) and 21 April 2011 (b) in trial 2. Standard deviations are indicated by bars. Results of the Bonferroni test are indicated by letters.

Leaf curl and *Monilinia spp.* damages were very high in 2011. They caused partial or total defoliation of the trees. To express the ability of the cultivars to recover from a severe disease pressure, we have represented the proportion of defoliated shoots just after main contaminations and at the end of the season (figure 3).

The calculated ratio evaluates the recovery of the cultivar after high disease damages by measuring newly-emitted vegetative growth. Damages on Summer Lady, Royal Pride and Whitered cultivars were high and these cultivars hardly withstood 2011 disease pressure. Bellerime, Onyx, Ivoire and Coraline expressed a better ability to produce new leaves after a defoliation event.

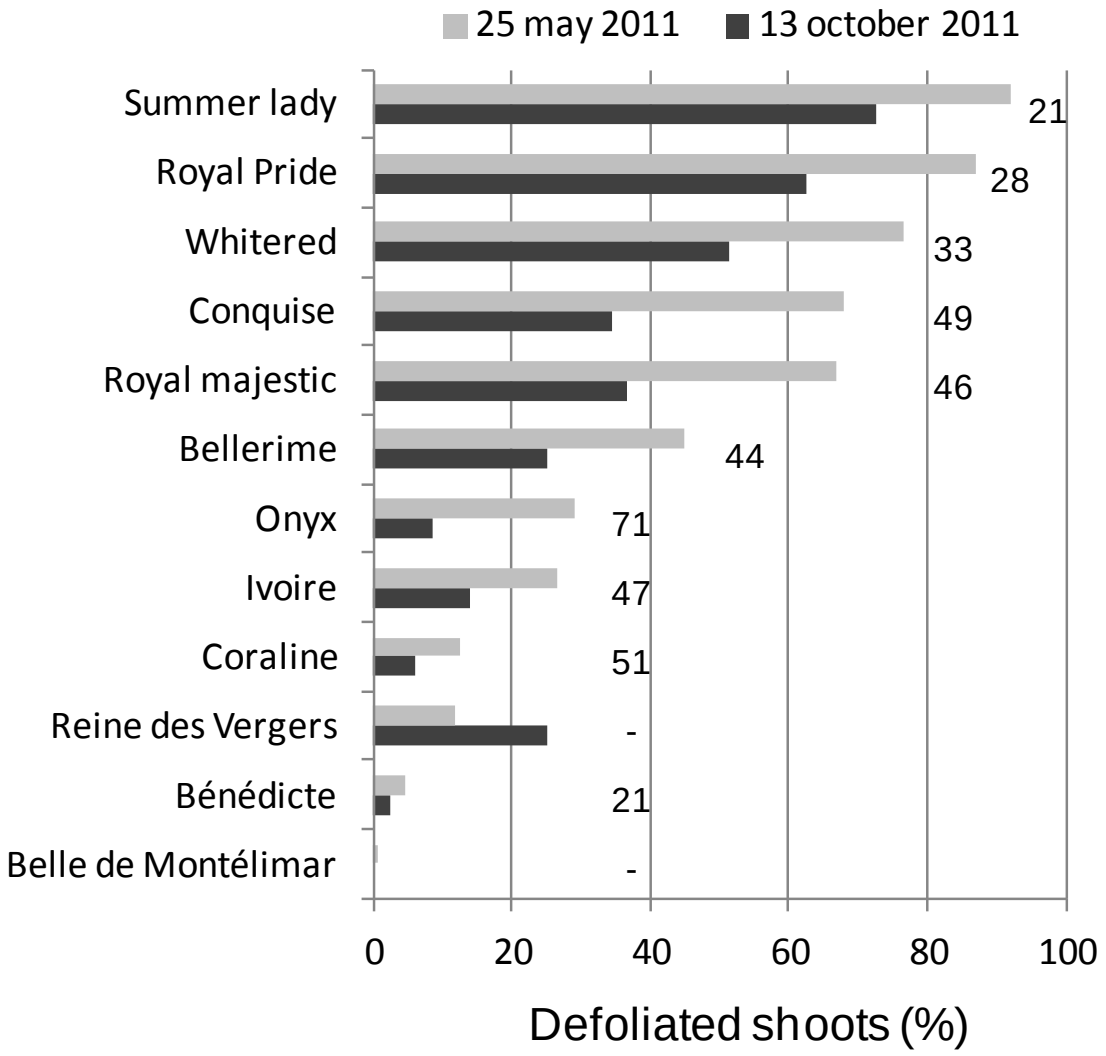


Figure 3: Proportion (%) of defoliated shoots on 25 May 2011 (called *x*) and 13 October 2011 (called *y*). Numbers in the right part of the figure are computed as *(x-y)*100 / x* to evaluate the recovery (measured by vegetative growth) of the cultivars. Main severe damages of leaf curl and *Monilinia spp.* occurred before 25th May 2011.

Powdery mildew damages were low in 2010 and severity scores ranged from 0.0 to 1.0 (figure 4a). Conversely, severity scores ranged from 3.5 to 4.0 in 2011. Because defoliation caused by leaf curl and *Monilinia spp.* was very high for some cultivars (figure 4b), powdery mildew assessment was sometimes difficult or impossible (*e.g.* Summer Lady).

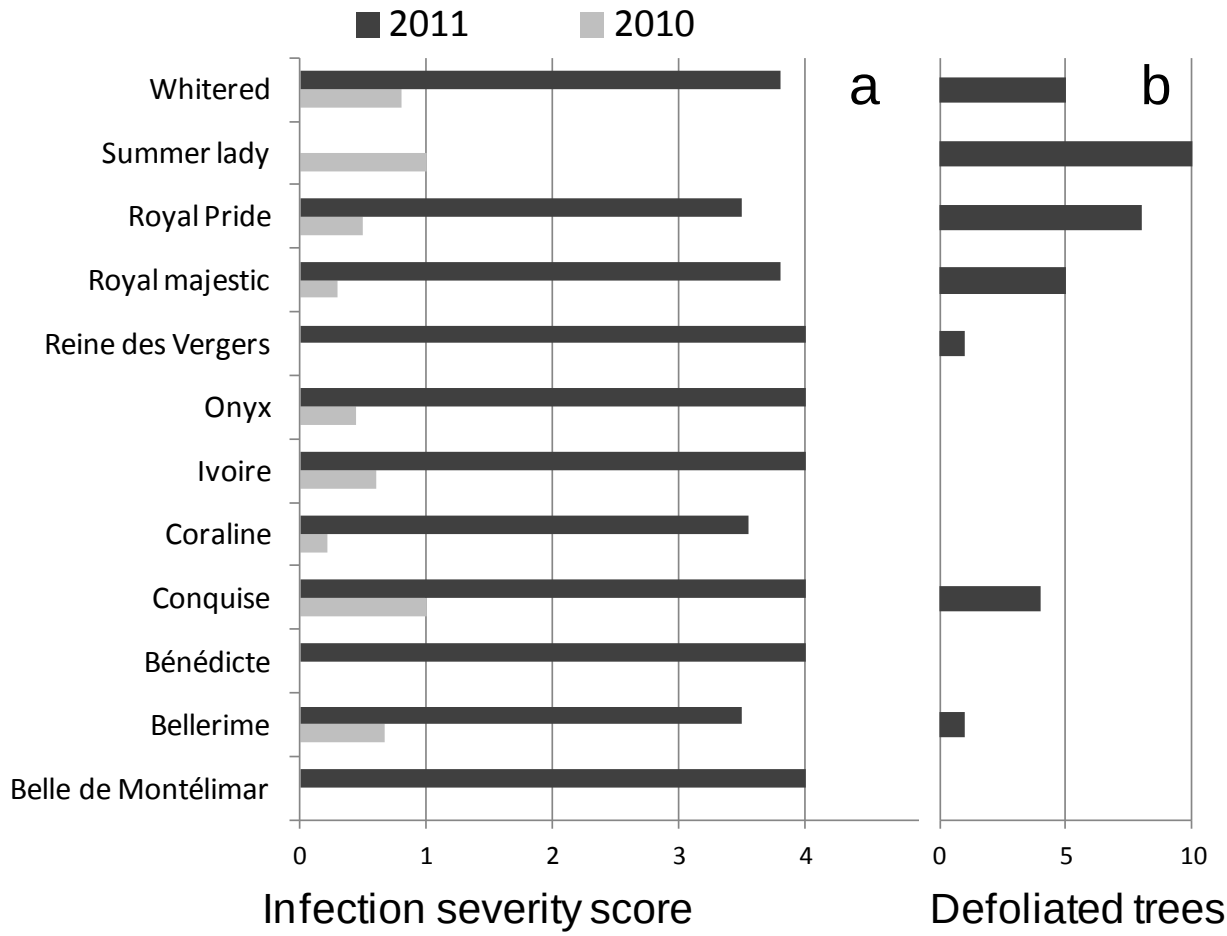


Figure 4: Mean powdery mildew severity scores on 23 June 2010 and 6 June 2011 in trial 2 (a). The number of defoliated trees (more than 95% without leaves) out of 10 trees on 6 June 2011 is represented in (b).

## Discussion

This study shows that these two experimental trials are complementary: trial 1 is more suitable for a screening purpose whereas trial 2 permits a more accurate analysis of the differences in susceptibility between cultivars. When considering a set of agronomical features such as the susceptibility to several diseases and fruit quality, the identification of suitable cultivars for a given context becomes a long-term task. In trial 1, 29 cultivars were initially planted; usable data were available for 18 of them (because of tree mortality) and only 2 cultivars were finally selected as suitable for low-input orchards.

The experimental plot design (block *vs.* randomized), the methodology of observation (shoot *vs.* whole tree as a sample unit) and the strategy to control (or not) pests and diseases in the experimental plot raise many methodological questions. On the one hand, choosing a no treatment strategy permits to observe the cultivar behavior. However, the development of several diseases with possible interactions (e.g. leaf curl x powdery mildew) makes the susceptibility assessment tricky. Moreover, severe damages occurring one year can affect the tree growth of susceptible cultivars, which makes a long-term comparison difficult. On the other hand, applying treatments on a range of cultivars is not satisfactory in a random plot because the optimum spraying date depends on the cultivar.

The variability of diseases pressure and the probability for flower contamination needs to be considered as well. Indeed, we have observed that the differences in leaf curl susceptibility between cultivars decreased when leaf curl pressure increased (figure 1). The link between diseases pressure and the gradient of susceptibility between cultivars is unknown. Relations between these two variables could be linear or with threshold effects, which makes the experimental assessment of multi-disease susceptibility a hard task. For *Monilinia spp.*, we have observed that climatic conditions were not always fulfilled for a similar contamination of all the cultivars studied. When contaminations partially affect the cultivars, comparisons become difficult.

In 2011 in trial 2, no fruits were harvested except on Bénédicte cultivar, which demonstrates the low hardiness of the studied cultivars when disease control is strictly limited. As the trees of the cultivars Reine des Vergers and Belle de Montélimar were one or two years younger, the observations have to be continued.

Several studies have assessed the susceptibility to pests and diseases of plum cultivars (*e.g.* Garcia-Galavis et al., 2009) and peach cultivars (*e.g.* Assmann et al., 2010; Ivascu et al., 2006). However, methodological considerations (such as interactions treatment x cultivar) are not tackled. Therefore, there is a need for studies dealing with methodological considerations in order to provide reliable results.

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# The impact of flower and fruit thinning methods on yield, diseases and fruit quality of sweet cherries (*Prunus avium*) under organic growing conditions

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**Abstract**

*The aim of this project was to find out if fruit thinning in cherry production can contribute to preventing diseases and increasing fruit quality. Therefore, between 2009 and 2011, the impact of various thinning methods on yield, diseases and fruit quality was tested in organically managed cherry orchards at the experimental farm of BOKU. In 2009, the pruning of flower branches during blossom reduced yield and growth, but had no effect on fruit size and fruit quality of mainly early ripening cultivars. In 2010, a portable thinning machine (Electroflor) and a method to remove young cherries at the stage of pea size by scissors were tested. Furthermore, between beginning and full flowering we treated twice with wettable sulphur (4%), lime sulphur (3%) as well as with IP- standard ATS (1.8%). Additionally to the thinning effect, the impact on fruit size, fruit quality and diseases (*Monilinia ssp.*) was assessed. Some treatments (Electroflor, manual thinning of young fruits, lime sulphur, ATS) showed a strong thinning effect and an increase of fruit size. Nevertheless, yield losses due to the lower number of fruits caused by thinning could only partly be replaced by higher fruit size. From the mechanical variants, the Electroflor is more suitable for farmers than hand thinning of flowers or fruits because of lower costs. The treatments with lime sulphur and wettable sulphur showed a reducing effect on flower infestations with* Monilinia laxa*. We could not find any influence on fruit diseases and internal fruit quality due to the treatments.*

*In an orchard where we used the Electroflor in 2010 and 2011 an increasing effect on the fruit weight and also some significant changes in fruit quality characteristics could be found.*

**Keywords:** sweet cherry, flower thinning, mechanical thinning, fruit quality, *Monilinia laxa*

## Introduction

Organic sweet cherry production in Austria has so far been a niche with about 20 ha of production area (Ama, 2011), however, there has been a rising demand on the market. The control of the cherry fruit fly (*Rhagoletis cerasi*) is one limiting factor of production, but especially with the use of self fertile cultivars in combination with weak rootstocks in the past years fruit size has to be improved. Thinning could also help reducing fruit rots caused by *Monilinia ssp.,* which is very difficult to control, especially for organic growers.

Compared to other fruits like the apple, there have so far only been a few experiences with the thinning of cherries, especially in organic growing. Mechanical removing of flowers at the beginning of blossom could reduce the fruit number and fruit rot, at the same time increase the weight per fruit (Weber, 2009).

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Repeated treatments with lime sulphur during full blossom resulted in a reduction of yield and an increase of fruit size (Kelderer & Zago, 2010). Also flower treatments with sulphur showed a strong thinning effect, but no influence on fruit size (Hangarter, 2006).

Therefore, our aim was to examine some new thinning methods for cherries in field trials.

## Material and methods

The field trials took place in two organically managed orchards of the research garden of the Institute in the north east of Vienna. The climate is pannonian, warm and dry, with usually less than 600 mm of annual precipitation.

In orchard A, a total of thirteen sweet cherry cultivars (‘Bigarreau Burlat’ type 1, ‘Bigarreau Burlat’ type 2, ’Bigarreau Moreau’ type 1, ’Bigarreau Moreau’ type 2, ’Early Lory’, ’Hybrid 222’, ’Merchant’, ’Merton Premier’, ’Valeska’, ’Sweetheart’ and the local cultivars ’Langstielige’, ’Marzer Kirsche’ and ’Schachl’), was planted in autumn 2003 on Gisela 5 rootstock and distributed among 8 blocks (with single trees as repetitions for each variety) in a completely randomised system. Every year the tested thinning variants were applied in 4 blocks on all cultivars, the other 4 blocks served as untreated control.

In 2009 (14$^{th}$ April), during full blossom (BBCH 65) of most of the cultivars, 20-25% of the flower branches were removed by pruning in the treated blocks. The weather conditions during flowering were ideal in that year.

In 2010 and 2011, a portable thinning machine (Electroflor, Infaco, France) was used during flowering to remove about 40 % of flowers at the stage of full blossom of the trees (BBCH 65).

The influence of treatments on growth, diseases (*Monilinia ssp.*), yield, size and quality of fruits was assessed on all trees.

In another orchard (B) with the cultivars ’Blaze Star’ and ’Merchant’ (planted in spring 2003 on rootstock Gisela 5, with 4.5 x 2.5 m distance), some chemical and mechanical flower thinning methods were tested in 2010. At the beginning of flowering on 7$^{th}$ April 2010 100 flowers per branch were marked (in total 15 branches per variant). The already mentioned thinning machine (Electroflor) and a method to remove young cherries at the stage of pea size with scissors were compared to spraying treatments on flowers with wettable sulphur (4%), lime sulphur (3%) as well as the IP- standard ATS (1.8%). The trees were treated twice between the beginning and full flowering, on 11th April (BBCH 65) and on 16th April (BBCH 65-66), with a Solo sprayer (1.000 l/ha).

After blossom, the infestation with *Monilinia laxa* on flowers was assessed (200 flowers per tree). The thinning effect on number, size and quality of fruits was evaluated during yield at the marked branches, not marketable fruits were counted separately.

All fruit quality tests were done in the laboratory of the Institute with standardized methods.

The data was statistically analysed with SPSS 17, with a variance analysis (ANOVA) with subsequent S-N-K-test ($P < 0.05$), if the conditions of normal distribution and homogenity of variance were given; if not, a non parametrical procedure (Kruskal Wallis-test, Mann-Whitney-test, resp.) was used.

## Results and discussion

*The pruning of flower branches during blossom*

The growth of stem (2.8 cm) and the average yield (6.5 kg/tree) of the pruned trees were significantly lower than in the control (4.0 cm and 10.0 kg/tree, resp.). However, there was no influence on the percentage of marketable fruits and on fruit size (Table 1). Also

considering the fruit quality characteristics, no differences could be found (data not shown).

Table 1: Influence of a late pruning during blossom on growth and yield of early ripening cherry cultivars in orchard A (over all average of 13 cultivars) in 2009

| Parameter | Variant | | p-value |
|---|---|---|---|
| | Not pruned | Pruned | |
| | $\bar{x} \pm \sigma_x$ | $\bar{x} \pm \sigma_x$ | |
| Growth of stem circumference [cm] | 4.0 ± 2.39 | 2.8 ± 1.67 | 0.002[1] |
| Yield/tree [kg] | 10.0 ± 4.38 | 6.5 ± 2.59 | 0.000[1] |
| Marketable fruits [%] | 92.3 ± 13.61 | 93.5 ± 12.53 | 0.170[2] |
| Average fruit weight [g] | 5.5 ± 1.06 | 5.5 ± 0.99 | 0.934[1] |
| Specific yield [kg/cm²] | 0.11 ± 0.052 | 0.10 ± 0.041 | 0.066[1] |

p-value from Anova[1] or Kruskal-Wallis-test[2]

The time between pruning during blossom (14th April) and yield for the mainly early ripening cultivars (the yield was between 26th May and 9th June, only 'Sweetheart' ripened later on 25th June) used in our trial was very short and maybe crucial for the lower growth and yield, with no influence on fruit size and quality. In order to improve fruit size like it was described for other stone fruit species (Costa & Vizzotto, 2000, Webster & Spencer, 2000), it could be more efficient to prune the flower branches at an earlier time.

*Mechanical thinning with Electroflor*

Table 2: Influence of mechanical thinning by Electroflor of flowers during blossom on growth, diseases and yield characteristics of cherries in orchard A (average of 13 cultivars) in 2010

| Parameter | Variant | | p-value |
|---|---|---|---|
| | No thinning | Thinning | |
| | $\bar{x} \pm \sigma_x$ | $\bar{x} \pm \sigma_x$ | |
| Total harvest/tree [kg] | 5.3 ± 2.50 | 4.2 ± 2.02 | 0.026[2] |
| Average fruit weight [g] | 6.10 ± 1.306 | 6.70 ± 1.311 | 0.000[1] |
| Not marketable fruits [%] | 22.1 ± 12.57 | 23.4 ± 12.35 | 0.493[1] |
| Flowering rate (1=low, 5=high) | 4.66 | 4.59 | |
| *Monilinia fructigena* infection on 26th May [% infested fruits] | 2.52 ± 2.84 | 1.20 ± 2.097 | 0.004[2] |
| *Monilinia laxa* [% infested flowers on 2nd May] | 9.0 ± 8.24 | 5.8 ± 3.71 | 0.204[2] |
| Cross sectional area in November 2010 [cm²] | 91.19 ± 40.806 | 98.84 ± 41.151 | 0.278[1] |
| Growth of stem circumference [cm] | 2.17 ± 1.100 | 2.16 ± 1.098 | 0.830[1] |
| Specific yield [kg/cm²] | 0.062 ± 0.0273 | 0.046 ± 0.0276 | 0.005[2] |

p-value from ANOVA[1] or Mann-Whitney Test[2]

In 2010, the mechanically thinned trees showed a slightly lower yield (4.16 kg/tree) compared to the control (5.34); also the specific yield was significantly reduced in the treated variant. On the other hand, the average fruit weight could be significantly increased in the thinned trees. Also a reduction of *Monilia* infestations could be observed in the mechanically thinned trees. The percentage of not marketable fruits and the growth were not differing between the variants (Table 2).

In 2011, growth, percentage of not marketable fruits, total harvest per tree and specific yield did not differ between the treatments. The average fruit weight was again significantly higher on the thinned trees (Table 3).

Table 3: Influence of mechanical thinning by Electroflor of flowers during blossom on growth, diseases and yield characteristics of cherries in orchard A (average of 13 cultivars) in 2011

| Parameter | Variant | | p-value |
|---|---|---|---|
| | No thinning | Thinning | |
| | $\bar{x} \pm \sigma x$ | $\bar{x} \pm \sigma x$ | |
| Total harvest/tree [kg] | 6.6 ± 2.92 | 6.2± 2.67 | 0.308[1] |
| Average fruit weight [g] | 5.7 ± 1.04 | 6.0 ± 0.97 | 0.004[1] |
| Not marketable fruits [%] | 3.1 ± 3.27 | 3.1 ± 2.79 | 0.695[2] |
| Flowering rate (1=low, 5=high) | 4.36 | 4.52 | |
| *Monilinia fructigena* infection (0=no, 9=high) | 0.59 | 0.64 | |
| Cross sectional area in November 2011 [cm²] | 102.9 ± 42.28 | 111.0 ± 47.71 | 0.504[2] |
| Growth of stem circumference [cm] | 2.7 ± 1.06 | 2.5 ± 1.18 | 0.339[2] |
| Specific yield [kg/cm²] | 0.067 ± 0.0268 | 0.059 ± 0.0250 | 0.133[2] |

p-value from ANOVA[1] or Mann-Whitney Test[2]

Table 4: Influence of mechanical thinning by Electroflor on fruit quality parameters of cherries in orchard A (average of 6 cultivars) in 2011

| | No thinning | Thinning | p-value |
|---|---|---|---|
| | $\bar{x} \pm \sigma x$ | $\bar{x} \pm \sigma x$ | |
| Stalk firmness [N] | 9,1 ± 3,70 | 9,5 ± 3,70 | 0,055[2] |
| Stone rate [%] | 7,3 ± 1,26 | 7,1 ± 1,35 | 0,431[2] |
| Fruit form ratio | 0,95 ± 0,090 | 0,95 ± 0,079 | 0,400[2] |
| Fruit flesh firmness [kg/cm²] | 1,03 ± 0,306 | 1,07 ± 0,291 | 0,008[2] |
| L* | 35,3 ± 7,03 | 35,4 ± 6,76 | 0,583[2] |
| a* | 30,5 ± 9,67 | 30,8 ± 9,34 | 0,863[2] |
| b* | 13,3 ± 8,11 | 13,5 ± 7,83 | 0,693[2] |
| C* | 33,6 ± 11,98 | 33,8 ± 11,58 | 0,837[2] |
| Ascorbic acid [mg/l] | 233 ± 65,0 | 223 ± 62,5 | 0,347[2] |
| Soluble dry matter [%Brix] | 14,6 ± 2,10 | 14,6 ± 2,12 | 0,881[2] |
| Acidity [g/l] | 5,25 ± 1,343 | 5,06 ± 0,675 | 0,956[2] |
| pH-value | 3,55 ± 0,063 | 3,57 ± 0,057 | 0,063[1] |
| Redox potential [mVl] | 329 ± 25,8 | 333 ± 17,8 | 0,673[2] |
| Conductivity [S/m] | 2,55 ± 0,284 | 2,70 ± 0,246 | 0,000[1] |
| P-value [µW] | 278 ± 51,2 | 301 ± 47,1 | 0,006[2] |

p-value from ANOVA[1] or Mann-Whitney Test[2]

Furthermore, some significant changes in fruit quality characteristics could be found. The cherries from the thinned trees showed a higher fruit firmness, also the electrochemical characteristics conductivity and P-value increased significantly (Table 4).

*Chemical and mechanical flower thinning*

In the experiment with the cultivars ’Blaze Star’ and ’Merchant’ the mechanical treatment with Electroflor showed the strongest thinning effect with 13.2 marketable fruits per 100 flowers. The hand thinning of fruits (20.2), lime sulphur (21.7) and ATS (23.2) were similar in the yield to the untreated control (27.1). Sulphur showed even a higher fruit setting (36.8 fruits per 100 flowers) and therefore, also the highest yield (176 g/branch).

Table 5: Fruit setting and yield at harvest and flower infestation with *Monilinia laxa* in the treated variants in 2010 in orchard B (mean of the cultivars ‘Blaze Star’ and ‘Merchant’)

| | Control | Hand thinning (fruits) | Electro-flor | ATS | Sulphur | Lime sulphur | p-va-lue |
|---|---|---|---|---|---|---|---|
| | $\bar{x} \pm \sigma_x$ | $\bar{x} \pm \sigma_x$ | $\bar{x} \pm \sigma_x$ | $\bar{x} \pm \sigma_x$ | $\bar{x} \pm \sigma_x$ | $\bar{x} \pm \sigma_x$ | |
| Harvested Fruits (total) [Fruits from 100 flowers] | 35 ± 15,9 c | 27 ± 9,4 abc | 19 ± 8,3 a | 29 ± 11,0 bc | 45 ± 16,8 d | 32 ± 12,3 c | 0,000[1] |
| Fruit setting (total) [% of control] | 100,0 | 76,0 | 53,1 | 82,4 | 128,0 | 89,3 | |
| Marketable fruits [Fruits from 100 flowers] | 27 ± 14,4 b | 20 ± 10,2 b | 13 ± 7,7 a | 23 ± 12,9 b | 37 ± 16,6 c | 22 ± 11,8 b | 0,000[1] |
| Not marketable fruits [Fruits from 100 flowers] | 8 ± 5,4 a | 7 ± 4,0 a | 6 ± 4,4 a | 6 ± 5,4 a | 9 ± 5,9 a | 10 ± 7,9 a | 0,075[1] |
| Markteable yield [g/branch, 100 flowers] | 144 ± 63 bc | 126 ± 57 b | 88 ± 50 a | 134 ± 57 bc | 176 ± 62 c | 139 ± 69 bc | 0,000[1] |
| Marketable yield [% of control] | 100,0 | 87,5 | 61,1 | 93,1 | 122,2 | 96,5 | |
| Ø Fruit weight [g/fruit] | 5,6 ± 0,9 a | 6,6 ± 1,4 b | 6,8 ± 2,0 b | 6,4 ± 1,5 b | 5,3 ± 1,4 a | 6,7 ± 1,6 b | 0,000[1] |
| Ø Fruit weight [% of control] | 100,0 | 118,8 | 122,4 | 114,7 | 94,3 | 120,3 | |
| *Monilinia laxa* [% infested flowers on 29th April] | 16,9 ± 8,8 b | 13,3 ± 8,2 b | 9,8 ± 3,6 ab | 8,9 ± 3,7 ab | 3,0 ± 1,8 a | 2,5 ± 1,0 a | 0,000[1] |

p-value from Anova[1]

Both mechanical variants hand thinning of fruits (126g) and Electroflor (88g) had a significant lower yield, all others were in between. The average fruit weight could be increased by all treatments except sulphur compared to the control. Our results are contradictory to Weber (2009), who could not find an increasing effect on fruit size by mechanical thinning ogf young cherries. Nevertheless, hand thinning of fruits by scissors

(15 minutes per tree) is more time expensive compared to the Electroflor (4 minutes per tree) and hence not affordable for farmers.

Both treatments with fungicides, sulphur (3.0%) and lime sulphur (2.5%) reduced the flower infestation by *M. laxa* compared to the control (16.9%) and the untreated hand thinning variant of young fruits (13.3). We found no differences in the percentage of marketable fruits between the variants (table 5).

Regarding fruit quality characteristics in the treated variants no important differences could be found compared to the control (data not shown).

## Conclusions

- Late pruning of fruit branches during blossom on trees of early ripening cherry cultivars reduced growth and yield without increasing fruit size and fruit quality.
- Mechanical thinning of flowers of early ripening cherry cultivars with Electroflor increased the single fruit weight and had also an effect on some fruit quality characteristics.
- Some treatments (Electroflor, manual thinning of young fruits, lime sulphur, ATS) showed a strong thinning effect and an increase of fruit size on the cultivars ’Blaze Star’ and ’Merchant’. Nevertheless, yield losses due to lower number of fruits caused by thinning could only partly be replaced through the larger fruit size. Looking at the tested mechanical variants, the Electroflor is more suitable for farmers than hand thinning of flowers or fruits because of lower time need. The treatments with lime sulphur and wettable sulphur showed a reducing effect on flower infestations with *Monilinia laxa*. No influence on fruit diseases and internal fruit quality could be found due to the treatments.

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# Black and red currant cultivars for organic production

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**Abstract**

*Organic or unsprayed production of black currants (*Ribes nigrum*) and red currants (*Ribes rubrum*) needs cultivars which are resistant or less susceptible to the most common pests and diseases like powdery mildew (*Sphaerotheca mors-uvae*) and leaf spot (*Gloeosporidiella ribis*). The aim of this study was to find promising less disease susceptible, high-yielding cultivars with an acceptable juice quality for organic production. 13 black currants and 10 red currants were evaluated in 2009 to 2011 in Denmark. The black currant cultivar 'Narve Viking' was the best cultivar for organic production. 'Narve Viking' had high yields, good juice quality and resistance to pests and diseases. However; also the more disease susceptible cultivars 'Ben Lomond', 'Ben Hope' and 'Ben Tirran' had high yields and good juice quality when grown unsprayed.*

*The red currant cultivar 'Red Poll' had the highest yield, the best juice quality and was most resistant to diseases when grown unsprayed. 'Roodneus' also had a high yield, acceptable juice quality and disease resistance.*

**Keywords:** Unsprayed, yield, susceptibility to pests and diseases, juice quality, industrial use.

## Introduction

Organic or low pesticide production of black currants (*Ribes nigrum* L.) and red currants (*Ribes rubrum* L.) for industrial use needs cultivars which are resistant or less susceptible to the most common pests and diseases like American gooseberry mildew (*Sphaerotheca mors-uvae* Schweinitz), leaf spot (*Gloeosporidiella ribis* Libert), white pine blister rust (*Cronartium ribicola* J.C. Fischer) and black currant gall mite (*Cecidophyopsis ribis* Westwood) or reversion virus (*Atavismus*). Disease infections may cause an early leaf drop followed by reduced yield (Lindhard Pedersen, 1998).

The cultivars must also be suited for mechanical harvest and obtain a satisfying juice quality for industrial use. To achieve a satisfying colour in black currant jam or juice a minimum level of 300 mg malvidine chloride per 100 gram berries must be reached and a level of 130 mg per 100 gram berries of ascorbic acid is important to ensure the healthiness of the product (Kaack and Groven, 1981).

In an earlier study the results from the first two fruiting years, 2005 and 2006 from an unsprayed trial including 13 black currants and 10 red currants were reported (Lindhard Pedersen, 2007). The conclusion was that the black currant cultivars 'Narve Viking', 'Tiben', 'Ben Hope' and maybe 'Ben Gairn' and the red currant cultivars 'Rolan' and maybe also 'Augustus' were promising new cultivars for organic or low pesticide production of currants.

The aim of this study was to follow up on these cultivars and to investigate if the promising less disease susceptible, high-yielding cultivars of black and red currants suited for organic industry production were the same when the planting grew older.

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## Material and Methods

In April 2003 at Department of Food Science, Aarslev, 13 black- and 10 red currant cultivars, respectively, were planted as one-year-old plants at a planting distance of 3.5 x 0.5 m. Plots consisted of 6 bushes per cultivar planted in three blocks and totally randomised inside each block for black and red currants. Planting was done in Mypex™ in the bush row and grass was established in the alleyways. Plants were irrigated the first two years after planting and manuring was done using poultry manure pellets due to leaf analyses. Plants were kept unsprayed from planting and until 2011.

Black currant gall mite and reversion spread by the mite are severe pests of black currants. Big buds infested with gall mites were removed from the planting two to three times during each winter by hand to try to control the pest.

Harvest time and machine harvested yield and berry size were recorded in 2009, 2010 and 2011. Scores for foreign elements in the harvested product, vegetative annual growth, leaf healthiness and infections of occurring pests and diseases were carried out in June and August in 2009-2011 on a scale from 1-9, where 1= nothing. Samples of the harvested berries were collected for analysing of the fruit juice. Samples were stored frozen and analysed in winter. 200 grams of berries were homogenised with 100 g distilled water and the content of soluble solids was analysed with refractometer (Bellinngham + Stanley LTD. RFM 330). Content of total titrable acid was measured according to Kaack (1988). Total acids were converted to citric acid, which is dominating in black currants. Content of total anthocyanins was analysed with spectrophotometer (Shimadzu MPS 2000,) according to Wrolstad (1976). Statistical analyses of data were performed using SAS (Version 9 www.sas.com). Differences among cultivars were analysed with GLM-procedure. Means were separated with Duncan's test and statistical significance was defined at $p< 0.05$.

## Results

The black currant cultivars 'Narve Viking', 'Ben Lomond', 'Ben Hope' and 'Ben Tirran' had the highest yields (Table 1). 'Ben Lomond' and 'Ben Avon' had the biggest berries and 'Ben Gairn' had the cleanest product. The highest content of soluble solids (sugar) was obtained by the cultivars 'Baldwind and 'Titania'. 'Ben Tirran' and 'Tiben' had the highest level of acid and 'Ben Gain' had the highest content of colour in the juice (Table 1). The harvest was carried out in second half of July and the beginning of August. 'Ben Gain' was the earliest cultivar and 'Baldwind' the latest (Table 1).

The black currant cultivars 'Titania', 'Tiben' and 'Narve Viking' had the strongest growth and together with 'Ben lomond' the healthiest looking green leaves in June (Table 2). 'Ben Avon' was most susceptible to aphids and 'Baldwin' and 'Ben Lomond' to mildew (Table 2). In August white pine blister rust developed 'Narve Viking' and 'Ben Dorain' were the most susceptible cultivars. Whereas 'Baldwin', '8944-4' and 'Ben Lomond' were most susceptible to leaf spot. Due to disease infection the leaf healthiness was reduced in August. The cultivars 'Narve Viking' and 'Ben Gairn' maintained the best looking healthy leaves in August (Table 2). No other important pests or diseases occurred during the three years.

The highest-yielding red currant cultivars were 'Red Poll', 'Roodneus', 'Rovada', 'Augustus', 'Rolan' and 'Tatran' (Table 1). 'Rovada', and 'Rosetta' had the biggest berries and juice of 'Rondom' had the highest sugar content followed by 'Red Poll'. 'Red Poll' juice had the highest content of acid and colour.

The red currant cultivars 'Roodneus' and 'Red Poll' had the strongest growth and together with 'Rovada' the healthiest looking green leaves in June (Table 2). No severe infestation of aphids occurred. In June the cultivars 'Red Lake' and 'Red Start' already had an

infection of leaf spot and in late August all the leaves of these two cultivars had dropped due to a severe leaf spot infection (Table 2). All the cultivars had severe infections of leaf spot in August. The cultivars 'Red Poll', and 'Roodneus' had the most healthy looking leaves in August (Table 2). No other important pests or diseases occurred during the three years.

Table 1: Origin, harvest date, yield, berry size, foreign elements in the harvested product and soluble solids, citric acid and colour content of the juice in 13 cultivars of black currants and 10 cultivars of red currants in average of 2009-2011.

| Black currants | Origin | Harvest | Yield | Berry size | Foreign elements in the harvested product | Soluble solids | Citric Acid | Colour, Malvidin Chloride |
|---|---|---|---|---|---|---|---|---|
| Cultivar | Country | Date | Tonne/ha | G/100 berries | Score 1-9 1=nothing | % | Mg/g | Mg/100g |
| Baldwind | Scotland | 7 Aug | 3.4 ef | 60 e | 5.4 a | 18.0 a | 34.6 h | 441 g |
| Ben Alder | Scotland | 3 Aug | 5.7 bcd | 62 e | 4.7 b | 16.4 g | 36.1 f | 691 b |
| Ben Avon | Scotland | 26 Jul | 6.1 bc | 81 ab | 5.0 ab | 15.4 i | 35.3 g | 485 f |
| Ben Dorain | Scotland | 27 Jul | 4.3 cdef | 63 e | 4.3 bc | 17.4 b | 38.4 c | 651 c |
| Ben Gairn | Scotland | 21 Jul | 4.1 def | 74 cd | 3.7 c | 17.2 c | 29.2 k | 772 a |
| Ben Hope | Scotland | 1 Aug | 7.8 a | 74 cd | 4.8 ab | 16.8 e | 36.3 e | 637 c |
| Ben Lomond | Scotland | 2 Aug | 8.8 a | 84 a | 5.0 ab | 16.6 f | 37.6 d | 542 e |
| Ben Tirran | Scotland | 3 Aug | 7.4 ab | 76 bc | 4.3 bc | 15.8 h | 40.6 a | 502 f |
| Narve Viking | Norway | 3 Aug | 8.9 a | 70 d | 4.7 b | 15.5 i | 29.6 j | 638 c |
| Tiben | Poland | 29 Jul | 5.2 cde | 77 bc | 5.0 ab | 17.0 d | 40.4 a | 640 c |
| Titania | Sweden | 23 Jul | 2.8 fg | 76 bc | 4.7 b | 18.0 a | 39.9 b | 526 e |
| 8944-4 | Scotland | 23 Jul | 1.6 g | 50 f | 4.3 bc | 15.0 j | 25.1 l | 498 f |
| 8944-13 | Scotland | 23 Jul | 4.8 cde | 63 e | 5.0 ab | 14.1 k | 31.5 i | 598 d |
| Red Currants | Origin | Harvest | Yield | Berry size | Foreign elements in the harvested product | Soluble solids | Citric Acid | Colour, Malvidin Chloride |
| Cultivar | Country | Date | Tonne/ha | G/100 berries | Score 1-9 1=nothing | % | Mg/g | Mg/100g |
| Augustus | Holland | 14 Aug | 14.4 ab | 40 e | 5.0 ab | 9.0 j | 27.9 b | 68 e |
| Red Lake | North America | 4 Aug | 0.8 d | 33 f | 4.0 d | 10.4 h | 25.2 e | 67 e |
| Red Poll | England | 7 Aug | 17.3 a | 53 cd | 5.0 ab | 13.2 b | 30.2 a | 123 a |
| Red Start | England | 31 Jul | 0.6 d | 37 ef | 3.8 d | 10.3 i | 27.7 d | 63 f |
| Rolan | Holland | 31 Jul | 13.0 abc | 57 bc | 5.0 ab | 12.6 d | 21.4 g | 71 c |
| Rondom | Holland | 4 Aug | 10.5 bc | 50 d | 5.1 a | 13.6 a | 23.8 f | 71 c |
| Roodneus | Holland | 7 Aug | 15.4 ab | 55 bcd | 5.1 a | 12.5 e | 27.6 c | 122 b |
| Rosetta | Holland | 7 Aug | 8.3 c | 60 ab | 4.6 c | 12.1 f | 21.0 h | 59 g |
| Rovada | Holland | 7 Aug | 14.5 ab | 63 a | 4.7 bc | 11.4 g | 28.2 b | 69 d |
| Tatran | Slovakia | 9 Aug | 12.5 abc | 51 cd | 5.1 a | 12.7 c | 27.5 c | 67 e |

Numbers followed by the same letter for the same species in columns do not differ significantly for P≤0.05.

Table 2: Scores for growth, leaf healthiness, infestations of aphids, infections of mildew, rust and leaf spot in 13 cultivars of black currants and scores for growth, leaf healthiness, infestations of aphids and leaf spot in 10 cultivars of red currants in average of 2009-2011.

| Black currants | Growth, June | Leaf Healthiness June | Aphids, June | Mildew, June | Rust, August | Leaf Spot, August | Leaf Healthiness August |
|---|---|---|---|---|---|---|---|
| Cultivar | Score 1-9 1= no growth | Score 1-9 1= no leaves | Score 1-9 1= no infestation | Score 1-9 1= no infection | Score 1-9 1= no infection | Score 1-9 1= no infection | Score 1-9 1= no leaves |
| Baldwind | 4.8 d | 5.5 e | 1.7 f | 2.8 b | 2.8 f | 8.2 a | 1.8 d |
| Ben Alder | 4.7 d | 6.2 d | 2.7 bc | 1.2 cd | 3.9 de | 6.4 cd | 3.2 c |
| Ben Avon | 6.2 c | 6.6 c | 3.3 a | 1.1 cd | 4.4 c | 6.0 def | 3.5 c |
| Ben Dorain | 4.9 d | 5.9 de | 2.3 de | 1.2 cd | 5.1 ab | 5.6 f | 3.5 c |
| Ben Gairn | 5.1 d | 5.7 de | 3.0 ab | 1.0 d | 3.0 f | 4.2 g | 5.1 a |
| Ben Hope | 6.1 c | 6.9 bc | 1.7 f | 1.0 d | 4.7 bc | 6.3 cde | 3.3 c |
| Ben Lomond | 6.0 c | 7.1 abc | 2.1 ef | 3.1 a | 3.5 e | 7.3 b | 2.1 d |
| Ben Tirran | 5.8 c | 6.7 c | 2.2 de | 1.2 cd | 4.4 cd | 6.4 cd | 3.1 c |
| Narve Viking | 6.4 abc | 7.3 ab | 2.0 ef | 1.0 d | 5.5 a | 1.7 h | 5.2 a |
| Tiben | 6.8 ab | 7.5 a | 2.9 ab | 1.2 cd | 2.6 f | 6.9 bc | 3.4 c |
| Titania | 6.8 a | 7.1 abc | 2.8 bc | 1.0 d | 1.0 h | 5.7 ef | 4.2 b |
| 8944-4 | 4.8 d | 4.8 f | 2.5 cd | 1.0 d | 2.0 g | 7.3 b | 3.0 c |
| 8944-13 | 5.9 c | 6.9 bc | 2.5 cd | 1.3 c | 2.7 f | 6.6 cd | 4.1 b |
| Red Currants | Growth, June | Leaf Healthiness, June | Aphids, June | Leaf Spot, June | | Leaf Spot, August | Leaf Healthiness, August |
| Cultivar | Score 1-9 1= no growth | Score 1-9 1= no leaves | Score 1-9 1= no infestation | Score 1-9 1= no infection | | Score 1-9 1= no infection | Score 1-9 1= no leaves |
| Augustus | 5.4 bc | 7.1 cd | 1.2 abc | 2.3 b | | 8.6 ab | 1.4 de |
| Red Lake | 3.9 e | 4.4 g | 1.3 abc | 3.1 a | | . | 1.0 e |
| Red Poll | 6.4 a | 7.6 ab | 1.6 a | 1.5 de | | 6.7 d | 3.2 a |
| Red Start | 4.1 e | 3.9 h | 1.3 abc | 2.9 a | | . | 1.0 e |
| Rolan | 4.7 d | 6.7 ef | 1.2 bc | 1.6 de | | 8.8 ab | 1.7 d |
| Rondom | 5.6 bc | 7.3 bc | 1.5 ab | 1.8 cd | | 7.7 c | 2.6 bc |
| Roodneus | 6.6 a | 8.0 a | 1.4 abc | 1.6 de | | 7.1 d | 3.0 ab |
| Rosetta | 4.8 d | 6.3 f | 1.1 c | 1.3 e | | 8.3 bc | 2.3 c |
| Rovada | 5.9 b | 7.8 a | 1.1 c | 2.0 c | | 9.0 a | 1.1 e |
| Tatran | 5.2 cd | 6.7 de | 1.4 abc | 1.6 de | | 8.3 bc | 1.8 d |

Numbers followed by the same letter in columns do not differ significantly for P≤0.05

## Discussion

The highest yielding Norwegian cultivar 'Narve Viking' was also the highest yielding cultivar when the planting was young (Lindhard Pedersen, 2007). The Scottish cultivar 'Ben Hope' was also among the highest yielding cultivars both as young and older plants, whereas 'Ben Lomond' and 'Ben Tirran' were more on the average (Lindhard Pedersen, 2007). The four highest yielding cultivars also had satisfactory juice quality and strong growth in the period. Especially 'Narve Viking' was rather resistant to the common diseases and kept the healthy green leaves until late August. 'Ben Hope' is a promising cultivar for organic production as it is resistant to gall mites. However, it is more susceptible to leaf spot than 'Narve Viking'. The two cultivars 'Tiben' and 'Ben Gairn', which were promising as young plants (Lindhard Pedersen, 2007), had rather low yields in 2009 to 2011 (Table 1). 'Ben Gairn' is an interesting cultivar for organic production as it is resistant to the reversion virus. In 2009 it had a good juice quality with high sugar and very high colour content and 'Ben Gairn' is also rather resistant to the common diseases. However, it had a low yield. 'Ben Lomond', 'Ben Hope' and 'Ben Tirran' had high yields even they were rather susceptible to diseases (Tables 1 and 2).

'Titania' and 'Ben Alder' were earlier recommended for organic production (Lindhard Pedersen 1998), but in this investigation and in Lindhard Pedersen (2007) the yielding of 'Titania' was very low. Ben Alder is no longer among the highest yielding cultivars. 'Ben Alder, had a satisfactory juice quality, but the leaf healthiness was rather low and it was susceptible to rust and leaf spot.

'Ben Lomond' and 'Ben Tirran' are two rather old Scottish cultivars, which are used in conventional production in Denmark. Unsprayed they are among the highest yielding cultivars and have a satisfactory juice quality. Their leaf healthiness was not very good and 'Ben Lomond' was one of the most susceptible cultivars to mildew and leaf spot. Only the variety 'Baldwind' was more susceptible. Despite that 'Ben Lomond' and 'Ben Tirran' were rather high-yielding, this was also found by Lindhard Pedersen (1998).

The red currant cultivars 'Roland', 'Rovada' and 'Augustus' were promising cultivars for organic production when the plants were young (Lindhard Pedersen 2007). Also in this evaluation these three cultivars are among the highest yielding. However, these cultivars were very susceptible to leaf spot and had poor leaf healthiness in August (Table 1 and 2). The highest yielding cultivars over time were 'Red Poll' and 'Roodneus'. These cultivar also had a fine juice quality with high sugar and acid content and the highest content of colour and the best looking leaves and the lowest susceptibility to leaf spot.

## Conclusion

The black currant cultivar 'Narve Viking' was the best cultivars for organic production. This cultivar had high yield, good juice quality and resistance to pests and diseases. But also the more disease susceptible cultivars 'Ben Lomond', 'Ben Hope' and 'Ben Tirran' had high yields and good juice quality when grown unsprayed.

The red currant cultivar 'Red poll' had the highest yield, the best juice quality and was most resistant to diseases when grown unsprayed. 'Roodneus' also had a high yield and acceptable juice quality and disease resistance.

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# Breeding of resistant strawberry cultivars for organic fruit production – Diallel crossing strategies and resistance tests for *Botrytis cinerea* and *Xanthomonas fragariae*.

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**Abstract**

*Organic strawberry production suffers from high yield losses caused by numerous fungal and bacterial diseases. Two of the most important diseases are the grey mould disease caused by* Botrytis cinerea *Pers. (teleomorph* Botryotinia fuckeliana*), and the bacterial angular leaf spot disease caused by* Xanthomonas fragariae *(Kennedy & King). Beside cultivation methods and organic plant protection measures, the development of resistant cultivars seems to be the most promising strategy in order to improve the productivity in organic strawberry cultivation. Therefore, we established resistance tests to determine resistant and susceptible strawberry cultivars and breeding selections. In a first run, 40 different cultivars and selections were tested for their susceptibility towards* B. cinerea *by artificial inoculation of fruits and leaves and evaluation of the disease symptoms. Plants of 40 cultivars were tested for susceptibility to* X. fragariae *by artificial inoculation in the greenhouse. In a diallel crossing approach, 12 commonly cultivated strawberry cultivars have been crossed reciprocally and propagated in a field trial. Important characteristics of the progeny such as ripening time, yield, morphological traits and occurrence of diseases have been evaluated for a period of two consecutive years and lead to the determination of general (GCA) and specific (SCA) combining abilities. Together with the results of the resistance tests we identified a set of genotypes that show resistant characteristics towards* B. cinerea *and might be suitable for use in organic cultivation systems. Furthermore, they can be used for targeted breeding experiments in the future.*

**Keywords:** *Fragaria* × *ananassa*, grey mould, angular leaf spot, combining ability

## Introduction

The necrotrophic fungus *B. cinerea* causes severe damage in a broad spectrum of host plants (Elad *et al.,* 2007; Williamson *et al.,* 2007) and the control requires high efforts especially in organic farming (Boff *et al.,* 2001) due to the fact that botryticides are not permitted. Warm temperatures and high humidity lead to a high rate of sporulation (Sosaalvarez *et al*., 1995). Hence, the released conidia are spread widely by wind and cause infections of flowers, leaves and fruits. Once the conidia germinated in strawberry flowers, the mycelial growth is temporarily suppressed by high levels of the Flavan-3-ols Catechin, Epicatechin and Proanthocyanidin (Puhl & Treutter, 2008). The content of those substances decreases during ripening of the fruits and the fungus continues its life cycle. Due to the fact that there are numerous factors influencing the disease progress and the pathogen reacts in a rather unspecific way, there are no incidences for a monogenic

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resistance against *B. cinerea* (Chandler *et al.,* 2006). Therefore breeding of resistant cultivars is challenging and as a first step we tried to find sources of resistance by testing a high number of genotypes.

*X. fragariae* was first reported by Kennedy and King (1962) in Minnesota, USA. From there it spread worldwide, dispersal occurred mainly through trade and propagation with infected strawberry runner plants. The gram negative bacterium is highly host specific for strawberries. For Europe the disease was first reported in Italy in 1973, later in Greece, Portugal, Spain, Romania and since the 1990's also in Germany. It is listed as a quarantine pest at the European and Mediterranean Plant Protection Organization (EPPO) with the status A2 (OEPP/EPPO, 1986). Yield loss is reported from 5-8 % (Roberts *et al*., 1997), up to 75 % (Epstein, 1966). The main yield reducing effect is caused by brown spotted calyx which reduces the marketability of the fruit. Until now, there are neither suitable plant protection products nor resistant strawberry cultivars available. Resistant breeding selections US4808 and US4809 have been found (Maas *et al*., 2000; Maas *et al*., 2002) but there are no targeted resistance breeding activities. Therefore, our approach is to test a large number of cultivars, selections and Fragaria species to generate beneficial data about the susceptibility towards *X. fragariae* and potential usability in breeding experiments.

In a diallel crossing approach, different genotypes are crossed in a reciprocal way. Each genotype is used both as mother and as father. This approach gives us insight into the inheritance of different morphological traits by calculating the general combining abilities (GCA) of the parents and the specific combining abilities (SCA) of the progeny (Aalders & Craig, 1974; Vieira *et al*., 2009).

## Material and Methods

The field trial area is located near Dresden, Germany at an altitude of 113-118 m. The soil belongs to the soil class Luvisol and the soil type can be characterized as a sandy loam with a pH of 6.0 (2010) and an average soil value of 65. The previous crops were a grass mixture followed by *Tagetes*. The experimental plots were fertilized by 30 t/ha manure in spring 2010 and supplemented with 60 kg/ha P (TSP) and 40 kg/ha N (CAN) in spring 2011. The plant protection strategy follows the guidelines of integrated pest management. In 2011, fungicides were avoided in order to evaluate fungal diseases in the experimental plots.

Diallel crosses of the cultivars 'Antea', 'Arosa', 'Clery', 'Daroyal', 'Darselect', 'Elsanta', 'Florence', 'Galia', 'Madeleine', 'Marmolada', 'Polka', 'Sonata' and 'Yamaska' were carried out in march 2009 in the greenhouse. Each cultivar was used as mother and father except 'Yamaska' which has only female flowers. The seedlings were raised in the greenhouse and planted in the field in august 2009. The total number of crosses made was n=144, with 13 mother and 12 father genotypes. For each cross 15 seedlings were planted in a randomized block design with two replications. Yield, fruit firmness, flowering time, leaf health, position of inflorescences, and number and color of fruits have been measured and evaluated in 2010 and 2011.

The general (GCA) and specific (SCA) combining abilities of the parents and the progeny in the diallel crossing approach was calculated according to Falconer (1984). For the main effects, the GCA corresponds to the difference between the line mean of the cultivar and the total mean of all crosses. Conferring to the interaction between the parents, the SCA can be calculated as the difference between the mean of each crossing and the expected value. Therefore, the expected value consists of the paternal $GCA_P$, the maternal $GCA_M$, and the total mean.

For the resistance test against *B. cinerea* we inoculated 15 fully ripened fruits of 40 strawberry genotypes with 5µl droplets of a $10^5$ CFU/ml conidial suspension after surface sterilization. We tested 32 cultivars and 8 selections from the Dresden-Pillnitz strawberry breeding program (Figure 1). Inoculated fruits were placed upon wet filter paper in aluminum boxes and incubated at 20°C (14h light, 10h dark) in a climate cabinet for nine days. The boxes were randomized and their positions changed within the cabinet. The degree of fruit rot by *B. cinerea* was evaluated on a scale from 0 (no symptoms), 1 (symptoms <10%), 2 (symptoms 11-25%), 3 (fruit rot 26-50%) to 4 (full fruit rot >50%).

Resistance to *X. fragariae* was evaluated for 40 strawberry cultivars (Figure 2) by targeted infection of the plants in the greenhouse. Frigo plants of the cultivars were brushed with a $10^9$ CFU/ml inoculum of the bacteria on the abaxial side of the leaves. For each cultivar, eight to nine plants were tested in three replicates using a randomized block design. After the inoculation guttation was forced by increasing humidity and temperature at the afternoon and cooling down the cabin over night, so that the bacteria could enter the plants through open hydathodes. The infections were evaluated 15, 21, 35 and 62 days after inoculation using a scale from 1 (no symptoms) to 9 (necrotic leaf).

Statistical analysis was carried out with SAS 9.2 using the procedure NPAR1WAY with the Wilcoxon operation (Kruskal-Wallis-Test, α=0.05) and ANOVA (α=0.05). To fulfill the requirements for the analysis of variance, the data from the *B. cinerea*-test were transformed according to Fisher and Yates.

## Results

In the *Botrytis cinerea* resistance test the artificially inoculated fruits showed first symptoms of fruit rot after one to three days post-inoculation, depending on the cultivar. The symptoms of rotting increased during the incubation period and after six days the differences between the cultivars were clearly visible. As expected, we could not find totally resistant cultivars.

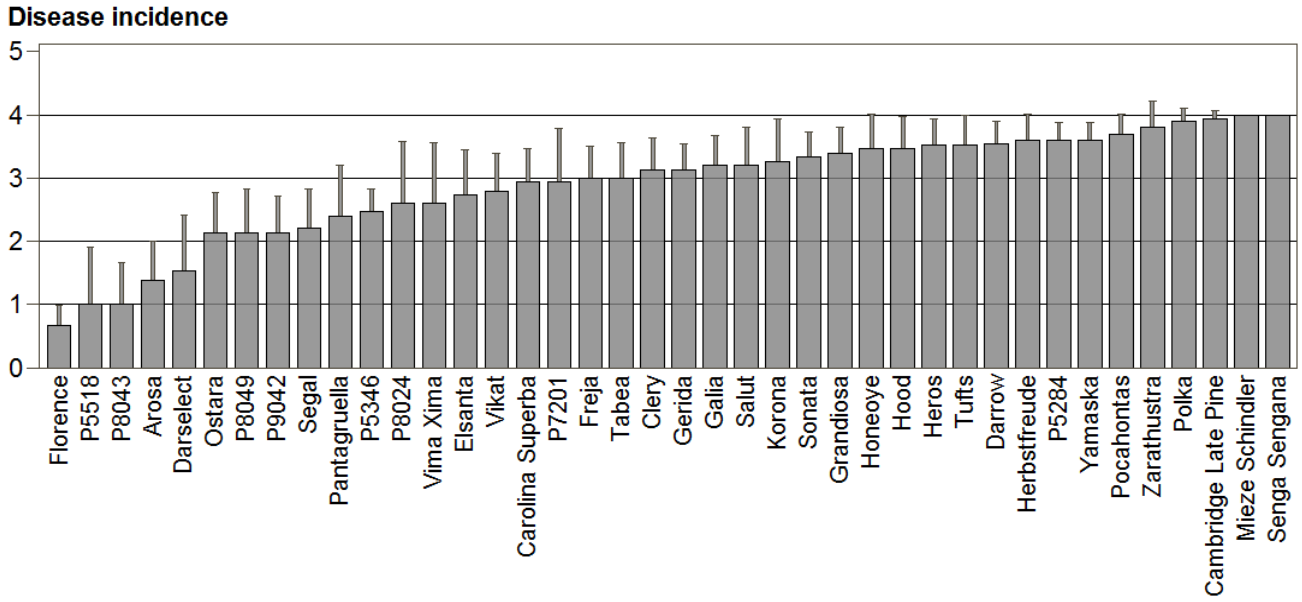


Figure 4: Susceptibility of 40 different strawberry genotypes towards grey mold caused by *B. cinerea*, 6dpi. Scale from 0 (no symptoms) to 4 (full fruit rot), error bars show positive standard error of the means.

The lowest fruit rot symptoms showed the cultivar ‘Florence’ with a mean score of 0.7 which refers to <10 % disease incidence. For P5580, P8043, ‘Arosa’ and ‘Darselect’ we found mean scores for fruit rot between 1 and 2. They are still significantly less susceptible than the cultivars ‘Mieze Schindler’ and ‘Senga Sengana’ with a fruit rot score of 4 (data not shown). The fruits of these cultivars were completely rotten after six days of incubation (Figure 1).

Resistance to *X. fragariae* was evaluated 15, 21, 35 and 62 dpi. The typical symptoms of the angular leaf spot disease became visible after 15 dpi and spread slowly on the infected leaves. The largest differences in susceptibility between the tested cultivars were found at the end of the experiment after 62 dpi. The highest level of disease incidence was recorded for the cultivar ‘Malwina’, followed by ‘Darselect’ and ‘Korona’. With a mean disease score of 3.9 the early flowering ‘Clery’ is the least susceptible cultivar in the test. A similar level of disease incidence showed the cultivars ‘Diana’, ‘Donna’ and ‘Florin’ (Figure 2). We found no resistant cultivar towards *X. fragariae*.

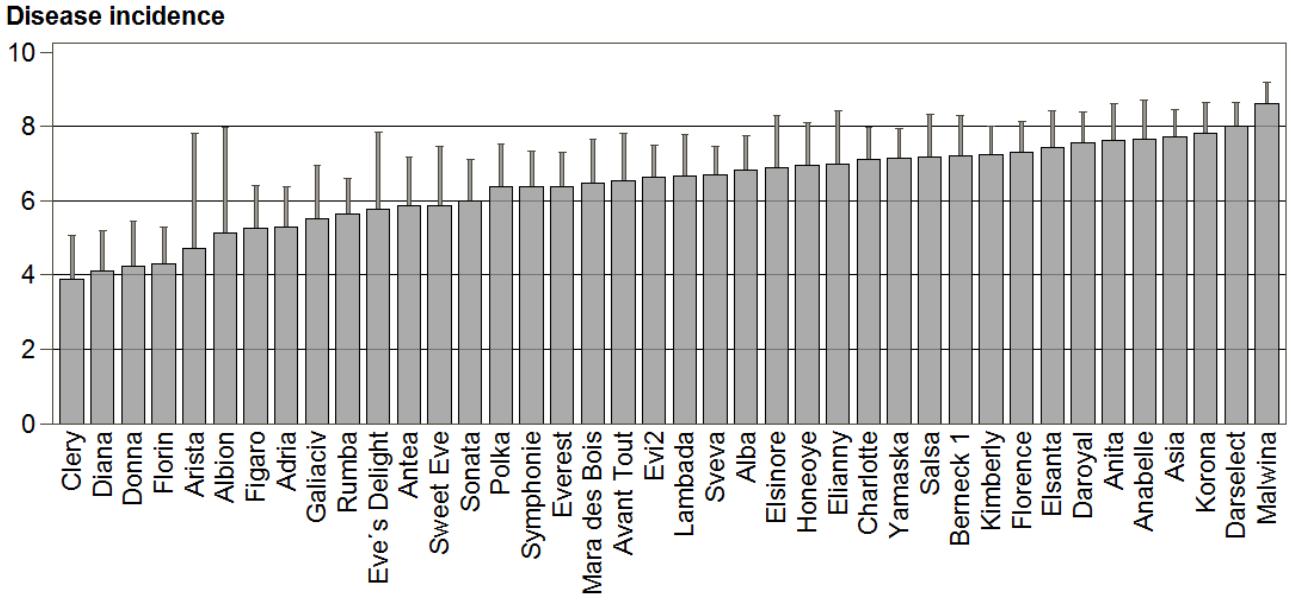


Figure 5: Susceptibility of 40 different strawberry genotypes towards bacterial angular leaf spot disease caused by *X. fragariae*, 62dpi. Scores from 1 (no symptoms) to 9 (necrotic), error bars show positive standard error of the means.

For the ten commonly grown cultivars ‘Clery’, ‘Darselect’, ‘Elsanta’, ‘Florence’, ‘Galia’, ‘Honeoye’, ‘Korona’, ‘Polka’, ‘Sonata’ and ‘Yamaska’ results from the resistance tests to both pathogens, *B. cinerea* and *X. fragariae* are shown in detail in Table 1.

The data show that there is no cultivar with low susceptibility against both pathogens. The cultivars ‘Darselect’ and ‘Florence’ show significantly the slightest fruit rot symptoms, whereas ‘Darselect’ is highly susceptible against the angular leaf spot disease. For ‘Clery’, which is less susceptible to *X. fragaria*, we have to face a *B. cinerea*-score of 3.1 that is not significantly different from the highly susceptible ‘Polka’. ‘Elsanta’ is significantly more susceptible to the grey mold disease than ‘Darselect’ and ‘Florence’ but not different compared to ‘Clery’, ‘Galia’, ‘Honeoye’, ‘Korona’, ‘Sonata’ and ‘Yamaska’ and less susceptible than ‘Polka’. ‘Clery’ and ‘Galia’ have a significantly lower disease score for *X. fragariae* than ‘Darselect’ whereas the differences between ‘Elsanta’, ‘Florence’, ‘Honeoye’, ‘Korona’, ‘Polka’, ‘Sonata’ and ‘Yamaska’ are not significantly different.

Table 4: Susceptibility of selected strawberry cultivars towards *X. fragariae* (*Xf*) and *B. cinerea* (*Bc*) after artificial inoculation.

| Cultivar | Score *Xf* 62 dpi (1-9) * | Score *Bc* 6 dpi (0-4) * |
|---|---|---|
| Clery | 3,9 a | 3,1 bc |
| Darselect | 8,0 c | 1,5 a |
| Elsanta | 7,4 bc | 2,7 b |
| Florence | 7,3 bc | 0,7 a |
| Galia | 5,5 ab | 3,2 bc |
| Honeoye | 6,9 bc | 3,5 bc |
| Korona | 7,8 bc | 3,3 bc |
| Polka | 6,3 bc | 3,9 c |
| Sonata | 6,0 abc | 3,3 bc |
| Yamaska | 7,1 bc | 3,6 bc |

* Means indicated with different letters are significantly different (Tukey-HSD, α=0.05).

The results of the diallel crossing approach shown in Table 2 are summarized for the paternal and maternal GCA values of the characteristics yield, fruit firmness and leaf health. The general combining ability can be interpreted as a prediction for the mean crossing performance of a cross between two cultivars.

Table 2: General combining abilities (GCA) of 13 strawberry cultivars in a diallel crossing system, GCA is given for each cultivar as mother (maternal) or father (paternal) in the reciprocal cross.

| Traits: | **Yield** | | **Fruit firmness** | | **Leaf health** | |
|---|---|---|---|---|---|---|
| **Cultivar** | **GCA** paternal | **GCA** maternal | **GCA** paternal | **GCA** maternal | **GCA** paternal | **GCA** maternal |
| Antea | -2 | -19 | -0,16 | 0,27 | -0,40 | -0,24 |
| Arosa | -4 | **77** | 0,22 | **0,74** | -0,04 | -0,11 |
| Clery | -34 | -48 | -0,12 | -0,33 | -0,25 | -0,17 |
| Daroyal | 0 | -38 | -0,08 | -0,34 | 0,21 | 0,25 |
| Darselect | 1 | 21 | **0,44** | 0,23 | -0,25 | -0,52 |
| Elsanta | 22 | 19 | 0,42 | -0,35 | -0,16 | -0,42 |
| Florence | -30 | -10 | -0,05 | -0,13 | 0,05 | 0,02 |
| Galia | -58 | -34 | -0,44 | -0,06 | **0,45** | **0,43** |
| Madeleine | -5 | -52 | 0,23 | 0,21 | -0,27 | -0,04 |
| Marmolada | 6 | -43 | -0,26 | -0,26 | 0,01 | 0,03 |
| Polka | **85** | **81** | -0,22 | -0,37 | **0,48** | 0,23 |
| Sonata | 21 | 44 | 0,02 | 0,15 | 0,18 | 0,20 |
| Yamaska | | 2 | | 0,24 | | 0,31 |

For the different GCA-effects we find that crosses using ‘Polka’ as a father resulted in yield of 85 g/plant more than the mean of all crosses, which was 245 g/plant. With ‘Arosa’ as mother, fruit firmness was 0.74 scores higher than the mean of all crosses. Leaf health in progenies from crosses with ‘Polka’ and ‘Galia’ as father is 0.48 scores / 0.45 scores

higher than the allover mean. In context with the GCA's we calculated the specific combining abilities (SCA's) for each of the 144 crosses (data not shown). Small SCA effects of a cross mean that the prediction of a trait can be estimated by GCA-effects of the parents in a reliable way. For 'Polka' x 'Galia' regarding trait 'yield' we find a very small SCA of 2 and a yield of 269 g/plant; in this case the performance of the cross is realized based on GCA-effects. For the combination 'Darselect' x 'Polka' with a SCA of 1 and a yield of 362 g/plant, the performance of the combination is mainly influenced by the parental GCA effects. In contrast, the progeny of the cross 'Sonata' x 'Madeleine' reach the highest yield in the test field with 410 g/plant and a very high SCA of 117; in this case the performance of the cross could not be estimated truly by GCA-effects of the parents. For fruit firmness, 'Arosa' x 'Elsanta' with a SCA of -0,03 and a firmness index of 6,28 also indicates, that in this case the trait is mainly influenced by the parental GCA effects.

## Discussion

As a conclusion it can be expected that the cultivation of less susceptible cultivars like 'Florence', 'Arosa' and 'Darselect' might be a chance to overcome high yield losses due to *B. cinerea* in organic farming, when botryticides are not permitted. In comparison to this approach with artificially inoculated fruits the results may differ under natural conditions. Disease incidence of *B. cinerea* also depends on environmental factors and on the morphology of the plants. High leaf density and the position of inflorescences under the leaves and close to the soil increase the amount of infestation. Furthermore, the results revealed storage characteristics of the fruits of different cultivars at room temperature. Fruits of the mentioned cultivars can be stored longer, in case of 'Florence' six days at 20°C with very little symptoms of fruit rot. To improve the statistical power of the data, the resistance tests have to be repeated over several years. Additionally we investigate the resistance of leaves and flowers to *B. cinerea*.

For *X. fragariae* we found no sources of resistance among the 40 cultivars in the test. Therefore, we will continue of the test using *Fragaria* species from strawberry genetic resources of the Fruit genebank at JKI in Dresden-Pillnitz. In contrast to descriptions in literature, a systemic distribution of bacteria in the plants could not be detected. Subsequently, molecular analysis will be carried out using PCR methods according to Zimmermann *et al*. (2004).

Further crossing experiments based on the best combinations from the diallel crossing approach will be performed and other traits will be evaluated, such as early flowering and susceptibility to the widespread pathogens *Mycosphaerella fragariae* and *Diplocarpon earliana* that cause the common leaf spot disease in strawberry plants. Although the conditions on the experimental field do not fit the guidelines for organic farming, the results improve a targeted resistance breeding process for organic farming.

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# Dispersal of *Aureobasidium pullulans* by pollinating insects to control *Botrytis* infection in strawberries

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**Abstract**

*Infections with* Botrytis cinerea *cause serious damage in strawberry production. The infection starts already at blossom. For* Botrytis *control several treatments are necessary and the spray applications increase the humidity in tunnels and favour* Botrytis *development.*

*If an active ingredient could be dispersed by pollinating insects the substance will reach the flowers directly without using water. Within a research project Bio-Protect GmbH and Koppert BV developed a method combining pollinating with plant protection. The usefulness of the dispersal of* Aureobasidium pullulans *through bumble bees and the control of* Botrytis *infection in a protected strawberry production was shown over a two years period. The trials were carried out in the Ortenau region, Baden-Württemberg, on the early variety Clery. The dispersal was measured by a strain specific qPCR and the disease control by evaluation*Botrytis *infection in comparison with untreated control tunnels.*

*It was shown that the bumble bees dispersed between $10^3$ and $10^5$*A. pullulans cells per *blossom and the efficiency in* Botrytis *control was between 60 and 80%.*

**Keywords:** *Aureobasidium pullulans*, antagonist, *Botrytis cinerea*, bumble bees, strawberries

## Introduction

Fruitrotsare among the most serious infections in strawberries and most strategies to control the disease are just more or less successful, especially in organic production.The main infections in strawberries are caused by the grey mold pathogen (*Botrytis cinerea*) through blossoms. The occurrence of an infection mainly depends on the weather conditions during harvest.Therefore any kind of treatment hasto be protective and as an ideal case every blossom has to be treated. This optimal protection is under practical use with conventional treatment methods hardly to conduct.

Bio Protect GmbH developed biocontrol agents, which contain antagonistic strains of *Aureobasidium pullulans,* against several bacterial and fungal pathogens (Kunz 2004; Weiss, Mögel, and Kunz 2006). One of the *A. pullulans* formulations controls *Botrytis cinerea* in grapes (Achleitner 2010) and the application of *A. pullulans* to strawberry blossoms reduced infections by *Botrytis cinerea* (Adikaram, Joyce, and Terry 2002). Therefore first trials with spray applications of Boni Protect forte were done in organic strawberries, revealing increase in harvest and longer shelf life of the fruit (Mayr and Späth 2008).

Aim of the following study was to develop a method to treat as many blossoms as possible without enhancing treatment dates. If pollinating insects dispersed aplant protection agent during pollination, it should be possible to protect a maximum number of blossoms.

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Koppert BV was working on beneficial organisms for a long period of time, distributes bumble bees and developed several dispensing systems (Fig. 1) for the use with bumble bees.*Aureobasidium pullulans* in an appropriate formulation is predestinated to be dispersedin such a system. With this new innovative technique, it would be possible to improve the efficacy of the product and also reduce the application rate by bringing the product directly to the blossom.

Figure 1 Bumble bee hive with dispenser filled with a powdery formulation of *A. pullulans* in a strawberry tunnel.

**Material and Methods**

The trials were carried out in protected production of a Bioland farm in Sinzheim/Müllhofen, Ortenau region Baden-Württemberg on the early variety Clery. Trials were done over a two years period. In 2010 trials were realized in ten tunnels (length between 100 and 200m, total acreage 1.5 ha) and in 2011 in twelve tunnels (length 180-200m, total acreage 2.2 ha).

Trials were started at the beginning of bloom. Bumble bee hives were placed in the middle of each tunnel and the dispensers were filled with a modified formulation of Boni Protect forte containing the two strains of *A. pullulans* DSM14940 and DSM14941.

In vertical spacing from the bumble bee hives at different distances (in 2010 up to eight distances in 2011 fourdistances) at a time ten blossoms were collected to measure the dispersal of *Aureobasidium pullulans* by the bumble bees.The determination of DSM14940 was measured by a quantitative real time PCR (qPCR).

qPCR analyses were performed using a CFX96 Real-Time PCR Detection System (BioRad, Munich). Manual threshold was set to 180. As qPCR chemistry the QuantiTect® SYBR® Green PCR Master Mix (QIAGEN, Hilden) together with primers CF10-RAPD-F4 and CF10-RAPD-R6 in a reaction volume of 25 µl was used(Sickinger 2008). Maximum sample volume to be tested was 10 µl. The PCR protocol consisted of an initial denaturation step of 5 min at 95°C, followed by 40 cycles of denaturation at 94°C for 20 sec, annealing at 54.5°C for 20 sec and extension at 72°C for 20 sec. A melt analysis followed each PCR run to verify identity and homogeneity of the amplicon.

Samples consisted of washing fluids containing intact blastospores. No DNA extraction was performed prior to PCR analysis. For the analyses strawberry blossoms (in general 10) were collected in Whirl-Pak bags (Carl Roth GmbH, Karlsruhe), and incubated with 20 ml$H_2O$ for 15 min. A 1 ml aliquot was removed and centrifuged for 1 min at 15,000 g. The supernatant was discarded and pellets resuspended in an equal volume of $H_2O$. Samples were either analyzed directly or stored at -20°C.

Absolute quantification of blastospores in samples was done by standardization with respect to serial dilutions of washed pure cultures of strain DSM14940.

Statistical analysis of log transformed data was done using one-way analysis of variance, and mean separation was accomplished using Tuckey's Multiple Comparison test ($p \leq 0.05$). Arithmetic means were retransformed to be described in the text.

Disease incidence was evaluated by counting *Botrytis* infection sites on flowers and fruits of 200 plants in each plot. At least four plots (different rows) were counted in each treatment (untreated, treated with new dispenser, treated with old dispenser). Symptomatic flowers and fruits were removed.In 2010 primary and also secondary *Botrytis*infections were distinguished.

Efficiencies of treatments against primary *Botrytis* were calculated according to Abbott (Abbott 1925). Statistical analyses of the data were done using one-way analysis of variance, and meanseparation was accomplished using either Student's t-test (($p \leq 0.05$) or Tuckey's Multiple Comparison Test ($p \leq 0.05$).

## Results

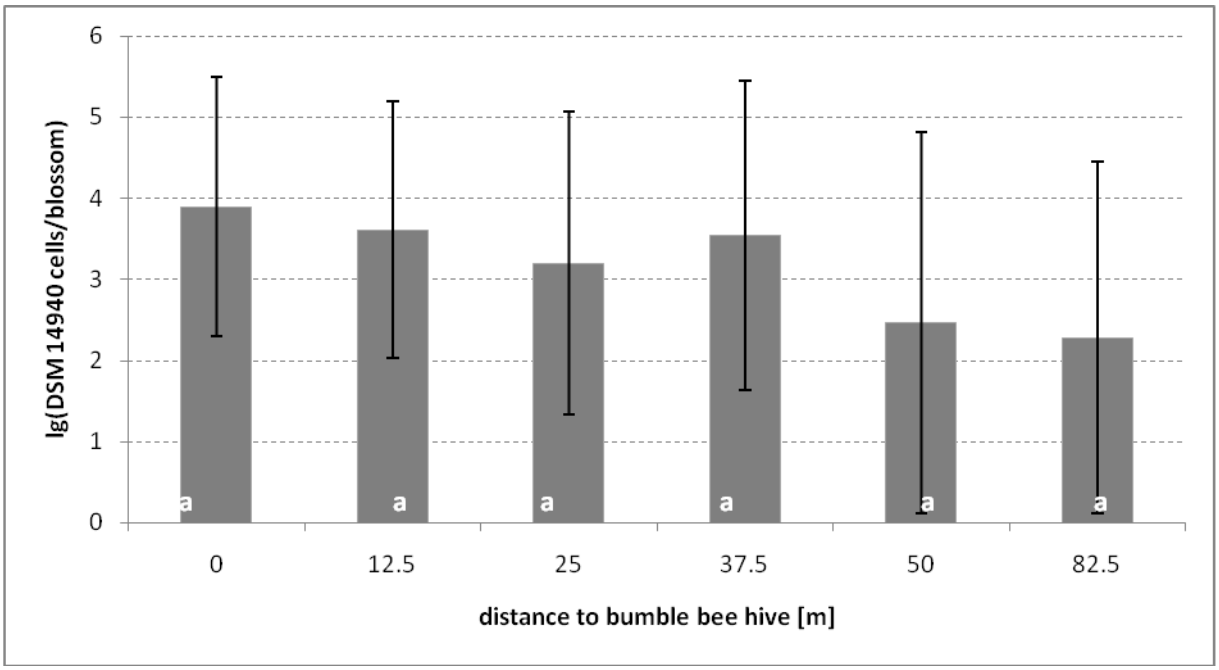


Figure 2: Average abundance of DSM14940 on blossoms of all treated tunnels in relation to the distance of the flowers to the bumble bee hives. The same letters in columns indicate no significant difference in Tuckey'sMultiple Comparison test ($p \leq 0.05$). n = 9-23

In 2010 dispersal of *A. pullulans* was evaluated in ten tunnels. Bumble bees reached all parts of the tunnels and a laminar distribution of *Aureobasidium pullulans*occurred. There was no significant influence of the distance to the bumble bee hive on DSM1940 cell numbers present on the blossoms (Fig. 2).

In both years during the whole sampling time in more than 80% of all taken samples from tunnels with dispensers DSMZ14940 was detected. On some sampling days even 97% of the samples were positive. The abundance of strain DSM14940 was up to $10^5$ per blossom. On average between $10^2$ and $10^4$cells per blossom were found.

In 2010 evaluation of infections by *Botrytis cinerea* was done on 12.05.2010in the control tunnels 25 and 27 as well as in the treated tunnels 26, 28 and 29. These five tunnelswere comparablein size, configuration and exposure. In addition to the primary *Botrytis* infections caused through infected blossoms, secondary fruit infections caused by *Botrytis cinerea* were evaluated.

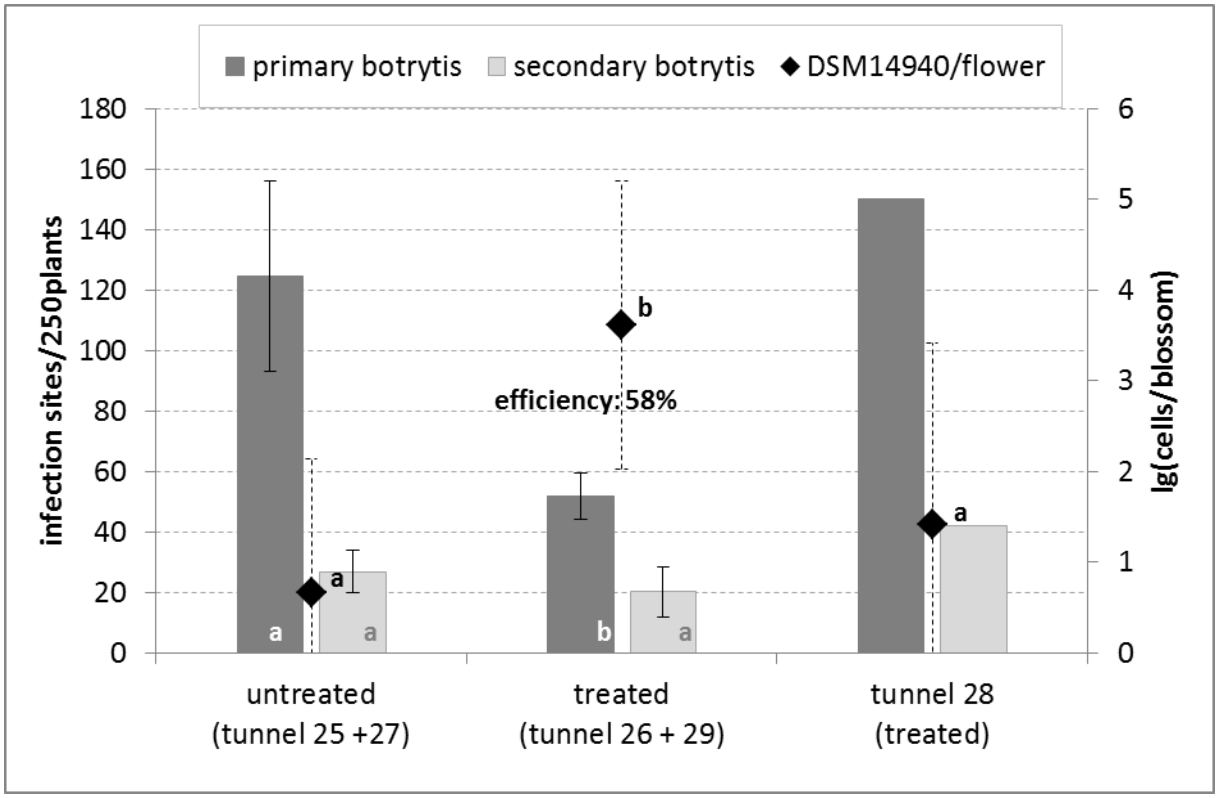


Figure 3: *Botrytis* infection sites and average abundance of DSM14940 on blossoms of treated and untreated tunnels in 2010. Tunnel 28 was not included in statistical analysis of disease incidence (n=2). Different letters in columns indicate a significant difference in Student's t-test (p≤ 0.05); n = 4. Different letters next to rhombs indicate a significant difference in Tuckey's Multiple Comparison test (p≤ 0.05); n = 18-40.

In tunnel 28 low activity of the bumble bee population was noticed during the trial. In consequence, the abundance of *A. pullulans* was significantly lower than in the other treated tunnels and the number of primary infections was higher. Therefore this tunnel was not included in calculations of means and in the statistical analysis (Fig. 3). In untreated tunnels in average less than 10 DSM14940 blastospores per blossom and in average 125 infection sites per 250 plants were found. In treated tunnels more than 1,000 DSM14940 blastospores per blossom and in average 52 infection sites per 250 plants were found (Fig.3). The dispersal of *Aureobasidium pullulans* was able to significantly reduce infections caused by *Botrytis cinerea* with an efficiency of 58%. The number of secondary infections was low and there was no difference between the treatments concerning secondary infections.

In 2010 function of the dispensers sometimes were restricted by high humidity. Therefore a second typeof new developed dispenser was included in the trials in 2011. Exposure, size and configuration of the tunnelswere morehomogenous than in 2010 but plant development in the first three tunnels (20-22) was lightly earlier than in the remaining tunnels. A first evaluation was carried out in tunnels 20-22 on the 19.04.2011(Fig.4).

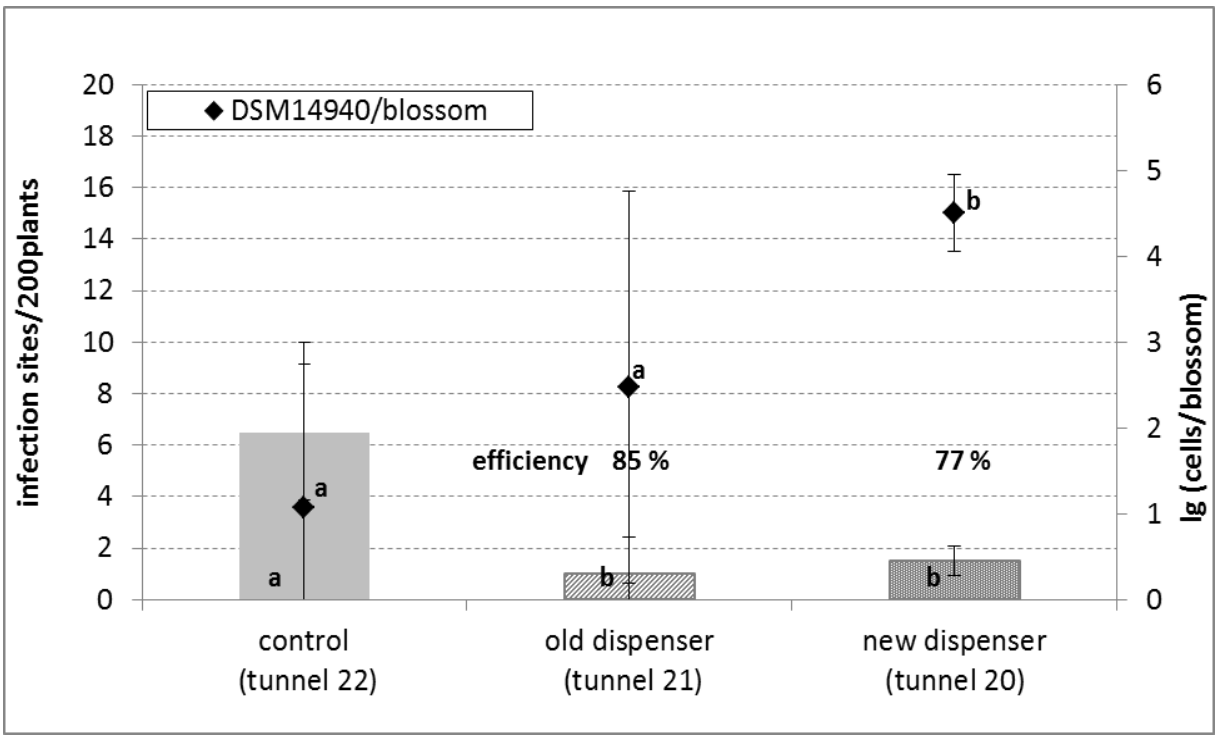


Fig.4: *Botrytis* infection sites and average abundance of DSM14940 on blossoms of treated and untreated tunnels in 2011 (1st evaluation). Different letters in columns (n=4) or in rhombs (n=16) indicate significant differences in Tuckey's Multiple Comparison test ($p \leq 0.05$).

In untreated tunnels in average 6 infection sites were found per 200 plants. Unless the DSM14940 cell number per blossom was significantly lower in tunnel with the old dispenser compared to tunnel with the new one, with both dispenser types a significant reduction of incidence was observed (Fig.4)showing efficiencies of 77% and 85%.

On 28.04.2011 a second evaluation in eight tunnels of similar shape (two control tunnels 22 and 25, three tunnels with old dispensers21,24 and 27 and three tunnels with the new dispenser type:20,23 and 26)was conducted (Fig.5).

Disease incidence increased to 18 infection sites per 200 plants in untreated tunnels.and was significantly reduced in the treated tunnels (Fig. 5) showing efficiencies of 55% and 65%. Again the cell count of DSM14940 was significantly higher in treated tunnels. In this case, cell counts in three tunnels with old dispenserswere compared to cell counts in three tunnels with new dispensers and no significant difference between the dispenser types were found. May be the difference between tunnel 21 and 20 (Fig. 4) was caused by differences in the activity of bumble bees.

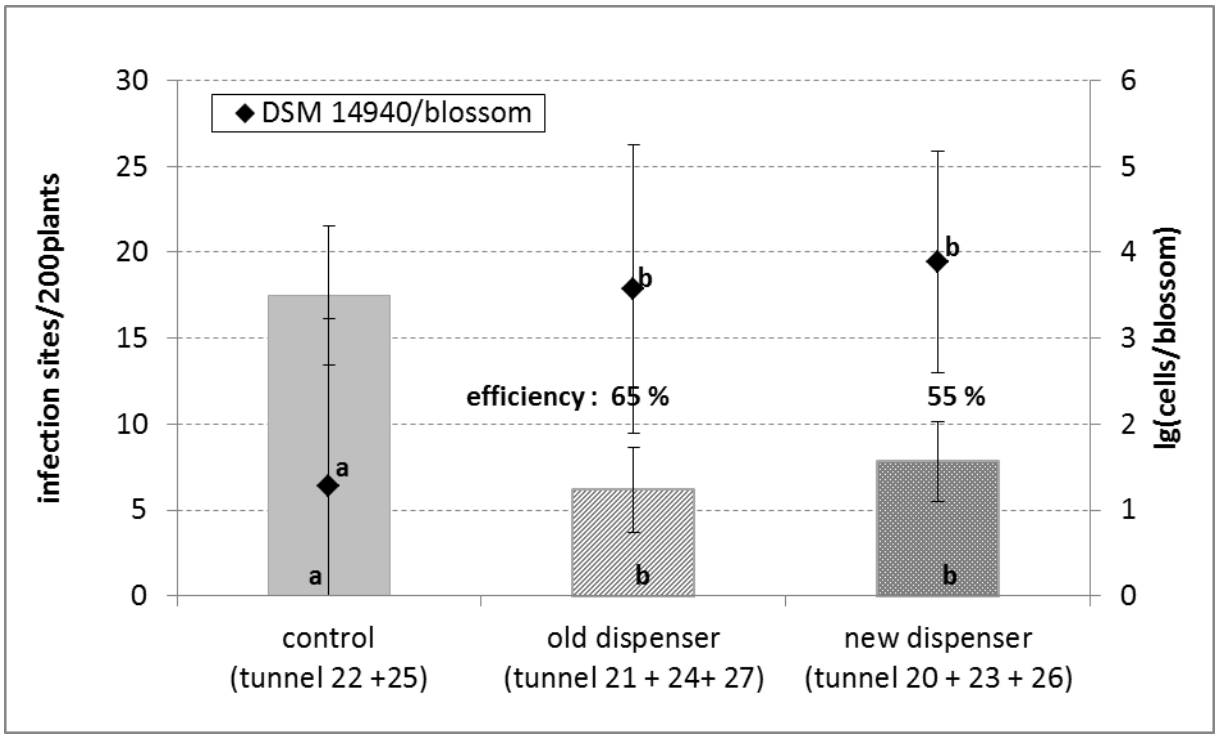


Fig.5: *Botrytis* infection sites and average abundance of DSM14940 on blossoms of treated and untreated tunnels in 2011 (2nd evaluation). Different letters in columns (n=6) or next to rhombs (n=32-48) indicate significant differencesin Tuckey's Multiple Comparison test (p≤ 0.05).

**Discussion:**

The results of thistwo year trials in protected strawberry production showed that it is possible to disperse *A. pullulans,* the active ingredient of the plant protection agentBoni Protect forte, to strawberry blossomsusing pollinating insects. As indicated in other studies (Adikaram, Joyce, and Terry 2002; Mayr and Späth 2008), inserting *A. pullulans* into strawberry blossoms reduced infections by *Botrytis cinerea.* Regarding the fact that in organic strawberry growing *Botrytis*control is difficult up to now, this innovative strategyshowing an efficiency in *Botrytis* control between 60-80% offers an alternative way in future plant protection.Using this method a reduction of amount of product compared to conventional treatment methods and also a reduction of labor for the grower can be achieved. Due to the fact that resistance of strainsof *Botrytis cinerea* against most common chemical plant protection agentsincreases (Weber 2011), this method will also obtain importance in integrated production.

**Acknowledgements:**

We thank Georg Schmälzle for his support and allocation of the strawberry tunnels,Ralf Vögele for providing the qPCR facilities, Monika Schwarz and David Flügel for technical assistance and the BMWi,(FKZ,AIF-ZIM KU2335101 SK9) for funding.

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# Short Contributions

# Evaluation of Polish scab-resistant apple cultivars in organic orchard.

D. E. Kruczyńska[1], K. P. Rutkowski, A. Wawrzyńczak, H. Bryk

**Abstract**

*In the years 2005-2011 the quality and storability of three scab resistant apple cultivars of Polish origin 'Gold Milenium', 'Free Redstar' and 'Melfree' were investigated. The differences in tree growth were noticed. Tree size of 'Free Redstar', expressed as TCSA, was the highest compared to the others, and it gave the highest yield in the experiment. The fruit size varied between cultivars and depended on weather conditions in a season. The smallest fruits, in all seasons, born 'Free Redstar', and the biggest 'Gold Milenium'. During the first five years of observations the symptoms of apple scab did not occurred on the leaves or fruits of evaluated cultivars. No symptoms of sooty blotch and flyspeck of apples were found on any of the apple tree cultivars.*

*Quality of fruits at harvest and after storage depended on the growing season. Regardless of the season apples of 'Melfree' cultivar characterized the highest total soluble solids content (except 2008 season) and titratable acidity in comparison to the other tested cultivars. 'Duration of storage is limited by incidence of storage disorders and diseases. Superficial scald seems to be a problem for 'Melfree' and 'Free Redstar' cvs.*

**Keywords:** organic production, diseases, fruit quality, storability

## Introduction

The breeding of apple cultivars resistant to scab started in last century. However, the taste of many of them was not widely accepted by the consumers. Nowadays the taste of some scab resistant apple cultivars is comparable to highly accepted "standard" cultivars (Kuhn and Thybo, 2001). Weibel et al. (2000) concluded that apples from organic orchards had higher taste marks than conventional ones.

The aim of the study was to investigate the quality and storability of three scab resistant apple cultivars of Polish origin 'Gold Milenium', 'Free Redstar' and 'Melfree'.

## Material and Methods

In the years 2005-2011fruits of three scab resistant apple cultivars of Polish origin 'Gold Milenium', 'Free Redstar' and 'Melfree' were harvested at the organic orchard belonging to Research Institute of Horticulture, Skierniewice, Poland. Trees on M.9 rootstock were planted in 2004, in a complete randomised design with four replications, consisting of five trees per plot.. Each year the crop protection programme involved 3 treatments with a copper preparation carried out before flowering and 2 treatments with a sulphur preparation carried out in May/June.

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Trunk diameter at 30 cm above the ground level was measured and fruit yield was recorded yearly. Trunk cross sectional area (TCSA) and cropping efficiency index (CEI) were calculated. Fruit size and percentage of blush were evaluated.

The scab and powdery mildew symptoms were observed on 400 leaves (for each cultivars). Leaves with symptoms of the diseases were counted and the surface of lesions was measured. The symptoms of sooty blotch and flyspeck of apples were investigated during harvest time.

Quality parameters of fruits (total soluble solids - TSS, titratable acidity –TA, and flesh firmness) were measured at harvest, after storage and after shelf life. Internal ethylene concentration in the core of fruits and starch index were measured at harvest as well.

Fruit firmness was measured on the opposite sides of fruit (blushed and unblushed) using the EPT-1R pressure tester (Lake City Technical Products, Canada), equipped with a 11.1 mm tip. TSS was measured in the juice collected from individual apples with the digital refractometer (ATAGO, PR-101, Japan). TA was measured using the automatic titrator (Mettler-Toledo, DL-21, Swiss), standard titration method, 0.1 N NaOH, pH=8.1 – end point.

Additionally, after storage and after shelf life the incidence of storage disorders and diseases were assessed.

The results were subjected to analysis of variance, and the differences between the means were estimated by LSD ( $\alpha$=0.05).

**Results and Discussion**

The differences in tree growth were noticed. Tree size of ‘Free Redstar’, expressed as TCSA, was the highest compared to the others, and it gave the highest yield in the experiment. The fruit size varied between cultivars and depended on weather conditions in a season. The smallest fruits, in all seasons, born ‘Free Redstar’, and the biggest ‘Gold Milenium’. Data presented by Czynczyk et al. (2004) suggested that ‘Gold Milenium’ (previous name ‘Early Freegold’) is the scab resistant apple cultivars most suitable for sustainable fruit production in modern orchard.

During the first five years of observations the symptoms of apple scab did not occurred on the leaves or fruits of evaluated cultivars. In 2010 the symptoms of apple scab were observed on the leaves of ‘Free Redstar’ cv. The severity of powdery mildew on the leaves of all cultivars was low. No symptoms of sooty blotch and flyspeck of apples were found on any of the apple tree cultivars.

Quality of fruits at harvest and after storage depended on the growing season. Regardless of the season apples of ‘Melfree’ cultivar characterized the highest total soluble solids content (except 2008 season) and titratable acidity in comparison to the other tested cultivars. ‘Melfree’ apples from organic orchard seems to be more acid than from integrated fruit production (Rutkowski et al., 2005). Percentage of blush varied from ca 20% on ‘Gold Milenium’ to 100% on ‘Free Redstar’ apples. Duration of storage is limited by incidence of storage disorders and diseases. Superficial scald seems to be a problem for ‘Melfree’ and ‘Free Redstar’ cvs.

‘Melfree’ and ‘Gold Milenium apples are not only suitable for fresh apples, but also for producing apple juices with a high health-giving value. Those cultivars provide high levels of phenolics in their juice (Markowski et al., 2009).

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# Efficacy of alternative substances to control apple scab by leaf litter treatment

F. Rüdiger[1], N. Nietsch[2], A. Kollar[1], B. Pfeiffer[2]

**Abstract**

*Currently, a frequently used and effective strategy to control apple scab in organic fruit growing is the application of copper in combination with sulphur. With regard to the intended EU-wide prohibition of copper as a plant protectant, it is thus of the utmost importance to find comparable alternatives. This study therefore focuses on the decrease of the infection threat during spring by the reduction of sporulation from the leaf litter. This reduction is to be achieved by treatments of the leaf litter with substances, that potentially have a direct fungicidal effect on the apple scab pathogen itself or enhance microbial competition, and, secondly, show an indirect effect by increasing the attractiveness of the treated apple leaves for earthworms and consequently their decomposition.*

*In addition to several plant extracts, two yeast extracts, a soya-containing medium and different individual components were tested. First results show, that casein-peptone, TSB (Tryptic Soy Broth) and casamino acids can reduce the ascospore potential by more than 90 % compared to the untreated control. Furthermore, the first experiments indicate, that one of the yeast extracts leads to an accelerated decomposition of the leaves by earthworms.*

**Keywords:** *Venturia inaequalis*, apple scab, leaf litter, ascospore potential, organic fruit production

## Introduction

Apple scab (*Venturia inaequalis*), which is a serious problem in apple growing regions worldwide mainly overwinters on fallen leaves. During rain events in spring, pseudothecia release ascospores, which constitute the principal source of primary inoculum (MacHardy *et al.* 2001; MacHardy 1996). Aim of this study is a reduction of the ascospore potential in spring without removal of the organic matter from the orchard. This especially offers a solution for smaller organic fruit growers to reduce ascospore dose of fallen leaves without removing the leaf litter from the orchard as it is done with vacuum foliage collectors. Several media and plant extracts were tested considering a direct fungicidal effect on the apple scab pathogen and an acceleration of the leaf decomposition by attraction of earthworms. Germination tests of conidia were made prior to the selection of plant extracts and media to estimate their potential fungicidal effect. The experimental design of the presented work is based on the previous study of Kollar and Pfeiffer (2003) and several results of former years (Pfeiffer, 2010; Pfeiffer *et al*., 2004; LVWO Weinsberg, 2006; LVWO Weinsberg, 2009). These investigations revealed that yeast extract seems to have a beneficial effect on the palatability of apple leaves for earthworms.

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**Material and Methods**

In November 2010 dried leaves that were heavily infected with apple scab were exposed in the open field in two different ways.

In the orchard of Weinsberg, the individual leaf depots (200g/0,5m²) were placed directly onto the ground and covered with wire meshes. From January until March 2011 (January 17th 2011, February 1st 2011, February 23th 2011, March 11th 2011 and April 25th 2011) different plant extracts, nutrition media and yeast extracts were applied to the individual leaf litter depots (each 150 ml, corresponds to 300l/ha) (table 1). Each treatment was replicated three times.

To determine a potential fungicidal effect of the applicated media, ascospore dose was measured with the water bath method published by Kollar (2000). Therefore, samples were taken between March 3rd 2011 and June 6th 2011 in regular intervals of 4-12 days.

Leaf litter was in direct contact to the soil to determine the palatability of the treated apple leaves for earthworms. Between January 11th 2011 and June 21st 2011 a visual rating to record degradation of the leaf litter and activity of earthworms was done every 6-26 days, according to weather and soil conditions (% of amount at the beginning of the experiment).

Table 5: Extracts, media, concentrations and number of treatments on the trials of Dossenheim and Weinsberg 2010/2011.

| agent | Weinsberg | Dossenheim |
|---|---|---|
| Water control | | 4x |
| Untreated control | x | |
| Tryptic soy broth 9% | 5x | 4x |
| Tryptic soy broth 9%, | | 4x |
| Tryptic soy broth ethanol extract 80 % | | 4x |
| Tryptic soy broth ethanol precipitate 80 % | | 4x |
| Tryptic soy broth 9%, heat treatment | | 4x |
| Yeast extract *Foodgreen* 1,1 % | 3x, 5x | |
| Yeast extract *Leiber* 1,1 % | 3x, 5x | 4x |
| Yeast extract *Leiber* ethanol extract 80 % | | 4x |
| Yeast extract *Leiber* ethanol precipitate 80 % | | 4x |
| Yeast extract *Leiber* heat treatment | | 4x |
| Yucca saponin, 1,5 % (vol %) | 5x | 4x |
| Primrose root extract 6%* | 5x | 4x |
| Saponaria officinalis 10% (6% vol)* | | 4x |
| Quillaja officinalis (bark)10% (6% vol)* | | 4x |
| TSForte 1 % | | 4x |
| Palmarosa 0,1% + TSForte 1% | | 4x |
| Peptone casein 6 % | | 4x |
| Peptone Soy 6 % | | 4x |

* water extract of 10 g plant material/100 ml water, diluted to 6 % aqueous solution

In Dossenheim contact of the leaves to the ground was avoided by putting them into sowing-boxes (each filled with 70 g) and covering the soil with saran tissue. Treatment of the leaves was done on January 11th 2011, February, 16th 2011, February 27th 2011 and March 7th 2011. In total 18 different treatments were tested with one replication each. Samples to record ascospore potential were taken between March and June 2011 every

week. To determine the amount of microorganisms via CFU, a sample (each 1g) of every leaf depot was taken at the beginning of march. Leaf samples were crushed in liquid nitrogen and shaken in 99 ml distilled water for 15 min. A series of dilution of this solution was plated on TSA agar enriched with cyclohexemid (0,4 µg ml-1) and incubated for three days at 20 °C. The number of CFUs was calculated according to the formula *CFU/ml= number of colonies*solution*10*.

Intention of this work was to test a broad range of substances, which potentially reduce infection pressure during spring. According to this the experimental design constitutes a temptative test with up to 18 variants and n ≤ 3 and statistical assessment is not provided.

## Results

Weinsberg: A considerable reduction of the leaf litter by earthworms could be detected in February. At this date, more than 50 % of the leaf litter treated with *Leiber* yeast extract was already degraded, while all other variants showed a slower reduction. This tendency, a promoting effect of *Leiber* yeast extract, could be observed over the period of the whole experiment. With the end of March, an accelerated leaf decomposition was also visible for the TSB variant. In the mid of April more than 95 % of the leaf litter treated with *Leiber* yeast extract or TSB were decomposed. The value of all other variants was between 15 and 20 %. In general, the ascospore potential was very low during the experimental period of spring 2011. Compared to the control, all of the media tested in Weinsberg showed a reduction of the ascospore potential. Due to the fast leaf decomposition of *Leiber* yeast extract and TSB, the ascospore potential of these variants could only be determined until mid of April. The most effective media were *Foodgreen* (3x) and Yucca saponin with a reduction rate of about 50 %. *Foodgreen* (5x) and Primroot showed a lower efficacy with a ascospore potential of 70 and 85 % compared to control, respectively.

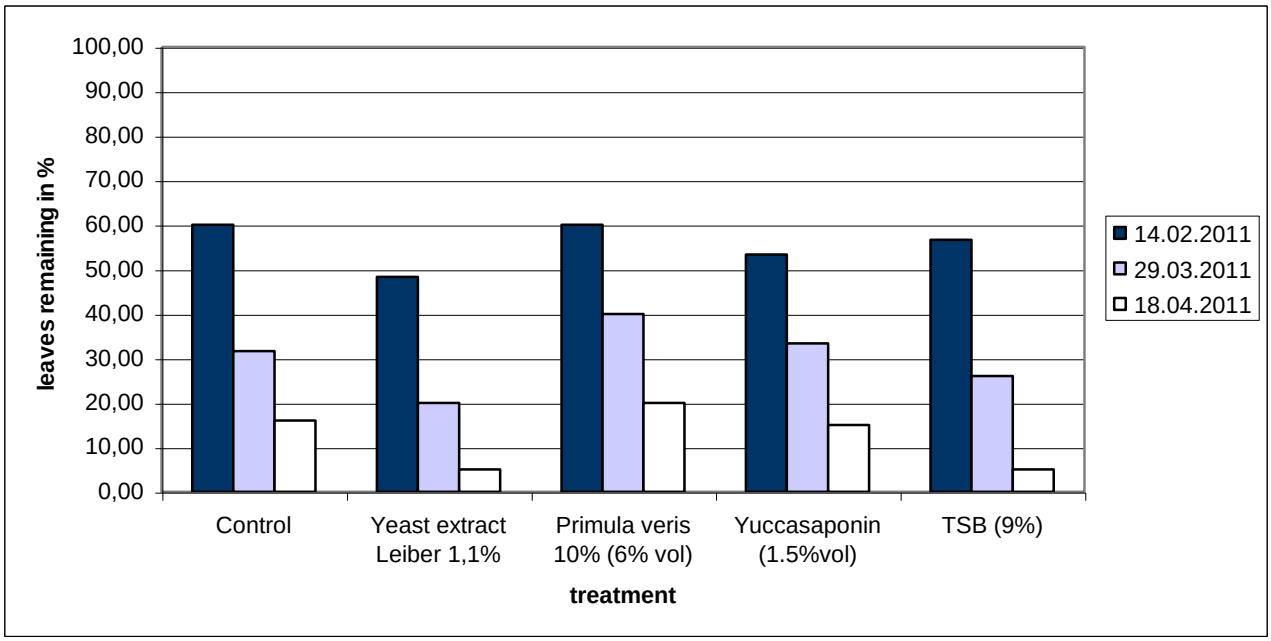


Figure 6: Leaf decomposition trial in Weinsberg 2010/2011. Untreated control in comparison with yeast extract *Leiber*, *Primula versis*, Yuccasaponine and TSB. Until 14.02: 2 treatments, 29.03 and 18.04: 5 treatments.

Dossenheim: Compared to Weinsberg the ascospore dose in Dossenheim was very high. The most effective reduction of discharged ascospores was observed for Peptone casein, TSB (9%) and Casamino acids with an ascospore potential of 2, 4 and 5 % compared to the control. By treatment with *Saponaria officinalis* and Yuccasaponin discharge of ascospores was lowered to 60-65 %. *Quillaja officinalis*, yeast extract *Leiber* and extract of *Primula veris* were the less effective variants, with an ascospore potential of more than 80 % compared to control.

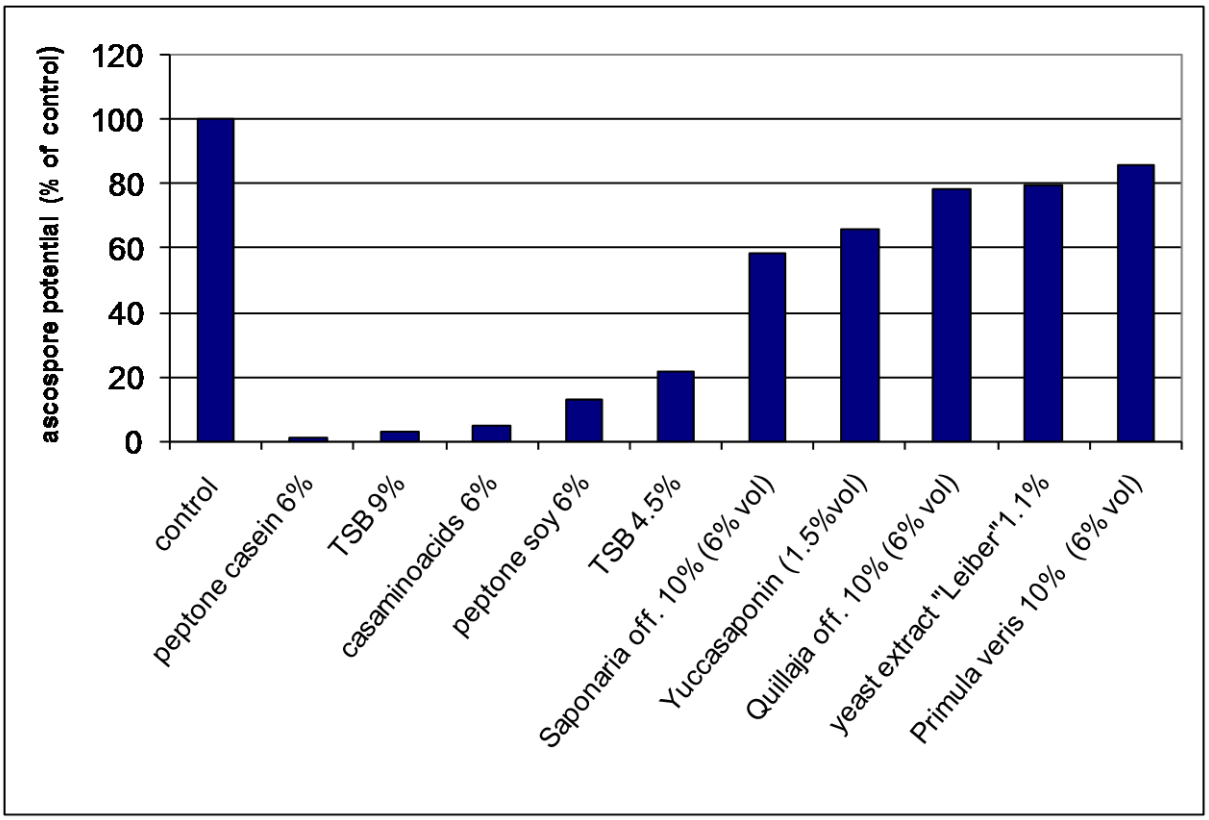


Figure 7: Cumulated ascospore potential (in % of the control) in Dossenheim up to June 2011.

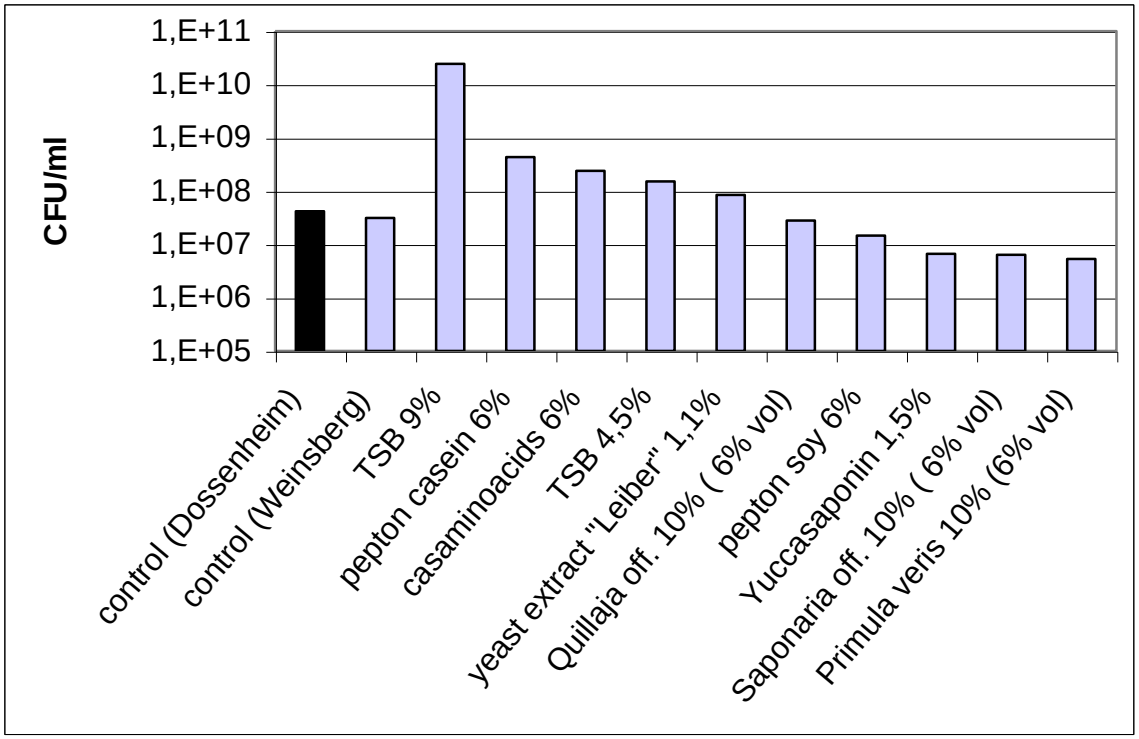


Figure 8: Microbial colonization of leaves depending on treatment. Results for the trial in Dossenheim supplemented by control value of Weinsberg.

Evaluation of the CFUs showed, that the number of bacterial colonies was significantly increased for TSB 9%, peptone casein, casamino acids and TSB (4,5 %) compared to control. The saponin containing plant extracts (Yucca, Saponaria, Primula) as well as the yeast extract did not have any effect on the colonisation of the apple leaves with bacteria.

## Discussion

The initial results have shown, that peptone casein, casamino acids and TSB are promising substances to reduce the ascospore potential of fallen leafs. The trial in Weinsberg additionally showed an accelerated leaf decomposition by treatment of the leaves with TSB or *Leiber* yeast extract. Because of this, in the mid and end of April decomposition of the leaf depots with contact to the ground was proceeded so far, that no more leaves were present when ascospore potential increased clearly at the end of April. The results for *Leiber* yeast extract confirm with the research work of the former years, where activity of earthworms was enhanced as well. The results of ascospore discharge align with the microbiological examination, where the variants with the most effective reduction of the ascospore potential had the highest number of bacterial colonies. In the following period of the project, additional substances are to be tested for their direct or indirect effect on the apple scab pathogen. Furthermore the results of the most promising media of the research work 2010/2011 have to be confirmed. Also a combination of media with fungicide effects and those which promote leaf decomposition will be done.

## Acknowledgements

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# Strategic irrigation against apple scab (*Venturia inaequalis*)

M. Korsgaard[1]

**Abstract**

*In Denmark there are several organic orchards, that do not spray at all, and they seek non-spraying methods to prevent apple scab. In this pilot trial, irrigation with water at strategic moments was tested to prevent scab-infection.*

*In April-June 2011 in five organic orchards we irrigated the orchard floor, to force the ascospores to be released. We irrigated with at least 0.2 mm of water in dry periods, at least 12 hours before rain forecast. The idea was to empty the stock of ascospores during dry spells, so they would dry out without infecting the leaves. To find the best strategic times for irrigation, we used the local weather forecast and the scab-warning programme Rimpro based on data from climate stations located in the orchards.*

*In this first year of trial we experienced difficulties in spreading the water evenly. A water wagon turned out to work better than sprinklers. We found, that the irrigation did result in ascospore-release, but the effect on the scab-attack was not significant. We also experienced, that the fruit growers found this way of preventing scab very interesting and easy to carry out. The pilot-trial was financed by "Fonden for Oekologisk Jordbrug" and in 2011 it was conducted by the organic advisory-service. It is continued in 2012 and 2013 at the University of Copenhagen.*

**Keywords:** *Venturia inaequalis,* non-spraying, prevention, organic apple production.

## Introduction

Apple scab is often causing serious losses in apples, especially if springtime is humid and in susceptible varieties.

In 2008 and 2009 the apple scab was not a problem in Denmark. This was due to dry weather during spring and early summer, and the fact, that dry spells got interrupted by short showers of rain.

These rain showers released the overwintering ascospores. But the showers were short and the leaves dried rapidly, hence the spores never got humidity enough to infect the leaves. They simply dried out.

The two seasons with none or very low level of scab-infections gave the idea of utilizing the climate by irrigating at strategic moments. Such a scab preventing method would be helpful to the Danish organic fruit growers, who produce unsprayed apples, and could also reduce the number of scab-sprays in sprayed orchards.

## Material and Methods

In five organic apple orchards we established an irrigation system to irrigate the orchard floor. We simulated short showers of rain during dry spells from beginning of April till the beginning of June.

The growers decided themselves when to irrigate. Their decision was supported by the local weather forecast combined with the scab warning-system Rimpro, based on data from their own climate station.

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The irrigation was turned on in situations, when Rimpro showed that many ascospores were ripe, and the weather was dry, and rain was forecasted later than within12 hours.

The growers irrigated with at least 0.2 mm water, which is 0.2 l/m².

Three of the hosts put up sprinklers to irrigate the orchard floor. Two of the hosts created a water wagon.

The trials were conducted in 6 rows of apples. In the orchards with sprinklers, we irrigated every other plot of 15m row.

In the two orchards with water wagon the 6 rows were either irrigated or not irrigated.

The degree of scab attack on the leaves and on the apples got registered in July on 5 trees in the middle of every plot. The infection level on the apples got registered again in September, right before harvest.

The release of ascospores got registered in a spore trap in April, to see if the ascospores did react to the irrigation.

The five hosts irrigated against scab from 4 till 12 times during April and May, see table 1.

The differences reflect the different climatic situations in the five orchards and their slightly different strategies of irrigation. Some of the hosts choose to irrigate once a week during dry spells to empty the stock of ascospores combined with irrigation the day before rain forecast. Other hosts choose only to irrigate the day before rain forecast.

Table 1: Description of five trials with irrigation against apple scab.

| Host No. | Location | Varieties and age of trees | Other types of scab control | Irrigation method | Number of irrigations in 2011 | Dates of irrigations |
|---|---|---|---|---|---|---|
| 1 | Slangerup, Nord-sjælland | 'Aroma', 20 years | Biodynamic preparations | Water wagon | 4 | 11/4, 25/4, 29/4, 7/5 |
| 2 | Kyse, Syd-sjælland | 'Holsteiner Cox', 3 years | None | Sprinklers | 12 | 4/4, 11/4, 15/4, 18/4, 26/4, 31/4, 2/5, 10/5, 12/5, 21/5, 31/5, 5/6, 6/6 |
| 3 | Regstrup, Vest-sjælland | 'Aroma', 5 years | None | Sprinklers | 9 | 11/4, 18/4, 25/4, 28/4, 2/5, 8/5, 10/5, 21/5, 5/6 |
| 4 | Otterup, Nordfyn | 'Gravenstein', 11 years | Sulfur-sprays | Sprinklers | 7 | 7/4, 10/4, 11/4, 18/4, 25/4, 1/5, 9/5 |
| 5 | Harndrup, Midtfyn | 'Elshof', 15 years | Sulfur-sprays | Water wagon (from old sprayer) | 7 (x 2 each time) | 14/4, 17/4, 30/4, 2/5, 14/5, 21/5, 5/6 |

**Results**

There was less than 1% scab in orchard no. 4 and 5. These orchards had a very dry spring, and in both of the orchards sulphur sprays were also used to control scab.

In orchards no. 1-3, 39 - 74 % of the apples had spots in September. The spots were caused by both scab and an undefined “spot” disease.

In orchard 2 and 3 there was a tendency to a small, but positive effect of the irrigation, but the level of scab rose very fast in July and August, while the weather turned extremely wet (Figure 1 and 2).

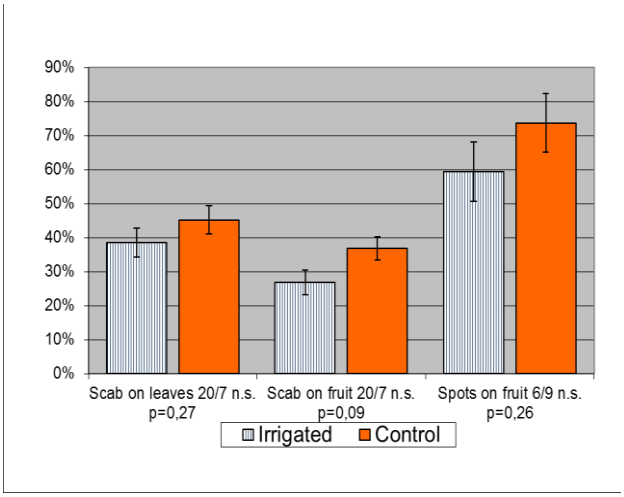


Figure 1: Effect of strategic irrigation on apple scab 2011. Orchard no. 2. Variety: Holsteiner Cox

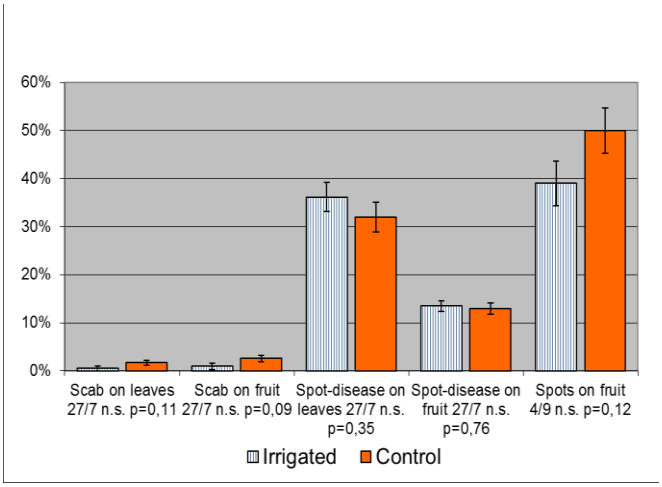


Figure 2: Effect of strategic irrigation on apple scab and "spot"-disease 2011. Orchard no. 3. Variety: Red Aroma

In orchard 1 the water wagon was top sprinkling, so the whole tree got wet. That gave a tendency to a slightly negative effect of the irrigation, when monitored in July, but in September, there was no difference between irrigated and non-irrigated plots.

The irrigation of the orchard floor did result in ascospore-release, this was confirmed by counting ascospores in a spore trap.

Another result of the trial was the gaining of technical experiences on the irrigation equipment; see the evaluation in table 2.

The quality of the weather forecast is important for this method. We found, that in some cases the Norwegian weather forecast (www.yr.no) service is more accurate, than the Danish (www.dmi.dk).

We also experienced, that the growers found it very interesting to conduct the experiment and to decide when to irrigate. All five hosts would like to use the method in general, if we find the right “recipe”.

Table 2: Evaluation of the equipment for irrigation against apple scab

| Equipment | Description | Advantages | Disadvantages |
|---|---|---|---|
| Water wagon, rebuilded manure spreader | The manure spreader was mounted with a pump and shortened, to be able to turn in the rows. | It has a large water tank, and needs not to be filled often. It spreads the water over several rows, which is faster. | The irrigation is wetting the trees, which requires a longer dry period after the treatment. The chance of good moments for scab-irrigation are reduced. |
| Water wagon, rebuilded mist sprayer. | 1000 l mist sprayer, where the compressor was removed and a bar with sprinklers*) was mounted in front. The pump was geared up. | Evenly distribution of the water on the orchard floor without "shadow" effect from the trunks. The trees does not get wet at all. Fairly cheap to rebuild. | The treatment takes time.The water tank is small and needs to be filled often. The long treatment time dimishes the chances of optimal irrigation moments. |
| Sprinklers hanging in the rows, underneath the branches. | A hose is hung up app. 0,5 m above ground in the tree rows. Microsprinklers**) was mounted between every 2.-3. tree. | Easy, you just turn on the water for 3-10 minuts (depen-ding on the water pressure). The short treatment time gives better chances of optimal irrigation moments. | The trunks ar shadowing the irrigation, leaving dry spots. The water pressure was too low to cover the orchard floor. Hanging branches are in risk of being irrigated. |

*) The sprinklers are Hardi Round nozzles no. 1553-40, 10 l/min at 2 bars.
**) Micro sprinklers no. 022201 from Dansk Vandingsteknik.

## Discussion

The strategic irrigation against apple scab did actually provoke the ascospores to be released. But the method has to be improved to gain a satisfactory effect.

The limitations of the method got clear in 2011: If scab is not completely prevented at midsummer, a following wet summer will result in a too heavy scab attack on the fruit.

It is also a question how much impact conidiaspores from neighbouring areas are having on the late scab attack.

It is important to find a technical design, which works fast and easy and covers the whole orchard floor. We will work on improving the technical design in 2012 and also work with higher amounts of water.

## Acknowledgements

I would like to thank the following: "Fonden for Oekologisk Jordbrug" who financed the trial in 2011, conducted by the Organic advisory service. The five organic fruitgrowers, hosting the trials. The Danish Ministry of Food Agriculture and Fisheries, which are funding the trials in 2012 and 2013, conducted by the University of Copenhagen.

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# Effect on abundance of important pome fruit pests and diseases by mixing four apple varieties in one orchard

B. Benduhn[1], S. Brauer[2], C. Adolphi[3]

**Abstract**

*In 2007 a mixed cultivation consisting of four little genetically related varieties of apple was planted in an ecologically managed orchard. There is a monitoring for any changes in abundance of important pome fruit pests and diseases. This will include a review of the biological consequences of a mix of varieties. In addition, economic data of culture are measured and evaluated with known data.*

**Keywords:** mix of varieties, apple scab, *Venturia inaequalis*, pests

## Introduction

Most commercial fruit growing farms handle just a few Malus species over a large area. These genetically identical plants have an increased risk for the occurrence and spread of insects and fungal organisms especially *Venturia inaequalis* (Kellerhals et al., 2003). Aim of the project was to determine whether mixed orchards can contribute the abundance of pests and diseases. Additionally the work processes of mixed orchard and monoculture are compared to show whether possible economic disadvantages could be balanced by advantages of a variety mix like lower use of plant protection agents.

## Material and Methods

The trial orchard is located in a typical organic fruit farm at the area “Altes Land”. The orchard was divided into a mixed orchard and a monoculture system (Fig. 1). Each system consisted of four *Malus* species growing on M9 rootstock with a planting distance of 1.2 m in rows and 3.8 m between rows. The mixed orchard consists of 1.800 trees; the pure orchard consists of four species, each consisting of 120 trees planted in two rows. In the mixed orchard the varieties are the same as in the monocultures. Within the variety-mix always two trees of one cultivar standing next to another were combined to one unit. This "two-tree units" have the greatest possible distance within and between the rows.

Caused by the young age of the orchard, the differences are actually comparatively small. For this reason, only the actual situations concerning to apple scab as an example for fungal diseases as well as economically data are shown below. Furthermore investigations concerning powdery mildew *(Podosphaera leucotricha),* apple sawfly *(Hoplocampa testudinea)* and red spidermite *(Panonychus ulmi)* are done.

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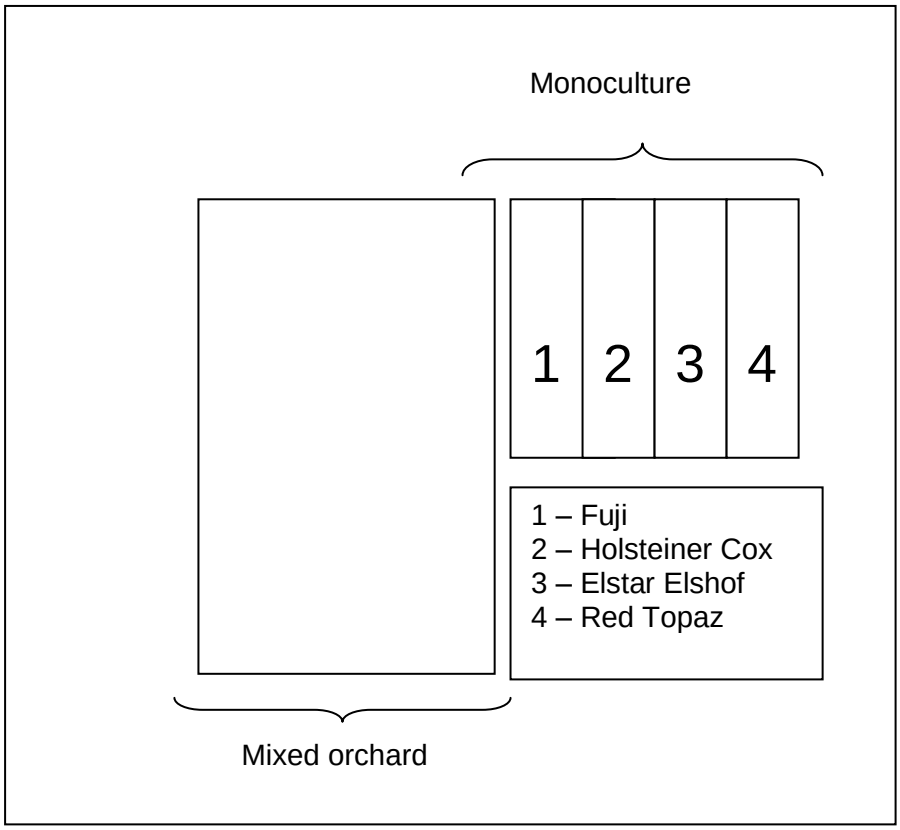


Figure 1: Planting plan mixed orchard and monoculture (not to scale) (Fieger-Metag, N., 2009, modified)

Orchard management and plant protection was done by the fruit grower. Treatments were applied with a tractor mounted sprayer. During the first year of planting no plant protection products were used. 2008 and 2009 plant protection products and application rates in the trial and the remaining fruit farm were the same. In 2010 plant protection measures in the secondary scab season were reduced.
The collection of economical data and the monitoring of possible pest infestation is the most important matter to be attended.

**Results**

The results of investigation, based on observed changes in pest infestation and apple scab are not affected by extreme differences in data. In 2010 the primary apple scab infections were particularly high. The number of scabbed leaves and fruits were rather low even so the application of plant protection products was reduced in the orchard (Fig. 2). The high susceptibility to scab infections of `Fuji´ was recognized in all observations.

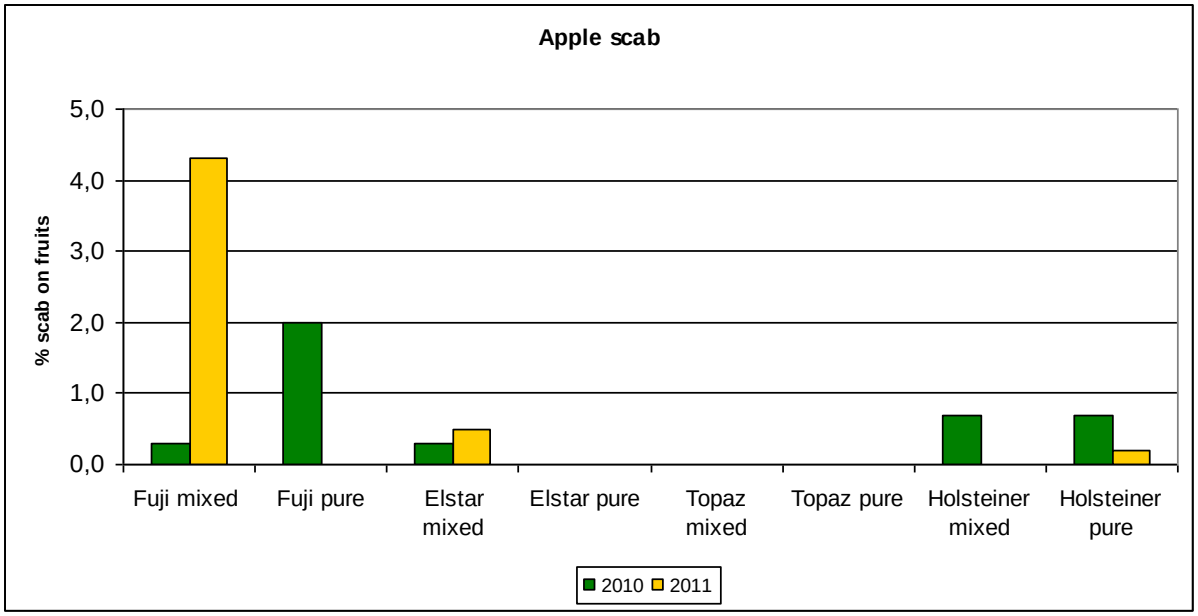


Figure 2: Scab on fruits in 2010 and 2011

Economic data are also used to analyse the effort of tree planting, which was twice as high in the mixed culture as in the monoculture (Beer, M., 2011). Productivity of fruit picking was measured by the picking rate. Differences in rates of pick are due to volume of fruit per tree, tree height and fruit size. The fruit picking rate in the mixed orchard of the *Malus* species Topaz was 1 % lower than in the monoculture. In `Fuji´, however, the difference was about 11 % (Fig. 3).

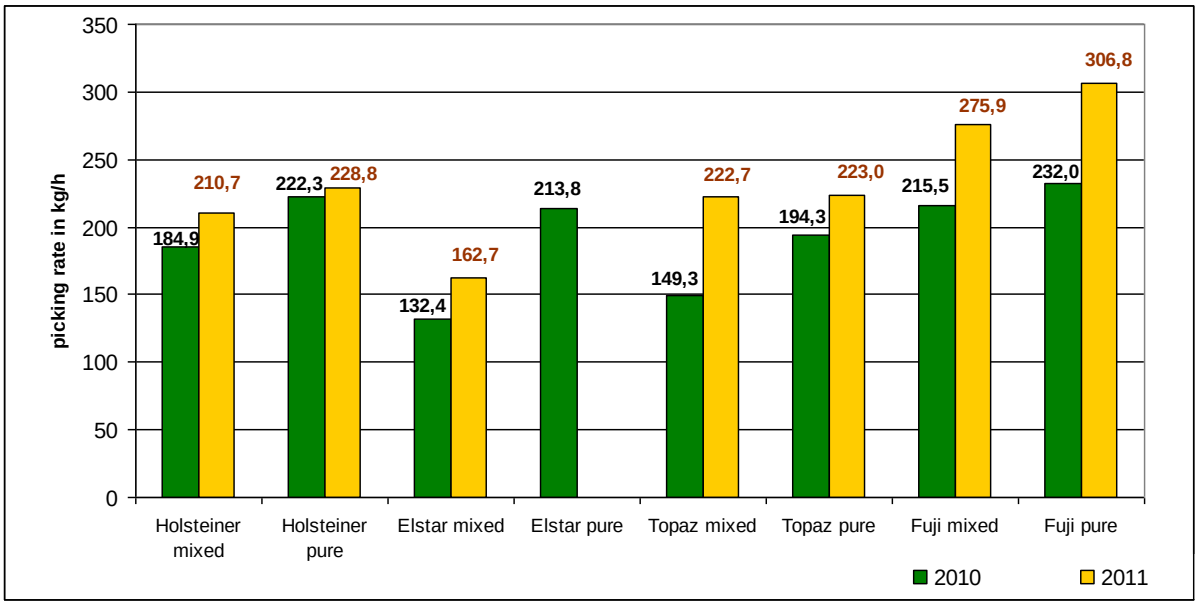


Figure 3: Economic data in both systems 2010 and 2011

Investigations on powdery mildew *(Podosphaera leucotricha),* apple sawfly *(Hoplocampa testudinea)* and red spidermite *(Panonychus ulmi) do not show any differences in degree of infestation,* so far.

## Discussion

During the five-year trial, the differences in pests and diseases between the pure culture and the mixed culture stayed on a low level. The high picking rate of `Fují´ in the monoculture about 307 kg per hour is due to the volume of fruit per tree and the tree height which made harvesting convenient. This will decrease in the following years because those plants grow into larger trees. The effort in the mixed culture will also increase due to the different varieties. Apple trees must be accurately labelled in order that seasonal workers can reap the fruits correctly.

Over the years no significantly differences between mixed plantation and monoculture in consideration of pest infestation and apple scab were observed. It is essential to continue the observation to see whether monocultures have a higher level of pest population than mixed orchards.

Due to the various *Malus species* management of mixed orchards in comparison to monoculture is very time consuming and labour intensive. For mixed orchards efficient harvesting and good logistics are fundamental to be profitably.

The results strongly suggest that further investigations concerning on fruit yield data, efficiency of fruit pickers, time and effort during pest management, plant protection and pruning may need to be done, to give a critical evaluation of the economical characteristic of mixed and mono cultures.

## Acknowledgements

We would like to thank Jörg Quast for his willingness to plant a mixed apple orchard and his cooperation as well as the BÖLN (Bundesprogramm Ökologischer Landbau und andere Formen nachhaltiger Landwirtschaft) for funding the project.

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# Differences in leaf litter, ascospore production and infection of pear scab (*Venturia pirina)* in Dutch organic orchards

B.G.H. Timmermans[1] and P.J. Jansonius[1]

**Abstract**

*The last two years we measured the amounts of leaf litter and ascospore production per unit of leaf litter area in 7 organic pear orchards throughout the Netherlands. In one of the orchards, adapted managements strategies were implemented two years ago, being grass/clover that is grown as ground cover on the tree-strip, and organic cattle-manure that replaces chicken manure pellets, in order to stimulate the earthworm population and change the palatability of the leaf litter.*

*First results indicate large differences between orchards in percentage of ground covered by dead leaves at the time of major ascospore infections, but also in number of ascospores per cm$^2$ leaf litter and in resulting potential ascospore dose. We used these data, together with weather data (temperature, rainfall), in a simple multivariate analysis to gain insight in the dynamics of the system. In 2010, 85 % of the variation in pear scab was explained with a model with rainfall during summer and the amount of ascospores per unit of leaf area. In 2011, 81 % of the variation was explained by a model with the amounts of ascospores per unit leaf area and the potential ascospore dose. In the adapted management experiment we measured no changes in leaf litter in the treatments yet.*

*We discuss that our first results show that, to a limited degree, leaf litter was indeed important for the scab epidemic in 2011, whereas in 2010 the high amount of rainfall in the second part of the growing season must have led to a high conidial infection pressure. Surprisingly, in both years ascospore number per unit leaf area was of more importance than leaf litter area or potential ascospore dose. This raises questions on for example the correlation with branch-lesions that we did not measure, and whether the number of ascospores per unit of leaf litter is a direct or an indirect factor that steers the scab incidence in the orchards.*

**Keywords:** Conference, farmyard manure, leaf decomposition, disease cycle, rainfall

## Introduction

In The Netherlands, pear scab (*Venturia pirina* Aderh.) is a major fungal problem in organic pear orchards, and a constant worry for the farmers. During the last few years, a number of organic pear farmers, who formerly had only small percentage of scab infected fruits in their orchards, have seen increases in the scab incidence. This irrespective of their intensive management that formerly seemed enough to control the fungus.

To control scab, one way to handle the problem can be to search for new and more effective additives that can be used. These can be sprayed directly on trees and fruits, or used as indirect system-controls to steer towards a more scab-suppressing orchard condition. Two years ago, we reported about an experiment in which we used beetroot Vinasse on leaf litter to decrease potential ascospore dose in Conference (Timmermans *et al*., 2010). Preliminary experiments in more controlled conditions (Köhl, 2007) reported promising results but after translating these to practical measures on field scale, we

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measured the opposite, which could be due to variation in Vinasse quality or the application method we used.

In contrast with apple scab (caused by *Venturia inaequalis* Cooke) pear scab can overwinter not only on the leaf litter covering the soil, but also on the braches of the pear trees as lesions. Therefore, ascospores formed on leaf litter are not the only starters of infection in spring.

In order to gain insight in the disease cycle of pear scab and the role of ascospores, we developed a means of measuring leaf litter and number of ascospores per unit of leaf litter area at the time of the first major ascopore infections in spring. First visual impressions were that there were quite big differences between orchards, and we devised adapted managements strategies to see whether we could steer differences, or whether they are (partly) intrinsic to local soil and (micro)climate.

**Material and Methods**

The orchards: collecting leaves

This project has been performed with leaves from 8 commercial orchards, throughout The Netherlands, in which standard commercial fungicide management has been applied. In the winter of 2009-2010 and 2010-2011 fallen leaves have been collected in the 8 orchards, by walking and collecting around 2-3 kg of fallen leaves from the soil throughout each of the orchard-fields. Many small samples were taken to obtain a reliable sample. The leaves have been transported to the Louis Bolk Institute and after mixing placed in a field in wire-mesh cages with a small mesh at the bottom that prevented earthworms from entering. In spring, during a heavy rain shower a plastic foil was placed at 50 cm height above the mesh cages, to prevent ascospore discharge.

Measurement of leaf litter

In spring of 2010 and 2011, at the time we presumed the major ascospore discharges would take place (21, 22, 26 April, 15, 17 May 2010; 14, 19, 20 and 21 April 2011) the remaining leaf litter present in the 8 orchards was quantified. This was done by using a wire mesh (mesh size 4 mm) and placing it on the soil every 10-20 meters (depending on the length the orchards) in several rows throughout the orchards. The mesh was adapted in length to be half the row distance of each orchard, and placed from tree-base to mid-row. On each placing location the amount of wire-squares that were filled with leaf litter, in one line of the mesh, was counted (totals of 54 – 163 counts per orchard).

Measurement of the potential ascospore number per unit of leaf area

After a period of warm and dry weather (around 20°C during the day) leaves in each mesh cage were sampled (April, 2010 and 2011): the loose and slightly moist leaves at the top were mixed and a 25 g sample was taken from each mesh cage. The wet and compacted lowest level of leaf litter was not sampled. Leaves were then incubated at room temperature in trays lined with wet tissue and covered with a porous plastic sheet for two weeks.

Measurement of the ascospore number was done in a method according to Kollar (2000): the 25 g leaf material from each mesh cage was put into 1 l glass jars and submerged in 500 ml demineralised water, in which the leaves were shaken for 1 hour. Then, the material was poured out of the pots and sieved with a 0.25 mm sieve to remove the course material and subsequently with a 53 µm sieve to remove finer detritus. The filtrate was placed in a centrifuge for 5’ at 3300 rpm. The supernatant was pipetted into 1 ml of water

and kept at -20°C until counting, that was done on subsamples in a Bürker counting chamber under a microscope at 400x magnification. A handful of the incubated leaves were spread out and a digital image was taken, from which the area per g leave was measured using image-J.

Measurement of scab infection

In each orchard, also the scab infection was measured. In 2010 this was done by randomly picking 1000 fruits (picking one side of a tree fully, every 10-20 m) in 2 of the rows were the leaves were collected and the leaf litter was measured in spring. The fruits were then scrutinized for scab infections. In 2011, the scab infections were measured by walking through each orchards and randomly turning 500 pears per orchards, and scrutinize them for infections, by Marc Trapman (Biofruit advies b.v.) and the authors.

Adapted management experiment

In one of the orchards with a history of high scab infection and a visibly bad decomposition of overwintering leaves, two adapted management strategies were applied starting in winter 2009-2010. The orchard is located in the province of Flevoland on clay soil and has older V-shaped Conference trees and little light on the soil. Row distance is 3.5 m and the trees are planted 1.2 m apart from each other and have 4 branches each. Each management strategy was performed in two repetitions of 54 m length and 3 rows width. Treatments were:

- Control: autumn 750 kg/ha organic chicken pellets (3% N), spring 500 kg/ha organic chicking pellets enriched with beetroot vinasse (8% N), tillage 2 times a year (autumn, spring)
- Adapted manure treatment: march 20 ton/ha cattle manure (in 2010 mixed with compost) instead of the chicken pellets
- Adapted tillage: no tillage
- Adapted undergrowth: grass/clover (*T. repens* cultivar Alice, sown in spring 2010 and resown in spring 2011)

Statistical analysis was done using R (R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org).

## Results

Leaf litter on the soil in the 8 orchards varied a lot (Figure 1A.). Orchards 1,2,4 and 8 had very small amounts of leaf litter in both years, whereas others (6,7,5) had quite some leaf litter in both years. Strikingly, two orchards in Zeeland seemed to have much more leaf litter in 2011 than in 2010.

Also, there were large differences in the potential amount of ascospores on the leaf litter as was measured under conducting conditions in the lab (Figure 1B.). Multiplying these measurements gives the potential amount of ascospores per ha (potential ascospore dose) in each of the orchards, as a measure of potential infection pressure by ascospores (Figure 1C.). Finally there was also large variation in scab infection in the two years (Figure 1D.).

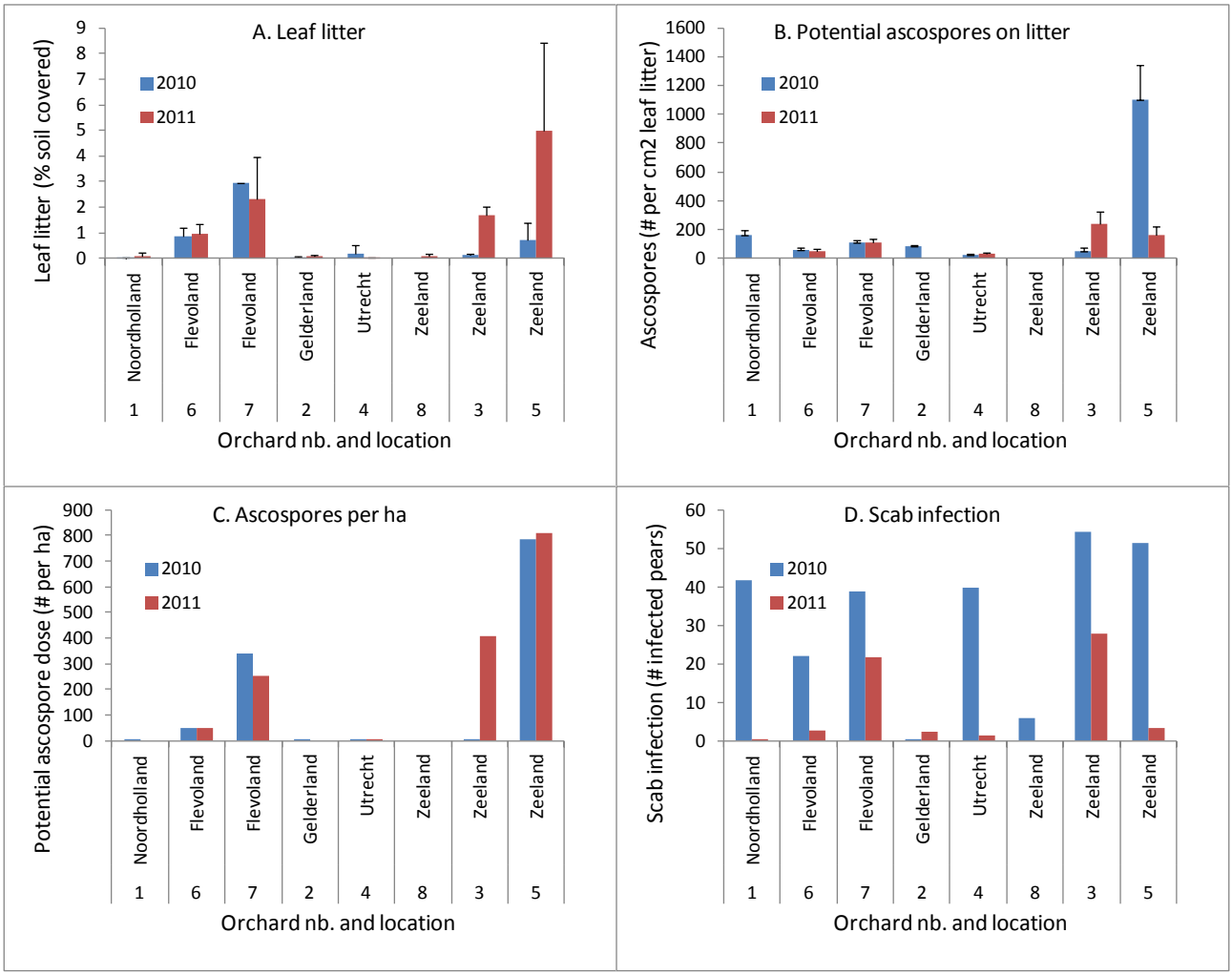


Figure 1. Leaf litter (A) (error bars indicate standard deviation), potential ascospore density on leaf litter (B) (error bars indicate standard error), potential number of ascospores per ha (C) and scab infection (D). Scab infection between the two years cannot be compared as it was measured somewhat differently (see materials and methods).

To show the intricacy of the system, scab infection was plotted against potential ascospore dose (Figure 2): there was no clear relation in both years. In 2010, there seems a sort of triangular shape in the data, indicating a potential multivariate relationship. In 2011, the situation is different: in one of the orchards (number 5) a high infection pressure by ascospores seems to coincide with a low number of infected fruit.

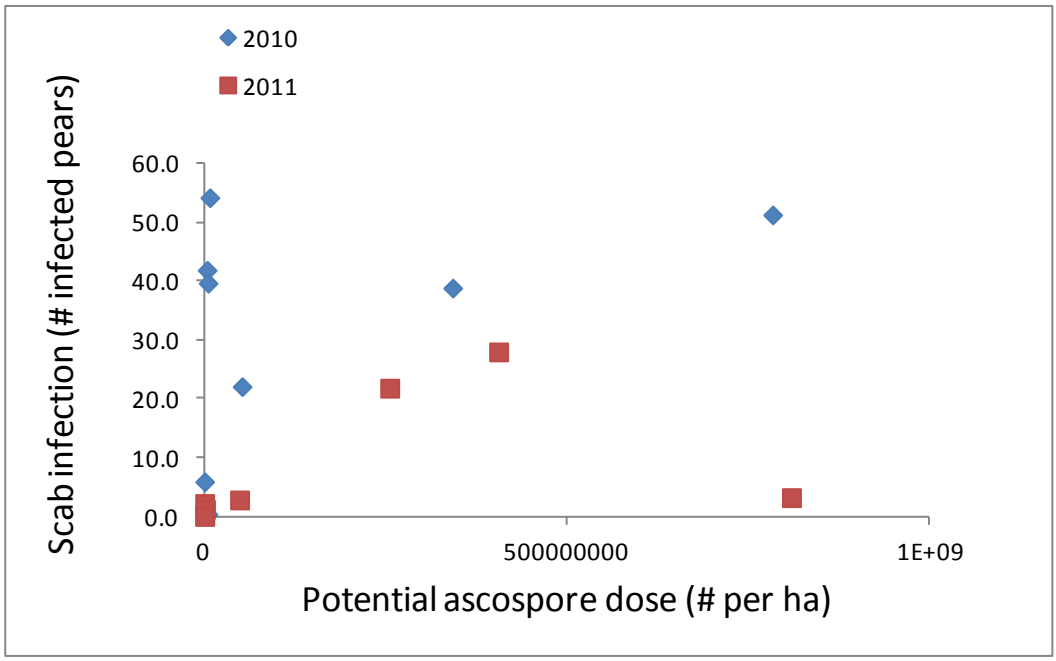


Figure 2. Scab infection plotted against potential ascospore dose in 2010 and 2011. The general level of scab infection between the two years cannot be compared as it was measured somewhat differently (see materials and methods).

That data presented above were used to do some multivariate statistics (Figure 3). In 2010, we find a statistical model that can explains 86% of the variation in the scab infection in the orchards, that contained rainfall from July till September (p=0.01) and the potential number of ascospores on the leaf litter in the orchards (p=0.046). Third important factor was the amount of leaf litter but this was not significant (and is not shown). In 2011 we find a statistical model that explains 82% of the variation of the scab containing again the potential number of ascospores on the leaf litter (p<0.01) and the potential ascospore dose (p=0.07). The two points dominating the relation are orchards 7 and 3, having both a high potential ascospore dose in 2011. In our analysis of scab incidence in the year 2011, we also tested the amount of variation explained by the scab incidence measured in 2010, but it had no significant impact.

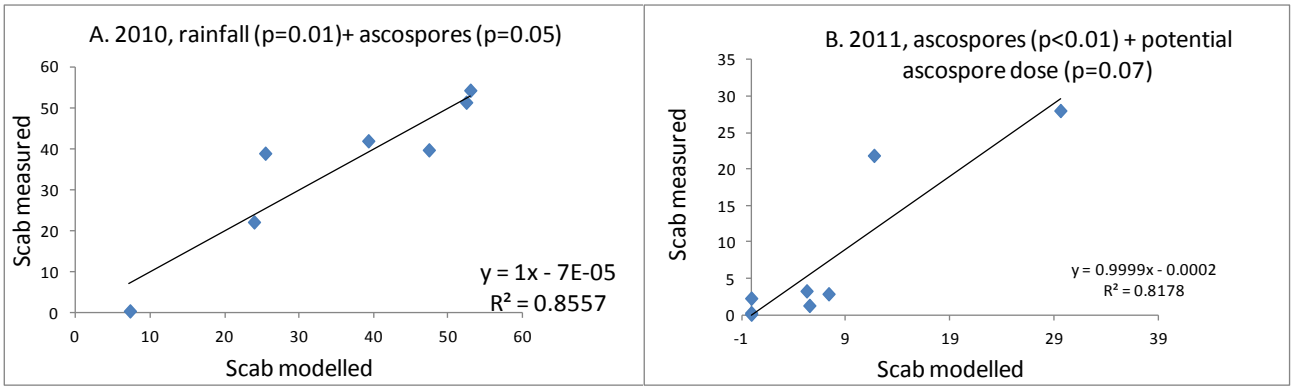


Figure 3. Variation in scab modelled by the best statistical models, containing rainfall from July till September and the potential ascospore density on leaf litter in 2010, and the potential ascospore density on leaf litter and the potential ascospore dose in 2011.

Finally, we measured the amounts of leaf litter separately in the various adapted management strategies in our experiment (located in orchard 7 in the former figures) (Figure 4). Within the orchard there are quite some differences in percentage of soil area covered. However, the were no clear changes by the management strategies yet.

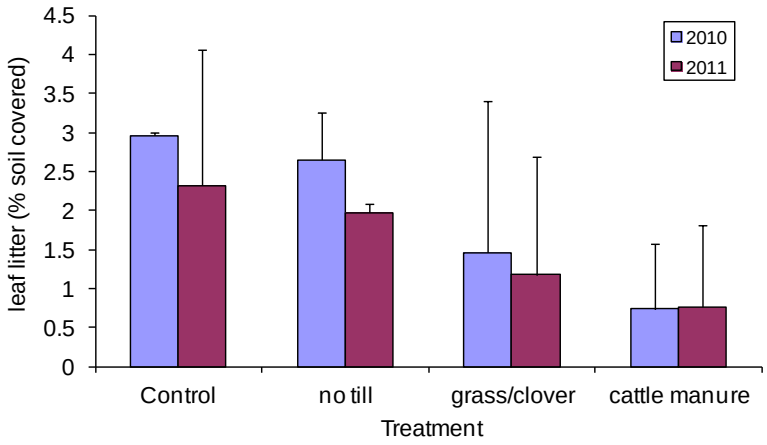


Figure 4. Leaf litter in de adapted management experiment in 2010 and 2011. Error bars indicate standard deviation.

## Discussion

Back to our research aim: what insights in the disease cycle of pear scab do we get from these preliminary results? First of all, we can see that there are large variations, both in infection parameters and in scab incidence, within the range of orchards in our research. Both can have large annual variations: others reported the relations between autumn scab incidence and spring ascospore production (Horner & Horner, 2002). We find some correlations here: scab incidence in 2010 explained 45 % and 30 % of the variation in ascospore per unit leaf litter and potential ascospore dose, but no relation with scab incidence in 2011.

The presented data suggests that in 2010, a year with a very wet end of the summer in The Netherlands, rainfall in this period has been the main factor that explained variation in the scab incidence. Rainfall during the first part of the growing season explained only a small part of the variation. Furthermore, our results show that ascospores formed on leaf litter play a role but this is not the whole story. Strikingly, the ascospore number per unit of leaf litter area was of more importance in explaining variation in scab incidence than our calculated potential ascospore dose. There are at least two possible explanations. One could be that in the field, conditions for ascospore formation and/or infection success are not very favourable, and that the large differences in number of ascospores become a regulation factor. A second explanation can be that the number of ascospores on leaf litter is correlated with conidia from branch lesions in the same orchard. If we highlight for example orchard 1 in 2010 this becomes more clear: here, there were almost no leaves on the soil and therefore only a very low potential ascospore dose. There were still quite a number of ascospores per unit of leaf litter area, and scab incidence in 2010 was quite high. This would suggest an influence of branch lesions in this orchard. It would be very interesting to measure this additionally.

We have to mention that our analyses are based on correlations, and can never proof a causal relationship. Therefore, we included an experiment in one of the orchards, orchard

7, in our research. With adapted management, we try to increase leaf litter decomposition here, and hope to report some results in the future.

**Acknowledgements**

We want to thank the members of the organic pear working group greatly for the help en support in doing this work. We thank Marc Trapman for his advice and help in measuring the scab infection in a number of orchards in 2011.

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# Application of Beetroot Vinasse – Influence on Foliage Decomposition and Ascospore Potential on Remaining Leaf Material

A. Bohr[1], S. Buchleither[1], S. Späth[1], U. Mayr[1]

**Abstract**

*The beetroot vinasse products "Biorga", "Biofa" and "Provita" were tested on* Jonagold *cultivars. All three products were sprayed shortly before natural leaf fall in November 2010, each diluted to 33 % (400 l vinasse per 800 l water per hectare). The chosen orchard had a uniformly high incidence of apple scab (*Venturia inaequalis*). After the spraying a predefined amount of leaves (150 g) with strong symptoms of apple scab was picked from the trees and put outdoor into wire cage depots to overwinter. Each treatment was replicated threefold, including an untreated control and a unit sprayed with a 10 % sugar solution.*

*The two aspects of the trial required two different settings. Firstly, a set of cage depots was put directly on the ground in order to monitor the decomposition process of the leaves. Secondly, another set of cages was put on a synthetic canvas cover in order to stop decomposition and thus keep the leaf material as intact as possible to examine its ascospore potential. Leaf degradation was observed regularly between November 2010 and March 2011. To evaluate potential inoculum, ascospores were discharged using the water bath method according to Kollar (2000). This was first done in early April 2011 (beginning of the ascospore release season) and repeated in mid April and early May 2011 (peak of the season).*

*The vinasse products "Biorga" and "Provita" reduced the amount of leaves left in March 2011 by around a third. For "Biofa" this accelerating effect was not found. Concerning the amount of ripe ascospores, all three vinasse treatments showed no effect at the first sampling date early in the spore release season. However, at the two latter sampling dates during the main phase of the release period, a reduction of at least 55 resp. 75 % compared to the untreated control was found.*

*The described findings are only based on one-year results and should be interpreted with appropriate cautiousness. The trial will be repeated with the same setting in 2011/12.*

**Keywords:** beetroot vinasse, apple scab, *Venturia inaequalis*, inoculum, decomposition

## Introduction

Some organic apple growers in the Bodensee region in Southern Germany use vinasse, sprayed on the leaves shortly before leaf fall, as part of their strategy towards apple scab (*Venturia inaequalis*). The idea of a vinasse application is to accelerate the decomposition of the leaves and therefore reduce the potential scab inoculum for the following year.

The accelerating effect of vinasse is largely agreed upon. However, the application of vinasse might also stimulate the maturation process of ascospores, thus leading to a higher percentage of ascospores on the remaining leaves.

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The research results concerning this question vary extremely. Köhl (2007) found an ascospore reduction of 95 % when dipping leaves in vinasse solutions which would equate to doses of 200 l/ha and 400 l/ha respectively when recalculated for a commercial orchard application. Contrary to this result, Timmermans et al. (2010) tested vinasse in Conference pears and found an increase in ascospore potential (concerning *Venturia pirina*) by 45 % after an application of 500 l/ha vinasse diluted 1:1 with water. Pfeiffer et al. (2004) observed a moderate increase of ascospore potential after a vinasse treatment with a concentration of 3 %.

All in all, the subject is not finally clarified yet. The results may also differ due to the various vinasse products and different concentrations used. This article summarises the implications of a one-year trial at the Kompetenzzentrum Obstbau-Bodensee with the three different vinasse products “Biorga”, “Provita” and “Biofa”. The products were compared in regard to acceleration of decomposition and ascospore potential on the remaining leaves. Also part of the trial was an untreated control and a treatment with sugar solution.

## Material and Methods

The trial was placed in a *Jonagold* orchard with a uniformly high level of scab-infections throughout the site. The treatment was carried out on the 3rd of November 2010 when the most severely infected leaves at the end of the long shoots started to fall. The vinasse products “Biorga”, “Provita” and “Biofa” were applied in a dilution of 33 % (400 l vinasse and 800 l water per hectare). After the spraying, the trees were dripping wet on both upper and lower side of the leaves. Additionally a treatment with a 10 % sugar solution and an untreated control were added. Directly after the spraying was completed, leaves with obvious and uniform scab infections were picked off the trees and put into wire cages to overwinter in the open field. Each cage was filled with 150 g fresh leaf material and each treatment was replicated threefold. The depots prevented the leaves to fly away but allowed natural exposure to the weather. One set of wire cages was put on a synthetic canvas cover in order to prevent earthworm activity (together with some soil on top of the canvas to re-enact natural conditions) and another set was put on the bare soil (see Fig.1).

Figure 1: Wire mesh cages with treated leaves in the fall of 2010 put on synthetic canvas cover to prevent earthworm activity (left) and on the bare soil to monitor natural decomposition (right).

The latter set was examined visually between November 2010 and March 2011 in regard to differences in speed and timing of decomposition. At the end of the trial the weight of the remaining leaf material was assessed.

The set on the canvas cover was used to count the amount of ripe ascospores on the leaves at three different points in time. In order to prevent ascospore discharge prior to the scheduled date of counting, the cages were roofed some days before the anticipated rain. Ascospores were counted at the beginning of the spore ripening in early April and again in

the middle of April and in early May during the peak period of ascospore release. To determine the number of ripe spores, the water bath method of Kollar (2000) was used. Leafs were dried at room temperature overnight and torn into pieces of approx. 1 $cm^2$. Strong veins were taken out. 1 g of the remaining leaf material was suspended in 50 ml of distilled water and shaken at 100 rpm for one hour. Immediately after the shaking the fluid was mechanically decanted into another flask to stop the discharge process. In the next step the spores were quantified with a 0.5 ml Kolkwitz planktoncytometer (Hydro-Bios, Germany). Each sample was stirred up again directly before it was put into the planktoncytometer in order to prevent spores from sinking to the bottom or clinging to the flask. Then the ascospores were allowed to settle for at least 10 minutes and 30 squares were counted under a microscope.

## Results

Decomposition process

Leaves treated with “Biorga” resp. “Provita” were decomposed faster which lead to a considerably reduced amount of leaves left at the beginning of the ascospore release season. At the beginning of March the untreated control still had 37 % of the leaves left in the cage. For “Biorga” and “Provita” this percentage was 12 % resp. 5 %. Thus the leaf matter was reduced to around one third compared to the control. The treatment sprayed with the vinasse “Biofa” showed no reduction and the sugar solution rather slowed than fastened the decomposition process in this trial: In the middle of March 50 % of the initial leaf matter was still in the wire cages (see Fig. 2).

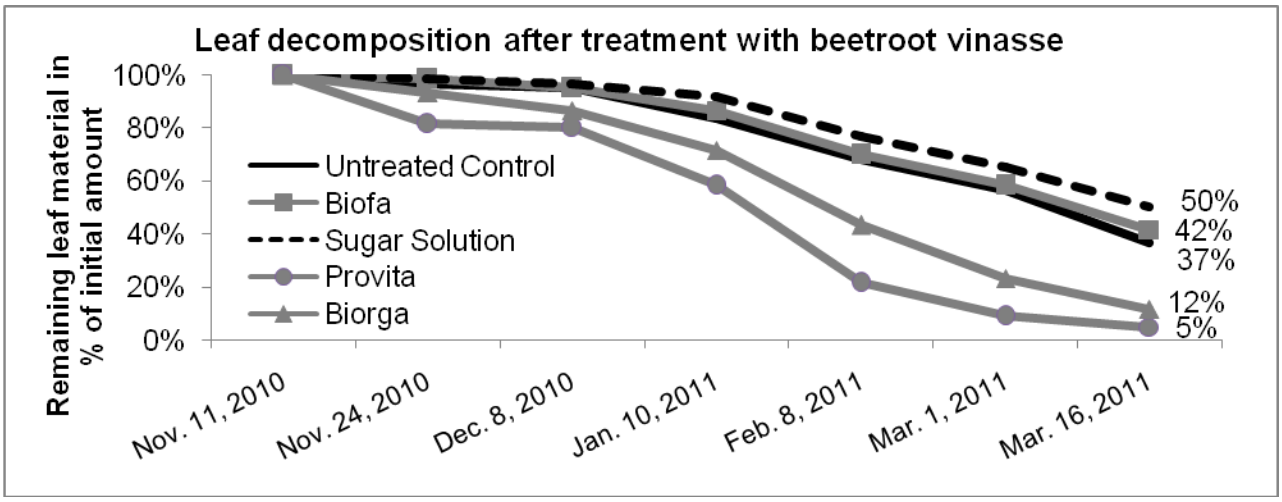


Figure 2: Process of leaf degradation after autumn treatment with three different beetroot vinasse products and sugar solution.

Ascospore ripening process

At the first date of analysis there was no significant difference between the amount of ascospores in the three vinasse treatments and the untreated control. At the second date of analysis (middle of April), the leaves of the untreated control discharged around 640,000 ascospores per gram leaf matter. The corresponding number on the vinasse-treated leaves was between 260,000 and 283,000, i.e. the spore potential was reduced by 55 to 60 %. At the third and last sampling date, the vinasse-treated leaves contained at least 75 % less ascospores than the untreated leaves – the strongest reduction measured in this trial but with an unexplained high spread in the untreated control (see Fig. 3).

The sugar solution showed no clear effects. At the first and second sampling date, the leaves treated with sugar solution again discharged more spores than the control-leaves. However, at the last date of analysis a lower spore potential was found.

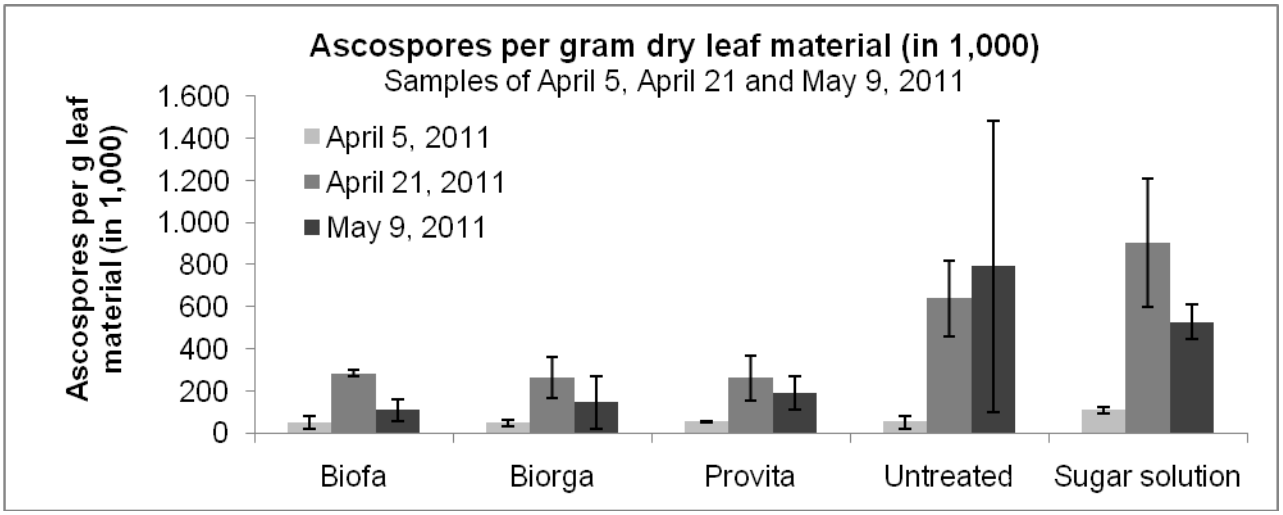


Figure 3: Mean amount of discharged ascospores at the three dates of water bath analysis (n=3 replications per treatment and date). Error bars represent standard deviation.

## Discussion

The vinasse products “Biorga“ and “Provita” accelerated the leaf decomposition process considerably. This effect could so far not be shown for “Biofa”. This might result from the difference in composition of the vinasse products which will be analyzed in the next year of the trial. The hypothesis that the application of vinasse has a negative influence on the ascospore potential of the remaining leaves could not be affirmed – for two of the three dates of analysis the opposite was found. Nevertheless, these conclusions are based on a one-year field trial only. Due to a high standard deviation in the result of the untreated control at the sample taken in May, the results are only significant for the sample of April 21, 2011. The research will be repeated in 2012, possibly with more replications, in order to verify the results.

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# Armicarb® - a new bio-fungicide for use in organic and conventional fruit growing in Europe

R. Milling[1], J-P. Laffranque[2] and M. Orpella[3]

## Abstract

*Armicarb® is a bio-fungicide being developed across Europe by Agronaturalis Ltd. for use in a wide range of crops; grapevines, hops, pome fruit, soft fruit, stone fruit, vegetables and ornamentals. Armicarb® is a specially optimised formulation of potassium bicarbonate; the result of extensive testing by researchers at Cornell University, USA, for use as an agricultural fungicide. The proprietary wetting system ensures a complete and thorough covering of plant surfaces, whilst at the same time limiting wash-off of the highly water-soluble active substance by rain. It is this critical balance of 'sticking' and 'spreading' by the formulation that delivers a level of field performance, outdoors as well as in protected conditions, equivalent to conventional fungicide standards.*

*Armicarb® has a broad spectrum of activity against important fungal pathogens, including but not limited to powdery mildews,* Botrytis *and* Venturia spp*. While it acts exclusively through direct contact to spores and fungal hyphae on the plant surface, laboratory studies on apple seedlings maintained under high relative humidity conditions demonstrated a limited curative activity against infections of* Venturia inaequalis*. The mode of action of the fungicide active substance is very rapid, allowing infections to be prevented as long as fungal hyphae have not yet penetrated through the cuticle. Results of development trials against* Botrytis *in grapevines and powdery mildew in protected vegetables are presented here.*

**Keywords:** Apple scab, Armicarb®, *Botrytis*, potassium bicarbonate, powdery mildew

## Introduction

The fungicidal activity of bicarbonate salts has been known for at least 80 years (Marloth, 1931). Specific control of numerous different fungi has been reported subsequently (Punja & Grogan, 1982; Horst *et al*., 1992; Palmer *et al*., 1997; Jamar *et al*., 2007). The mode of action of bicarbonate salts is linked to the perturbation of pH, osmotic pressure and the bicarbonate/carbonate ion balance of sensitive fungi. Bicarbonate acts by contact to fungi in aqueous solution and inhibits the development of fungal mycelium and spores. Due to its multi-site mode of action it is thought that the risk of resistance developing is low.

Potassium bicarbonate is now included in Annex I of Directive 91/414/CEE for use as a fungicide, and is authorised for use in organic production by the European Commission ruling n°. 404/2008. Indistinguishable from natural potassium and bicarbonate, residues are considered not to be relevant, and the product is exempt from MRLs in the EU. Available commercially in Switzerland since 2008, Armicarb®, a soluble powder containing 85 % (w/w) potassium bicarbonate, is being developed by Agronaturalis Ltd. and its partners across Europe for use against target diseases in a wide range of fruit and vegetable crops.

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## Material and Methods

To evaluate the effective timing of Armicarb® applications against *V. inaequalis*, apple seedlings of Golden Delicious at 4-6 leaf stage, 6 plants per treatment, were treated with 0.5 % (w/v) Armicarb® at different times post-inoculation with spores of *V. inaequalis* (100,000 spores/ml). Plants were incubated at 100 % relative humidity, 21 ˚C, for 24 h post-inoculation, and then at 60 % relative humidity, 20 ˚C, for 10 days until disease assessments.

Armicarb® was tested against *Botrytis cinerea* in grapevines in 3 trials in Austria in 2010. Three applications were made at the classical treatment timings, A, B and C; full flowering to end of flowering (BBCH 65-69), pre-bunch closure (BBCH 77), and beginning of colour change (BBCH 81-83). Unformulated potassium bicarbonate was tested alongside, and in one trial the reference product, fludioxonil+cyprodinil, was tested at stages B and C only. Spray volumes were 1000 l/ha at all treatment timings, with applications made to the whole crop canopy. Efficacy was assessed on at least 100 bunches per plot.

Four field trials were carried out in Spain in 2011 against powdery mildew, *Leveillula taurica*, in protected tomatoes (2 trials) and peppers (2 trials). Armicarb® was applied 4 times at 7-10 day intervals, starting applications when first symptoms were observed. Spray volumes at all application timings were 1000 l/ha. Disease assessments were made before the first application (0-DAA), at each application timing (0-DAB, 0-DAC, 0-DAD), and 7 days after D (7-DAD). Disease severity was assessed on 25 leaves per plot.

## Results

Disease incidence and severity of *V. inaequalis* on untreated apples seedlings were 56 % and 24 % respectively. Armicarb® applied 2, 5 or 8 h after inoculation resulted in the highest efficacy against the pathogen, always above 90 % for both disease incidence and severity (Fig. 1). From 12 to 24 h post-inoculation applications, the efficacy started to decline, and by 36 h post-inoculation, efficacies were reduced to only 38 % and 52 % for disease incidence and severity respectively.

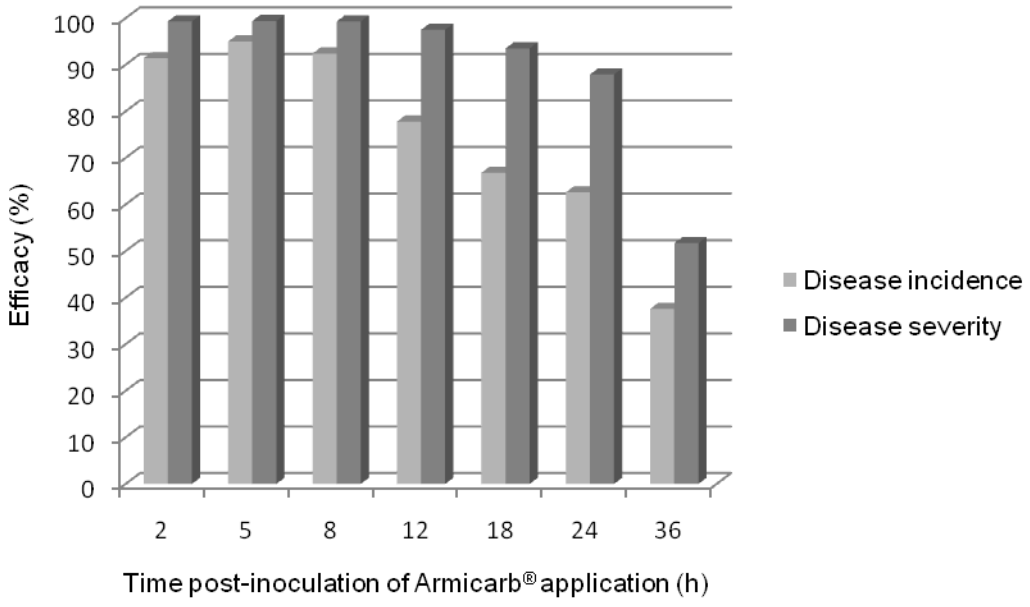


Figure 1 Effects of post-inoculation applications of Armicarb® against *V. inaequalis* infections of apples seedlings

Levels of infection with *B. cinerea* in the 3 trials in grapevines varied from 'low' to 'medium' to 'high'. Armicarb® gave very good levels of disease control, 64-85 % efficacy on disease severity, independent of disease pressure, and comparable to the conventional fungicide standard. In contrast unformulated potassium bicarbonate, applied at 2.3 times the active substance rate, was significantly less effective under medium-high disease pressure conditions. Armicarb® did not cause any phytotoxic effects either on leaves or bunches.

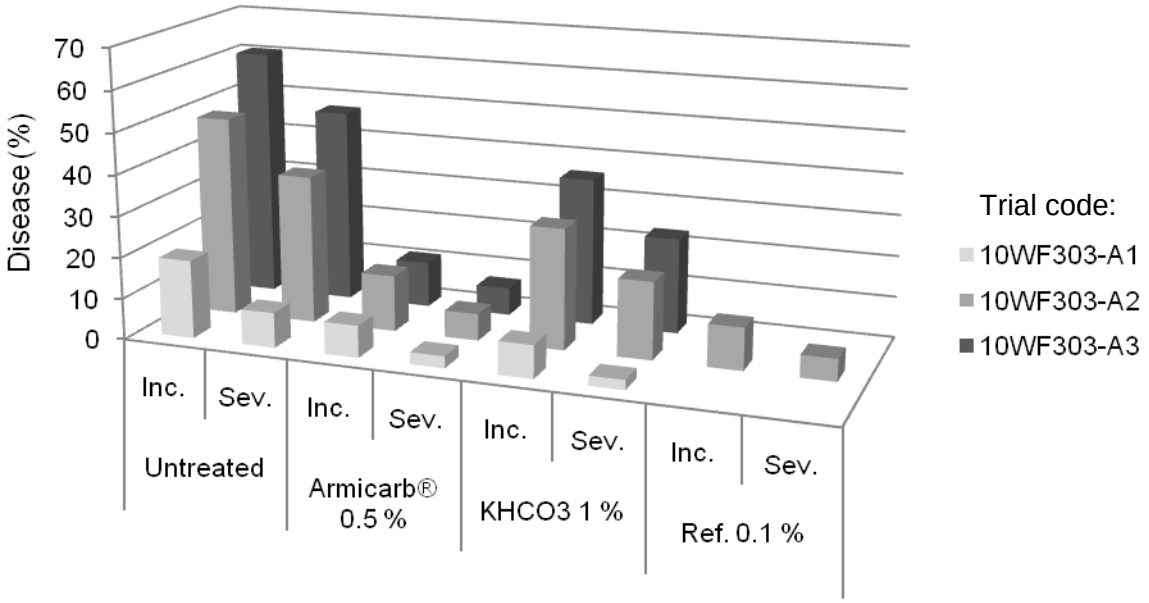


Figure 2 Efficacy of Armicarb® against *B. cinerea* in grapevines in 3 trials in Austria, 2010. Disease incidence (Inc.) and severity (Sev.) data are shown

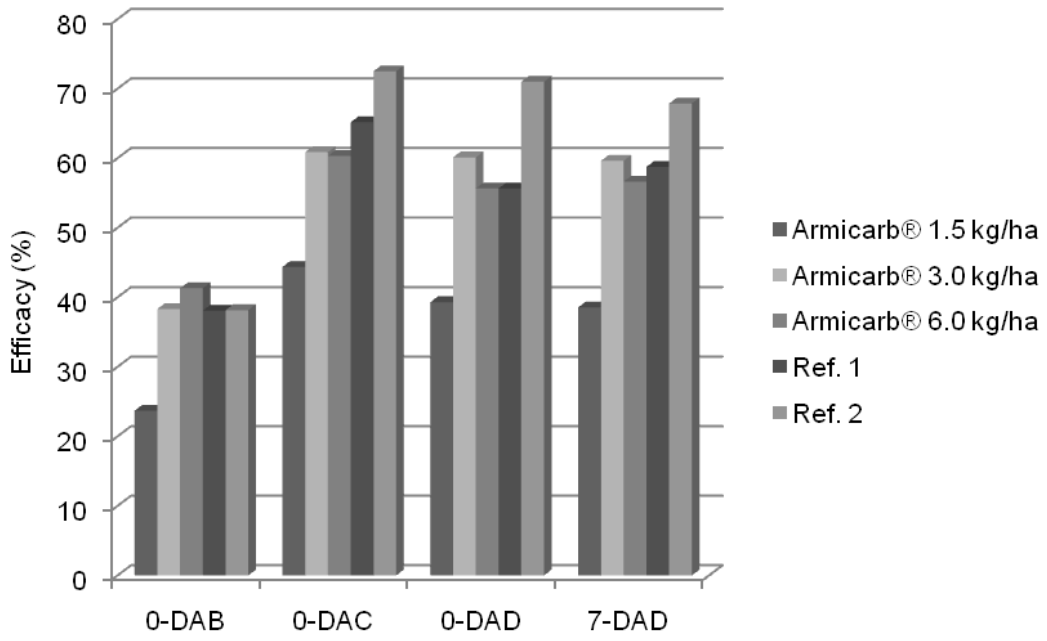


Figure 3 Efficacy of Armicarb® against powdery mildew of tomato and pepper (average of 4 trials) in Spain, 2011

Disease severity of *L. taurica* in the Spanish tomato and pepper trials increased steadily during the course of the trials, rising from just 2 % before the first fungicide treatments to reach 37 % at the end of the trials (average of 4 trials). A strong dose-effect was observed with Armicarb® between 1.5 and 3.0 kg/ha (Fig. 3). No difference was found between 3.0 and 6.0 kg/ha. Armicarb® achieved a good level of disease control, 60-61 % efficacy at the three later assessment timings, comparable to cyproconazole (ref. 1) and somewhat less than azoxystrobin (ref. 2). No symptoms of phytotoxicity were observed in any of the trials, at the 3 dose-rates of Armicarb® evaluated.

## Discussion

Results of timed applications with Armicarb® against apple scab demonstrated the effectiveness of the product for up to 8 h post-infection (disease incidence and severity), and for as long as 12-18 h in terms of disease severity. These data suggest there may be some flexibility in application timing with respect to weather conditions in the field, but Armicarb® should always be applied as close to infection events as possible. In was noted that in these trials, plants were maintained under very high humidity for 24 h after infection, meaning that the potassium bicarbonate would have remained dissolved on the leaf surfaces during this time.

The improved performance of Armicarb® in comparison to unformulated potassium bicarbonate was demonstrated in the field in grapevines against *B. cinerea*. The enhanced rainfastness and more uniform coverage of plant surfaces delivers a level of efficacy comparable to conventional fungicide standards.

Similarly, in glasshouse trials in tomatoes and peppers, the efficacy of Armicarb® against powdery mildew was on the level of the triazole reference product. With its exemption from EU MRLs, Armicarb® will be especially suitable for use in programmes with conventional fungicides, as well as in organic fruit production.

## Acknowledgements

Laboratory trials with Armicarb® on apple seedlings were sponsored at the FiBL by M. Refardt, Stähler Suisse SA. Trials against *B. cinerea* in grapevines were sponsored by D. Kranz, Stähler Austria GmbH. Field trials in Spain on tomatoes and peppers were sponsored by P. Michitte, Certis Europe BV.

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# Evaluation of products suitable for scab (*Venturia inequalis* (Cke.) Wint.) control in organic fruit production and implementation of sea algae based product Alginure to the IP scab control in Czech Republic.

M. Bagar[1], V. Psota[1]

**Abstract**

Products for scab control in organic apple production and different scab control systems were evaluated in three separate trials in Czech Republic. Results are presented.

Small plot field trial on solo applications of different product for scab control suitable for organic fruit growing was conducted in 2010 in South Moravia. The evaluated products were lime sulphur Polisenio, Sulikol K (sulphur), VitiSan, Alginure. Lime sulphur and Alginure achieved efficacy 81 and 87 % respectively.

The influence of Alginure was tested in semi-field trials on three localities in Czech Republic in 2011. IP scab control schedule was compared to the same schedule where Alginure was added 3-5 times together with contact fungicide. The use of Alginure markedly increased the efficacy of the treatment. The infestation on the variation with Alginure was 57 % lower on average.

Organic scab control schedule was compared to the IP control schedule in field trial at another locality in South Moravia, Czech Republic. Products used in organic variation were: copper, sulphur, lime sulphur, Alginure, Prev-B2, VitiSan, Myco-Sin, calcium hydroxide. The results were comparable or better than the standard chemical fungicide control.

**Keywords**: scab control, ecological, apple, fruit

## Introduction

The ecological fruit production in Czech Republic does not have a big tradition. The surface of organic orchards increased after government support to ecological agriculture was established. However, there is still a lack of experience and technical information, especially in pest and disease control. Situation has improved within the last few years, as some institutions started to pay more attention to this topic.

In presented experiments we focused especially to the scab control on apple, which is the main crop and the main disease in our region.

## Material and Methods

1. Small plot trial for evaluation of different products on scab

Small plot trial was conducted in Nosislav, South Moravia in the season 2010 in 30 years old orchard with variety Idared, span 3x4,5 m. There were 6 variations in three repetitions. The product were applied preventively as a solo application by backpack sprayer Stihl with the start on April 16th (BBCH 15), following on Apr. 22, 27, May 3, 6, 11, 15, 19, 24, 28, 31, June 7, 17, 25 and July 19 and 29th. The variants were: Untreated control, Sulikol K (sulfur 50%) 6 kg/ha, lime sulfur Polisenio (lime sulfur 27 %) 8 l/ha, VitiSan (Potassium hydrogen carbonat) 5 kg/ha, Alginure (algae extract 24%, plant aminoacids 7%, phosphates 20%) 3 l/ha and Alginure 5 l/ha. Evaluation was done on June 11th and August 2nd. Efficacy was counted by Abbott formula, the differences between variations were evaluated by Fischer LSD test.

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2. Influence of Alginure placed into a regular IP chemical spraying program against scab

Three semi field trials were carried out in Czech Republic in 2011 in localities Určice, Central Moravia (3 applications of Alginure), Stošíkovice, South Moravia (4 applications of Alginure) and Bašnice, East Bohemia (5 applications of Alginure). Sea algae based product Alginure (algae extract 24%, plant aminoacids 7%, phosphates 20%; producer Tilco Biochemie GmbH) was applied in tank mix with the other fungicide products in IP spraying schedule. Trials were in commercial apple orchards with a regular spraying schedule. The testing plot of size 3-4 ha was within the orchards just beside the standard plots. Alginure was applied around the flowering time within the intensive infection period. Spraying Schedule:

Bašnice: 19.4. (DD.MM.) (Alginure) + Kumulus, 28.4. (Alginure), Dithane, 4.5. (Alginure), Captan, 16.5. (Alginure), Delan, 2.6. (Alginure), Tercel, 11.6. Syllit , 23.6. Delan, Kumulus, 13.7. Captan, Kumulus, 11.8. Flint

Určice: 7.4. FUNGURAN, 14.4. FUNGURAN, 21.4. POLYRAM, 28.4. MYTHOS, 6.5. DISCUS, DELAN, (ALGINURE), 13.5. POLYRAM, (ALGINURE), 19.5. TERCEL, (ALGINURE), 26.5. SYLLIT 65 WP, 31.5. SYLLIT 65 WP, 7.6. POLYRAM, 14.6. SCORE, DELAN, 21.6. SYLLIT, 29.6. POLYRAM, 14.7. DELAN, 26.7. DELAN

Stošíkovice: 31.3. Champion 50 WP, 4.5. Novozir MN 80 New, Kumulus WG, 11.4. Thiram granuflo, Kumulus WG, 22.4. (Alginure), Delan 700 WDG, Mythos 30 SC, 29.4. (Alginure), Captan 80 WG, Kumulus WG, 5.5. (Alginure), Delan 700 WDG, 13.5. (Alginure), Zato 50 WG, Score 250 EC, 26.5. Novozir MN 80 NEW, Dithane DG Neo, Kumulus WG, 30.5. Merpan 80 WG, Score 250 EC, 13.6. Kumulus WG, 22.6. Kumulus WG, 8.7. Kumulus WG, 26.7. Thiram granuflo, Kumulus WG, 27.7. Captan 80 WG, 4.8. Discus

Evaluation of infection on leaf and fruits was done three times in Určice and Stošíkovice and twice in Bašnice. First evaluation was a short time after the last application of Alginure, and the last evaluation was before the harvest. Leaf infection was evaluated by 0-4 scale, fruit infection by 0-1 scale.

3. Comparison of organic and IP scab control system

Scab control spraying schedule based on products for organic agriculture was compared with an IP chemical spraying schedule in Buchlovice, South Moravia in 2011. Apple orchard with variety Idared of size 2 ha and age 25 years was chosen to be tested with the organic treatment. Neighbouring orchard with the same variety but younger age of 15 years was evaluated for a comparison. The treatment was following:

Organic schedule 2011: 31.3. Funguran (CuOH 77%) 1,5 kg + rape seed oil 2%, 8.4. Alginure 3l/ha, Kumulus (sulphur 80%) 3,5 kg, 20.4. Kumulus 4 kg, Prev-B2 (B 2%, d-limonen 4,2%) 0,3 l, 27.4. Lime sulfur Polisenio 6 l/ha, 29.4. Kumulus 3 kg, Alginure 6 l, 5.5. Kumulus 3 kg, Funguran 0,5, Alginure 5 l, 11.5. Kumulus 10 kg, Prev-B2 0,25 l, 16.5. Kumulus 7 kg, Prev-B2 0,25 l, 19.5. Kumulus 5 kg, Prev-B2 0,2 l, 27.5. Kumulus 5 kg, Myco-Sin 8 kg, 31.5. Ca(OH)2, 6.6. lime sulfur Polisenio 6 l/ha.

Chemical fungicide schedule 2011: 31.3. Funguran 1,5 kg, Olej 2%, 8.4. Syllit 1,2 l, Kumulus 3 kg, 19.4. Syllit 1,2 l, Kumulus 3 kg, 28.4. Mythos 1 l, Kumulus 3 kg, 2.5. Zato 0,15, Delan 0,5 kg, 11.5. Syllit 1,4 l, Kumulus 5,8 kg, 17.5. Zato 0,15, Delan 0,35 kg, 23.5. Thiram 3 kg, Kumulus 3 kg, 31.5. Punch 0,3 l, Captan 80 1,8 kg, 2.6. Zato 0,15 kg, Syllit 1,2 l

Evaluation was done after the end of the main infection period on June 7th.

## Results and discussion

1. Small plot trial for evaluation of different products on scab

Table 1.: Average number of infected fruit per variant and efficacy % by Abbott.

| Variation | Evaluation June 11th | | Evaluation Aug. 2nd | |
|---|---|---|---|---|
| | infestation % | **efficacy %** | infestation % | **efficacy %** |
| Lime sulphur | 1,00B | **85,01** | 2,00B | **80,64** |
| Sulikol K | 5,00A | **25,04** | 4,00B | **61,28** |
| VitiSan | 3,66A | **45,13** | 5,33A | **48,40** |
| Alginure 3 l $ha^{-1}$ | 4,00A | **40,03** | 3,00B | **70,96** |
| Alginure 5 l $ha^{-1}$ | 1,33A | **80,00** | 1,33B | **87,12** |
| UTC | 6,67A | | 10,33A | |

The products lime sulfur Polisenio and Alginure showed quite high efficacy on the scab even in the high infection conditions of that season. The lower efficacy of VitiSan was probably due to its preventive application in this trial as the product is recommended as a stop treatment.

2. Influence of Alginure placed into a regular IP chemical spraying program against scab

Table 2.: Comparison of leaf infestation of fungicide treatment with and without Alginure

| variety | **Locality/treatment** | Date of evaluation/leaf infestation degree | | |
|---|---|---|---|---|
| | **Locality: Bašnice, (ANOVA: F 0.0029 P 0.997090)** | | | |
| | Variant | June 23 | | September 15 |
| Idared | Alginure + fungicides | 4,2A | | 7,3A |
| | Fungicides alone | 8,5A | | 11,3A |
| Gloden delicious | Alginure + fungicides | 1A | | 1,3A |
| | Fungicides alone | 2A | | 1,8A |
| Jonagold | Alginure + fungicides | 1A | | 9,8A |
| | Fungicides alone | 2A | | 12,3A |
| | **Locality: Stošíkovice, (ANOVA F 1.4326 P 0.239104)** | | | |
| | | June 6 | July 27 | September 8 |
| Gala | Alginure + fungicides | 0A | 2,5A | 2A |
| | Fungicides alone | 2,8A | 7B | 7,5B |
| Golden delicious | Alginure + fungicides | 1A | 2A | 4,3A |
| | Fungicides alone | 6,3B | 6,3B | 10,3B |
| | **Locality: Určice, (ANOVA F 2.6104 P 0.016661)** | | | |
| | | June 7 | July 28 | September 13 |
| Golden delicious | Alginure + fungicides | 6A | 6,5A | 4,5A |
| | Fungicides alone | 9,5B | 11,8B | 10,5A |

The results show that the incorporation of Alginure into the conventional spraying program brings important reduction of infestation. The average reduction on all localities was 57 %

comparing to a standard treatment. The differences on the locality Bašnice were not significant, but on the other two localities the differences were significant (on level 0,05). Influence of the Alginure treatment on the infection decrease lasted till the harvest time. Using this kind of product can have great importance for low residue production and also for antiresistance strategies. It is planned to continue with this study by looking for optimal dosage and timing of the product.

3. Comparison of organic and IP scab control system

Table 3.: Comparison of leaf and fruit infection and cost of the ecological and conventional treatment in 2011

| variation | leaf infection degree | % infected fruits | scab control costs | % comparison of costs |
|---|---|---|---|---|
| ecological | 0,3 | 0 | 10 409 CZK (416 EUR) | 103,9 |
| conventional | 1,3 | 0,75 | 10 009 CZK (400 EUR) | 100 |

The trial shows that in this season the ecological scab control got slightly better results than the IP spraying program. That corresponds to the previous results in Czech Republic (Falta, 2010) which showed that the ecological scab control got comparable results to the chemical. Also the treatment costs were in Czech conditions comparable, when the ecological scab treatment was about 3,9 % more expensive than the IP. This also corresponds to the previous results from Czech Republic (Vávra, 2010).

**Acknowledgements**

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# Penicillium decay on stored apple fruits after microbiological treatments

J. Kowalska[1], D. Remlein Starosta[1] and J. Bocianowski[2]

**Abstract**

*Postharvest diseases cause considerable losses to harvested fruits during storage. Biocontrol of postharvest diseases is an alternative means of management of blue mould pathogen. Control of apple postharvest diseases caused by* Penicillium expansum *on apple fruit was evaluated with three different commercial products: Yield Plus®, Trifender® and Polyversum®. Biocontrol agents of these products were yeast like fungus* Cryptococcus albidus, Trichoderma asperellum *and* Pythium oligandrum*, respectively.*

*In the first experiment, under field conditions,* P. oligandrum *and* T. asperellum *were applied three times during the vegetation season and the last one was made just before harvest. Suspension of both products, in recommended concentration, was introduced into organic apple orchard. After harvest apples were stored in the paper bags in cooling room ($4^0$C, 70%RH). The incidence of blue mould as a percentage of infected of fruit area was assessed after 4 months.*

*The second test was conducted in the laboratory on harvested apple fruits. Each bioagents were used at different concentration 10 X, 2,5 X and 1 X of recommended dose by dipping of fruits in water suspension before storage. Treated fruits were storied at $4^0$C, in the plastic bags to maintain high humidity (98%RH). In test disease assessment was quantified by the four month period of storage as a percent of mean decay area.*

*As the results were noted that the percentage of decayed fruits after field treatment with T. asperellum was reduced by 10% compared to* P. oligandrum*.*

*On apples fruits treated with the highest concentration of all biocontrol agents was observed the best reduction effect of* P. expansum *(0,93; 0,73 and 0,97% of decay area for* C. albidus*,* T. asperellum *and* P. oligandrum*, respectively). The best result was obtained after Trifender® treatment (0,73; 0,67 and 2,07 % of decay symptoms, respectively for each used concentrations), while 5% of spoilage was observed on untreated by microorganisms fruits. In case of recommended dosage (1X) of* C. albidus *was noted increase of decay symptom area (30,33%) compared to control. Polyversum® in recommended (1X) and 2,5X dose was also recognized as concentration with weak or lack of blue mould reduction.*

**Keywords:** organic production, apple fruits, blue mould, storage ability, biocontrol

## Introduction

Postharvest diseases cause considerable losses to harvested fruits during storage. Decay damage is calculated as 20-30 % of the world fruits production (Sharma *et al*. 2009). The fruit infection can occur either prior to harvest or during harvesting and subsequently during handling and storage. Public awareness on pesticide residues issues as well as European policies are creating the need for searching of alternative control methods of postharvest diseases caused by different bacterial and fungal pathogens. There is no doubt that biocontrol of postharvest diseases is an alternative means of management of blue mould, one of the most common postharvest apple diseases.

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Public awareness on pesticide residues issues as well as European policies are creating the need for searching of alternative control methods of postharvest diseases caused mainly by *B. cinerea* and *Penicilium* sp. Microorganisms to be used for control of post-harvest diseases (the yeast *Candida oleophila, Aureobasidium pullulans, Pseudomonas* sp.*)* are under assessment for inclusion into the list of active substances foreseen in Reg. 1107/2009. According to knowledge of authors only *Coniothyrium minitans* and *Pseudomonas chloraphis* MA342 are included. The aim of investigations was to assess the ability of three different microbiological products, used directly in orchard or as postharvest treatment to reduce blue mould infection and incidence in storage conditions.

**Material and Methods**

Control of apple postharvest diseases caused by *Penicillium expansum* on apple fruit was evaluated with an three different commercial products: Yield Plus ®, Trifender® and Polyversum®. Biocontrol agents of this products was yeast like fungus *Cryptococcus albidus*, *Trichoderma asperellum* and *Pythium oligandrum,* respectively.

The first experiment was carried out in years 2009-2010 in the organic orchard composed of Gloster cultivar. In the experiment under field conditions, *P. oligandrum* and *T. asperellum* were applied three times during the vegetation season and the last treatment was made just before harvest. Suspension of both products, in recommended by the producers concentration, was introduced into organic apple orchard. The incidence of blue mould as a percentage of infected of fruit area was assessed after 4 months of storage. Antagonists' fungi suspension was sprayed uniformly onto the plants using a shoulder-carried sprayer "Kwazar" (vol. 10 L). Fruits were harvested carefully and put in paper bags and were directly transported to the laboratory where they were immediately cooled to $4^0$C in the storage room. After the period of storage (4 month, $4^0$C, 70% RH), fruits were checked for appearance of blue mold symptoms. The incidence of *Penicillium* disease was presented as a percentage of fruit decay area.

The experiment in laboratory was carried out in 2009/10 to assess the ability of used products to protect fruits against *Peniclillum* spoilage. The second purpose in this test was to evaluate the proper concentration of used fungi to obtain the best control efficiency. This experiment was conducted on harvested apples of the same Gloster cultivar. Apples were delivered from the organic orchard and before harvest were no treated with any chemicals or products. As a comparative sample some of fruits were treated by captan 14 days before harvest (at dose 1.9 kg per hectare). Only undamaged and unwounded fruits were chosen for the experiment. One day after harvest, organic fruits were dipped in water suspensions containing three different concentrations of spores of used products, i.e. 10 X; 2,5 X and 1 X of recommended dose. According to the producers, the recommended dose for Trifender, Polyversum and YieldPlus is $5 \times 10^8$ cell $\cdot ml^{-1}$, $1 \times 10^6$ cell $\cdot ml^{-1}$ and $1 \times 10^6$ cell $\cdot ml^{-1}$, respectively. The concentrations of the suspension were checked out with Brücker chamber and then adjusted to required concentration. After treatment apples were put on paper towels to remove the excess of the suspension. Fruits were then placed into plastic boxes and stored at $4^0$C and 98% RH. In test disease assessment was quantified by the four month period of storage as a percent of spoilage area. As a statistical analysis firstly, the normality of distribution of the trait was tested using Shapiro-Wilk's normality test (Shapiro & Wilk, 1965). Non-normal trait was transformed using the power (Box-Cox) transformation (Quinn & Keough, 2002) with $\lambda$=0.45. Two-way fixed model analysis of variance was carried out to determine the effect of treatment, concentration and treatment $\times$ concentration interaction on the variability of the studied trait. The Tuckey's honestly significant differences (HSDs) of trait were calculated, and on this basis homogeneous

groups (not significantly from each other) were determined. One-sample *t*-test was used for statistical verification of the hypothesis of lack of differences between mean value for each level of treatment × concentration interaction and control (s.a. captan). Data analyses were performed using the statistical package GenStat v. 7.1 (Payne *et al*., 2003).

## Results

Results of the field experiment indicated that application of microorganisms during vegetation could be important for quality of stored fruits. The best results were obtained after field treatment with *T. asperellum*. Under storage condition the blue mould symptoms was reduced by 10% compared to *P. oligandrum* (Table 1).

Table 1: The influence of antagonistic fungi application into organic orchard on blue mould incidence on apples fruits after storage period.

| No. | Treatments | Mean % of dacay area |
|---|---|---|
| 1 | *T. asperellum* | 33,31 |
| 2 | *P. oligandrum* | 44,30 |

As a results of laboratory test analysis of variance indicated that the main effects of treatment (P=0.014) and concentration (P=0.028) as well as treatment × concentration interaction (P=0.045) were significant for studied trait (Table 2). It means that every of used fungi or concentration had significant different influence on development of *Penicilium* spoilage. Also the interaction between every used concentration and antagonist were significant differ. In this case every obtained result could be discussed as important value.

Table 2: Mean squares from analysis of variance for studied effect of treatments of antagonist and used concentration and their interaction.

| Source of variation | Number of degrees of freedom | Mean squares |
|---|---|---|
| Treatment | 2 | 1.616* |
| Concentration | 2 | 3.440* |
| Treatment × Concentration | 4 | 1.225* |
| Residual | 18 | 9.841 |
| * significant at 0.05 level | | |

Table 3: The influence of antagonist and used concentration and their interaction on area of spoilage calculated as mean values and standard deviations (SD).

| Microorganism | Concentration | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1X r.e. | | 2,5X r.e. | | 10X r.e. | | 1X, 2,5X and 10X | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| *C. albidus* | 30.333 | 35.388 | 1.667 | 1.528 | 0.933 | 0.577 | 10.978A | 22.904 |
| *P. oligandrum* | 13.867 | 22.633 | 13.267 | 18.583 | 0.967 | 0.351 | 9.367A | 15.943 |
| *T. asperellum* | 2.067 | 1.328 | 0.667 | 0.577 | 0.733 | 0.681 | 1.156B | 1.053 |
| C, P and T | 15.422a | 24.347 | 5.200b | 11.126 | 0.878c | 0.492 | | |
| $HSD_{0.05}$ | for treatment: 6.512, for concentration: 4.101, for treatment × concentration interaction: 9.815 | | | | | | | |

C – *C.albidus*, P- *P. oligandrum* and T- *T. asperellum*
r.e. – recommended dose

In experiment conducted with different antagonistic fungi obtained results were significant differ from obtained with apples immersed in captan control. After four months of storage the decay area of blue mould reached 5 % of captan treated fruits area. In case of some concentrations and microorganisms was noted that 1X of *C. albidus* affected in 30.33% of spoilage area, in case of *P. oligandrum* and its concentration 1X and 2.5X was observed the spoilage area 13.96 and 13.27%, respectively. It was significant higher percent comparing to captan control (5%). Surprising was that in case of recommended dosage of *C. albidus* and in case of *P. oligandrum* in recommended and 2,5X dose was recognized as ineffective treatments. The best result was obtained after *T. asperellum* treatments (0.73; 0.67 and 2.07 % of decay symptoms in different concentrations). The highest used concentration of microorganisms was the most effective (0.93; 0.73 and 0.97% of decay area).

## Discussion

One of the major unanswered questions is when and how the antagonistic microorganisms should be applied to assure their survival and maximum control of postharvest diseases. According to this Wiśniewski & Wilson (1992) it is believed that biological control by means of microbial antagonist have greater potential for success when applied after harvest. Obtained results suggested that treatment before or after harvest could be effective in control blue mould. However, very important impact on reduction of *Penicillum* decay had used cell concentration for fruit protection. Zhang *et al.* (2005) also noticed that the concentration of antagonist had significant effects on biocontrol effectiveness. They obtained completely inhibition of oranges Penicilium decay with antagonist spores suspension 1 $x10^9$ cell x $ml^{-1}$. The results of laboratory trial suggesting that recommended by producers' antagonists' concentration had significant lower influence on decay incidence than commercially used chemical (captan) treatment. The observed spoilage fruit areas after postharvest treatment underline the importance of the suspension spore density to assure the efficacy of the application. We believe that for long term cold storage should be used even ten times higher concentration that is recommended to received the best control of apples blue mould.

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# The influence of bioproducts on root growth and mycorrhizal occurrence in the rhizosphere of strawberry plants cv. 'Elsanta' under controlled conditions

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**Abstract**

*An experiment was carried out in a greenhouse of the Research Institute of Horticulture (RIH) to evaluate the effect of new organic fertilizers and amendments on root growth and mycorrhizal abundance and biodiversity in the rhizosphere of strawberry plants cv. 'Elsanta'. The plants were grown in rhizoboxes (sized 37 cm x 1.8 cm x 20 cm), filled with 1.85 kg of a podsolic soil collected from an uncultivated field of an experimental organic orchard of the RIH. The soil characteristics were: pH 5.5, organic matter content 1.5%, P content 51 mg P · kg$^{-1}$, K content 158 mg K · kg$^{-1}$. The plants were treated with different organic fertilizers and amendments: dry granulated bovine manure (Doctor O'grodnik), extract of vermicompost (Humus UP), extract of humates (Humus Active + Aktywit PM), plant extract (BioFeed Amin), extract from several seaweed species reinforced with humic and fulvic acids (BioFeed Quality), a consortium of beneficial soil organisms (Micosat), a stillage from yeast production (Vinassa) and a solution of titanium (Tytanit). Plants treated with BioFeed Amin, BioFeed Quality, Micosat, Vinassa and Tytanit received also half dose of dry manure. A standard NPK fertilization (NPK control) and a not fertilized control were also included. The following parameters were measured: root growth and morphological parameters, number of Arbuscular Mycorrhizal Fungi (AMF) spores, mycorrhizal frequency of AMF in the roots. The chemical composition of the applied products and of soil were also determined. The treatment inducing the highest development of mycorrhizas in the roots of strawberry plants cv. 'Elsanta' were Micosat and BF Amin. The treatments BF Quality and BF Amin had the most beneficial effect on the formation of AMF spores in the rhizosphere. Application of the bioproducts had a positive effect on root growth parameters in comparison with the plants fertilized with NPK. The use of BF Quality and Humus UP induced a reduction of the amount of mineral nutrients in the soil.*

**Keywords:** bioproducts, mycorrhizal frequency, AMF, root morphology, strawberry

## Introduction

Organic farming is considered an important element of the Polish and EU's strategy for the development of the agricultural sector. The production of organic fruits has been increasing in recent years. However, the limited availability of traditional organic fertilizers (i.e. manure), and scarce information about the effects of new kinds of organic fertilizers, such as plant extracts (Sas Paszt *et al.*, 2009) or microbial inocula (Malusá *et al.*, 2007), are serious obstacles threatening the future development of the sector. Application of mycorrhizal inocula can increase species diversity of these fungi in the rhizosphere and consequently improve the growth, yielding and yield quality of cultivated fruit crops (Sas Paszt & Żurawicz, 2005; Sas Paszt & Głuszek, 2007). Establishing the best conditions for plant-fungus symbiosis is the key factor to obtaining efficient mycorrhization (Estaún *et al.*, 1994).

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The continuous cultivation of one crop reduces the diversity and richness of AMF species in the soil (An *et al.*, 1993). Studies of indigenous mycorrhizal fungi colonizing the roots of strawberry plants have also been conducted (Nemec, 1974; Didier *et al.*, 2003; Derkowska *et al.*, 2008; Botham *et al.*, 2009). Much of the research work has concentrated on the effects of mycorrhizal fungi on the growth and yield characteristics of strawberry plants (Niemi & Vestberg, 1992). Plants of the cultivar 'Elsanta' have not yet been the subject of many studies on their symbiosis with AM fungi (Varma & Schüepp, 1994). Roots of the strawberry cultivar 'Elsanta' readily form symbiotic associations with arbuscular fungi (Varma & Schüepp, 1994). Maintaining species diversity of these fungi in the rhizosphere environment of an organic orchard may have an effect on the growth and health status of the plants and the quality of the fruit they produce. New innovative products and technologies for organic fruit production are under development, including the use of beneficial soil microorganisms and fertilizers or amendments of organic origin (Sas Paszt *et al.*, 2008; Malusà & Sas Paszt, 2009). The influence of organic amendments on the development of mycorrhizal fungi has been reported by several authors. The technologies rely on the use of these bioproducts as they are or in a form enriched with beneficial soil microorganisms (Malusà *et al.*, 2007). Several organic fertilizers and amendments have been recently tested and introduced, which are also acting as natural stimulators of plant growth and development (Chelariu & Ionel, 2005; Gousterova *et al.*, 2008; Khan *et al.*, 2009; Chelariu *et al.*, 2009; Meszka & Bielenin, 2009). These are preparations of natural (plant or animal) origin, harmless to humans and the environment, which contain nutrient elements and biologically active substances (i.e. plant hormone-like substances, enzymes) as well as other compounds that stimulate plant growth, yield, quality or tolerance to abiotic stresses. Enrichment of organic bioproducts with beneficial soil bacteria and mycorrhizal fungi could enhance the effectiveness of the organic products in plant growth stimulation and fruit production.

The study, carried out within the framework of a project aimed at developing new products and technologies for organic fruit production in Poland, evaluated the effect of new organic fertilizers and amendments on root growth and mycorrhizal abundance and biodiversity in the rhizosphere of strawberry plants 'Elsanta'.

## Material and Methods

The experiment was carried out in a greenhouse over a 5-month period with the use of frigo-plants of the strawberry cultivar 'Elsanta'. The plants were planted in rhizoboxes (37 x 1.8 x 20 cm), filled with 1.85 kg of a podsolic soil collected from an uncultivated field of an experimental organic orchard of the RIH. The plants were subjected to the following growing conditions: photoperiod 16/8 h (day/night), light intensity 70 µM $m^{-2}$ $s^{-1}$, temperature 25/20 C and air humidity approx. 50% (Dinkelaker *et al.*, 1993; Sas *et al.*, 2003; Sas Paszt and Żurawicz, 2005). The levels of nutrient elements in the soil were: organic matter 1.5%, P 51 mg P $kg^{-1}$, K 158 mg K $kg^{-1}$, pH 5.5.

The following experimental treatments were applied:

1) Control (no-treatment) (organic matter 1.5%, P 51 mg P $kg^{-1}$, K 158 mg K $kg^{-1}$, pH 5.5).

2) Standard NPK soil fertilization: 4 g $NH_4NO_3$ $plant^{-1}$, 3 g triple superphosphate and 6 g $K_2SO_4$ per rhizobox.

3) Dry granulated bovine manure for organic farming (Doktor O'grodnik) – 1 g per rhizobox.

4) Micosat (CCS Aosta s.r.l.) – a mixture of AM fungi: *Glomus* species, *Trichoderma viride* and rhizosphere bacteria species (*Bacillus subtilis, Pseudomonas fluorescens, Streptomyces* spp.) – 10 g per rhizobox.

5) Humus UP (Ekodarpol) – an extract from a vermicompost – 2% solution to the soil per rhizobox.

6) Humus Active + Aktywit PM (Ekodarpol) – Humus Active is a soil improver with active humus and a population of beneficial microorganisms – 2% solution of Humus Active and 1% solution of Aktywit PM to the soil per rhizobox.

7) BioFeed Quality (Agrobio Products B.V.) – an extract from several seaweed species reinforced with humic and fulvic acids – 0.5% solution + 0.5 g manure to the soil per rhizobox.

8) BioFeed Amin (Agrobio Products B.V.) – an extract of 100% vegetal amino-acids – 0.5% solution + 0.5 g manure to the soil per rhizobox.

9) Tytanit (Intermag) – titanium (Ti) 0.8% (5 g Ti in 1 l of working solution), pH 3.4, containing 3163 mg $kg^{-1}$ Ti – 0.05% solution + 1 g manure to the soil per rhizobox.

10) Vinassa – molasses residue from yeast production – 0.5% solution + 0.5 g manure to the soil per rhizobox.

Determination of root growth and morphological parameters:

- Dry weight (Ostrowska *et al.* 1991).
- Root morphological parameters (total root length, root diameter, root surface area, root volume and total number of root tips) - image analysis system with an Epson scanner, controlled by WinRhizo software (Regent Instruments Inc.).

Determination of soil chemical parameters:

- The amount of N and C in the soil - Dumas method using a TruSpec CNS analyzer. Available P and K - the Egner-Rhiem method of emission spectrometry (Cygański 1997). The electrochemical method in KCl was used to measure soil pH.

Determination of mycorrhizal frequency:

- Roots were cold-stained using the Phillips & Hayman method (1970).
- The microscopic analysis of the roots - Trouvelot's method (1986).
- The mycorrhizal frequency (F%) and mycorrhizal intensity (both relative – M%, and absolute – m%) were assessed in each root segment.
- The mycorrhizal parameters were calculated using the Mycocalc software: http://www2.dijon.inra.fr.mychintec/Mycocalc-pgr/download.html

Identification of spores:

- Trap cultures were set up with narrowleaf plantain in 0.5 L pots filled with a mixture of rhizosphere soil and autoclaved sand, at a ratio of 1:1 v/v (Błaszkowski 2003). Pots were placed in SunBags (Sigma).
- After six months, 200g samples of the pot substrate were taken from the trap culture combinations and spores were isolated by wet sieving and centrifuging in a sucrose gradient (Brundrett *et al.* 1996).
- The isolated spores were divided into morphotypes according to size, shape, and colour of spores.
- The layer thickness of spore walls and germination walls was measured in freshly isolated spores which were crushed in PVLG or PVLG+Melzer's reagent (1:1, v/v) and observed under a light microscope equipped with a micrometer eyepiece (Błaszkowski 2003, 2008).
- The observed AMF species were named according to Schüßler *et al.* (2001) and Błaszkowski (2003).

All the results were statistically evaluated with analysis of variance. Comparisons of means were at $p \leq 0.05$ with the Duncan test.

**Results**

The highest values of mycorrhizal frequency and relative intensity were recorded in the roots of the plants inoculated with the microbial consortium (Micosat), followed by the plants from the treatments with: BF Amin + manure and Humus UP (Tab. 1). The lowest value of mycorrhizal frequency was determined for the NPK standard fertilization. Treatment of strawberry plants with bioproducts increased formation of mycorrhizal structures in the roots.

Table 1: Mycorrhizal colonization parameters determined in the roots of strawberry plants 'Elsanta' grown in rhizoboxes. (Means of 90 replicates; different letters referring to the same parameter indicate statistically significant difference $p \leq 0.05$).

| Experimental treatments | F [%] | M [%] | m [%] |
|---|---|---|---|
| Control | 11.11 ab | 0.11 a | 1.0 ab |
| NPK | 2.22 a | 0.02 a | 0.67 a |
| Manure | 3.33 a | 0.03 a | 1.00 ab |
| Micosat | 45.55 d | 1.63 b | 3.33 bc |
| Humus UP | 21.11 bc | 0.48 a | 2.37 b |
| Humus Active + Aktywit PM | 15.56 b | 0.70 a | 4.00c |
| BF Quality + ½ manure | 3.33 a | 0.03 a | 1.00 a |
| BF Amin + ½ manure | 28.89 c | 1.67 b | 6.74 d |
| Tytanit + manure | 3.33 a | 0.08 a | 0.78 a |
| Vinassa + ½ manure | 16.67 b | 0.26 a | 1.74 ab |

Table 2: Root growth and morphological parameters of strawberry plants cv. 'Elsanta' treated with different bioproducts (means of 3 replicates; different letters referring to the same parameters indicate statistically significant differences $p \leq 0.05$)

| Treatment | Root dry weight [g] | Root surface area [$cm^2$] | Root diameter [mm] | Root volume [$cm^3$] | Root length [cm] | Number of root tips |
|---|---|---|---|---|---|---|
| Control | 2.2 bc | 726 ef | 0.64 b | 11.93 d | 3582.49 bc | 6723.5 d |
| NPK | 0.93 a | 574 d | 0.66 c | 4.54 a | 1348.82 a | 2787.7 a |
| Manure | 2.7 d | 277 a | 0.64 b | 9.85 c | 3007.95 b | 5609.8 bc |
| Micosat | 2.7 d | 608 cd | 0.61 a | 10.19 c | 3323.66 b | 6236.2 c |
| Humus UP | 2.5 cd | 648 e | 0.62 ab | 9.17 c | 2945.50 b | 6202.7 c |
| Humus Active + Aktywit PM | 1.6 ab | 578 d | 0.66 c | 8.00 b | 2297.18 ab | 4388.3 b |
| BF Quality + ½ manure | 1.7 b | 479 bc | 0.60 a | 9.28 c | 3198.24 b | 6978.0 d |
| BF Amin + ½ manure | 1.9 b | 609 cd | 0.61 a | 10.14 c | 3323.34 b | 6708.4 d |
| Tytanit + manure | 3.2 e | 649 e | 0.62 ab | 12.16 d | 4087.93 c | 9028.2 e |
| Vinassa + ½ manure | 2.4 c | 802 f | 0.67 c | 9.56 c | 3582.49 bc | 5298.8 bc |

The highest dry weight of roots was recorded in the treatment with Tytanit + manure, followed by Manure and Micosat (Tab. 2). The plants fertilized with NPK had the lowest dry root weight. The largest surface area was found in the roots of the plants treated with Vinassa + manure, followed by the Control, Tytanit + manure, and Humus Active with Aktywit PM, but the smallest surface area of roots - after the application of Manure (Tab.2). Root diameter ranged from 0.60 to 0.66mm with the largest value found in the plants treated with NPK, Humus Active + Aktywit PM and Vinassa (Tab. 2). The plants not fertilized and those treated with Tytanit + manure showed the largest volume of roots, while those treated with NPK had the lowest (Tab. 2). The highest induction of elongation of roots was produced by Tytanit + manure; all other treatments except the NPK, also favoured to some extent elongation of roots (Tab.2). Consequently, Tytanit showed the highest numbers of root tips, also in this case, all organic products increased the branching of roots, while this parameter was not influenced by NPK treatment (Tab. 2).

In comparison to the control, BF Quality + manure, BF Amin + manure, Micosat, Humus Active + Aktywit PM, and manure induced the formation of the highest number of AMF spores in the trap cultures containing the rhizosphere soil of strawberry plants 'Elsanta' grown in rhizoboxes (respectively: 199.32; 173.53; 160.04; 155.47 and 150.66). The lowest average and total numbers of spores were recorded for the treatments Vinassa + manure, NPK, and the no-treatment control (respectively: 89.68; 117.42 and 101.97). Several AM fungi species, ranging from 3 to 6, were found in the trap cultures containing rhizospheric soil from the diverse treatments. The most common species of AM fungi found in the experimental combinations were: *Glomus claroideum* (found in all 10 treatments), *Scutel-lospora dipurpurescens* (found in 9 treatments), *G. mosseae* (identified in 7 treatments), *G. fasciculatum* (present in 6 treatments). *Glomus caledonium* was found in three treatments and *G. macrocarpum* in two. On the other hand, four AMF species were found in only one treatment: *Glomus microaggregatum* and *G. constrictum* were present only with Manure, *G. pallidum* with Vinassa, and *G. drummondii* in the NPK control.

## Discussion

The highest mycorrhizal frequency (45.55) was achieved for the application of the microbial consortium Micosat. Such result supports findings showing the efficacy of inoculation of plants with AMF (Jeffries *et al.*, 2003; Malusá *et al.*, 2007). The inoculation of strawberry plants with Micosat also resulted in an increased number of spores produced by the fungi. However, only one species *Glomus mosseae* of the four present in the inoculum was found as a spore. This species was also among the indigenous fungi present in the podsolic soil, used for all of the experimental treatments. This fact could be due to the differences in either sporulation or various ability of the fungal species to inoculate the plant roots. However, the plants treated with BF Quality (a seaweed extract) had one of the lowest mycorrhizal frequency rate (3.33%), whereas the plants treated with BF Amin (a plant extract enriched with humic acids) had a higher mycorrhizal frequency rate (28.89%).

The largest number of spores in the trap cultures was obtained after the combined applications of manure and plant-derived preparations: BF Quality or BF Amin (obtained from processed seaweed or herbaceous plants). The results show a favourable impact of the organic products on the sporulation of AM fungi. Results of other studies confirm the positive effect of seaweed-derived substances (Kuwada *et al.*, 2005; Kuwada *et al.*, 2006a,b) and other compounds of plant origin (Poulin *et al.*, 1993; Ishii *et al.*, 1997; Gryndler & Hršelová, 1998; Gryndler *et al.*, 2005; Horii *et al.*, 2009) on the development of AMF.

All bioproducts had a positive effect on the growth parameters of the roots, particularly in comparison to NPK. Interestingly, these effects were found also from the treatments with bioproducts applied to the leaves (Vinassa and Tytanit). Effect of foliar fertilization on root growth and morphology was already noted (Malusá *et al.*, 2007). Interestingly, Tytanit, which is supposed to enhance the photosynthetic metabolism, induced the highest root elongation and also number of root tips. Previous experiments with preparations containing titanium concerned primarily the impact of this element on the size and quality of the yield produced by various fruit plants (Serrano *et al.*, 2004), including strawberry (Laszlov-szky-Zmarlicka, 2006; Skupień & Oszmiański, 2007ab; Michalski, 2008), and also on the chemical composition of plants (Borkowski *et al.*, 2007; Wallace *et al.*, 1977). The positive effect of the biofertilizer Vinassa, which is the stillage resulting from the processing of sugar beet molasses in the production of yeast, on the development of the root system of strawberry plants confirms the data from the other studies (Chelariu & Ionel, 2005; Chelariu *et al.*, 2009). Vestberg (1992ab) evaluated suitability of *Glomus mosseae* and also *G. intraradices* for inoculation of strawberry and reported that the latter was found to be the most efficient fungus species as it increased shoot growth several-fold.

Generally, the organic fertilizers induced a considerable branching of the root system, as derived from the high total root length and number of tips. On the contrary, the NPK treatment was always causing the smallest root system, which is consistent with the observations of the other authors (Haynes & Goh, 1987; Sas *et al.*, 2003; Glinicki, verbal communication, 2011). This feature is common in chemically fertilized plants and it is considered also a possible factor affecting the plant ability to tolerate environmental stresses (e.g. water deficiency).

We found that in the rhizosphere of strawberry plants 'Elsanta' fertilized with various bioproducts there were spores of arbuscular mycorrhizal fungi of several species of the genus *Glomus* and also of the fungus *Scutellospora dipurpurescens*. However, their occurrence was not uniform. For example, *Glomus claroideum*, a species frequently found in Polish sandy soils (Błaszkowski, 2003), was present in all the experimental treatments. The *Glomus mosseae*, a very common species found also in the rhizosphere of strawberry plants (Didier *et al.*, 2003), was present in soil from several treatments. This species is also used as inoculum to increase the resistance of strawberry plants to stress caused by excess phosphorus (Stewart *et al.*, 2005) and drought (Yin *et al.*, 2010). Four other species, namely *Glomus microaggregatum*, *G. constrictum*, *G. pallidum* and *G. drummondii*, were found in the soil treated with only one bioproduct. This difference in the presence of AMF species in rhizospheric soil could be related to the effect of the treatment. Indeed, the plant is actively interacting with the soil to promote the establishment of mycorrhizal symbiosis for nutritional purposes (Hartmann *et al.*, 2009). Changes in its physiology caused by differences in availability of nutrients could thus modify the chemical communication between the plant and the different AMF species, leading to a selective establishment of the symbiosis (Allen *et al.*, 2003). Such hypothesis could be supported by findings from several authors. *Glomus macrocarpum* had also been recorded by Didier *et al.* (2003) in trap cultures of the rhizosphere soil of strawberry plants, but they found no spores of this species in soil samples from strawberry field crops. *Glomus caledonium*, identified in the rhizosphere of the strawberry plants fertilized with Tytanit, was found to occur in intensively and semi-intensively cultivated agricultural soils (Oehl *et al.*, 2003).

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# Effect of composts based on brown coal on the growth of roots of strawberry plants cv. 'Elsanta' and their colonization by arbuscular mycorrhizal fungi

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**Abstract**

*In June 2010, strawberry plants cv. 'Elsanta' were planted in stoneware pots in the Experimental Field of Warsaw University of Life Sciences in Skierniewice. The following fertilization combinations were included in the experiment: control (NPK), brown coal with whey with oyster mushroom, brown coal with Vinassa with oyster mushroom, brown coal with whey with peat, brown coal with Vinassa with peat, brown coal with oyster mushroom, brown coal with whey, brown coal with whey with oyster mushroom with peat, brown coal with whey with shiitake mushroom, brown coal with Vinassa with shiitake mushroom, Brown coal with Vinassa, brown coal with shiitake mushroom, brown coal with shiitake mushroom with peat. The combination of brown coal with whey increased the fresh and dry weight of roots as well as root length and volume compared to the control combination with NPK. The application of brown coal with Vinassa and peat had a positive effect on increasing the root biomass, and the number of roots tips. The use of the composts and brown coal had a positive effect on root growth characteristics and mycorrhizal frequency in the roots of plants cv. 'Elsanta'. The combinations of brown coal with Vinassa and peat and brown coal with whey and oyster mushroom had the greatest impact on root growth and the colonization of the roots of strawberry plants cv. 'Elsanta' by arbuscular mycorrhizal fungi. Application of composts (based on brown coal) alone or in combination with reduced rates of NPK mineral fertilization can be an effective alternative in the growing of fruit and other horticultural crops.*

**Keywords:** mycorrhizal frequency, AMF, root morphology, strawberry

## Introduction

Sustainable and pro-ecological cultivation measures are considered as an important element of the Polish and EU's strategy for the development of the agricultural sector. The production of organic fruits has been increasing in recent years. However, the limited availability of traditional organic fertilizers (i.e. manure), and scarce information about the effects of new kinds of organic fertilizers, such as plant extracts (Sas Paszt *et al.*, 2009) or microbial inocula (Malusá *et al.*, 2007), are serious obstacles threatening the future development of the sector. Application of mycorrhizal inocula can increase the species diversity of these fungi in the rhizosphere and consequently improve the growth, yielding and yield quality of cultivated fruit crops (Sas Paszt & Żurawicz, 2005; Sas Paszt & Głuszek, 2007). We are thus evaluating the feasibility of different organic sources that are included in the list of products allowed for use in organic farming, as well as new materials that have the potential to be included in Annex I of Commission Regulation (EC) 889/2008 for the production of composts that could be used in organic fruit production.

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The aim of the study, carried out within the framework of a project intended to develop new products and technologies for organic fruit production in Poland, was to evaluate the quality of new composts and their effect on the growth and yield of strawberry plants.

## Material and Methods

Strawberry plants cv. 'Elsanta' were planted in June 2010 in stoneware pots in the Experimental Field of Warsaw Agricultural University in Skierniewice. The experiment is carried out in 12 combinations in 4 replications. In each stoneware pot there were planted 3 strawberry plants.

The following fertilization combinations were used in the experiment:

- Standard (NPK) Control
- Brown coal + whey + oyster mushroom (1 : 0.1 : 0.08)
- Brown coal + Vinassa + oyster mushroom (1 : 0.1 : 0.08)
- Brown coal + whey + peat (1 : 0.1 : 0.1)
- Brown coal + Vinassa + peat (1 : 0.1 : 0.1)
- Brown coal + oyster mushroom (1 : 0.08)
- Brown coal + whey (1 : 0.1)
- Brown coal + whey + oyster mushroom + peat (1 : 0.1 : 0.08 : 0.1)
- Brown coal + whey + shiitake mushroom (1 : 0.1 : 0.08)
- Brown coal + Vinassa + shiitake mushroom (1 : 0.1 : 0.08)
- Brown coal + Vinassa (1 : 0.1)
- Brown coal + shiitake mushroom (1 : 0.08)
- Brown coal + shiitake mushroom + peat (1 : 0.08 : 0.1)

In order to determine the extent of colonization of strawberry roots by mycorrhizal fungi, root samples were taken and submitted for laboratory analysis in June, 2011.

Determination of root growth and morphological parameters:

- Dry weight (Ostrowska *et al.* 1991).
- Root morphological parameters (total root length, root diameter, root surface area, root volume and total number of root tips) – image analysis system with an Epson scanner, controlled by WinRhizo software (Regent Instruments Inc.).

Determination of mycorrhizal frequency:

- Roots were cold-stained using the Phillips & Hayman method (1970).
- Microscopic analysis of the roots – Trouvelot's method (1986).
- Mycorrhizal frequency (F%) and mycorrhizal intensity (both relative – M%, and absolute – m%) were assessed in each root segment.
- The mycorrhizal parameters were calculated using the Mycocalc software: http://www2.dijon.inra.fr.mychintec/Mycocalc-pgr/download.html

All the results were statistically evaluated with analysis of variance. Comparisons of means were at $p \leq 0.05$ with the Duncan test.

## Results

The use of the composts based on brown coal had a positive effect on the colonization of the roots of strawberry plants cv. 'Elsanta' by arbuscular mycorrhizal fungi. The highest mycorrhizal frequency (22.22%) was obtained in the roots of strawberry plants cv. 'Elsanta' after the application of brown coal + whey+ shiitake mushroom. Mineral NPK fertilization (control) reduced the occurrence of AM fungi.

Table 1: Comparison of mycorrhizal frequency [F%], relative mycorrhizal intensity [M%] and intensity of the mycorrhizal colonization in the root fragments [m%] in the roots of strawberry plants cv. 'Elsanta' (Experimental Field of Warsaw University of Life Sciences, Skierniewice, 2011).

| Experimental treatments | F % | M % | **m** % |
|---|---|---|---|
| (NPK) Control | 1.11 | 0.01 | 0.33 |
| Brown coal + whey + oyster mushroom | 2.22 | 0.02 | 0.66 |
| Brown coal + Vinassa + oyster mushroom | 15.55 | 0.38 | 2.57 |
| Brown coal + whey + peat | 7.78 | 0.12 | 1.44 |
| Brown coal + Vinassa + peat | 15.55 | 0.20 | 1.33 |
| Brown coal + oyster mushroom | 3.33 | 0.03 | 0.33 |
| Brown coal + whey | 5.55 | 0.05 | 1.0 |
| Brown coal + whey + oyster mushroom + peat | 4.44 | 0.09 | 2.33 |
| Brown coal + whey + shiitake mushroom | **22.22** | 0.40 | 1.91 |
| Brown coal + Vinassa + shiitake mushroom | 21.11 | 0.30 | 1.38 |
| Brown coal + Vinassa | 3.33 | 0.03 | 1.0 |
| Brown coal + shiitake mushroom | 10.0 | 0.1 | 1.0 |
| Brown coal + shiitake mushroom + peat | 0.0 | 0.0 | 0.0 |

The highest fresh weight of roots was obtained in the combination with brown coal + whey, where root weight was higher by 26% compared to the control (Table 2). The other combinations with brown coal used in the experiment did not increase significantly the weight of roots. Also, root dry weight of the plants composted with brown coal with the addition of whey had the highest value (Table 2).

Brown coal used in the combinations: brown coal + Vinassa + peat and brown coal + whey + shiitake mushroom contributed to an increase in the number of root tips by more than 23%. With the other composts from brown coal and additives there was also an increase in the number of root tips, but not as significant as in these two combinations mentioned (Table 2).

Root surface area was affected most favourably by the use of brown coal + whey + shiitake mushroom, which increased this parameter by 30% (Table 2).

The use of the composts from brown coal and additives had no effect on increasing the diameter of the roots nor their volume.

In comparison with the NPK control, the use of brown coal + Vinassa + peat and brown coal + whey increased root length by more than 31%, while brown coal with the addition of whey and shiitake mushroom increased root length by 56% (Table 2).

Table 2: Effect of composts based on brown coal on the growth parameters of the roots of strawberry plants cv. 'Elsanta' (Experimental Field of Warsaw University of Life Sciences, Skierniewice, 2010.)

| Combination | **Root fresh weight** [g/L of soil] | **Root dry weight** [g/L of soil] | **Number of root tips** [per L of soil] | **Root surface area** [$cm^2$/L of soil] | **Root diameter** [mm/L of soil] | **Root volume** [$cm^3$/L of soil] | **Root length** [cm/L of soil] |
|---|---|---|---|---|---|---|---|
| (NPK) Control | 3.0 a-c | 0.76 b | 1398.4 a-d | 129.05 a-d | 0.52 d | 1.70 b-d | 782.20 ab |
| Brown coal + whey + oyster mushroom | 2.0 a | 0.43 a | 1339.9 a-c | 104.60 ab | 0.40 a | 1.04 a | 831.69 a-d |
| Brown coal + Vinassa + oyster mushroom | 3.1 bc | 0.80 b | 1567.5 a-d | 134.88 a-d | 0.45 a-c | 1.54 a-d | 937.36 a-d |
| Brown coal + whey + peat | 3.1 bc | 0.73 b | 1596.5 a-d | 125.73 a-d | 0.45 a-c | 1.42 a-d | 886.15 a-d |
| Brown coal + Vinassa + peat | 2.8 a-c | 0.76 b | **1725.6 cd** | 141.14 b-d | 0.43 ab | 1.56 a-d | **1025.58 b-d** |
| Brown coal + oyster mushroom | 2.5 ab | 0.63 ab | 1651.2 b-d | 131.86 a-d | 0.45 a-c | 1.48 a-d | 931.51 a-d |
| Brown coal + whey | **3.8 c** | **0.90 b** | 1614.7 a-d | 152.71 cd | 0.47 a-d | 1.80 cd | **1029.45 cd** |
| Brown coal + whey + oyster mushroom + peat | 3.2 bc | 0.76 b | 1593.5 a-d | 131.60 a-d | 0.49 b-d | 1.60 a-d | 863.67 a-d |
| Brown coal + whey + shiitake mushroom | 3.2 bc | 0.86 b | **1744.7 d** | **168.80 d** | 0.43 ab | 1.85 d | **1227.04 d** |
| Brown coal + Vinassa + shiitake mushroom | 2.5 ab | 0.73 b | 1478.9 a-d | 126.57 a-d | 0.44 av | 1.38 a-d | 925.91 a-d |
| Brown coal + Vinassa | 2.6 ab | 0.76 b | 1164.5 a | 114.70 a-c | 0.47 b-d | 1.34 a-c | 788.53 a-c |
| Brown coal + shiitake mushroom | 2.5 ab | 0.80 b | 1352.0 a-d | 125.09 a-d | 0.45 a-c | 1.39 a-d | 895.06 a-d |
| Brown coal + shiitake mushroom + peat | 2.8 a-c | 0.76 b | 1263.6 ab | 99.89 a | 0.51 cd | 1.29 ab | 615.34 a |

## Discussion and Conclusions

Brown coal is a new medium in plant cultivation. This material is used as a soil improver or as a medium in soilless cultures (Bartczak *et al.* 2007). Brown coal is a good source of humic compounds and mineral nutrients. However, during mineralization of clean brown coal it contributed no N in an available form (Robertson & Morgan 1995). Products derived from brown coal and added to the soil can decrease the negative influence of soil aluminium on plant root growth (Yazawa *et al.* 2000). Earlier reports showed that pure brown coal, as opposed to peat or wood chips, is not a good substrate for growing plants. Brown coal in a mixture with rockwool has been used in strawberry cultivation as a soilless growth medium (Bartczak *et al.* 2007) and found to cause the lowest growth in comparison with clean rockwool or peat-pine bark mixture. Starck and Oswiecimski (1985) observed that tomato plants var. Revermun cultivated on brown coal medium produced a significantly lower yield than plants cultivated on pine bark or peat. Asli & Neumann (2010) showed that humic acid at 1g $L^{-1}$ inhibited shoot and leaf growth in maize but did not reduce root growth.

There are not many reports on composted brown coal used as a fertilizer in crop cultivation. In our study, we used composts derived from brown coal with several nitrogen sources (e.g. whey) and with two different species of lignin degrading fungi. Whey as a by-product of the dairy industry is a good source of organic nitrogen compounds. Brown coal used as a growth medium supplement has a positive impact on plant growth, eg. in tomatoes (Gagnon & Berrouard 1994). Humic substances have a protective effect on aminoacids and the protein complex, preventing its further decomposition in the soil (Trojanowski 1973). Shiitake and oyster mushroom were used as compost amendments because they have a good lignin degrading properties (Tuor *et al.* 1995; Boyle 1998; Cohen *et al.* 2002).

In our study, some of the composts derived from brown coal increased the frequency of mycorrhizal occurrence in strawberry roots and increased the number and biomass of roots. The highest mycorrhizal frequency was obtained in the roots of strawberry plants cv. ‘Elsanta’ after composting with brown coal + whey + shiitake mushroom. Mineral NPK fertilization (control) reduced the occurrence of AM fungi in the roots of strawberry plants.

The highest fresh weight of roots was obtained in the plants fertilized with the compost based on brown coal + whey, where root fresh weight was higher by 26% compared to the NPK control. The dry weight of roots of the plants composted with brown coal + whey was also the highest. The use of the composts from brown coal modified root growth characteristics. In comparison with the NPK control, the use of brown coal + Vinassa + peat and brown coal + whey increased root length by more than 31%, while brown coal with the addition of whey and shiitake mushroom increased root length by 56%.

Composts based on brown coal can be an effective alternative in sustainable fertilization of strawberry plants compared to fertilization with mineral NPK fertilizers, due to their enhancing influence on plant growth development and yielding (presented in another abstracts to the conference Ecofruit 2012 by Stępień et al. 2012). These compost can also be used in combination with the reduced rates of mineral fertilizers in fruit crop species cultivation.

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# Effect of bioproducts on root growth and the occurrence of arbuscular mycorrhizal fungi in the rhizosphere of strawberry plants cv. 'Elsanta' and 'Elkat' in field conditions

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**Abstract**

*The aim of the study was to determine the effect of biofertilizers on the growth of strawberry roots and their colonization by mycorrhizal arbuscular fungi. Plants of two strawberry cultivars, 'Elkat' and 'Elsanta', were planted in May 2010 in an experimental orchard in Dąbrowice. The experiment was set up in a randomised block design in four replications. Each replication consisted of a plot of 20 strawberry plants planted 0.25 m apart with a 1-metre-wide gap between the rows. The experiment included 10 combinations: Control 0 (no fertilization), Control NPK, manure, Micosat + manure, Humus UP, Humus Active + Aktywit PM, BF Quality + manure, BF Amin + manure, manure + Tytanit, Vinassa + manure. In June 2011, the roots of the plants were collected for a laboratory assessment of mycorrhizal frequency. Regardless of the cultivar, the applied biopreparations had a positive effect on the colonization of the roots of strawberry plants cv. 'Elkat' and 'Elsanta' by arbuscular mycorrhizal fungi. The combined application of the preparation Micosat + manure caused a 6-fold increase in mycorrhizal frequency in the roots of strawberry cv. 'Elkat' in relation to the control fertilized with NPK. Mineral NPK fertilization caused a reduction in mycorrhizal frequency in the roots of strawberry plants of the two strawberry cultivars.*

**Keywords:** strawberry, arbuscular mycorrhizal fungi (AMF), mycorrhizal frequency

## Introduction

The study, carried out within the framework of a project aimed at developing new products and technologies for organic fruit production in Poland, evaluated the effect of new organic fertilizers and amendments on root growth and mycorrhizal abundance and biodiversity in the rhizosphere of strawberry plants 'Elsanta' and 'Elkat'. The influence of organic amendments on the development of mycorrhizal fungi has been reported by several authors. The technologies rely on the use of these bioproducts as they are or in a form enriched with beneficial soil microorganisms (Malusà *et al.*, 2007; Derkowska *et al*. 2008; Günes *et al.* 2009; Kirad *et al.* 2009). Several organic fertilizers and amendments have been recently tested and introduced, which are also acting as natural stimulators of plant growth and development (Chelariu & Ionel, 2005; Gousterova *et al.*, 2008; Khan *et al.*, 2009; Chelariu *et al.*, 2009; Meszka & Bielenin, 2009; Spinelli *et al.* 2010). These are preparations of natural (plant or animal) origin, harmless to humans and the environment, which contain nutrient elements and biologically active substances (i.e. plant hormone-like substances, enzymes) as well as other compounds that stimulate plant growth, yield, quality or tolerance to biotic and abiotic stresses. Enrichment of organic bioproducts with beneficial soil bacteria and mycorrhizal fungi could enhance the effectiveness of the organic products in plant growth stimulation and fruit production.

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## Material and Methods

Plants of two strawberry cultivars ‘Elkat’ and ‘Elsanta’ were planted in May 2010 in an experimental orchard in Dąbrowice. The experiment was set up in a randomised block design in four replications. Each replication consisted of a plot of 20 strawberry plants planted 0.25 m apart with a 1-metre-wide gap between the rows. In June 2011, the roots of the plants were collected for a laboratory assessment of mycorrhizal frequency.

The following experimental treatments were applied:

1) Control (no-treatment) (organic matter 1.5%, P 51 mg P $kg^{-1}$, K 158 mg K $kg^{-1}$, pH 5.5).

2) Standard NPK soil fertilization: 4 g $NH_4NO_3$ $plant^{-1}$, 3 g triple superphosphate and 6 g $K_2SO_4$ per rhizobox.

3) Dry granulated bovine manure for organic farming (Doktor O’grodnik) – 1 g per rhizobox.

4) Micosat (CCS Aosta s.r.l.) – a mixture of AM fungi: *Glomus* species, *Trichoderma viride* and rhizosphere bacteria species (*Bacillus subtilis, Pseudomonas fluorescens, Streptomyces* spp.) – 10 g + 0.5 g manure per rhizobox.

5) Humus UP (Ekodarpol) – an extract from a vermicompost – 2% solution to the soil per rhizobox.

6) Humus Active + Aktywit PM (Ekodarpol) – Humus Active is a soil improver with active humus and a population of beneficial microorganisms – 2% solution of Humus Active and 1% solution of Aktywit PM to the soil per rhizobox.

7) BioFeed Quality (Agrobio Products B.V.) – an extract from several seaweed species reinforced with humic and fulvic acids – 0.5% solution + 0.5 g manure to the soil per rhizobox.

8) BioFeed Amin (Agrobio Products B.V.) – an extract of 100% vegetal amino-acids – 0.5% solution + 0.5 g manure to the soil per rhizobox.

9) Tytanit (Intermag) – titanium (Ti) 0.8% (5 g Ti in 1 l of working solution), pH 3.4, containing 3163 mg $kg^{-1}$ Ti – 0.05% solution + 1 g manure to the soil per rhizobox.

10) Vinassa – molasses residue from yeast production – 0.5% solution + 0.5 g manure to the soil per rhizobox.

Determination of mycorrhizal frequency:

- Roots were cold-stained using the Phillips & Hayman method (1970).
- Microscopic analysis of the roots – Trouvelot’s method (1986).
- Mycorrhizal frequency (F%), mycorrhizal intensity (both relative – M%, and absolute – m%), abundance of arbuscules (both relative – A%, and absolute – a%) were assessed in each root segment.
- The mycorrhizal parameters were calculated using the Mycocalc software: http://www2.dijon.inra.fr.mychintec/Mycocalc-pgr/download.html

All the results were statistically evaluated with analysis of variance. Comparisons of means were at $p \leq 0.05$ with the Duncan test.

## Results

The highest mycorrhizal frequency was recorded in the roots of plants cv. ‘Elkat’ (F = 38.89%) in the combination Micosat + manure. Relative mycorrhizal intensity was the highest in the roots of plants cv. ‘Elkat’ (M = 2.26%) in the combination BF Amin + manure. The highest abundance of arbuscules was recorded in the roots of plants cv. ‘Elkat’ (A = 0.03%) in the combination with manure. The lowest values of mycorrhizal frequency,

mycorrhizal intensity and abundance of arbuscules were observed in the control combination fertilized with NPK (‘Elsanta’: F = 4.44%, ‘Elkat’: F = 6.67%).

Table 1. Comparison of mycorrhizal frequency (F%), relative mycorrhizal intensity (M%), intensity of the mycorrhizal colonisation in the root fragments (m%), arbuscule abundance in mycorrhizal parts of root fragments (a%) and relative abundance of arbuscules (A%) in the roots of strawberry plants cv. ‘Elsanta’ and ‘Elkat’ (Dąbrowice, 2011) (on the left – ‘Elsanta’, on the right – ‘Elkat’).

**‘Elsanta’**

| Treatments | F% | M% | m% | a% | A% |
|---|---|---|---|---|---|
| Control | 8.89 | 0 | 1 | 0 | 0 |
| NPK Control | 4.44 | 0.04 | 1 | 0 | 0 |
| Manure | 27.78 | 0.45 | 1.59 | 0 | 0 |
| Micosat + manure | 34.44 | 1.02 | 3.01 | 0 | 0 |
| Humus UP | 18.89 | 0.78 | 4.3 | 0 | 0 |
| Humus Active + Aktywit PM | 22.22 | 0.47 | 1.83 | 0 | 0 |
| BF Quality + manure | 24.44 | 1.2 | 4.7 | 0 | 0 |
| BF Amin + manure | 20 | 0.88 | 4.35 | 0 | 0 |
| Manure + Tytanit | 18.89 | 0.6 | 3.1 | 0.93 | 0 |
| Vinassa + manure | 18.89 | 0.32 | 1.71 | 0.93 | 0 |

**‘Elkat’**

| Treatments | F% | M% | m% | a% | A% |
|---|---|---|---|---|---|
| Control | 14.44 | 0.28 | 1.8 | 0 | 0 |
| NPK Control | 6.67 | 0.58 | 2.33 | 0 | 0 |
| Manure | 27.78 | 1.88 | 6.89 | 3.21 | 0.03 |
| Micosat + manure | 38.89 | 0.79 | 2.03 | 0 | 0 |
| Humus UP | 33.33 | 0.69 | 2.11 | 0 | 0 |
| Humus Active + Aktywit PM | 23.33 | 0.63 | 2.71 | 0 | 0 |
| BF Quality + manure | 12.22 | 0.21 | 1.67 | 0 | 0 |
| BF Amin + manure | 16.67 | 2.26 | 1.53 | 0 | 0 |
| Manure + Tytanit | 23.33 | 1.05 | 4.94 | 0 | 0 |
| Vinassa + manure | 20 | 1.07 | 4.85 | 0 | 0 |

The observed mycorrhizal structures:

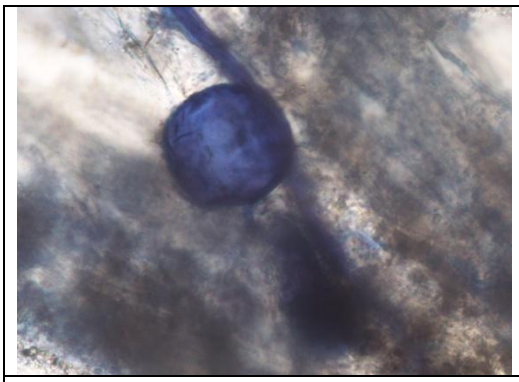

Photo 1. A spore in the roots of strawberry plants cv. ‘Elsanta’ (Dąbrowice, 2011)

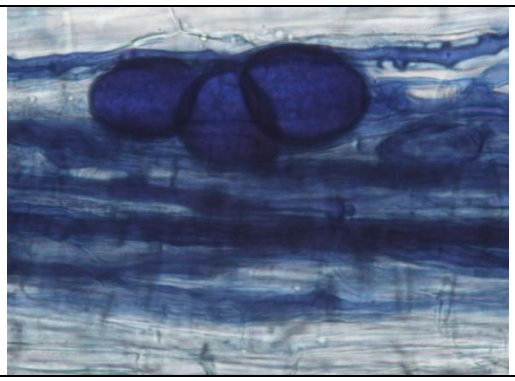

Photo 2. Vesicles in the roots of strawberry plants cv. ‘Elkat’ (Dąbrowice, 2011)

The combined application of the preparation Micosat + manure caused a 6-fold increase in mycorrhizal frequency in the roots of strawberry cv. ‘Elkat’ in relation to the control fertilized with NPK. Mineral NPK fertilization caused a reduction in mycorrhizal frequency in the roots of strawberry plants of the strawberry cultivars.

Regardless of the cultivar, the applied biopreparations had a positive effect on the colonization of the roots of strawberry plants cv. ‘Elkat’ and ‘Elsanta’ by arbuscular mycorrhizal fungi.

## Discussion and conclusions

The influence of different types of products of biological origin on plant growth and fruit quality has been widely reported by many authors (Żurawicz *et al*. 2006, Frąc *et al.* 2009*;* Roussos *et al*. 2009). Also, the occurrence of AMF in plant roots and the rhizosphere affected by these products has been observed by many authors in different kinds of plants (Celik *et al*. 2004; Kuwada *et al.* 2006b).

The beneficial influence of products of seaweed origin on the presence of arbuscular mycorrhizal fungi was observed in our experiment and was reported earlier by Kuwada *et al.* (2005; 2006a) for *Gigaspora margarita*. The positive influence of manure application was observed by Harinikumar & Bagyaraj (1989) in cropping sequence with maize, sunflower and other plants. The higher percentage of mycorrhizal colonization of dry bean (*Phaseolus vulgaris* L. cv. Viva) in manure-treated soil was observed by Tarkalson *et al.* (1998). In some cases increased amounts of manure can decrease the positive effect of AMF on plant growth parameters (Brechelt 1990). The results of our study revealed that NPK fertilization significantly decreases the number of mycorrhizal structures in strawberry roots due to the lowest percentage of mycorrhizal associations in the roots of NPK-fertilized plants. Other researchers have also reported the harmful influence of synthetic NPK fertilizers on mycorrhizas (Harinikumar & Bagyaraj 1989; Plenchette 1989). The results of this field experiment showed that mycorrhization has a positive effect on root colonization with arbuscular mycorrhizal fungi, which is in agreement with the previous studies conducted in various environmental conditions (Varma & Schuepp 1996; Vosatka *et al.* 2000).

Our results revealed that the highest mycorrhizal frequency was recorded in the roots of strawberry plants cv. ‘Elkat’ in the combination of Micosat + manure. The highest abundance of arbuscules was recorded in the roots of plants cv. ‘Elkat’ fertilized with manure. The combined application of the preparation Micosat + manure caused a 6-fold increase in mycorrhizal frequency in the roots of strawberry cv. ‘Elkat’ in relation to the control fertilized with NPK.

Mycorrhization and application of bioproducts can be recommended as an alternative to standard NPK fertilization, for use in strawberry cultivation, due to its positive influence on plant growth, development and higher colonization of strawberry roots with mycorrhiza (Sas Paszt *et al.* 2011).

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# The collection of beneficial soil microorganisms held in the SYMBIO BANK

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**Abstract**

*R **Results of studies to date have shown that there are large differences in the occurrence of mycorrhizal fungi depending on the species and plant cultivation method. At present, the material collected in the bank of isolated spores of mycorrhizal fungi and the bank of PGPR bacteria comes from the nearby ecological orchards and plantations and from ecological orchards and plantations in the Bieszczady and Białowieża region. Trap cultures have been used to isolate and identify spores of the following species of arbuscular mycorrhizal fungi:* Ambispora fennica, A. gerdemannii, Gigaspora margarita, Glomus aggregatum, G. caledonium, G. claroideum, G. constrictum, G. drummondii, G. fasciculatum G. macrocarpum, G. microaggregatum, G. mosseae, G. pallidum, G. rubiforme, Scutellospora dipurpurescens. *The collection in the SYMBIO BANK contains (approximately): Spores isolated from the soil of the following plant species: strawberry 16.0 thousand, apple 6.5 thousand, sour cherry 1.1 thousand pear 8.1 thousand. Isolates of bacteria:* Pseudomonas fluorescens *-170, dissolving phosphorus compounds - 40, digesting cellulose - 40, producing spores - 110, fixing atmospheric nitrogen - 10, Actinomycetes - 40. Isolates of microscopic fungi - 50, including Trichoderma sp. - 30. The work of isolating and identifying species and strains of AM fungi and PGPR bacteria is continued. They are collected, catalogued and stored in a Bank of Symbiotic Microorganisms, called SYMBIO BANK, specially established for this purpose. The collected strains and species are identified, characterized and stored in a cryoprotectant (glycerol) at the temp. of -80°C. In the near future, a website of the SYMBIO BANK will be launched, which will contain a list of the isolates held in the collection and their descriptions, which will serve as a source of key information for the identification of the species of AM fungi and PGPR bacteria in Poland. This will contribute to the knowledge of the biodiversity of these symbionts and help in the formulation of microbiologically-enriched bioproducts for use in fruit-growing practice. The most effective strains and species of microorganisms will be registered in Poland as bacterial and mycorrhizal inocula to be used in fruit production and in phytoremediation of heavy metal pollution. The establishment of the bank of spores of mycorrhizal fungi will contribute to the understanding and maintenance of the biodiversity of these symbionts, the knowledge of their biology and ecology, as well as to the development of ecological technologies of fertilization of fruit plants in Poland and the protection of the natural environment and human health.*

**Keywords:** bacteria, microscopic fungi, AMF, isolates

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## Introduction

Plant-soil microorganisms can modulate the uptake of mineral nutrients through feedback processes that reflect plant responses to environmental conditions. The intimate interrelation between the root and symbiotic arbuscular mycorrhizal fungi and the resulting enhancement in plant uptake of N and P, is further expanded by the interactions between the fungus and bacteria present in both the rhizosphere and mycorrhizosphere. Numerous species of plant growth promoting bacteria form biofilm when colonizing roots, which can affect bio-geochemical processes and can result in increased availability of poorly available mineral nutrients. The complexity of the relationship between plant and rhizosphere microorganisms is further complicated by the effects on both rhizobacteria and mycorrhizas of protozoan grazers, which are also directly affecting the root system. The soil biotic component include complex communities with different trophic levels that function in very heterogeneous environments. These communities are formed by several kinds of microorganisms that can live symbiotically or in association with roots. Four major groups of microorganisms are considered as beneficial to plants: arbuscular mycorrhizal fungi (AMF) (Jeffries *et al.* 2003), plant growth promoting rhizobacteria (PGPR) (Vessey 2003), nitrogen-fixing rhizobia, which are usually not considered as PGPR, and microbial biocontrol agents, which are composed of several kinds of microorganisms (viruses, bacteria, yeasts and fungi).

An important part the project called EkoTechProduct, which is carried out at the Research Institute of Horticulture in Skierniewice (Poland), is to establish and maintain a Bank of Symbiotic Microorganisms, called the SYMBIO BANK. At present, the collected material of isolated spores of mycorrhizal fungi and PGPR bacteria comes from organic orchards and plantations located around Skierniewice (Poland), and organic orchards and plantations in the Bieszczady and Białowieża areas (Poland).

## Material and Methods

To distinguish more than 80 isolates of *Pseudomonas* bacteria and bacteria dissolving phosphorus compounds, acquired from the soil in the root zone of apple and sour cherry trees, the technique of rep-PCR was employed, based on an analysis of DNA polymorphism. The tests allowed the selection of bacterial isolates that were different or belonged to the same strain. For the detection of arbuscular mycorrhizal fungi (AMF) in the roots of strawberry plants, the nested PCR technique was used, based on the amplification of fragments of the large subunit ribosomal gene (LSU rDNA) using specific primers. The analyses were performed on the DNA extracted from the roots of strawberry cultivars ‘Honeoye’, ‘Elsanta’ and ‘Elkat’ that had been treated with bio-preparations and fertilized with NPK. The tests helped to determine the presence or absence of mycorrhizal fungi of the genera *Glomus, Acaulospora* and *Scutellospora* in the roots. The results were used to determine the effect of the bio-preparations on the presence of mycorrhizal fungi in the roots of strawberry plants.

Over 600 strains of *Pseudomonas fluorescens* and other beneficial bacteria and fungi (*Rahnella aquatilis*, *Bacillus* sp., *Trichoderma* sp.) have been collected. *Pseudomonas fluorescens* strains were isolated from selective S1 medium (Gould *et al.* 1984). Other beneficial bacteria were isolated from differentiating CAS agar medium (siderophores production) or Pikovska medium (dissolving phosphate compounds) (Husen *et al.* 2003).

Ten bacterial strains with most potent beneficial abilities were identified and characterized by the BIOLOG system (Holmes *et al.* 1994; Pires & Seldin 1995) and used in the further screening studies under greenhouse conditions. Screening studies under greenhouse conditions indicate that three strains (Ps49A - *Pseudomonas fluorescens*, Pi3A and Pi5A -

*Rahnella aquatilis*) enhance growth of strawberry plants. However further and more complex studies must be conduct.

Identification of spores:

- Trap cultures were set up with narrowleaf plantain in 0.5 L pots filled with a mixture of rhizosphere soil and autoclaved sand, at a ratio of 1:1 v/v (Błaszkowski 2003). Pots were placed in SunBags (Sigma).
- After six months, 200g samples of the pot substrate were taken from the trap culture combinations and spores were isolated by wet sieving and centrifuging in a sucrose gradient (Brundrett *et al.* 1996).
- The isolated spores were divided into morphotypes according to size, shape, and colour of spores.
- The layer thickness of spore walls and germination walls was measured in freshly isolated spores which were crushed in PVLG or PVLG+Melzer's reagent (1:1, v/v) and observed under a light microscope equipped with a micrometer eyepiece (Błaszkowski 2003).
- The observed AMF species were named according to Schüßler *et al.* (2001) and Błaszkowski (2003).

All the results were statistically evaluated with analysis of variance. Comparisons of means were at $p \leq 0.05$ with the Duncan test.

**Results**

As part of the SYMBIO BANK, trap cultures were set up and used to isolate and identify spores of the following species of arbuscular mycorrhizal fungi: *Ambispora fennica, A. gerdemannii, Gigaspora margarita, Glomus aggregatum, G. caledonium, G. claroideum, G. constrictum, G. drummondii, G. fasciculatum G. macrocarpum, G. microaggregatum, G. mosseae, G. pallidum, G. rubiforme, Scutellospora dipurpurescens.*

The collection in the SYMBIO BANK contains (approximate numbers):

Spores isolated from the soil of the following plant species:

- strawberry 16.0 thousand
- apple 6.5 thousand
- sour cherry 1.1 thousand
- pear 8.1 thousand

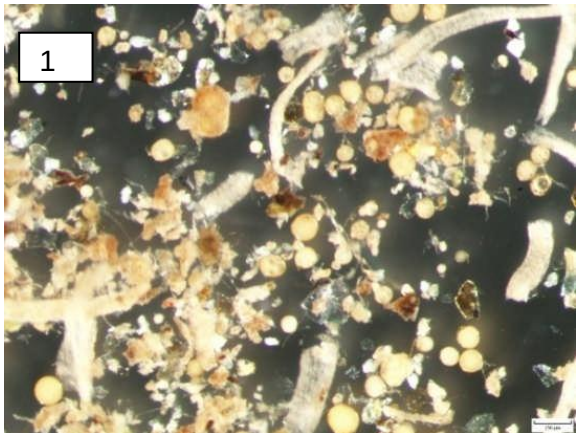


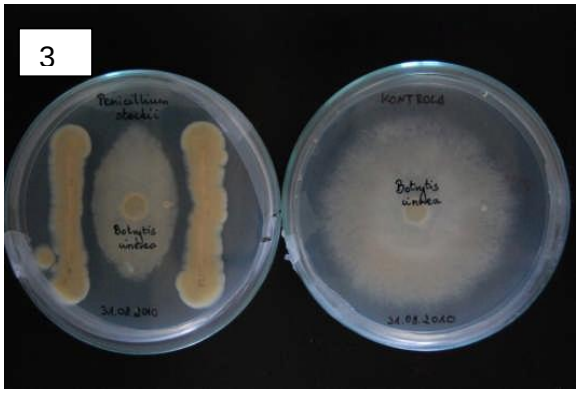
Photo. 1 and 2. Spores of mycorrhizal fungi (AMF) during isolation

Isolates of bacteria:

- *Pseudomonas fluorescens* 170
- dissolving phosphorus compounds 40
- digesting cellulose 40
- producing spores 110
- fixing atmospheric nitrogen 10
- actinomycetes 40

Isolates of microscopic fungi, a total of 50, including

- *Trichoderma* sp. 30



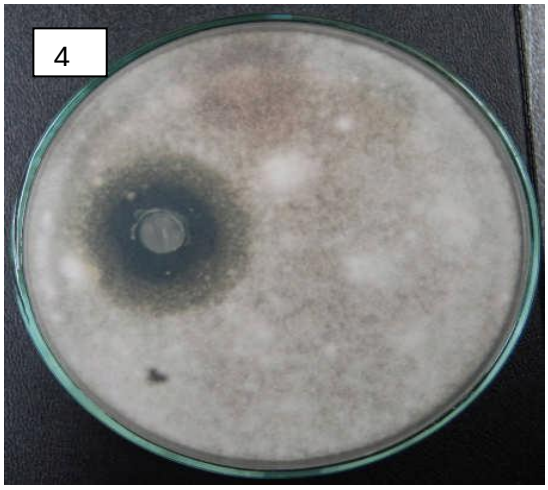




Photo. 3. Inhibition of the growth of *Botrytis cinerea* fungus by antagonistic fungi

Photo. 4. Isolates of rhizosphere bacteria, potential antagonists of soil pathogens (zone of growth inhibition caused by the bacteria)

Isolation and identification of the species and strains of AM fungi and PGPR bacteria is continued. After they have been collected, the microorganisms are identified, characterized, catalogued and stored. The strains of beneficial bacteria are stored in a cryoprotectant (glycerol) at -80°C. The AM fungi are stored in trap cultures in a greenhouse and in the form of spores immersed in various cryoprotectants and frozen at -80°C.

## Discussion

A website of the SYMBIO BANK will be launched very soon and will contain a list of strains and species of beneficial soil microorganisms held in the collection. Their descriptions will serve as a source of key information for the identification of the species of AM fungi and PGPR bacteria in Poland. This will contribute to the knowledge of the biodiversity of these symbionts and will help in the formulation of microbiologically-enriched bioproducts for use in fruit-growing practice.

Within the EkoTechProduct project the performance of all the rhizosphere components influenced by microbial inoculation practices will be also studied, e.g. modification of the rhizospheric environment and microbial development by the plant root system. For example, roots may release chemical signals that can be recognized by the microbes, which in turn respond either by modifying morphological features (Buee *et al*. 2000) or by producing feedback signals that set the plant to allow root colonization (Bais *et al*. 2006;

Hirsch *et al*. 2003; Vierheilig & Piché 2002). Furthermore, several compounds exudated by roots can mimic quorum sensing signals that affect the bacterial communities (Bauer & Mathesius 2004). On the other hand, plants respond to microbial root colonization by increasing the release of exudates and by modifying their composition (Phillips *et al*. 2004; Kamilova *et al*. 2006; Steinkellner *et al*. 2007).

All the plant-soil-microbial interactions will be studied after application of soil microbial inocula and their beneficial influence on plant growth, development and yielding.

The most effective strains and species of microorganisms will be patented and registered in Poland as bacterial and mycorrhizal inocula. They will be used in horticultural production and in phytoremediation of degraded soils. The establishment of the bank of spores of mycorrhizal fungi and rhizospheric PGPR bacteria will contribute to the understanding and maintenance of the biodiversity of these symbionts, the knowledge of their biology and ecology, as well as to the development of ecological technologies of fertilization of fruit plants in Poland and the protection of the natural environment and human health.

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# Effect of brown coal-based composts produced with the use of white rot fungi on the growth and yield of strawberry plants

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**Abstract**

*Composts were produced using brown coal from the Brown Coal Mine in Belchatow (Poland), with the following additions: a) an inoculum of either* Pleurotus ostreatus *or* Lentinus edodes *– white rot fungi (1% of the total weight of the compost matrix); b) Vinassa – a by-product of the production of bakery yeasts (10% of the total weight of the compost matrix); c) whey – a dairy by-product (10% of the total volume of the compost matrix).* The *composts were analyzed for nitrogen and carbon content, organic carbon fractions (TEC, HA and FA), and humification indices were calculated.The composts were used in a trial where strawberry plants were grown under field conditions, but in mesocosms made of terracotta pots (about 0.12 m$^3$) buried in soil.*

*The two species of fungi and the two by-products affected differently the decomposition of the organic matrix during the composting process, resulting in composts with different characteristics of the organic matter and different content of mineral elements. Those obtained with Vinassa had the highest N content and the highest amount of soluble organic C forms.*

*The use of the different composts as soil fertilizers induced a similar overall growth of strawberry plants cv. 'Elsanta'. However, fruit yield was differently affected by the applied treatment. Considering all the parameters measured, the compost obtained with the use of vinassa and* Pleurotus ostreatus *were the most promising among the different composts used.*

**Keywords**: Compost, strawberry, lignino-cellulosic fungi

## Introduction

Composted mixtures of vegetable and animal materials are allowed to be used in organic farming. Farms dedicated to horticultural productions are frequently lacking compostable raw materials. The limited availability of classical organic fertilizers (i.e. manure) even in not specialized farms, and scarce information about the effects of new kinds of organic fertilizers like plant extracts (Sas-Paszt et al. 2011) or microbial inocula (Malusà et al. 2007) are serious bottlenecks for the development of the organic horticultural productions in Poland. We are thus evaluating the feasibility of different organic sources, that are included in the list of products allowed to be use in organic farming, as well as new materials that have the potential to be included in Annex I of Commission Regulation (EC) 889/2008, for the production of composts that could be used in organic fruit production.

The aim of the study, carried out within the framework of a project intended to develop new products and technologies for organic fruit production in Poland, was to evaluate the quality of new composts and their effect on the growth and yield of strawberry plants.

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## Materials and Methods

Composts were prepared using brown coal as lignin substrate (Kopalni Węgla Brunatnego Bełchatów, Poland) with the addition of one of the following waste residues and one of the fungus species to produce four different mixtures:

a) Vinassa – a by-product of the production of bakery yeasts (10% of the total mass of the organic matrix)

b) whey – a dairy by-product (10% of the total volume of the compost matrix)

c) an inoculum of either *Pleurotus ostreatus* (pleurotus) or *Lentinus edodes* (shiitake) – white rot fungi (1% of the total weight of the compost matrix)

The addition of the fungi aimed at accelerating the decomposition of the organic compounds found in the brown coal, while the addition of the organic by-products enriched the compost matrix with mineral compounds (especially nitrogen) and amino acids increasing the efficiency of organic carbon transformation and the content of nutrients in the final product.

The composting process lasted 3 months. The products were thus analysed for their N and C content by C-N analyser, and the organic matter was characterized by alkali and acid extraction of the different fractions ($C_{ext}$, HA and FA) (Dell'Abate et al. 2002), and humification indices were calculated according to Ciavatta et al (1990).

The composts were used in a trial with strawberry plants cv Elsanta grown under field conditions, but in mesocosms made of terracotta pots (about 0.12 $m^3$) buried in soil. The four composts were compared to untreated (not fertilized) plants. The plants received an amount of compost standardized on the amount of N content in the compost, thus allowing to provide all treatments with an amount equivalent to 80 kg $N \cdot Ha^{-1}$.

Each treatment was applied to 10 plants each grown in a single pot placed on a completely randomized design. Growth was assessed by determining the number and weight of runners and yield was recorded.

## Results

The total C content of the four composts was quite similar, ranging from 51.1 to 53% (Tab. 1). The addition of vinassa to the composting matrix doubled the amount of N in the final compost and consequently resulted in a more suitable C:N ratio for fertilization purposes (Tab. 1) .

The characterization of the organic matter of the composts reveled significant differences linked with the kind of fungi used during the composting process and the substance added as a N source in the compost matrix (Tab. 2). The compost obtained with pleurotus contained a higher amount of total C in comparison to the one obtained with shiitake, irrespective of the additional component added (whey or vinassa). With the addition of whey, the compost made with pleurotus contained more organic C in comparison of the similar compost produced with shiitake. On the other hand, total organic C was little affected by the addition of vinassa.

The addition of vinassa affected more the C extractable fraction ($C_{ext}$) of the compost in comparison with the compost produced with whey as N source (Tab. 2). $C_{ext}$ in the composts with the addition of vinassa was 20% and 250% higher, for pleurotus and shiitake respectively, in comparison to the compost obtained with whey.

The composts obtained with shitake contained a higher fraction of humic and fulvic acids (HA+FA) in comparison to that obtained with pleurotus, irrespective of the additional N-rich ingredient (Tab. 2). However, vinassa induced the formation of a higher concentration of

these fractions with both fungi when compared to the compost obtained with the addition of whey.

The differences in the C fractions of the composts were paralleled by differences in three humification indexes (Tab. 2). The composts obtained with shiitake were characterized by higher values of HR and DH indexes in comparison to the ones produced with pleurotus. However, the compost obtained with the addition of vinassa showed a HU index 2.5 times higher than the compost with whey.

Table 1: C and N content and C:N ratio of the four composts.

| Compost | N % | C % | C:N |
|---|---|---|---|
| Brown coal + pleurotus + whey | 0,40 | 51,10 | 126 |
| Brown coal + pleurotus + vinassa | 0,76 | 51,80 | 69 |
| Brown coal + shiitake + whey | 0,42 | 53,00 | 125 |
| Brown coal + shiitake + vinassa | 0,86 | 51,85 | 60 |

Table 2: Extractable C, humic and fulvic carbon fractions, and humification indexes of the four composts.

| Compost | $C_{ext}$ g $kg^{-1}$ | HA+FA g $kg^{-1}$ | HR % | DH % | HU % |
|---|---|---|---|---|---|
| Brown coal + pleurotus + whey | 352.60 b | 2.30 b | 0.27 b | 0.65 c | 152.30 a |
| Brown coal + pleurotus + vinassa | 422.40 a | 2.68 b | 0.37 b | 0.63 c | 156.61 a |
| Brown coal + shiitake + whey | 175.1 c | 3.48 a | 0.57 a | 1.99 a | 49.32 c |
| Brown coal + shiitake + vinassa | 451.2 a | 3.64 a | 0.51 a | 0.81 b | 122.96 b |

The growth of the plants treated with all four composts was higher than the control, but similar among the different treatments (Tab. 3). No differences were found in case of the number of runners among the four composts; however, the weight of the runners was higher in plants treated with composts containing vinassa, and the highest weight was recorded for the plants receiving the compost made with vinassa and shiitake. The production of fruits was higher in the plants treated with the composts made with vinassa, and the highest production was observed in the plants receiving the compost produced with the addition of vinassa and pleurotus (Tab. 3).

Table 3: Growth parameters and yield of strawberry plants cv Elsanta treated with the four different composts.

| Treatments | Length of runners cm | Weight of runners g | Total weight of fruits g |
|---|---|---|---|
| Control | 246,7 b | 10,7 c | 36,7 c |
| Brown coal + pleurotus + whey | 333,2 a | 15,5 b | 89,7 b |
| Brown coal + pleurotus + vinassa | 336,5 a | 15,9 ab | 104,0 a |
| Brown coal + shiitake + whey | 311,7 a | 16,2 ab | 76,5 b |
| Brown coal + shiitake + vinassa | 332,7 a | 17,2 a | 80,7 b |

**Discussion and conclusions**

Differences in the fractions of the organic matter are related to the rate of its mineralization and hydrolysis. The two fungi used to enhance the transformation of the C fraction appear to function differently in this respect, probably due to different enzymatic machineries utilized for lignin hydrolysis (Toumela et al. 2000). The two organic by-products added to the mixture also differently affected the process of hydrolysis of the brown coal and the final composition of the composts, particularly for what concern the N content and C:N ratio. The addition of vinassa allowed to obtain a C:N ratio more suitable for fertilization purposes.

The addition of vinassa resulted also in a higher content of $C_{ext}$ and higher humification indexes in comparison to the composts produced with the addition of whey. The humification rate (HR%) provides quantitative information about the humic substances content normalised with respect to total soluble organic matter, while the degree of humification (DH%) provides the amount of the humified carbon in the extracted organic fraction (Ciavatta et al. 1990). More important, the HU index, which represent the total level of humification, was always higher in the composts obtained with the addition of vinassa, and particularly in those where pleurotus was used. Therefore, the composts obtained with the addition of vinassa presented a more suitable content of humified substances available for the plant. Considering the two white rot fungi, pleurotus allowed to obtain a more degraded compost. Degradation of lignin is carried out by by extracellular enzymes of different fungal species and then converted to phenols and quinones. All degraded fractions are then polymerized by a free-radical mechanism to generate the humus (Varadachari and Ghosh 1984; Horwath and Elliott 1996). The higher value of the HU index is pointing to a better functioning of *P. ostreatus* in the transformation of carbon from the brown coal matrix.

The plants treated with the composts always showed higher growth and yield than the untreated control, which points out the nutritional potential of these products. Nevertheless, the differences found in the chemical structure of the composts affected differently both growth and yield of strawberries. Indeed, even though the number of runners was not changed by the treatments, their total mass was higher in the plants receiving composts produced with the addition of vinassa (in combination with shiitake) in comparison to those produced with whey (with both white rot fungi). The best yield was obtained with the compost made with the addition of vinassa and pleurotus. Taking into account both growth and yield, the latter compost can thus be considered the product showing the best potential as fertilizer for strawberry production. The evaluation in this

respect is still undergoing. It should also be considered that the differences found in the characteristics of the studied composts might affect the soil biological quality and fertility and consequently indirectly influencing plant growth and yield (Bardi and Malusà 2012).

In conclusion, the differences in the chemical composition of the composts and in the effect on strawberry growth and yield are suggesting that for the production of organic composts based on brown coal, the use of *P. ostreatus* and of vinassa is providing a better nutrition of the plants in comparison to compost produced with the addition of *Lentinus edodes* and of whey.

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# Impact of supplementary plant preparations and organic fertilizers on yield of organic strawberries cv. 'Elkat' and 'Symphony'

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**Abstract**

*In 2011 the field experiments with organic strawberries cv. 'Elkat' and 'Symphony' were conducted. We evaluated the effects of product category supplementary plant preparations Lignohumate B and Synergin® and liquid organic fertilizers Prev-B2 and Hungavit A® on yield and other harvest data. The highest total and marketable yield was achieved in both varieties after Lignohumate B application. Lignohumate B also increased mean fruit weight. 'Elkat' had significantly higher yield and mean fruit weight compared to 'Symphony'. Prev-B2 significantly reduced the number of grey mould diseased fruits of 'Symphony'.*

**Keywords:** Strawberry, organic fertilizers, humates, yield, *Botrytis cinerea*

## Introduction

Traditional way to provide crops by nutrients in organic farming is application of voluminous organic fertilizers such as manure, compost or green manure in soil before planting. Because of relatively quick mineralization of raw organic material and thus potential risk of nutrients losses by washout during winter time (Gaskell *et al.*, 2009), some crops including strawberries may meet nutritional problems during restricted periods of the growth cycle (Neri *et al*., 2002). Foliar application of organic fertilizers based on humic acid substances, derived mainly from vermicomposts, seems to be perspective way to provide plants with suitable forms of nutrients during prolonged period (Singh *et al.* 2010). In addition vermicomposts contain humic acids and plant growth regulators like auxins, gibberellins and cytokinins, which support plant growth (Atiyeh *et al.*, 2002). Plant nutrition can be more efficient using supplementary plant preparations, which are not registered as fertilizers due to low nutrient content. Some of them together with organic fertilizers contain valuable biologically active components such as humic acids or plant growth regulators, others can help to protect crops from pests and disease incidence. Synergistic effect of humic acids and plant growth regulators are discussed (Arancon *et al.,* 2004, Verlinden *et al.*, 2009).

The aim of the present study was to prove the effect of two commercial supplementary plant preparation Lignohumate B (humate) and Synergin® (organic plant growth regulator) and two foliar fertilizers Hungavit A® (vermicompost) and Prev-B2 (boron fertilizer) on yield and quality of organic strawberries.

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**Material and Methods**

Experiments were carried out at Faculty of Horticulture in Lednice (Mendel University in Brno). The locality is situated in Southern Moravia (above sea level 180 m, average temperature 9 °C, average year precipitation 517 mm, silty soils). The soil was thoroughly prepared for two years according to the rules of organic agriculture using 3 types of green manure every year – phacelia, white mustard and mixture of field pea with oat, respectively. Young plants of cv. 'Elkat' and 'Symphony' were planted in September 2010. Double row planting system on ridges covered by black polypropylene plastic mulch (non-woven fabric) was used. The distance of double rows was 0.8 m, spacing of plants 0.35×0.25 m. The experimental plots were set as randomized block design with 3 replications per treatment (60 plants). Drop irrigation was installed above the plastic mulch. In spring 2011 the space between the ridges were mulched with wheat straw.

Four treatments were used consisting of different fertilizers and supplementary plant preparations, respectively. All of them were applied on the leaves with hand sprinkler five times in 7 day intervals:

1$^{th}$ application: 15. 4. 2011 (BBCH 55 – first flowers at the bottom of the rosette).

2$^{nd}$ application: 22. 4. 2011 (BBCH 58 – early balloon stage).

3$^{th}$ application: 10. 5. 2011 (BBCH 67 – flowers fading: majority of petals fallen).

4$^{th}$ application: 17. 5. 2011 (7 days after the end of flowering).

5$^{th}$ application: 4. 7. 2011 (7 days after the end of harvesting).

Treatments were following:

1. Lignohumate B.

Lignohumate B is a mixture of salts of humic substances (12 %) with a high content of fulvic acids component. It is an environmental-friendly supplementary plant preparation made from technical lignosulfonate during paper manufacturing (Amagro Ltd., Czech Republic). Foliar applications were applied at a dose 1.0 l of Lignohumate B in 600 l of water per hectar.

2. Synergin®.

Synergin® is a synergistic bioregulator of plant growth, which contains a number of physiologically active substances, namely the natural cytokinin and auxin precursors of organic origin produced from food raw materials (Juwital Ltd., Czech Republic). Synergin® was applied by spraying in recommended dose 2 l per hectare (spray liquid volume 600 l.ha$^{-1}$).

3. Lignohumate B + Hungavit A®.

Hungavit A® is a liquid fertilizer based on vermicompost extract (BioLife Ltd. Hungary). It contains following nutrients (mg.l$^{-1}$): N 140, K 650, Mg 7000, B 4950, Ca 83, P 290, Fe 7.7, Cu 0.2 and Zn 0.41 (Gaspar, 2002). Strawberry plants were treated with tank mix of Lignohumate B (1.0 l.ha$^{-1}$) and Hungavit A® – first two applications at a dose 7.7 l.ha$^{-1}$ followed by three apllication at dose 8.8 l.ha$^{-1}$ (spray liquid volume 600 l.ha$^{-1}$)

4. Prev B2®

Prev B2® is as boron liquid fertilizer and conditioner (Biofa AG, Germany). It contains 2.1% of boron, 4.2% of cold-pressed orange oil and 0.4% of fatty alcohol ethoxylate. The recommended dose 1.8 l.ha$^{-1}$ (spray liquid volume 600 l.ha$^{-1}$) was used.

5. Control

Untreated, free of any preparations.

During the harvest time we assessed the yield and quality of production. Data were recorded on all harvest dates, berries were picked twice a week. Mid-season ripening cv. 'Elkat' showed 8 harvests, the first one 26$^{th}$ May, the last one 22$^{nd}$ June. Late season

ripening cv. ‘Symphony’ was harvested during 2nd June and 27th June (total 7 harvests). Fruits were weighed and sorted into following qualitative classes according to the marketing standards of the European Union (commission regulation /EC/ no 843/2002): Extra class (regular shape and colour, diameter >25 mm), Class I (slight defect of shape, a white patch max 1/10 of the surface, diameter 18–25 mm) and Class II. class (defect of shape, a white patch max 1/5 of the surface), respectively.

The marketable yield consisted of berries assigned to the previous classes. Berries with smaller diameter, with malformations or other disorders, as well as those ones damaged by grey mould or other rots, formed unmarketable yield. Numbers of fruits infected by grey mould were calculated separately to determine the level of infestation (%) by *Botrytis cinerea* Pers. Mean fruit weight was calculated from weights of all berries. Collected data of different parameters were averaged and statistically processed by ANOVA and LSD test ($P<0.05$) using software Unistat version 5.1. Number of fruits diseased by grey mould (in percentage units) was analysed by using software UPAVplus, ver. 1.06 (Czech Phytosanitary Administration) taking into account arcsin data transformation.

## Results

Yield parameters were negatively influenced by late spring frosts. During flowering temperature dropped bellow 0 °C in four subsequent days. Critical temperatures were recorded 6th May, when it fell to -4.1 °C. It caused significant fruit set reduction and resulted in higher number of deformed berries taking part in unmarketable yield at both cultivars (Figure 1 and Figure 2). Cv. ‘Elkat’ had total yield 103.4 g per plant, while total yield of cv. ‘Symphony’ was only 42.9 g per plant.

Application of Lignohumate B produced highest total yield at both cultivars. In the case of ‘Elkat’, application of Lignohumate B significantly increased total yield not only compared to control but to all other treatments (Figure 1). Further, application of Lignohumate B significantly increased the weight of fruits sorted in Extra class, II. class, but also unmarketable portion of total yield. Application of other preparations slightly increased yield parameters compared to control, but the differences were not significant.

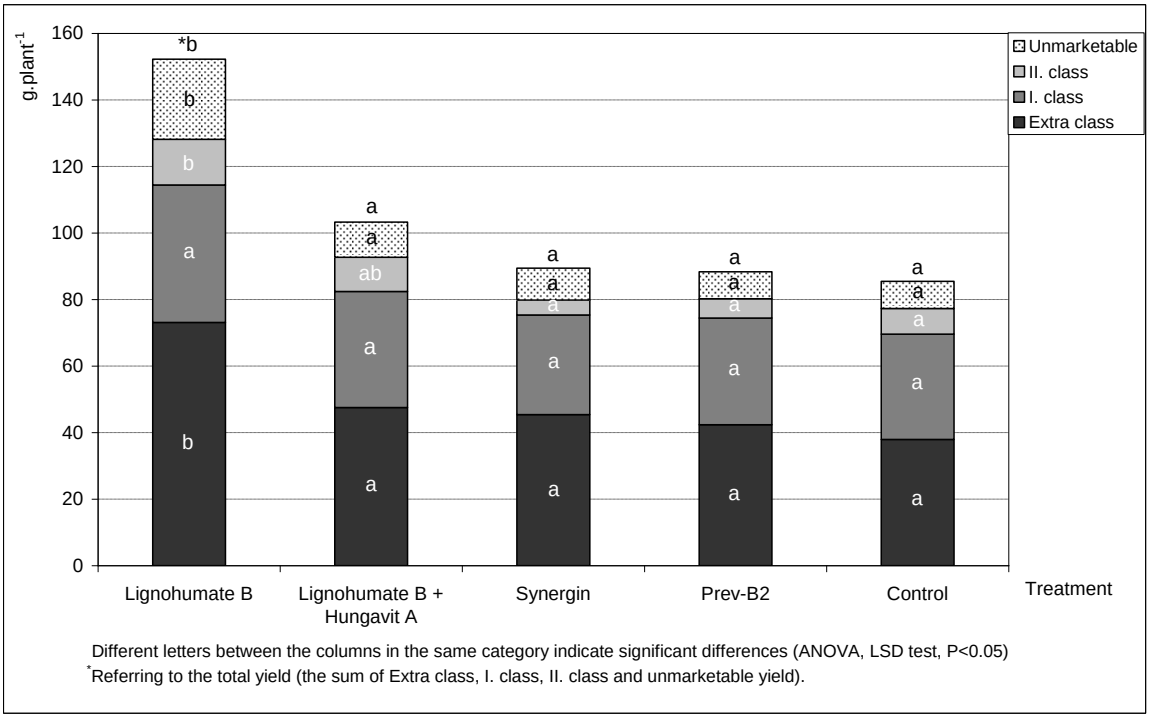


Figure 1: Harvest data for ‘Elkat’

'Symphony' showed lower portion of fruits in Extra class and higher number of fruits especially in class II, compared to 'Elkat'. Treatment by Lignohumate B resulted in the best yields as well, while it significantly increased total yield compared to control (Figure 2). All other treatments increased yields non-significantly. Combined application of Lignohumate B and Hungavit A® produced the highest number of fruits in Extra class.

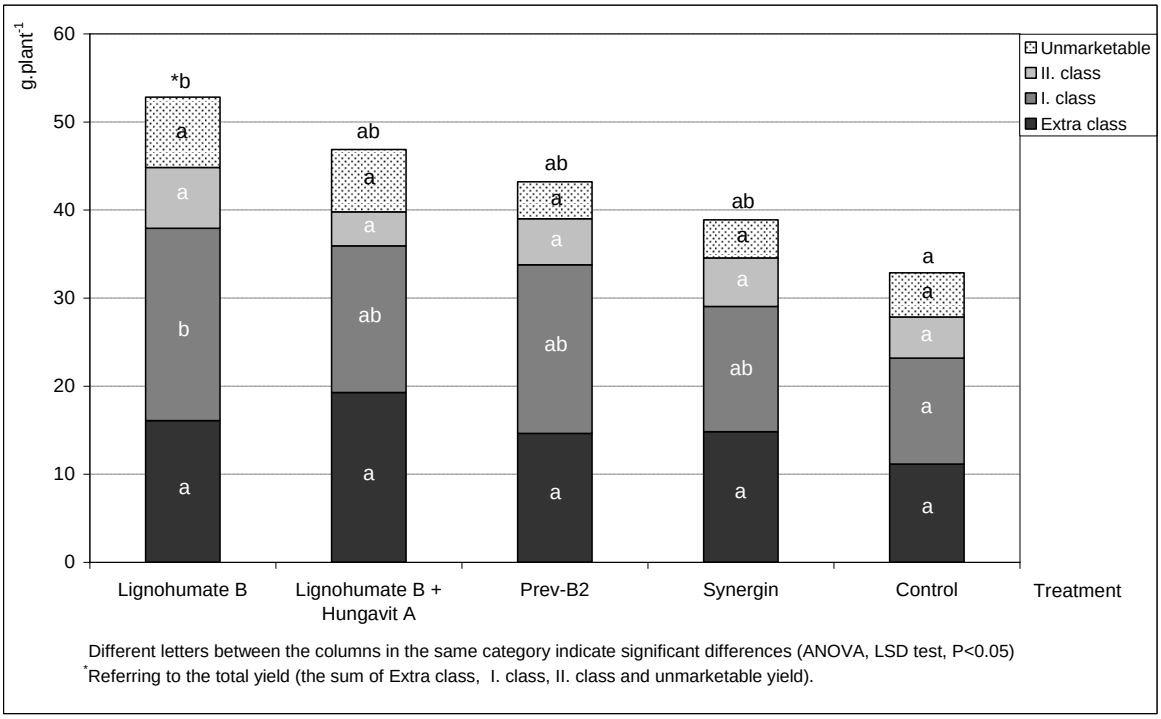


Figure 2: Harvest data for 'Symphony'

Table 1 submits economically important yield parameters like marketable yield and fruit size (expressed as mean fruit weight), respectively. All preparations increased marketable yield and fruit weight in both observed cultivars. Greater effect was found at cv. 'Elkat', when Lignohumate B produced significantly higher (65%) marketable yield compared to control. Spraying tank mix from humate (Lignohumate B) and vermicompost (Hungavit A®) resulted in 20% higher marketable yield. We cannot confirm significantly positive effect of variants on 'Symphony' yields, even though strawberries harvested from plots treated by Lignohumate B provided 60.5% higher marketable yield compared to control (Table 1).

'Elkat' showed to be more productive cultivar. It reached more than double, significantly higher marketable yield compared to 'Symphony'.

Table 1: Marketable yield (g.plant$^{-1}$) and mean fruit weight (g)

| Treatment | 'Elkat' | | 'Symphony' | |
|---|---|---|---|---|
| | Marketable yield (g) | Fruit weight (g) | Marketable yield (g.plant$^{-1}$) | Fruit weight (g) |
| Lignohumate B | 128.1 b | 11.1 a | 44.8 a | 8.4 a |
| Synergin® | 79.8 a | 10.9 a | 34.6 a | 8.4 a |
| Lignohumate B + Hungavit A® | 92.7 ab | 10.6 a | 39.8 a | 8.7 a |
| Prev-B2 | 80.2 a | 10.4 a | 39.0 a | 8.7 a |
| Control | 77.3 a | 9.7 a | 27.9 a | 7.5 a |

Different letters between rows indicate significant differences at P<0.05

Looking to the Table 1 we could see all treatments increased mean fruit weight of both cultivars, but none of them significantly. Regarding cv. 'Elkat', application of Lignohumate B gave the best results, when it provided 14.4% higher mean fruit weight compared to untreated control.

Comparing cultivars, 'Elkat' was characterized by bigger fruits. 'Elkat' has significantly higher fruit weight (26.5%) than 'Symphony'.

Further, we evaluated the effects of used preparations on grey mould, caused by *Botrytis cinerea*, on fruits. The results of observations of both varieties are quite different. While Lignohumate B significantly decreased number of infected fruits at 'Symphony', we observed significantly higher number of diseased fruits in plots of cv. 'Elkat' treated with the humate preparation. The best results gave Prev-B2 mainly at 'Symphony', where it significantly reduced the disease development compared to control (Table 2).

Table 2: Number of fruits (%) infected by *Botrytis cinerea*

| Treatment | 'Elkat' | 'Symphony' |
|---|---|---|
| Lignohumate B | 3.0 b | 1.1 a |
| Synergin® | 2.5 b | 1.3 ab |
| Lignohumate B + Hungavit A® | 1.0 a | 2.1 ab |
| Prev-B2 | 1.0 a | 1.0 a |
| Control | 0.4 a | 4.4 b |

Different letters between rows indicate significant differences at $P<0.05$

## Discussion

There are several studies reported the positive effect of vermicomposts to growth, yield and health status of strawberries. Arancon *et al.* (2003, 2004) proved that vermicompost significantly improved growth characteristics and increased yields compared to inorganic fertilizers. They found 35% higher marketable yield compared to control variant. In our study vermicompost (Hungavit A®) together with humate (Lignohumate B) increased marketable yields in both used cultivars 'Elkat' and 'Symphony' by 20,0% and 42,7%, respectively. American authors explained the positive effects of vermicompost not only because of direct supply of macronutrients but also due to hormones or humates in vermicomposts acting as plant-growth regulators independent of nutrient supply**.** Neri *et al.* (2002) and Singh *et al.* (2010) submitted similar conclusions presenting positive effect of foliar humic acid application on fruit quality, reducing the number of malformed and rotten (grey mould) fruits in organic strawberries. They assume an indirect positive physiological effect of humic acids on the whole plants.

Preparation Prev-B2 has a wide range of uses. In the Czech Republic it registered as boron fertilizers containing orange oil. This substance supports spray adhesion to leaves and directly suppresses pests and pathogens attacks. We found the less number of unmarketable strawberry fruits in plots treated by Prev-B2. It could be explained by positive effect both of orange oil and boron. While the first substance showed to be effective to suppress infection of several pathogens (Jamar *et al.*, 2010), the second one could reduce the risk of malformed fruits (Singh *et al.*, 2007). However in our study Prev-B2 proved to decrease grey mould only in cv. 'Symphony'. Jamar *et al.* (2010) found that Prev-B2 spraying effectively reduced scab incidence on fruits of apple trees. In the work of Singh *et al.* (2007) the reduction of number of malformed strawberries was confirmed only when boron was applied together with calcium.

Zahradníček *et al.* (2006) tested biostimulator Synergin® at several field crops and horticultural plants, e.g. vegetables and peaches. They proved positive effect on plant

growth and productivity. Their outputs correspond to a certain extent with our results, although Synergin® did not show significant effect in our study.

Soltani *et al.* (1999) tested Hungavit® fertilizers on tomato, pepper and potato. In accordance with our trial on strawberries, Hungavit® had non-significant effect on total yield of the vegetables and potato. Although there were 55% fewer diseased tomato and pepper fruit in Hungavit® treatments, this was not statistically significant from the control treatments, similarly to our observation.

In conclusion we have to admit that results of our experiments were strongly negatively influenced by unfavourable meteorological conditions during blossom. Late spring frosts caused pronounced reduction of number of evaluated fruits. The experiment will continue in 2012.

**Acknowledgements**

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# Bird protection in the State Research Station for fruit growing Heuchlingen

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**Abstract**

*Over the past few years up to 62 nesting boxes for birds have been provided in the 35-hectares area of the State Research Station for fruit growing Heuchlingen(State Institute for Viticulture, Oenology and Fruit Technology Weinsberg (LVWO)) in order to support several species of birds, especially cave breeders. Four species were found breeding in the one-hectare organically managed fruit orchard with six nesting boxes: Tree Sparrow* Passer montanus L.*, Great Tit* Parus major L.*, Common Redstart* Phoenicurus phoenicurus L. *and Black Redstart* Phoenicurus ochruros Gmel*.*

*The Tree Sparrow with a number of seven breeding pairs and 30 fully-fledged nestlings was dominating in 2011. In 2005, during a period of mass propagation of winter moth* Operophthera brumata L*. the clutches of Blue Tits* Parus caeruleus L*. and Great Tits were clearly larger than the year before due to the food quantity. In addition to the songbirds, the Kestrel* Falco tinnunculus L*., a falcon that hunts mice, was supported by a nesting box installed at the fruit storage building. It was breeding successfully the third time since 2007.*

*The Common Buzzard* Buteo buteo L*., another raptor has frequently been breeding in a walnut tree. During the bird's breeding period the use of heavy machinery around the nesting area was stopped in order to avoid disturbance of the brood.*

**Keywords**: bird protection, fruit orchard, nesting box, Blue Tit, Tree Sparrow

## Introduction

Several species of birds are very useful for the control of pests in addition to predatory mites and beneficial insects in orchards. Some birds depend nearly exclusively on aphids, caterpillars and mice. Thus, birds also preserve and – moreover - increase biodiversity and contribute to stabilize the agrarian ecosystem of a fruit orchard – just being present is a guarantee for the aforementioned. Some species of birds do not build nests themselves, but they are dependent on natural brood-caves (e.g. woodpecker cavities) in older high stem trees and some of them take over old nests from other birds. In the modern and intensively managed fruit orchards with small-crowned trees, characterised by slower growth, natural brood-caves do not exist. It is therefore necessary to provide artificial nesting boxes made of natural wood concrete (cement-bonded wood) for example, attached high up in a canopy or installed on posts or premises (farm buildings). By these means (or methods) various species of cave breeding birds can be located effectively in fruit orchards.

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## Material and Methods

Some years ago more than 60 nesting boxes have been installed in the orchards as well as in some buildings of the State Research Station for fruit growing Heuchlingen, six of them in the one-hectare organically managed fruit orchard. With diameters of 45, 32 or 26-27 millimetres and vertically oval in shape according to the requirements of each bird species, the entrance holes of the nesting boxes should be adjusted to southeast or south to catch the warm rays of the morning sun (Henze, 1991). They should not be adjusted westwards as the holes should not be exposed to direct rainfall. Furthermore, it is advisable not to adjust the entrance hole towards the tram line to avoid the spray dust while carrying out sprayings for plant protection measures. For a more effective monitoring and easier handling and cleaning, nesting boxes were hung at eye level on trees or posts. They were always thoroughly cleaned with a brush and any old nest were removed prior of the next breeding season. During the breeding periods the nesting boxes were monitored three times from May to July in regular intervals. Thereby the data and observations (i.e. number of eggs, nestlings, species) were noted.

## Results

1. Songbirds

The fouryears monitoring results (2008 to 2011) of the six nesting boxes of the organically managed fruit orchard of the Research Station for fruit growing in Heuchlingen are listed in table 1. Four species of breeding birds, namely Tree Sparrow, Great Tit, Common Redstart and Black Redstart were found more often. Nowadays, in 2011, the Tree Sparrow dominates with a number of seven successful broods and 30 fledglings. In 2010, two pairs of birds in two nesting boxes had two successful broods immediately one after the other. In former times the Tree Sparrow was regarded as a harmful species and was under severe pressure from hunting because it also feeds the buds and seeds of plants. However, during a long breeding season the sparrows feed lots of protein-rich food such as caterpillars and aphids from April to August. Over the last few years the population of the Tree Sparrows has been strongly decreasing and, as a result, it was set on the Red List of Threatened Species (Bauer, 2007).

Table 1: Results of the nesting box monitoring of six nesting boxes in the organically managed fruit orchard of the Research Station for fruit growing Heuchlingen (2008-2011)

| Species | N (2008) | | N (2009) | | N (2010) | | N (2011) | |
|---|---|---|---|---|---|---|---|---|
| | broods | nest-lings | broods | nest-lings | broods | nest-lings | broods | nest-lings |
| Tree Sparrow | 3 | 15 | 2 | 10 | 6 | 16 | 7 | 30 |
| Great Tit | | | 1 | 5 | 1 | 5 | | |
| Common Redstart | 1 | 3 | 2 | 8 | | | | |
| Black Redstart | 1 | 5 | 1 | 3 | | | | |

In 2011, the overall result of 328 fledglings of five different species of birds (Tree Sparrow, Great Tit, Blue Tit, Common Redstart and Common Starling *Sturnus vulgaris* L.) was proceeded by 61 successful broods taking place in 62 nesting boxes on the 35-hectares area Research Station for fruit growing Heuchlingen.

In 2005, the mass propagation of the winter moth reached its peak in the area. In June, untreated sweet cherry trees suffered from a total defoliation as a result of the caterpillars' feeding activity. The caterpillars of the winter moth did not even spurn walnut leaves.

Table 2: Results of the nesting box controls in the Research Station for fruit growing Heuchlingen (2004 and 2005)

| Species | N (2004) | | | N (2005) | | |
|---|---|---|---|---|---|---|
| | broods | nestlings | nestl./brood | broods | nestlings | nestl./brood |
| Tree Sparrow | 10 | 41 | 4.1 | 15 | 55 | 3.7 |
| Great Tit | 13 | 80 | 6.2 | 7 | 58 | **8.3** |
| Blue Tit | 3 | 20 | 6.7 | 3 | 29 | **9.7** |
| Common Redstart | 4 | 20 | 5.0 | 2 | 9 | 4.5 |

Three breeding pairs of the Blue Tit show a significant increase of nest-size from 2004 to 2005 (table 2). The average number of 6.7 fledglings in 2005 increased to 9.7 individuals per brood in 2005. According to personal observations, the main food source were caterpillars of the winter moth, that had been fed to the juveniles in the nesting boxes in intervals of 30 sec.. Low competitive species like the Small Blue Tit needs nesting boxes with a single (selective) entrance hole of 26 - 27 millimetres. This species was observed regularly in the same area with three to four broods/year.
In 2005, the clutches of the Great Tits were significant larger than 2004, probably due to the quantity of food. Taking the number of broods into consideration we can clearly see the competitive strength of the Tree Sparrow. Both of the two species breed in nesting boxes with an entrance hole of 32 millimetres in diameter. As Tree Sparrows start earlier into the breeding season, their first nest was not synchronized with the winter mothspopulation peak – this was reflected by the clutch size found for this species. A similar situation occurred to the Common Redstart. As a migratory bird it starts breeding not earlier than May, appr. at a period of time, when the winter moth caterpillars start to pupate.

2. Raptors

Likewise the cave breeders among the songbirds, falcons and owls do not build nests by themselves. They breed in old nests of raptors and crows as well as in wall niches of buildings. The Common Kestrel is known as a raptor keen on catching mice. By increasing nesting opportunities for kestrels it is proposed to provide special nesting boxes: large-scaled and with a semi-open front side. In 2007, a nesting box for kestrels was installed at the east side of the fruit storage building Station in Heuchlingen, mounted at a height of appr. five metres. It was well accepted by the birds and the kestrels have successfully bred three times.
The Common Buzzard also feeds on mice. It is a shy raptor and it builds large and stable nests consisting of twigs and branches in canopies of higher trees. Almost every year during the observations, it bred in a walnut tree at the area of the Research Station. To ensure an undisturbed breeding and rearing the nestlings, the use of disturbing machinery around the nesting site was not allowed.

## Discussion

By means of nesting boxes various songbirds such as Tree Sparrows, Great Tits, Blue Tits and Common Redstarts could be settled in orchards, intensely used for organic and integrated fruit growing of the Research Station for fruit growing Heuchlingen. The birds support fruit farmers in regulating pests. The Kestrel, a successful mouser breeds in a special nesting box at the fruit storage building, the Common Buzzard in a walnut tree. In 2004 and 2005, observations on Great Tits and Blue Tits showed an increase of the offspring due to a large food supply of caterpillars of the winter moth. Currently farmland birds are declining severely (Sudfeldt *et al*., 2009). Furthermore, in addition to supporting cave-dwelling species of birds, it is recommended to improve nesting and food conditions for birds building nests by themselves and for populations breeding in open spaces as well, such as Yellowhammer *Emberiza citrinella* L., European Goldfinch *Carduelis carduelis* L., European Serin *Serinus serinus* L., Red-backed Shrike *Lanius collurio* L. and others. This can be achieved by various means, for example by planting fruit-bearing shrubs and hedges (but no host plants for fire blight), by sowing wildflowers, by stone heaps and heaps of twigs and branches. Quite a number of beneficial insects are able to benefit from these measures.

## Acknowledgements

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# New species and new methods of application – a new chance for *Trichogramma* in Codling Moth Control?

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**Abstract**

*Diversification of methods of codling moth control is urgently needed in organic fruit growing. Since the application of sulphur products in late summer is not necessary on scab-resistant varieties, augmentative releases of the sulphur-sensitive beneficial* Trichogramma *species are possible in commercial growing.* Trichogramma evanescens*, occurring naturally in the orchards, showed promising capacities in codling moth control, as revealed in field trials with infestation simulated using "bait apples" with natural codling moth eggs deposited by females obtained from a laboratory rearing. Furthermore, an application method of spraying commercially produced parasitized eggs was developed using large nozzles, low pressure and a hydrocolloid based on 2 % Xanthan and 0,01 % Tween. Field tests with natural infestation are ongoing.*

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**Keywords:** *Trichogramma evanescens*, *Cydia pomonella*, Codling moth, application methods, apple

## Introduction

The utilization of *Trichogramma* species for codling moth control is known since more than twenty years (HASSAN et al., 1988). Up to now, the species *Trichogramma dendrolimi* and *T. cacoeciae* were mixed to assure an effective short and long term control. The method has not been implemented in practical use on larger areas because of high costs and erratic efficacy. Furthermore, *Trichogramma* species are sensitive to sulphur application used in scab control, which is the dominating fungicide in organic fruit growing. Problems arising from *Cp*GV- and insecticide resistance of codling moth in integrated fruit growing, the interest in using *Trichogramma*-egg parasitoids increased. From 2007 til 2011, a project coordinated by AMW Nuetzlinge in cooperation with Marktgemeinschaft Bodenseeobst, the Institute for Biological Plant Protection of the JKI and the University of Hohenheim was established to improve the application techniques for *Trichogramma*-releases. Different strains and species of *Trichogramma* have been tested on their efficacy in codling moth control. Furthermore, a new application system by spraying the beneficials in a liquid formulation was developed, based on results from Moser (1980).

## Material and Methods

In 2007 and 2008, first field trials to ***compare the efficacy of different strains and species*** of *Trichogramma* have been conducted. In a randomized block design 4 trees per plot with 4 replications on four varieties (*viz.* "Topaz", "Melrose", "Idared", "Berlepsch")

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were treated. Between the plots, 7 trees and between the tree rows, one “untreated” tree row were left as a barrier. On each treated tree, one cardboard with 500 *Trichogramma* pupae obtained from AMW (shortly before adult emergence) and 6 “bait apples” were fixed (max. 24 apples per replicate / 96 apples per variant). To obtain “bait apples”, the fruits were picked from the trees and exposed to *Cydia pomonella* gravid females for oviposition overnight. Until the next morning, usually 3 to 10 eggs were laid per fruit. Subsequently after oviposition, the apples were fixed on the trees with florist wire and marked with a clothespin.

At the “red ring” developmental stage of the eggs, the apples were removed and incubated in the laboratory until larval hatch of *C. pomonella*. When parasitization was observed (blackened eggs ) the respective part of the fruit skin was removed carefully, put into a glass tube and closed with cotton until the *Trichogramma* emerged. The *Trichogramma* specimens have been determined by electrophoresis of PCR-amplified fragments of the ribosomal DNA internal transcribed spacer 2 (ITS-2) (Silva et al. 1999, Li 2007; and own protocols). Baiting was done three times after *Trichogramma* release replicates in 5 days intervals not only the number of apples found by the different species but also to observe the efficiency as mediated by time.

To observe the distribution of the beneficials, cards with ca. 1,500 pupae of *Trichogramma* (shortly before emergence) were fixed at every 13$^{th}$ tree in four rows. In each of the two middle rows, in 13 adjacent trees two “bait apples” per tree were placed (see Fig. 2).

To develop a spray-application method**,** it was necessary to obtain a steady distribution of *Trichogramma* pupae in a liquid gel formulation (Zimmermann, 2010a, 2010b). On pure water as carrier, pupae were floating. Thus, a solution with the optimum viscosity had to be found, which would also not affect the emergence of *Trichogramma*. Furthermore, spraying pressure had to be optimized because a too high pressure may also reduce the emergence rate of the *Trichogramma* pupae.

In cooperation with the Institute fof Agricultural Engineering of the University of Hohenheim, an optimal solution for the carrier liquid and spray pressure should be found. Gel solutions with *Trichogramma* pupae were sprayed at different pressures and the sprayed pupae were collected using a mesh covered by a card web. The emergence rate was determined and compared with the emergence rate of “dry pupae” and of pupae that had just been dropped in pure water were taken as control.

## Results

After the determination of the baited *Trichogramma* species, it became obvious, that one species found, has not yet been released: *Trichogramma evanescens* occurred naturally. Because of the high natural density and the regularly observed abundance in vineyards, this species was included into the trials.

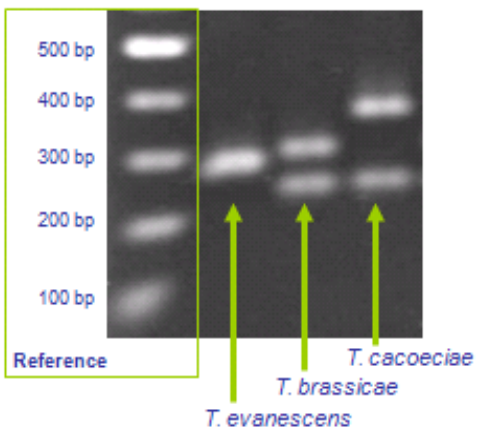


Figure 1: Gel electrophoresis of ITS-2 PCR products

In laboratory tests, this species showed best results in parasitization of codling moth eggs of all the 26 species and strains of *Trichogramma* tested.

In 2008, *T. evanescens* showed best results of all tested strains and species in the field. During the first exposure of “bait apples”, this species and one strain of *T. cacoeciae* showed a significantly better finding rate (as indicated by blackened =

parasitized eggs). Also in the second and third repeat of “bait apples”, *T. evanescens* showed a higher efficacy than the other species (Table 1). These results were confirmed by other trials in 2009. Even in the control, some eggs were parasitized (species not determined by PCR analysis).

Table 1: Percentage of “bait apples” found by different species/strains of *Trichogramma* (as indicated by any blackened eggs) during three periods starting 2 days after releasing *Trichogramma* pupae on cardboards. (Same indices in a column indicate no significant differences; oneway-ANOVA, followed by TUKEY-KRAMER HSD-test)

| *Trichogramma* species/strain | 20. - 25.7.2008 | 25. – 30.7.2008 | 30.7. - 6.8.2008 |
|---|---|---|---|
| *T. evanescens* DE 07 W | 100,0 a | 82,3 a | 41,7 a |
| *T. brassicae* | 46,9 b | 47,2 a | 25,4 a |
| *T. cacoeciae DE 90 O* | 46,6 b | 50,0 a | 33,3 a |
| *T. cacoeciae DE 07 CAC W* | 93,8 a | 40,6 a | 18,8 a |

Due to these observations, in the end of 2008 and in 2009 field trials were designed to observe the distribution of *T. evanescens* in the rows, if released with cardboard cards.

It could be observed, that *Trichogramma* dispersed rather good within the tree row, but also in the parallel tree row directly opposite to the trees with cards, parasitized eggs were found (figure 2).

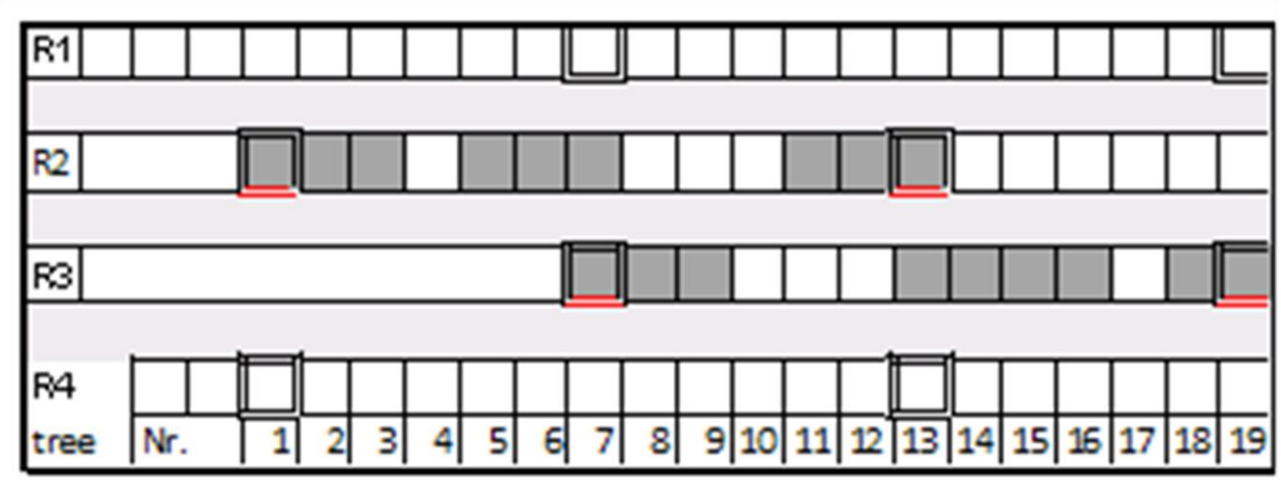


Figure 2: Trial to observe the distribution of *Trichogramma evanescens* in an orchard, variety Jonagold (cards with ca. 1,800 pupae per 13$^{th}$ tree): The double prime frame marks the trees with a *Trichogramma* card, dark colour marks trees where the “bait apples” were found.

**Application technique**

The comparison of three different hydrocolloids showed no significant difference. Because guar bean flour (E 412, from *Cyamopsis tetragonolobus*) may exert allergic reactions, Xanthan, (E 415, bacterial fermentation from *Xanthomonas campestris*) was preferred. The addition of Tween to Xanthan hydrocolloid resulted in smaller droplets and a better distribution of the spray plume. Since large droplets dry off very slowly and therefore might compromise the emergence rate, the addition of Tween seemed advisable.

In field trials with this hydrocolloid, the distribution of the droplets in the canopy was assessed. Using one TEEJET 11015 flat fan nozzle with a large opening, mounted in an approx. 45° upward facing angle at each side of a standard axial fan orchard sprayer,

operated at 3 bar spray liquid pressure, an air flow rate of approx. 25.000 $m^3$ $h^{-1}$ at 400 $min^{-1}$ PTO and a forward speed of 5 km $h^{-1}$, most of the hydrocolloid droplets have been deposited at the lower leaf surface. This is important to reduce losses from rainfall and predators since in trials not presented here, predation of the pupae was much lower when deposited at the lower leaf surface. The position of the nozzle depends on the canopy characteristics. Usually it should be mounted at the 4th or 5th nozzle position from the bottom.

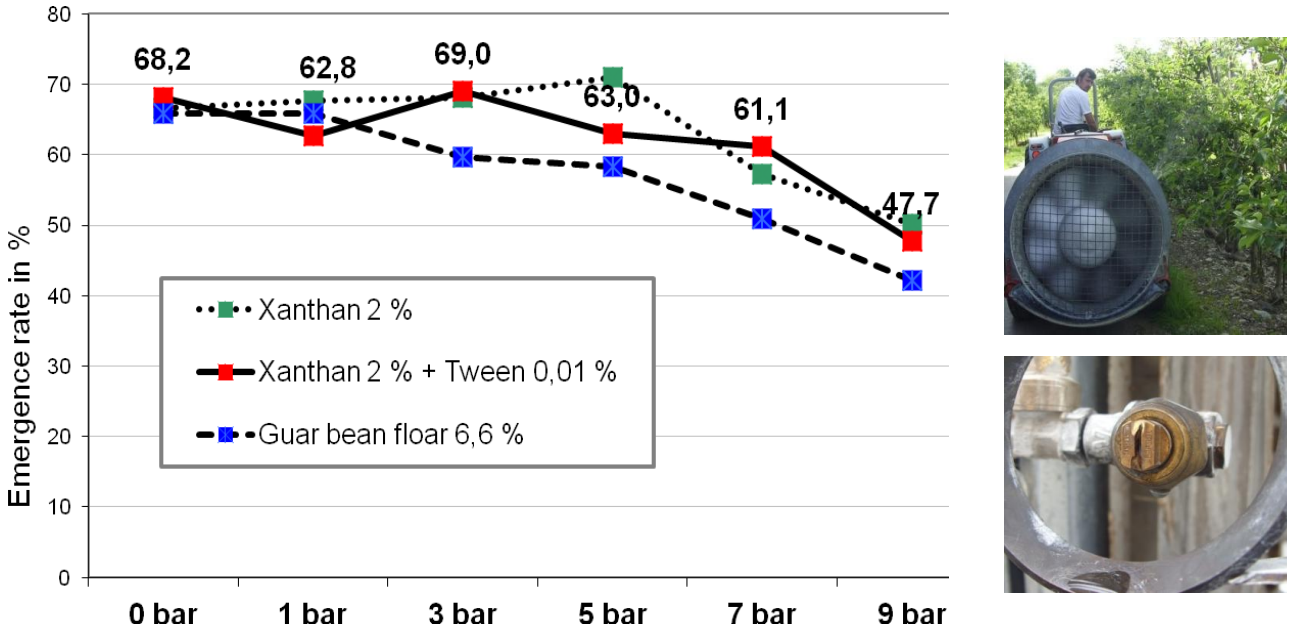


Figure 3: Emergence rate of *Trichogramma* (in %) with different hydrocolloids and pressure levels (in bar). The emergence rate out of dry eggs (control 1) was 88,2 %, out of eggs dipped in water (control 2) was 79,3 %. At the lower right side a picture of the nozzle (TEEJET 11015) used for the experiments. At the upper right side a picture of the application with orchard sprayer.

## Discussion

*Trichogramma evanescens* showed a promising potential for codling moth control in the field. Although this species was not mass released in orchards before these studies, it occurs frequently in apple orchards and vineyards. It was more effective than *T. cacoeciae*, although they reproduce sexually (only 50 % females released, compared to 100% in parthenogenetic species) and exhibit a shorter lifespan. Nevertheless, in the field tests, *T. evanescens* showed a better parasitization of the “bait apples” exposed at a certain distance from the point of release. In all trials, the weather conditions were not favourable with periods of rain. The better efficacy of *T. evanescens* in our tests might also be due to a better tolerance of this species to unfavourable weather conditions.

Generally, the persistence of *Trichogramma* was not longer than 14 days (stage of released pupae was shortly before adult emergence to reduce loss by predation).

Due to the potential of distribution observed for *T. evanescens*, which seems to be much larger than the distribution of other species (Wetzel et al., 1995; Sakr, 2002), *Trichogramma* cards seem an interesting option again. In this case, a mixture of different developmental stages is advisable to expand the persistence and compensate possible losses by adverse climatic conditions. For spraying application, the first trials have started with material with different development stages, a definitive conclusion, however, is actually not possible.

Generally, it seems that *Trichogramma* cards are more appropriate when the canopy is small and rainfall is to be expected. The spraying application seems best when the canopy is large, perhaps due to a better distribution of the parasitoids.. It is also more suitable for large areas and growers that have few attitude to manual work.

In organic fruit growing, especially on scab resistant varieties other products than sulphur can be used for disease control at the end of summer. Thus, actually, in the framework of the BOELN-project 2809OE098 the application of *T. evanescens* in *Trichogramma* cards and with spray application is tested for the control of fruit damage in the second generation of codling moth. Since the infestation level of codling moth in most orchards in 2011 was very low, definitive results are not yet available.

**Acknowledgements**

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# Natural regulation of codling moth populations in southern France

G. Libourel[1], P. Franck[2]

**Abstract**

*Biocontrol of the codling moth,* Cydia pomonella*, in the apple orchards from Provence (France) is so far considered as insignificant, due to (i) abundant codling moth populations because of the high orchard density, (ii) favourable climate allowing the completion of three generations each year, and (iii) very low abundance and efficiency of its natural enemies.*

*From 2009 to 2011, we measured biocontrol potential in low input apple organic orchards using codling moth 'sentinel' eggs. Predation and parasitism were estimated and compared based on eggs laid on paper sheets or directly on leaves. In this last case two fertilized females were kept under sleeve nets for three days previous egg exposition. Eggs were exposed during 6 days in 2010 and 3 days in 2011.*

*Predation was 25-30% higher on eggs laid on paper than on apple leaves, whereas parasitism was around 15% lower.*

*Mean predation and parasitism rates measured on the eggs laid on leaves were respectively 42% and 21% in 2010, and 48% and 2% in 2011.*

*Differences within and between years were discussed according to climatic conditions, orchard cultivation techniques, and protocol specificities.*

*However, these results already suggest that the conservation of natural enemies in orchards is a possible strategy to improve the control of codling moth populations.*

**Key words**: *Cydia pomonella*, codling moth, trichogramma, biological control, parasitism, eggs

## Introduction

Before mating disruption and granulosis virus availability, apple cultivation in organic farming in southern France was hardly thinkable, and very rare. Those techniques were a real advance for organic farming in the 80's. But with temperatures increasing, the biology of the pest changed: a new generation now occurs, adults became resistant to many active matters, including granulosis virus, leading to serious economical threats (Sauphanor, 2010). Therefore, the only remaining technique is the insect-proof net, giving very good results, although codling moth may be able to mate under nets.

Organic farming is pushing towards intensive use of ecosystemic services, therefore we investigated the possibility of using native trichograms already present in the agrosystem as parasites to control codling moth.

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## Material and Methods

### A/ Orchard

The 5000 m² apple orchard was planted in 2001, with 30 different cultivars grafted on EM7. Most of these cultivars were chosen because of their rusticity, especially to scab. The inputs have been minimized on this orchard from the beginning: full weed covering, very low fertilization, reduced watering. Plant protection is limited to use of granulosis virus (CpGV) and *Bt.* in 2010, the trees devoted to the trial have not been sprayed, whereas the whole orchard was not treated in 2011.

1) Year 2010

Four trees were selected and marked, according to their distance to a cabbage plot (*Brassica oleracea*), in order to see if these host species (attractive for a specific trichogram) could increase parasitism rate.

13 exhibitions have been repeated throughout the season. Virgin codling moth couples were placed under nets on each tree to mate and to egg deposit by females. Predation and parasitism were both afterwhile observed and measured in absence of net.

2) Year 2011

In order to see the possible role of weeds, the whole orchard has been divided in 2 identical blocks, each one with 2 different weed managements (regularly mowed/no mowing).

Five trees per block were marked, and 12 exhibitions have been done with codling moth placed under nets.

### B/ Codling moth eggs

Virgin adults were reared in INRA laboratories, and placed under nets on identified apple tree branches. After 3 or 6 days, eggs were counted and marked on leaves. After some more days, remaining eggs are quantified and brought back in lab, in order to estimate the destruction rates, and to identify emerging species.

In 2010, 2 couples were placed under each net, and eggs were exposed during 6 full days.

In 2011, only mated females were placed under nets, and eggs were exposed for 3 days only, in order to avoid hatching in orchard.

### C/ Observations on eggs

Leaves with eggs previously exposed were brought to lab, to check if eggs have not been sucked by predators such as mirids. Parasitized eggs start to turn black after 5 to 7 days.

Those eggs are classified as:

- predated
- parasitized by trichograms,
- safe,

and rates are calculated.

## Results

1) year 2010

The distance with cabbage plot were not found to have any effect on parasitism level. This could be explained by a high variability among different exhibition dates. Indeed, the predation rate varies from 0 to 89% with an average of 42%.

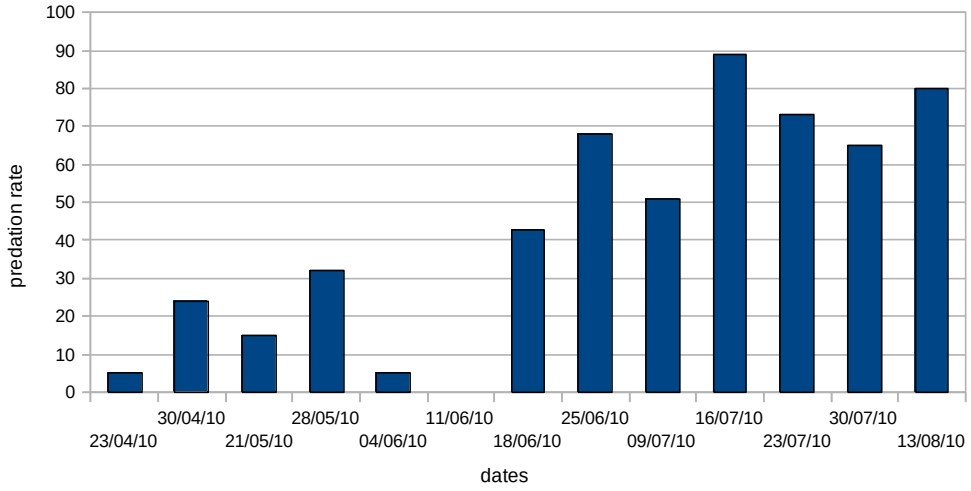


Figure 1: Predation rates on each of 13 exhibitions during 2010 season.

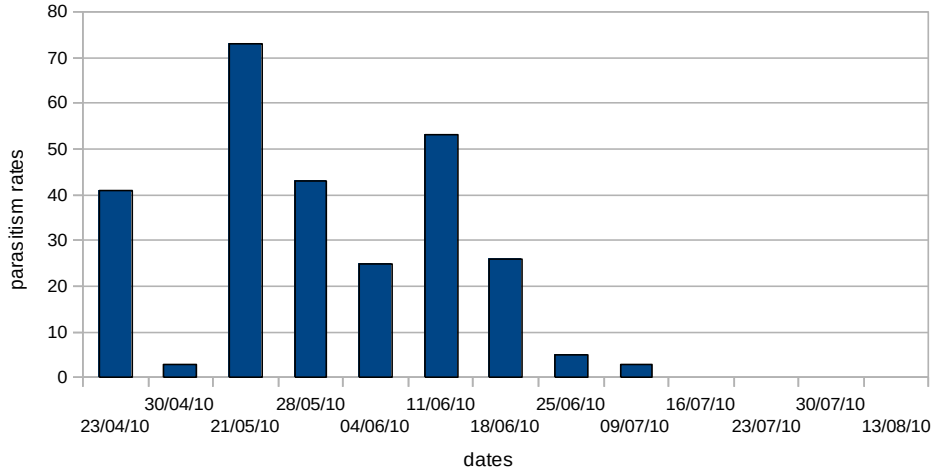


Figure 2: Parasitism rates due to trichograms, on each of 13 exhibitions during 2010 season

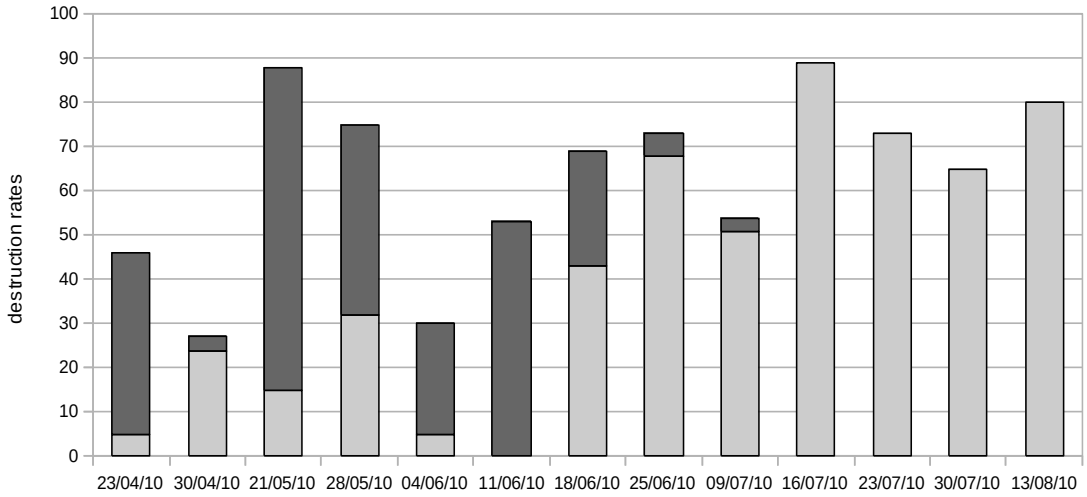


Figure 3: Total destruction rates (parasitism (in dark grey) + predation) on each of 13 exhibitions during 2010 season

2) - Year 2011

No statistical differences were found between both weed management strategies.

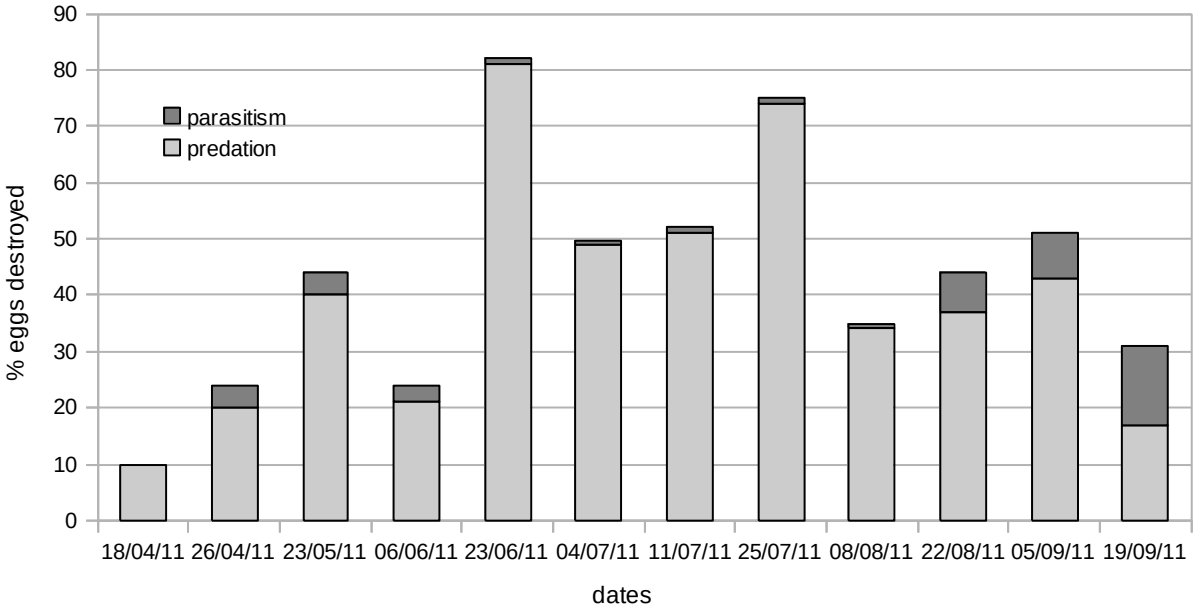


Figure 4: total destruction rates (parasitism (in dark grey) + predation) on each of 13 exhibitions during 2011 season

**Conclusions and perspectives**

1) On predation

Predation rates appear rather stable from on year to another. However, several parameters shall be considered as important:

- more nets were used in 2011, thus reducing the variability among dates
- eggs were exposed during 3 days in 2011, compared to 6 in 2010 : efficacy needs to be correlated to the number of exhibition days,
- although we assessed the eggs destruction later in 2011 than in 2010, predation was not found to be higher.

Higher predation in 2011, compared to parasitism, could be explained by several factors : favourable climatic conditions in 2011, favourable impact of the absence of pesticides on generalist predators, ...

Predation is very high in June and July, with rates reaching 80%. It may be permitted by warmer days and nights, as earwigs (favoured by a total weed covering) have a night activity, whereas aphids simultaneously migrate to host plants (Deï Tos, 2010).

2) On parasitism

Parasitism rates in 2010 have been very interesting (up to 70% !), and complementary to predation throughout the season. Parasitism was very active from April to June, and completely disappeared in July.

In 2011, rates were much lower, and also reduced in summertime. The increasing parasitism in late August and September needs to be confirmed.

This decrease may be explained by a shorter exhibition period, by the weather conditions, by many environmental conditions. This is why research shall be continued to better assess the regularity of beneficial efficacy.

Trichograms behaviour is not easy to follow, since they need very specific egg laying conditions, and their populations are very low.

Mean egg destruction rates reached 63% in 2010, and 51% in 2011, which is considered to be very encouraging, taking into account that egg parasitism due to *Ascogaster quadridentata* was not assessed, although it is known to be active in the region and in this orchard (parasitizing 6 to 8% of codling moth larvae on this orchard).

All additional parasites and predators (birds, bats included) of codling moth must therefore be added to these results.

With these specific conditions on this orchard (no sprays, high pressure), damages at harvest time varied from 6 to 32% according to cultivars and the apple load per tree. It remains too high for a fruit grower, but it has to be compared with organic orchards protected with CpGV, sometimes leading to more than 80% damages.

These promising results now need to be integrated in a reliable and sustainable orchard cultivation strategy, in order to optimize conservation biocontrol without endangering the economical result for the farmer.

**Special thanks** are addressed to Sandrine Maugin (INRA) for codling moth rearing and to François Warlop (GRAB) for translation of this paper.

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# Side effect of preparations used in organic farming on *Forficula auricularia* and *Chrysoperla carnea*

V. Falta[1], K. Holy[1], P. Nadenikova[2], J. Stara[1]

**Abstract**

*The side effects of selected botanical insecticides (*Quassia amara*, Rock Effect, Prev-B), spinosad (SpinTor) and other preparations used in organic farming (Alginure, Aqua-Vitrin, Myco-Sin, Vitisan) on* Forficula auricularia *and* Chrysoperla carnea *were tested in the laboratory bioassay. The most of preparations allowed or intended for organic growing were harmless to the tested species with the exception of spinosad which caused moderate mortality and a dramatic change in behaviour of earwigs. For the comparison, several synthetic pesticides or their combinations were tested too. Negative effects of bifenthrin, chlorpyriphos-m, thiacloprid and dodine were confirmed in the study.*

**Keywords:** organic fruit growing, pesticides, natural enemies, side effects

## Introduction

Effective pest control in organic fruit growing is based on the presence of a sufficient biodiversity of natural enemies in orchard. It has been known from IPM or conventional regimes that use of not selective pesticides (pyrethroids, organophosphates, neonicotinoids) can cause the outbreaks of pests such as aphids, spider mites, psyllids etc. However, similar situations can be expected even in organic farming if not suitable practices used. The examples of pyrethrins (Jansen *et al*. 2010) or of spinosad (Cisneros *et al*., 2002, Arthurs *et al*., 2007) shows that side effects of products must be also here taken into consideration to prevent failures of pest control.

## Material and Methods

*Forficula auricularia* individuals were collected in experimental apple orchard in Prague in July 2011. The corrugated paper belts placed on the trees were used to this purpose. The adults and nymphs were separated and in the groups of 10 individuals they were placed into cylindrical plastic containers with perforated lids. The piece of apple as a food for earwigs was placed on the filtration paper in the bottom of each container. Close before the treatment the earwigs were exposed to high $CO_2$ concentration to lower their activity to make the application possible. Earwigs were tested in two different ways: 1μl of the pesticide dissolved in acetone was applied by micropipette on the abdomen of insects as a topical treatment (i), cca 0.25 ml of a water solution of the pesticide was applied with hand sprayer on earwigs, filtration paper and piece of apple placed on the bottom of containers (ii). The mortality and the change in the behaviour of insects were evaluated 24, 48 and 96 hours after application. I addition, $LD_{50}$ and $LD_{90}$ of spinosad (topical application) was calculated (0.0125, 0.25, 0.5 and 1.25 of the base concentration) using probit analysis.

*Chrysoperla carnea* nymphs were obtained from commercial resources (Koppert Biological Sytems, Netherlands). 4x10 nymphs for each variant were separately placed into cells in plastic boxes (IWAKI & CO., Ltd., Tokyo, Japan).The filtration paper treated with water solution of pesticides was placed under the lid of boxes.

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The boxes were placed into environmental chamber at temperature 20°C and they were turned over to ensure the contact of nymphs with treated filtration paper. Evaluations were performed 1, 2, 5, 12, and 24 hours after treatment. On the base of mortality/change of behaviour the preparations were classified as follows (www.koppert.com): < 25 % = harmless (1), 25-50% = slightly harmful (2), 51-75% = moderately harmful (3), > 75% very harmful (4).

Table 1: Products used in the trial

| Product name | Active ingredients | Concentration [%] | Tested species* |
|---|---|---|---|
| Alginure | extract from seaweed | 1.00 | E, Ch |
| Calypso 480 SC | thiacloprid | 0.025 | E |
| Insegar 25 WG | fenoxycarb | 0.06 | E |
| Mospilan 25 SP | acetamiprid | 0.025 | E |
| MycoSin | Aluminium sulphate, deactivated yeast cell components, plant extract from horsetail (*Equisetum arvense*) | 1.00 | E, Ch |
| PREV-B | orange oil | 0.30 | Ch |
| *Quassia amara* chips | quassin, neoquassin | 6.00 | E |
| Reldan 40 EC | chlorpyrifos-methyl | 0.15 | E |
| Rock Effect | *Pongamia pinnata* oil | 1.00 | E, Ch |
| SpinTor | spinosad | 0.06** | E |
| Syllit 400 SC | dodine | 0.17 | E |
| Talstar | bifenthrin | 0.04 | Ch |
| Thiram Granuflo | thiram | 0.74 | E |
| Vitisan | potassium bicarbonate | 0.50 | E, Ch |
| Calypso 480 SC+Syllit 400 SC | thiacloprid+dodine | 0.025+0.17 | E |
| Calypso 480 SC+Syllit 400 SC | thiacloprid+dodine | 0.0125+0.085 | E |
| Thiram G.+Mospilan 20 SP | thiram+acetamiprid | 0.74+0.025 | E |

*) E=earwigs, C=chrysops **) basic spinosad concentration

## Results

The mortality of tested products on *earwigs* is shown in table 2. Reldan 40 EC caused mortality of nymphs 85% and 100% after 24 and 48 hours, respectively. Mortality of adults was 10% and 85% after 24 and 96 hours, respectively. Surviving insects showed dramatically changed behaviour (reduced mobility, convulsions) in comparison with control. Although the earwigs recovered in the end of the trial they seemed to die during the first hours. This effect is a very serious handicap for the survival of animals in nature conditions and it must be considered when preparations classified. Insecticides Calypso 480 SC and Syllit 400 SC did not cause mortality. However, the both pesticides significantly changed behaviour of earwigs. The both insecticides were classified as very harmful. Although the synergic effect of thiacloprid and dodine was not confirmed their combination influenced behaviour of earwigs even in half concentration variant. The similar situation was observed in the case of SpinTor which showed acute toxic effect on earwig behaviour (reduced mobility, convulsions) in 61.7% of individuals after 24 hours exposition. SpinTor was classified as moderately harmful on adults from the same reasons as Reldan

40 EC, Syllit 400 SC and Calypso 480 SC. $LD_{50}$ and $LD_{90}$ of spinosad was 0.349 and 4.494, respectively (table 3). Other tested products were classified as harmless to earwigs. The mortality of *chrysops* is presented in table 4. All tested products with the exception of inseciticide Talstar (100% mortality) were classified as harmless.

Table 2: Mortality of *Forficula auricularia* after treatments and classification of products.

| Treatment | Stage | Mortality [%] | | | | | Toxicity (1-4) |
|---|---|---|---|---|---|---|---|
| | | 24 hours | | 48 hours | | 96 hours | |
| | | topical | spray | topical | spray | topical | |
| Control | adults | 0 | 0 | 1.1 | 0 | 0 | - |
| | nymphs | 0 | 0 | 0 | 0 | - | - |
| Alginure | adults | 0 | 0 | 2.8 | 0 | - | 1 |
| Calypso 480 SC | adults | 0 | - | - | - | 0 | 4* |
| Insegar 25WP | adults | 0 | 0 | - | - | 0 | 1 |
| Mospilan 20 SP | adults | 0 | - | 0 | - | - | 1 |
| Myco.Sin | adults | 0 | 0 | 0 | 0 | - | 1 |
| PREV-B | adults | 0 | - | 0 | - | - | 1 |
| *Quassia amara* | adults | - | 0 | - | 0 | - | 1 |
| Reldan 40 EC | adults | 10 | - | - | - | 85 | 4 |
| | nymphs | - | 85 | - | 100 | - | 4 |
| Rock Effect | adults | 0 | 0 | - | 0 | 0 | 1 |
| SpinTor | adults | 61.7* | - | 15.4 | - | - | 3* |
| Syllit 400 SC | adults | 0 | 0 | - | 0 | 0 | 4* |
| Thiram Granuflo | adults | 0 | - | 0 | - | - | 1 |
| | nymphs | 3.3 | | 6.7 | - | - | 1 |
| VitiSan | adults | 2.5 | 0 | 2.5 | 0 | - | 1 |
| Calypso 480 SC +Syllit 400 SC | adults | 2.5 | 3.3 | 4.8 | 3.3 | - | 4* |
| Calypso 480 SC +Syllit 400 SC | adults | - | 3.3 | - | 3.3 | - | 4* |
| Thiram Granuflo. +Mospilan 20 SP | adults | 0 | - | 3.3 | - | - | 1 |

*) behaviour of insects dramatically changed

Table 3: Calculation of $LD_{50}$ and $LD_{90}$ in earwigs

| Probability | Log(factor) | Lower bound 95% | Upper bound 95% |
|---|---|---|---|
| 0,01 | - | - | - |
| 0,05 | 0,004 | 0,000 | 0,016 |
| 0,10 | 0,019 | 0,002 | 0,050 |
| 0,20 | 0,059 | 0,014 | 0,117 |
| 0,30 | 0,117 | 0,043 | 0,204 |
| 0,40 | 0,207 | 0,099 | 0,339 |
| 0,50 | 0,349* | 0,199 | 0,581 |
| 0,60 | 0,582 | 0,357 | 1,097 |
| 0,70 | 1,002 | 0,599 | 2,372 |
| 0,80 | 1,883 | 1,018 | 6,245 |
| 0,90 | 4,494** | 2,004 | 25,044 |
| 0,95 | 9,193 | 3,429 | 80,085 |
| 0,99 | 35,044 | 9,176 | 718,464 |

*) $LD_{50}$ **) $LD_{90}$

Table 4: Mortality of *Chrysoperla carnea* in the trial variants.

| Treatment | Mortality [%] | Toxicity (1-4) |
|---|---|---|
| Control | 0 | 1 |
| Alginure | 0 | 1 |
| Myco-Sin | 0 | 1 |
| PREV-B | 0 | 1 |
| Rock Effect | 0 | 1 |
| Talstar | 100 | 4 |
| Vitisan | 0 | 1 |

## Discussion

The most of products allowed or intended for organic fruit growing were harmless to earwigs and chrysops. The only exception was spinosad showing signifficant negative effect on earwigs. It confirmes that spinosad cannot be considered to have an environmental safety profile similar to most established biological insecticides (Cisneros *et al*., 2002). As was expected the reference chemical insecticides chlorpyriphos-methyl and thiacloprid were toxic to earwigs and bifenthrin to chrysops. Observed negative effect of dodine must be considered in IPM as well as the more frequent use of spinosad in IPM and organic farming.

**Acknowledgements**

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# Effect of mixtures with other products on the efficacyof codling moth granulovirus (CpGV)

E. Fritsch[1]; K. Undorf-Spahn[1]; J. Kienzle[2]; J. Huber[1] and J. A. Jehle[1]

**Abstract**

*The* Cydia pomonella *granulovirus (CpGV) is one of the most important agents for the control of codling moth in organic farming. In practice, CpGV products are often applied in tank mixtures together with other fungicidal or plant strengthening products to reduce the costs of separate applications. Some of these agents have a high pH value in solutions and therefore may have a negative effect on the stability of the CpGV occlusion bodies. Therefore, we examined whether such mixtures influence the activity of CpGV. In laboratory tests, CpGV was mixed with different fungicidal products (Cuprozin, Netzschwefel Stulln), plant strengtheners (Steinhauers Mehltauschreck, Wasserglas, VitiSan, Cocana, Myco-Sin, HF-Pilzvorsorge, CutiSan, Omniprotect and Armicarb), and other compounds, such as Molke, Düngal, lime sulphur, Ventex and Prev-B-2. After an exposure of four hours, virus activity was calculated from larval mortality determined in bioassays with neonate codling moth larvae. A significant loss of virulence was only found in mixtures under strong alkaline conditions higher than pH 11. Four agents, sodium silicate (Wasserglas) and lime sulphur as well as Omniprotect and Ventex should not be used with CpGV products in tank mixtures.*

**Key words:** Plant protection, codling moth granulovirus, *Cydia pomonella*, tank mixture, virus inactivation

## Introduction

The *Cydia pomonella* granulovirus (CpGV) is a highly important and widely used agent to control of codling moth, *C. pomonella*, in organic and integrated farming (Huber, 1998). In the past, it was common practice in organic fruit growing to use tank mixtures of CpGV products and wettable sulphur (e.g. Netzschwefel Stulln) for the combined control of codling moth and apple scab. The combination of both agents in mixtures had no detrimental effect on the viral activity. Hence, CpGV products could be applied in short intervals without the need of separate applications. Currently, not only fungicides like Cuprozin, Netzschwefel Stulln and lime sulphur are applied against powdery mildew and apple scab, respectively, but also several plant strengtheners are widely used in organic apple production. Treatments with potassium carbonate and bicarbonate (VitiSan and Omniprotect), sodium bicarbonate (Steinhauers Mehltauschreck) and water glass (sodium silicate) became important applications, too. Furthermore, products of clay (Myco-Sin and CutiSan) and a special kind of whey (Sprühmolkepulver) are currently tested for preventive application. Calcium chloride (Düngal) is often used for prevention of bitter pit during the hatching period of the second generation of codling moth. With the increasing number of products applied during the hatching period of codling moth larvae, the question arose, whether these products can also be applied in tank mix with CpGV without loss of virus activity. In general, CpGV is highly stable due to a proteinaceous occlusion body surrounding the virus particle.

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However, the occlusion body becomes destabilized at extreme (especially high) pH values and virus activity gets lost under such detrimental conditions (Jaques, 1977). To avoid any virus inactivation, mixing with some of the above mentioned anti-fungal agents is often not recommended because of the pH value of the tank mixture.

Recently, the effect of ten agents on the activity of CpGV was tested under standardized laboratory conditions at the Julius Kühn Institute (JKI) in Darmstadt (Fritsch *et al*., 2008). Here, we report the results of testing the miscibility of further ten commercial products with CpGV.

## Material and Methods

*Commercial products*

The commercial agents tested in mixtures with CpGV are listed in Table 1. For the performance of the bioassays the products were prepared in the same manner as recommended for field application but for a water volume of 200 ml. The calculated dosage is based on a water volume of 300 l per hectare and 2 m tree crown height.

*Virus*

The *Cydia pomonella* granulovirus (CpGV) used in the bioassays is a descendent from the isolate CpGV-M ("Mexican isolate") (Tanada, 1964). It was propagated in host insects and purified by the method described by Huber (1981). The isolate CpGV-M was provided from the DLR Rheinpfalz (Agricultural Service Center Palatinate, Neustadt/Weinstr.).

*Test insects*

Larvae of a laboratory strain (CpS) of the codling moth, *C. pomonella*, served as test insects in the bioassays. This laboratory strain is derived from Andermatt Biocontrol, Switzerland. The rearing method has been described by Bathon (1981).

*Bioassay method*

For the bioassays, each commercial agent was dissolved in 200 ml of water. After adding CpGV (2.4 x $10^5$ occlusion bodies/ml), the suspensions were incubated at room temperature for 4 hours. The pH of the each suspension was determined by colour-fixed indicator sticks (Roth, Art. Nr. C731 pH 4.5 – 10 and MERCK, Art. Nr. 9541 pH 2.5 – 4.5).

Larval mortalities of about 70% after 7 days and 100% after 14 days, respectively, were expected at the chosen virus concentration. Additionally, a water suspension of CpGV was prepared as a positive control. The virus activity in the mixtures was determined in bioassays with first instars (L1) of codling moth using the method described by Huber (1981). After incubation of the agents with CpGV occlusion bodies, aliquots of the prepared suspensions were mixed with an artificial diet (Ivaldi-Sender, 1974) and poured into bioassay trays (LICEFA, Bad-Salzuflen, Germany) with 50 separate cells (1.5 x 1.5 x 2 cm). The next day one neonate larva was placed into each cell. The covered trays were incubated at 26°C, 60-70% relative humidity and a 16 hr photoperiod. Larval mortalities were recorded after 7 and 14 days. The mortality data of larvae exposed to the mixtures and to the positive control were compared by using ANOVA (Proc GLM; SAS 9.2; Scheffe's test).

## Results and discussion

The effect of different commercial agents used in organic fruit production (Table 1b) on the activity of CpGV was investigated in bioassays by means of larval mortalities recorded after 7 and 14 days. After CpGV had been mixed with these agents for 4 hours, the mortality of most mixtures caused mortalities of about 70% after 7 days and about 98% after 14 days, respectively. These

data corresponded to those of the positive control (CpGV suspended in water) (Figure 1, Figure 2). A substantial reduction of mortality was only observed for the plant strengthener Omniprotect and the agent Ventex. In the bioassay evaluated after 7 days, no virus killed larvae were found for both products (Figure 1). After 14 days, the larval mortality reached 22% (Ventex) and 35% (Omniprotect), respectively (Figure 2). This suggested that the activity of CpGV was negatively affected by these two agents.

Table 1a: List of commercial products tested in 2007 in mixtures with codling moth granulovirus (CpGV) and the miscibility with CpGV in tank mixtures. Given are the field application rates of the products and the pH values of the mixtures determined by colour-fixed pH indicator sticks.

| ***a) Products tested in 2007 (Fritsch et al., 2008)*** | | | |
|---|---|---|---|
| **Commercial products (ingredients)** | **Application rate per ha for 300 liter water and 2 m crown height** | **Hydrogen ion concentration (pH)** | **Miscibility** |
| **Whey** (Sprühmolkepulver) | 14 kg | 4.0 | yes |
| **Steinhauers Mehltauschreck** (Sodium bicarbonate) | 5 kg | 8.5 | yes |
| **Water glass** (Sodium silicate) | 5 l | 11.0 | no |
| **Düngal** (Calcium chloride) | 20 l | 7.0 | yes |
| **VitiSan** (Potassium bicarbonate) | 5 kg | 9.0 | yes |
| **Armicarb*** (Potassium bicarbonate) * Registration pending | 5 kg | 8.5 | yes |
| **Cuprozin flüssig** (Copper hydroxide liquid formulation) | 0.66 l | 7.0 | yes |
| **Cuprozin WP** (Copper WP 24158) | 0.44 kg | 7.0 | yes |
| **Netzschwefel Stulln** (Sulphur) | 4 kg | 7.0 | yes |
| **Lime sulphur** (Polisenio) | 15 l | 11.5 | no |

The pH measurement showed low hydrogen ion concentrations (pH 11.5) for the virus suspensions containing Omniprotect and Ventex. No effect on viral activity was observed for the suspensions with higher hydrogen ion concentrations ranging from pH 3.8 to pH 10. Previous investigations showed that the chemical compounds water glass and lime sulphur resulted also in a high pH value of the mixtures and also significantly reduced the virus activity (Fritsch *et al.*, 2008) (Table 1a). Studies on the stability of a nucleopolyhedrovirus of *Heliothis zea* (corn earworm) reported also pH dependent effects. In this case, virus infectivity was reduced when the occlusion bodies were buffered at pH 2 or 12 but was unaffected at pH 5, 7, or 9 (Gudauskas & Canerday, 1968).

The stability of baculoviruses is due to the intact occlusion body surrounding the virus particles. At strong alkaline conditions the occlusion body protein will be dissolved.

In summary, the use of CpGV in combination with the products lime sulphur, water glass, Omniprotect and Ventex in tank mixtures is not recommended for application in organic production. A negative impact of the other tested products on CpGV was not observed under the described conditions.

Table 1b: List of commercial products tested in 2011 in mixtures with codling moth granulovirus (CpGV) and the miscibility with CpGV in tank mixtures. Given are the field application rates of the products and the pH values of the mixtures determined by colour-fixed pH indicator sticks.

| ***b) Products tested in 2011*** | | | |
|---|---|---|---|
| **Commercial products (ingredients)** | **Application rate per ha for 300 liter water and 2 m crown height** | **Hydrogen ion concentration (pH)** | **Miscibility** |
| **Cocana** (28% Potassium, soap based on coconut oil) | 8 kg | 10.0 | yes |
| **PREV-B 2** (Oil of orange) | 4 l | 7.0 | yes |
| **Myco-Sin** (clay) | 8 kg | 3.5 | yes |
| | 2.4 kg | 3.5 | |
| **HF-Pilzvorsorge** (Oil of fennel and other plant extracts) | 4 l | 6.5 | yes |
| **CutiSan** (Kaolin) | 2 kg | 7.0 | yes |
| **Omniprotect** (Potassium carbonate) | 6 kg | 11.5 | no |
| **Ventex** (Potassium carbonate) | 9 kg | 11.5 | no |
| **Düngal** (Calcium chloride) | 20 l | 7.0 | yes |
| | 10 l | 7.0 | |
| **Cuprozin Progress** (Copper hydroxide) | 2 l | 7.0 | yes |
| **Cuprozin 2720 WP** (Copper hydroxide) | 1.42 kg | 7.0 | yes |

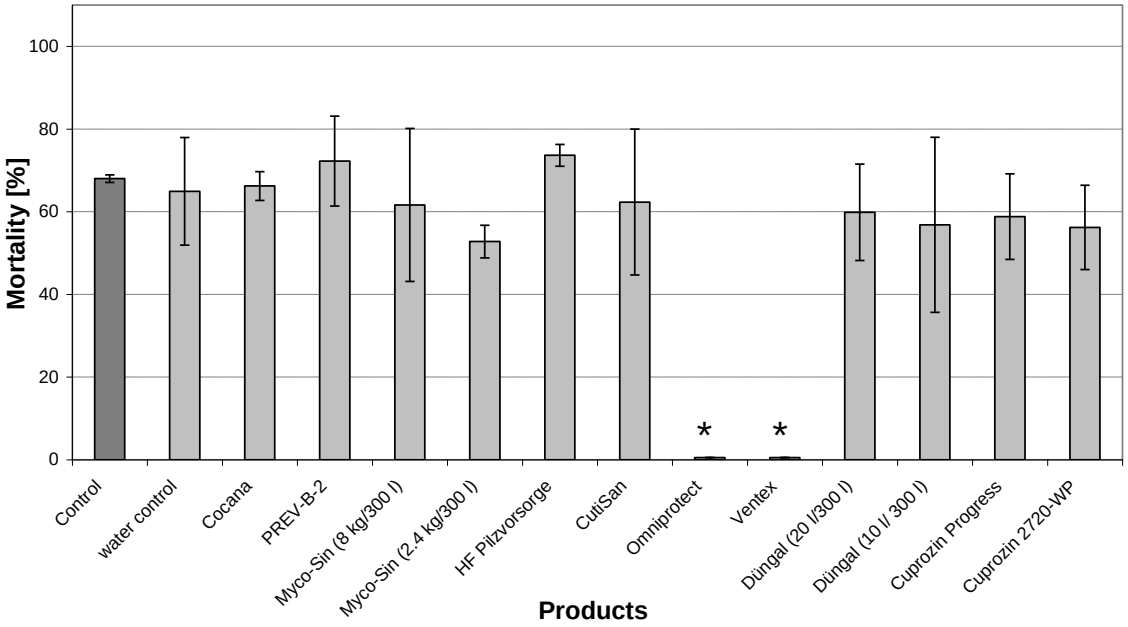


Figure 1: Activity of CpGV after 4 hours exposure in spray mixtures of different products at room temperature, calculated from larval mortality after 7 days in bioassays. Given is the mean mortality of neonate codling moth larvae from 3 replicates. The vertical lines indicate the standard error. For the control assay the CpGV was mixed directly into the bioassay medium. For the water control the virus was resuspended in water and exposed for 4 hours at room temperature before the bioassay was conducted. *The virus activity in these product suspensions differs significantly from the control and the other products at the level of $P< 0.05$ (Scheffe´s test, SAS 9.2; ANOVA, Proc GLM).

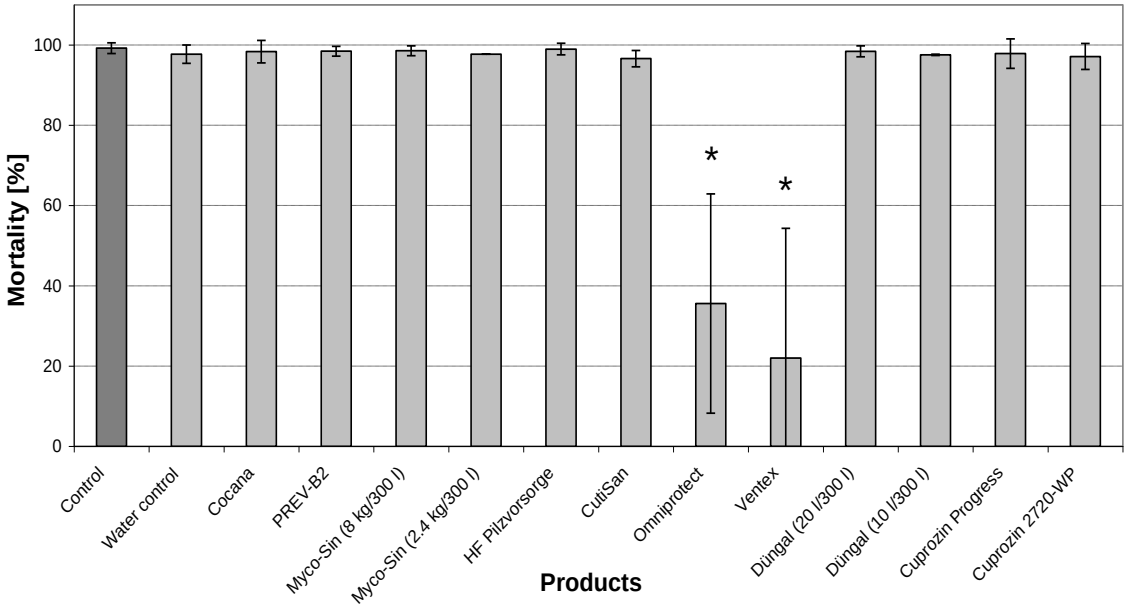


Figure 2: Activity of CpGV after 4 hours exposure in spray mixtures of different products at room temperature, calculated from larval mortality after 14 days in bioassays. Given is the mean mortality of neonate codling moth larvae from 3 replicates. The vertical lines indicate the standard error. For the control assay the CpGV was mixed directly into the bioassay medium. For the water control the virus was resuspended in water and exposed for 4 hours at room temperature before the bioassay was conducted. *The virus activity in these product suspensions differs significantly from the control and the other products at the level of $P< 0.05$ (Scheffe´s test, SAS 9.2; ANOVA, Proc GLM).

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# Use of nectar resources by *Asocgaster quadridentata* WESMAEL (Hymenoptera, Braconidae), an important egg-larval parasitoid of the codling moth: first evidence from laboratory studies

A. Herz[1], G. Eder[1], S. Feiertag[1] and S. Wittlich[1]

**Abstract**

*Functional biodiversity on different trophic levels can provide essential pest control in agroecosystems. Codling moth as a key pest in apple is the target host of several parasitoid species whose efficacy may depend from a diverse environment. For instance, plant-derived food (nectar, pollen) from flowering plants could be crucial for their survival and successful reproduction. In a first attempt, the effect of providing sugar resources and nectar providing plants on survival of males and females of the codling moth parasitod* Ascogaster quadridentata Wesmael *was estimated in laboratory studies. Plants were selected by their multifunctional qualities as nectar providers for pollinators and beneficials and as cover crop. Both sexes of* A. quadridentata *increased their survival considerably by access to different sugar sources. From the offered plants, however, only buckwheat could feed* A. quadridentata *successfully. Results will be used to design first field experiments in this ongoing study.*

**Keywords:** Functional biodiversity, plant provided food, conservation biological control, sustainable fruit growing

## Introduction

The involvement of natural regulative processes in pest control is an important prerequisite for sustainable production both in organic and integrated fruit growing. Codling moth, *Cydia pomonella* (Lepidoptera, Tortricidae) is the key pest in apple production due to direct damage of fruits and increasing resistance against insecticides as well as viral biopesticides. Conservation biological control by enhancing natural enemies may help to reduce pest pressure and improve control strategies. The egg-larval parasitoid *Ascogaster quadridentata* (Hymenoptera, Braconidae), a specialist of several Tortricid pests, occurs in most apple-growing regions and can be highly effective, especially in parasitizing the summer generation of the pest. Under laboratory rearing conditions, *A. quadridentata* lives several weeks when honey-fed. Thus, successful location and use of nectar and other sugar resources may also be an essential factor for sufficient survival and reproduction in the field. For active habitat manipulation, floral resource plants which favour the natural enemy but not the pest should be preferably selected (Lavandero *et al*., 2006). Furthermore, establishment of plants with confirmed multifunctional qualities (insectary plants, soil fertilization, use for human or animal food) or the preserving of the biodiversity of natural occuring wild plants may be more acceptable for the grower when aiming on the ecological engineering of the crop system (Fiedler *et al*., 2008; Walton & Isaacs, 2011). We describe laboratory experiments which test the use of sugar sources and nectar providing plants with multifunctional qualtities by *A. quadridentata* as the first step in designing suitable ecological infrastructures.

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## Material and Methods

Based on field collections from South-Hessia and Baden-Württemberg, a laboratory rearing of *A. quadridentata* was established in 2010. Parasitoid females were allowed to oviposit into codling moth eggs and hatched host larvae were subsequently reared in individual containers on artificial diet until parasitized host larvae emerged for cocoon spinning. Larvae were collected and added to plastic boxes containing corrugated cardboard rolls for pupation and adult emergence. Development took place within 5 to 6 weeks at 25°C and long day conditions (16:8 h). Plants were sown in pots and grown in the greenhouse until blooming.

For the first experiment, individual caged, freshly emerged parasitoids were offered one of the following options: 1) water, provided in a 5 ml plastic vial with a cotton wick, 2) water (as above) and honey (small drops smeared on pieces of Parafilm®), 3) water and fructose-solution (1 mol, provided in a 5 ml plastic vial with a cotton wick *ad libidum*), 4) water and glucose-solution (1 mol, in 5 ml plastic via with a cotton wick, *ad libidum*), 5) water and one freshly cut, water-immersed inflorescence of buckwheat (*Fagopyrum esculentum* Moench), changed daily, 6) water and one inflorescence of buckwheat, changed every second day. Survival of the wasps was recorded daily and sex was determined after death. For each treatment, 20 wasps were set up within a couple of days.

In the second experiment, wasps were provided with the following treatments: 1) water only, 2) water and honey, 3) water and one inflorescence of wild mustard (*Sinapis arvensis* L., 4) water and one inflorescence of Phacelia (*Phacelia tanacetifolia* Benth.), 5) water and buckwheat, 6) water and wild parsnip (*Pastinaca sativa* L.*)*, 7) water and red clover (*Trifolium pratense* L.*)*. Plants of treatments 3, 4, and 5 were grown in the greenhouse; plants of treatments 6 and 7 were taken from the field every morning. Inflorescences were changed daily. Survival and sex were recorded as above. For each treatment, 20 to 25 wasps were set up within a couple of days.

Survival data of wasps in the different treatments were analyzed using ANOVA with PROC GENMOD (SAS software version 9.2, 2008: Poisson distribution with logit link), followed by the LSD Multiple Range test or survival analysis with the Product-Limit method (PROC LIFETEST; SAS, 2008). No significant differences in longevity between sexes or interaction of sex with food were found and subsequent analysis was performed with pooled results of males and females in the different food treatments. Survival curves were compared between treatments using a log rank test and multiple comparisons between treatments were made using Šidák-corrected p-values (SAS, 2008).

## Results

Food had a significant effect on survival of *A. quadridentata* in the first ($\chi^2$ = 112.5, df = 5, $P < 0.0001$) and the second experiment ($\chi^2$ = 123.9, df = 6, $P < 0.0001$) as well. Access to sugar sources as honey or sugar solutions significantly increased the longevity (Figure 1). In the first experiment, wasps died after 3.9 days when offered water only, but lived nearly ten times longer (34.3 days) when having access to pure honey. Those wasps which were fed with glucose or fructose lived on average 19 and 25 days respectively. Offering buckwheat also clearly prolonged the survival of the wasps (16 days). However, changing the inflorescence only every second day had no significant effect in comparison to the water control and wasps died after 5.8 days in this treatment.

In the second experiment, overall survival was generally shorter in all treatments (Figure 2). In the water control, the wasps died after 3.3 days, but lived three times longer (10.5 days) when fed with honey. Having access to buckwheat also significantly increased the longevity of the wasps (5.4 days) in comparison to water. The other plants were obviously not used or not suitable to feed *A. quadridentata* as their provision did not prolong survival. Flower-visiting by individuals was observed only on buckwheat and wild parsnip, not in the other treatments.

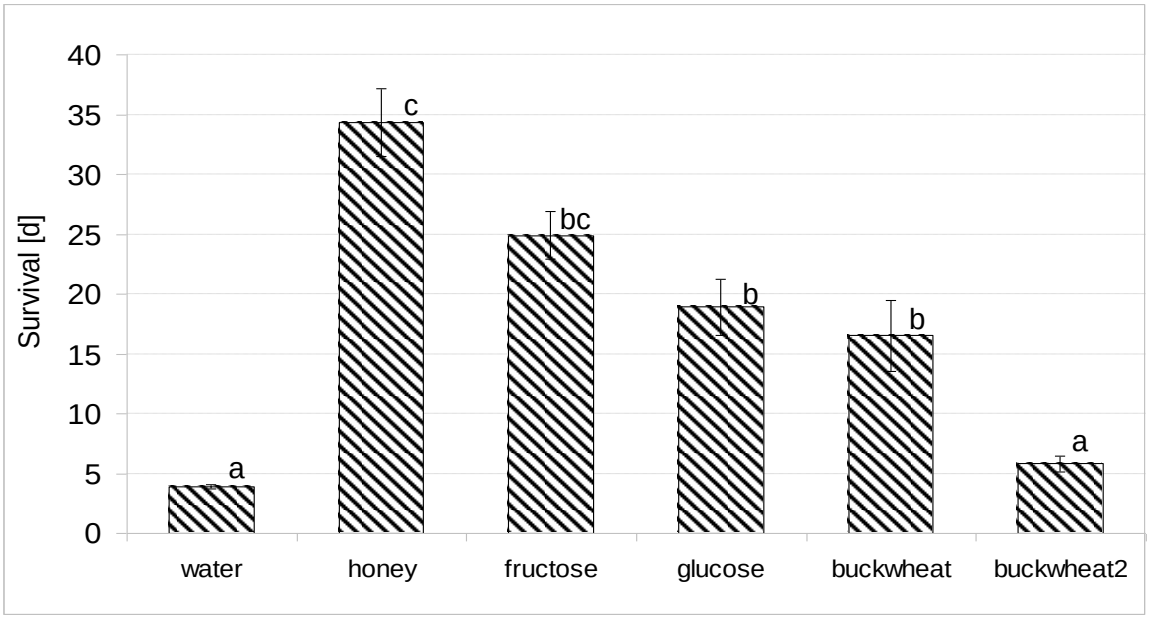


Figure 1: Longevity (mean ± SE) of *A. quadridentata* when provided with different sugar resources (1 mol solution) or buckwheat flowers, cut daily or every second day in comparison to water only. Significant differences between treatments are indicated by different letters at $p \leq 0.05$.

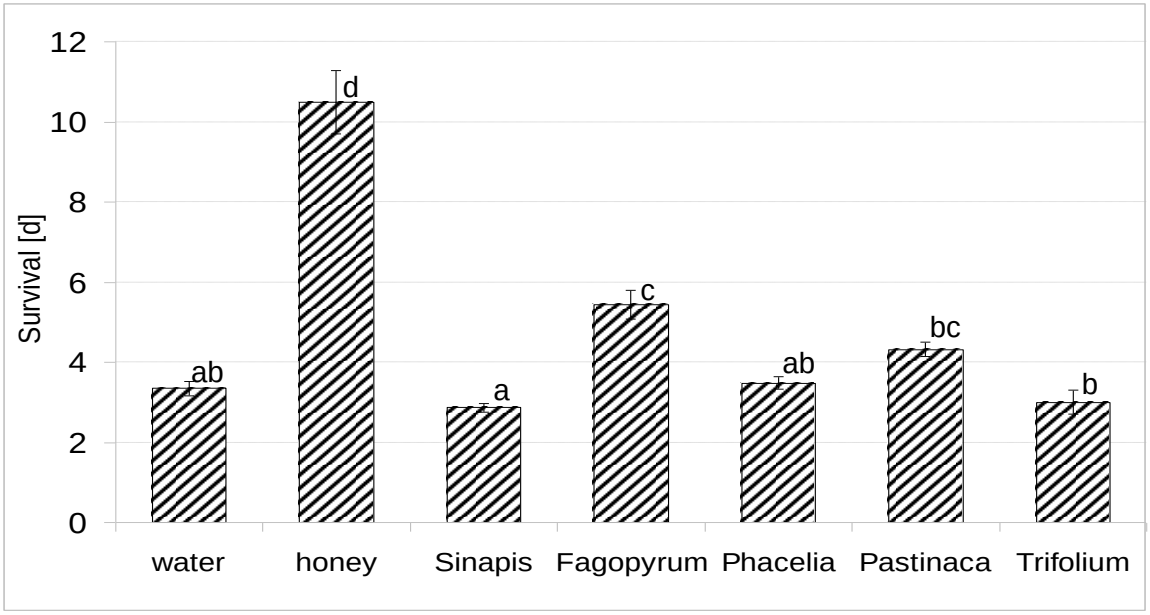


Figure 2: Longevity (mean ± SE) of *A. quadridentata* when provided with different sugar resources (1 mol solution) or buckwheat flowers, cut daily or every second day in comparison to water only. Significant differences between treatments are indicated by different letters at $p \leq 0.05$.

## Discussion

Lifespan of *A. quadridentata* increased to several weeks by feeding on honey or sugar solutions. Honey was the best food, suggesting that beside sugars other components (pollen, minerals etc.) may support the parasitoid`s survival. Wasps did also well from feeding on fructose and to some lesser extent from glucose and freshly cut buckwheat flowers. Changing buckwheat only every second day obviously leads to the starvation of the wasps, suggesting that the nectar provision was insufficient. Also Lee & Heimpel (2008) reported decrease in survival of *Cotesia glomerata* (L.) when buckwheat flowers were changed every second or third day instead of daily change. Methodology of presenting flowering plants (number of flowers, excised or intact inflorescences), plant quality *per se* as well as overall fitness of the parasitoids certainly affects the results of such experiments (Wade & Wratten, 2006).

Mustard, *Phacelia* and red clover had no enhancing effect on the wasps which died as quickly as those in the water control. Flowers of wild parsnip usually attract many insects with unspecialized mouthparts and were therefore expected to feed also this parasitoid. However, lifespan of *A. quadridentata* was only marginally prolonged by this plant. Flowers were collected from outside and their nectar might have already been exhausted and it may be valuable to repeat the test with greenhouse grown plants. According to these preliminary results, buckwheat can be considered as suitable cover crop in orchards for promoting *A. quadridentata*. This plant was already shown to be a suitable nectar resource for many beneficial insects also in other crop systems. It is usually planted due to its soil protecting and weed suppressing properties especially in vineyards, where *A. quadridentata* may be also active in parasitizing grape moths (Thiéry *et al*., 2011), thus presenting a cover plant with multifunctional qualities. Nevertheless, the need for additional plant-provided food for enhancing survival, but also fertility of this important parasitoid of tortricid pests will be studied in subsequent experiments. Also selectivity of buckwheat and other flowering plants in increasing fitness of natural enemies but not of the pest, e.g. the codling moth, needs to be clarified.

## Acknowledgements

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# Self evaluation for fruit growers: a cooperation project among growers, extension and research

E. Bravin[1], M. Blunschi[2]

**Abstract**

*Within the Interreg Project "Management of fruit-growing" (original German title "Betriebsmanagement im Obstbau") we developed an instrument for the self evaluation on the social and economic situation for organic and IP fruit growers. Two studies about controlling instruments for fruit growers published by Görgens (2003) and Mouron und Carint (2001[1]) served as a foundation for the self evaluation instrument. Sessions and interviews with chosen organic and IP growers, consultants and other experts from the region of Lake Constance (form both sides of the Swiss and German border) provided necessary information to further develop the already existing and used tools. Criteria for the new developed instrument were defined by experts: the new self evaluation instrument should be useful and user-friendly and help organic and IP growers on plot, farm and family level to address managerial issues. The self evaluation instrument allows fruit organic and IP growers to compare their own situation with benchmarks (quantitative part) and to evaluate the individual perspective of their situation (qualitative part). The self evaluation tool intends to provide benchmark based guidance to fruit growers that address important decisions – which otherwise they would decide based only on gut instincts. With the instrument fruit organic and IP fruit growers can make important decisions for the future of their orchard, farm and family based on validated industry benchmarks. The instrument is called ArboPlus and is available online.*

**Keywords:** Self evaluation instrument, fruit grower, management, social and economic situation.

## Introduction

The economic pressure on fruit growers increased over the last years. Within countries of the European Union production increased and consequently the price decreased (Schwartau, 2010). From 2007 the organic apple production area in Western Europe increased. 200 ha in Italy (ZMP, 2008), 200 ha in Austria (Wilhelm, 2008), 200 ha in Germany (ZMP, 2008) and 90 ha in France (Agence Bio, 2009). The offer of organic apples increased and the pressure on organic prices rises. In Switzerland, prices for IP and organic production were stable (AGRIDEA, 2007-2010). But with a possible liberalisation of the fruit market, due to the trade agreements for agriculture between Switzerland and the European Union, Swiss growers could experience more price-pressure (Büchele, 2007). The price paid to growers is a key factor that influences the family income above-average. The consequence of a price decrease is an income decrease (Mouron und Carint, 2001[2]). With lower prices for organic and IP apples farm income will also drop.

To change the difficult situation, fruit growers can follow different strategies: enlarge the production-area, switch to organic farming, specialize, and increase productivity and so on.

Management skills are essential for strategic change. Higher productivity needs an individual analysis of deficits in the production (Büchele, 2007).

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The objective of the Interreg Project “Management of fruit-growing” is to improve the managerial competence of fruit growers in the region of Lake Constance. Thus the project encompasses an exchange of information about management.

Strategic change is a very important decision. This decision can only be taken with useful information about the situation on the orchard, farm and family. To analyze the situation we developed the self evaluation instrument ArboPlus for organic and IP fruit growers.

## Method

*Self evaluation instrument*

Görgens (2003) describes a control system for fruit growers with different levels on the plot- and fruit production level with the evaluation of:

- numbers of the cultivars
- pest management / fertilizer
- thinning recommendation
- evaluation of the yield
- information to storage
- turnover (month/cultivar/plot)
- output of the plot/cultivar
- price of the cultivar
- harvest, grading and stock costs
- key indicator data about the farm

Mouron und Carint (2001[1]) developed a full cost calculation model for the economic evaluation of the plot for fruit growers and consultants. The instrument is called ArboKost and exist for organic and IP fruit growers. With this interactive tool growers can calculate the cash flow, the labor income, the gross margin and data about productivity, time and cost distribution.

To improve the situation of the organic and IP fruit growers in Switzerland and Germany in the region of Lake Constance we developed a user-friendly self evaluation instrument: a synthesis from Görgens (2003) and Mouron’s (2001[1]) work. The objective was to identify the key factors and to integrate them in a single tool.

In the quantitative part of the instrument organic and IP fruit growers can interactively evaluate their situation – on a traffic-light signalization system for each key factor: red – yellow – green (quantitative section on plot level).

In the qualitative section growers can evaluate possibilities for cooperation, performance of employees or division of work. The evaluation of the qualitative section is about contentment with the individual situation. This part of the evaluation is more suitable for the farm and family level.

ArboPlus is programmed on Excel 2007 (Visual Basic).

*Experts*

To choose the information in the check up instrument we used expert’s know-how. The criteria to choose the experts were their experience and contact with fruit growers. We asked six fruit production consultant for the organic and IP production, two farm management consultant, and two accounting analyst to participate in five evaluation sessions for the construction and evaluation of the tool. These experts were asked in sessions and/or interviews to evaluate different versions of the instrument – levels (plot, farm and family) and issues. Within explorative interviews expert have been asked if tools

with the same objectives already existed and if important issues were neglected. The feed-back of experts has been integrated in the self evaluation instrument for the next expert round. For the second evaluation, we hold two different types of experts' rounds. In one type of expert round the main objective was to test if the instrument was really user-friendly and if the information asked were easy to find for the growers. The other type of expert round was more based on the integration of expert inputs and important practical information to improve the quality of the self evaluation instrument.

To guarantee a user-friendly system and useful information the self evaluation instrument ArboPlus has been checked on a third evaluation level. 25 organic and IP fruit growers in Switzerland and Germany tested the instrument. Participating growers were selected by consultants from each region.

## Results

We defined three levels of the self evaluation instrument (called ArboPlus) using information collected from literature research (Görgens 2003, Mouron und Carint, 2001[1]) and with expert interviews and sessions:

- ArboPlus plot
- ArboPlus farm
- ArboPlus family

Additionally we developed the following tools that could assist fruit growers in managerial decision taking:

- planning of harvest employees
- decision tool for clearing the plot
- decision tool to choose a cultivar

*ArboPlus plot*

Plot level users (growers) can evaluate their apple plot using the self evaluation instrument. We chose the evaluation for apple growing because in Switzerland the apple orchard surface is 66% of the fruit surface (BLW, 2010). With the instrument users (growers) can evaluate the key factors of their orchard (apple) for different cultivars (Gala, Golden Delicious, Jonagold, Braeburn, Idared, other new cultivars, other traditional cultivars). The key factors of the apple production are the price (of the best class), the quality (grading results) and the yield (Mouron und Carint, 2001[2]). In the instrument more important factors (interview with experts) have been included: yield/harvest time, labour hours, productivity and revenues.

To set the traffic-light system on plot level up we defined the corresponding trigger points by using the average of on-farm results of a network of twenty professional IP and organic Swiss farmers (year 2004-2009) participating in the project Support Obst Arbo (SOA, 2011). As such a database does not exist for growers in the German part of the Lake Constance region the Swiss data for IP apples was adjusted by experts (consulters and growers of the region). With a sensibility analysis using a full cost model account we evaluated the colors of the traffic-light system by measuring the consequence on farm income.

- consequence on farm income <= -10% → green
- consequence on farm income >= -10%, <= - 20% → orange
- consequence on farm income >= -20% → red

*ArboPlus farm*
With the self evaluation instrument factors for accounting (gross margin, cash flow …) can be evaluated. Growers will have to collect the data themselves and then introduce it into the instrument. The results are also on this level defined by the traffic-light system. The value for green, yellow and red is determined by standards rates of accounting.
With ArboPlus farm it's also possible to evaluate the performance of employees, cooperation with other farmers, or possible cooperation, suppliers and distance to plots.

*ArboPlus family*
With the check-up instrument on family level apple growers can evaluate their situation in the family. In interviews with experts they often point out, the importance of such an instrument also on the family level. The family is very important for the success of the farm. The instrument addresses the following issues: living together of generations, division of work, family time together, competence & education and evaluation of external employment. The evaluation is based on the individual contentment of the family members. In addition, the instrument point to potential conflicts or future problems.

*Objectives and strategies with ArboPlus*
With the fulfilling of the self evaluation instrument fruit growers can consider their objectives and strategies to achieve their objectives. In the summary of the instrument the results of ArboPlus plot, ArboPlus farm and ArboPlus level are showed. This general view helps growers to watch forward out of the situation and set realistic objectives for the plot, farm and family. The most important results of the self evaluation instrument are then thematic arranged:

- Work-Life-Balance
- Finance
- Sale
- Work

For those important issues is than possible for the grower to choose their objectives and to find the strategies for their own objectives. After a year growers should control their objectives. If the objectives are not achieved, they should find new strategies or change the objectives. The analysis will so be fulfilled: choose the objective, find the strategy, and control the objectives.

## Discussion

It is very important for organic and IP growers to be able to analyze their situation. With better awareness they are in a better position to take the right decision. With this self evaluation instrument ArboPlus growers can analyse their situation, set objectives and control them. The can also find information on important issues such as check lists that help them to improve the farm situation. By involving organic and IP growers and consultants from the IP and organic fruit production, trust consulting, home economy we tried to interest growers in less common fields for real economic controlling instruments. The success of such instruments is only guaranteed if organic and IP growers really use it. The results will be even more promising if the self evaluation instrument is used on a regular basis. The implementation of the instrument in the Lake Constance region on Swiss and German side remains one of the most important tasks of the project.

The work in this research does not finish with the completion of the ArboPlus. The knowledge-transfer to growers is crucial. The percentage of use will increase with the implementation of the instrument in the education of fruit growers and in seminars for growers.

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# Artificial hideouts for control of fruit moths: Persistence of the entomopathogenic fungi *Metarhizium anisopliae* and *Isaria fumosorosea* under semi-field condition

D. Stephan[1]

**Abstract**

*The usage of corrugated cardboard and mulches as artificial hideouts for cocooning and pupating of different fruit moths is under discussion. Therefore, under semi-field conditions the persistence of two entomopathogenic fungi formulated in oil or water and two different application strategies on mulches were compared. Additionally, for* Isaria fumosorosea *the persistence of different produced spores was investigated. The results demonstrate that rape seed oil and* I. fumosorosea *persist on mulches for at least two months. But the persistence is influenced by environmental factors, especially rainfall. In contrast to* I. fumosorosea *the efficacy of* M. anisopliae *was not sufficient. Submerged spores of* I. fumosorosea *were as persistent as aerial conidia and its persistence was not influenced by the formulation, but by the application.*

**Keywords:** *Cydia funebrana*, biological control, entomopathogenic fungi, persistence

## Introduction

Within a national funded project for biological control of the Plum Fruit moth (*Cydia funebrana*) we investigated the potential of artificial hideouts treated with entomopathogenic fungi. In Germany plum fruit moths generate two generations with overwintering larvae of the second one. Assuming that larvae of both generations pupate between the bark of the tree or closed to the stem one control strategy by treating these or artificial hideouts with entomopathogenic fungi is under discussion. One constrain of the application of living microorganisms is the environmental stability of the microorganism. Therefore, experiments on the persistence of the two selected entomopathogenic fungi *Metarhizium anisopliae* and *Isaria fumosorosea* on mulch was investigated over two months under semi-field conditions. Because the persistence and efficacy of biocontrol agents is influenced by the produced inoculum and the formulation two type of spores produced in liquid or solid state fermenter and oil or water based formulations were compared. Because the applicability in the field is often a constrain for implementation two different application strategies were compared.

## Material and Methods

Because it was not possible to establish an artificial rearing of *Cydia funebrana* these experiments were carried out with larvae of *Cydia molesta* as a model species. For artificial rearing of *Cydia molesta* adults were kept in cylinder containing plastic foil for egg deposition. After three days the plastic foil with deposited eggs was transferred in boxes containing artificial diet (according to Ivaldi-Sender, 1974) and was kept at 25° C and 16 h light. Hatched larvae penetrated the artificial diet. Just before pupating the larvae ($L_5$) left the artificial medium and these larvae were used for all experiments.

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Production and formulation of the entomopathogenic fungi: Conidia of *Metarhizium anisopliae* (strain Ma43 = BIPESCO5 = F52) were produced in a Prophyta- laboratory solid state fermenter for 14 days at 25° C with a flexible aeration rate of 0.05-0.15 Nl/h and gram substrate (mixture of paraboiled rice and oat, 4:1). Conidia of *I. fumosorosea* (Strain Pfr4) were produced in 1-liter solid state fermenter for 14 days at 25° C with a fixed aeration rate of 0.15 Nl/h and gram substrate (mixture of paraboiled rice and oat, 4:1). For the production of submerged spores strain Pfr4 was grown in a liquid fermenter (Minifors, Infors, Switzerland) with 3.5 l medium (25g/l glucose, 20g/l Cornsteep, 4g/l NaCl) for 72h at 25° C. For separation of submerged spores the liquid medium was filtered over three layers of mull.

For preparing the oil based formulation the overgrown substrate of the solid state fermentation was suspended in rape seed oil and sieved with a mesh size of 150µm. For the water based formulation conidia were washed with deionised water containing 0.01% Tween 80. For comparison of formulations of Ma43 the formulations were adjusted to a concentration of $1x10^7$ conidia/ml (0.01 % Tween 80 in water) or $2x10^7$ conidia/ml (rape seed oil). For comparison of isolates (Ma43, Pfr4) and production system (aerial and submerged spores) concentrations of $1x10^6$ spores/ml were used. Per treatment 4000g of dried mulch (Plantop Dekormulch - natur, Ziegler, Germany) was mixed with 4000ml water based or 2000ml oil-based formulation. In 2011 spore suspensions were mixed or sprayed with a knapsack sprayer on the top of the substrate with an equivalent volume.

The treated mulch was transferred in a grid of nine plots each of 80x80cm size. Before, the grass vegetation was cut and covered with a weed-fleece. The plots were exposed to full sunlight and rainfall. Mulch samples of 20x20 cm (250g mulch) were taken weekly or in two week intervals over a period of two months. Three times 50 g of each sample were put in separate boxes and 25 $L_5$-larvae of *C. molesta* were added, incubated at 25° C darkness and the number of hatched moths was monitored over four weeks. Additionally, in the field the ambient temperature, humidity and rainfall, and additionally in 2011 the substrate and soil temperature and the substrate moisture was measured over the time. Because the experiments were not repeated over time, no statistical analysis was conducted.

## Results and Discussion

In 2010 and in 2011 experiments on the persistence of entomopathogenic fungi were conducted under semi field conditions. In both years mulch treated with rape seed oil alone resulted in a high reduction of number of hatched moths of *C. molesta* (fig. 1 and fig. 2). In contrast, the water based formulation did not show any clear effect. The effect of oil corresponds to results of laboratory experiments (Stephan and Herker, 2011). When conidia of *M. anisopliae*, strain Ma43, were suspended in water or oil especially the oil based formulation resulted in a reduction of hatched moths which can be explained mainly by the oil effect. But in the first three weeks (fig. 1B) a slight reduction of hatched moths compared to the blank formulation was visible. When suspended in water Ma43 showed a slight effect only in the first two weeks (fig. 1B). For both formulations the effect of Ma43 over the time was unsatisfying. When the effect of conidia of Ma43 and Pfr4 formulated in water were compared, for both isolates a high fluctuation of effects over time was measured (fig.1C). At the beginning of the experiment a clear effect was only visible for Pfr4. But after two weeks exposure in the field no effect was observed. On the other hand for Ma43 no effect was visible at the beginning of the experiment but after one week exposure, only less than 50% of moths hatched.

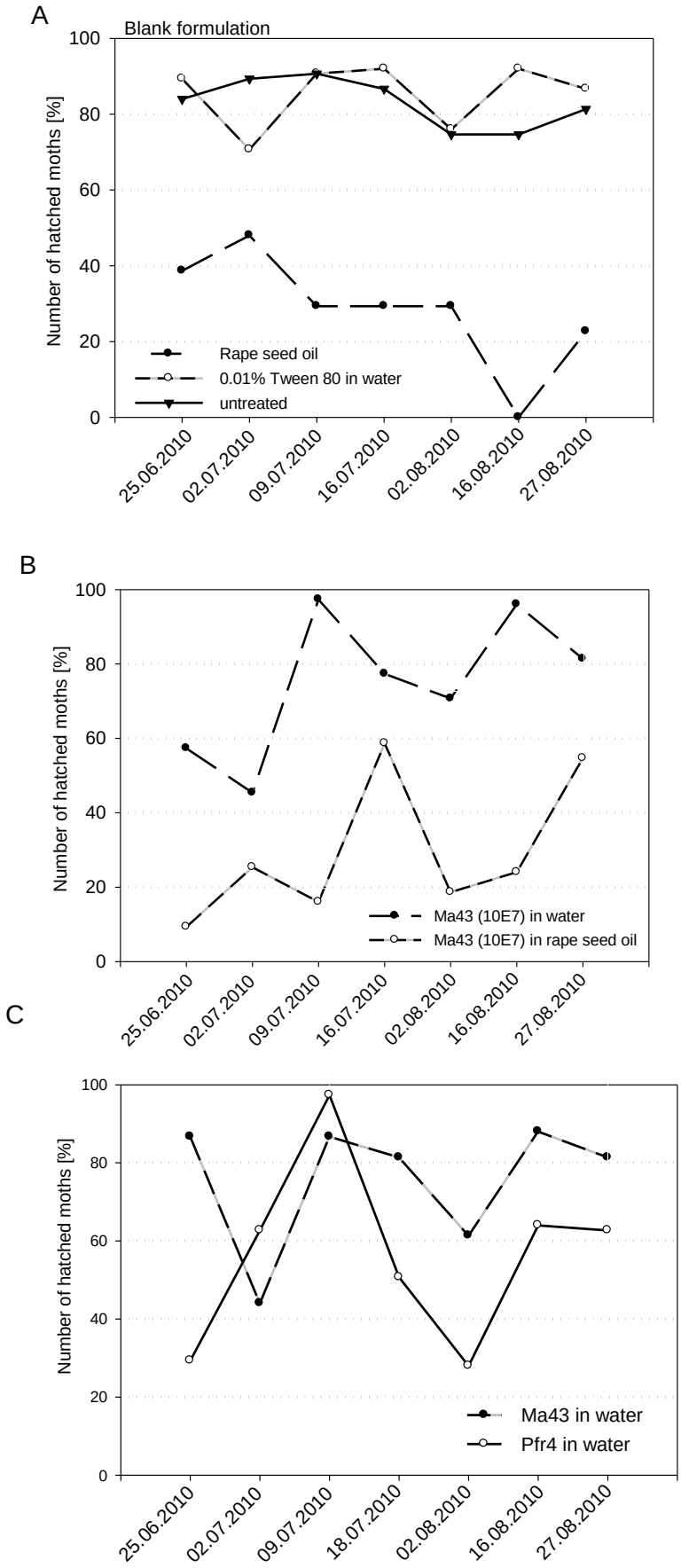


Figure 1: Persistence of oil or water based formulations of two entomopathogenic fungi mixed with mulches in 2010

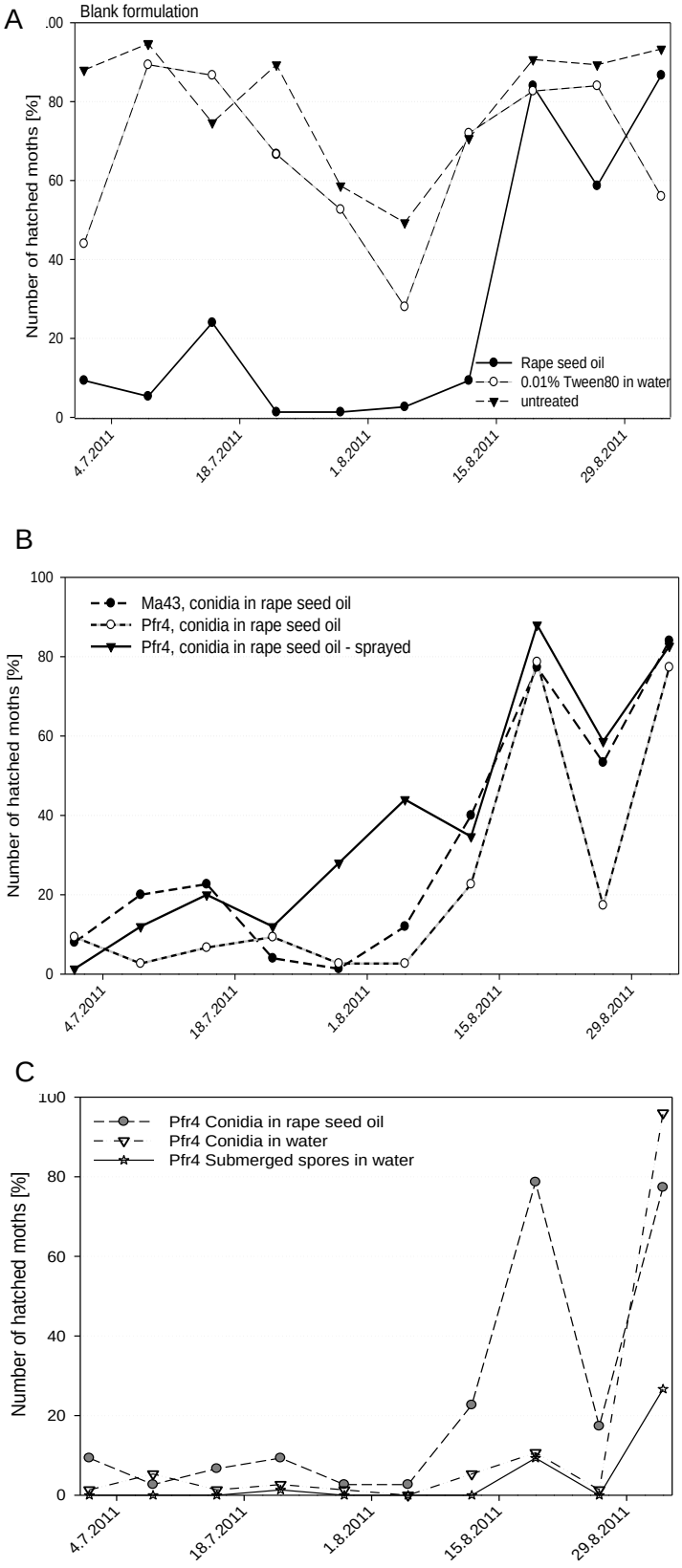


Figure 2: Influence of the fungal isolate, formulation, application and type of spores on the persistence on mulches in 2011

These results indicate that possibly, environmental factors like temperature and humidity influenced the persistence in field. The results of 2010 let assume that the environmental optima of the two isolate seem to be different (fig.1C). In 2011 again a clear oil effect was observed. The reduction of efficacy in mid of August can be explained by heavy rainfall (fig. 2A). In 2011 again the effect of oil formulated conidia of Ma43 was mainly caused by the oil formulation which was also the case for conidia of Pfr4 formulated in oil (fig. 2B). When the two application strategies - mixing or spraying - were compared a slight better effect was achieved with mixed material. This can be explained by a better distribution on the mulch. When conidia and submerged spores of Pfr4 were formulated in water nearly over the whole experimental time a dramatic reduction of hatched moths was achieved. Independently of using solid state produced conidia or liquid fermented submerged spores not more than 20% of the larvae developed to moths over the whole experimental time (except the last sample). Because water with 0.01% Tween 80 did not show any clear effect in laboratory experiments (data not shown) and in the persistence experiments of 2010 and 2011 the effect is caused by the entomopathogenic fungus. When the tests were analysed, *I. fumosorosea* grown out of the cocoons were found over the whole monitored period. Although in the field the mulch-temperature reached temperatures higher than 45° C the fungus was still effective. Additionally, submerged spores were as persistent as conidia. This is important, because this strain can be easily produced in liquid culture and an industrial production of this strain seems to be possible.

## Conclusion

In laboratory experiments mulches and corrugated cardboard are accepted as artificial hideouts for cocooning and pupation of various moth species (Herker et al. 2010). Bioassays have shown that rape seed oil and the entompathogenic fungi *M. anisopliae* and *I. fumosorosea* were effecting the development of Cydia *funebrana* (Stephan and Herker, 2011) and other fruit moths (unpublished data). The presented results on the persistence of rape seed oil and entomopathogenic fungi demonstrate that both, oil and *I. fumosoroseus*, have a long persistence under semi-field conditions over the cocooning period of the first and second generation of the plum fruit moth. For both treatments, the consistency of results over the time was better when mixed with the substrate instead of spraying on the substrate. Submerged spores were as good as conidia but have the important advantage that they can easily be produced in large scale. Further field experiments have to be carried out to proof whether oil and *I. fumosorosea* can be integrated in a control strategy for control of e.g. *C. pomonella*, *C, funebrana*, *C. molesta* or *Eupoecilia ambiguella*.

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Stephan, D. & Herker, M. (2011) Artificial hideouts with entomopathogenic fungi: A strategy for biological control of the plum fruit moth *Cydia funebrana*? IOBC/wprs Bulletin, 66: pp. 175-178.

# Apricot susceptibility to blossom brown rot (*Monilinia* spp.) and leaf rust (*Tranzschelia* spp.) under low-input production system

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## Abstract

*Disease-susceptibility of apricot cultivars is a key-stone towards low-input production systems. Among the main diseases, the susceptibility to* Monilinia *spp. and leaf rust of apricot cultivars are economically important but rarely assessed. A 5-years study in two sites was conducted to assess the susceptibilities to* Monilinia *spp. and leaf rust of 16 apricot cultivars under natural conditions (no inoculation). No fungicide and insecticide were applied during the experiment. In Torreilles site, no significant* Monilinia *spp. was observed. In Gotheron site, the percentage of shoots necrosed by* Monilinia *spp. ranged from 13% to 100% during 2010-2011. A Genotype x Environment interaction was observed for* Monilinia *spp. damages. Concurrently a high variability to leaf rust was observed in both sites. The ranking of cultivars susceptibility to leaf rust was similar between both sites.*

**Keywords:** Apricot, cultivar, susceptibility, *Tranzschelia* spp., *Monilinia* spp.

## Introduction

Production regularity in organic apricot orchards is highly constrained by blossom brown rot caused by *Monilinia* spp. infections on flowers. When the climatic conditions for *Monilinia* spp. infections are gathered during bloom, the development of this disease induces flowers decay and, in some cases, shoots necroses. Apricot leaf rust is caused by *Tranzschelia pruni-spinosae* or *Tranzschelia discolour*. This disease provokes brown leaves spots on the lower side of leaves and colour fading spots on the upper side of leaves. Rust development can induce premature defoliation, which is detrimental to tree vigour and production regularity. Copper-based treatments are partially efficient against the control of *Monilinia* spp. and leaf rust, but their negative environmental impact should be considered. In order to help growers in the choice of low disease-susceptible cultivars, we have assessed the susceptibilities to *Monilinia* spp. and leaf rust of 16 apricot cultivars observed during 5 years in two sites.

## Material and Methods

In 2006, 16 and 12 apricot cultivars were respectively planted in Gotheron experimental station (Saint-Marcel-les-Valence, Drôme, France) and Sica Centrex station (Torreilles, Pyrénées-Orientales, France). 9 cultivars were planted in both sites. In each site, 20 trees per cultivars were planted on *Prunus* rootstock (GF305 in Gotheron, Myrobalan in Torreilles) at a 4 x 4m distance randomly-located in the plot (Mercier *et al*., 2008). No fungicide and insecticide were applied during the study period (2003-2010). Fertilization and weed control were managed according to conventional agriculture practices.

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Shoots infected by *Monilinia* spp. were removed after the observations. The percentage of shoot necrosed by *Monilinia* spp. infections was assessed 100 days after bloom. The severity of leaf rust damages was assessed by one score ranking from 0 (no symptom) to 5 (81 to 100% of leaves infected or fallen). Statistical analyses were computed using Statgraphics plus 5.1 software (Statgraphics plus 5.1, Manugistics, Rockville, MD, USA).

## Results

In Torreilles site, no significant *Monilinia* spp. damage was observed because of dry climatic conditions during bloom (results not shown). In Gotheron site, the climatic conditions in 2007, 2008 and 2009 were favourable to *Monilinia* spp. development for some of the cultivars only (table 1). Conversely, the climatic conditions in 2010 and 2011 were favourable to *Monilinia* spp. development for all the cultivars, which allows a reliable ranking of cultivars' susceptibility. A high variability in *Monilinia* spp. susceptibility was observed: the percentage of shoots necrosed ranged from 13% to 100% (table 1). Bakour was the less susceptible cultivar and Bergarouge® Avirine, Candide and Frisson were the more susceptible ones. The probability for cultivars to be exposed to *Monilinia* spp. infection during bloom varied according to years. The increase of damages severity between 2010 and 2011 could be explained by the climatic conditions more favourable to *Monilinia* spp. development in 2011. Because shoots infected by *Monilinia* spp. were pruned out after assessment, a cumulative effect of the *Monilinia* spp. inoculums might not occurred.

Table 1: Mean percentage of shoot necrosed by *Monilinia* spp. in Gotheron site. Light grey cell indicates that the humectation period was less than 4 hours during bloom; dark grey cell indicates no humectation during bloom. (a): adjusted means after ANOVA. Values followed by different letters are significantly different ($P$<0.05) according to Newman-Keuls test.

| Cultivars | 2007 | 2008 | 2009 | 2010 | 2011 | Mean 2010-2011 [(a)] |
|---|---|---|---|---|---|---|
| **Bakour** (2137) | - | 6 | 0 | 3 | 23 | 13 A |
| **Goldrich** (2184) | 36 | 9 | 8 | 9 | 48 | 28 B |
| **TomCot**® (2669) Toyaco | 22 | 12 | 11 | 19 | 40 | 30 B |
| **Malice**® (2241) Avikot | 3 | 22 | 7 | 14 | 54 | 33 B |
| **Polonais** (1352) | 48 | 18 | 2 | 33 | 65 | 48 C |
| **Hargrand** (1814) | 4 | 18 | 9 | 31 | 72 | 50 C |
| **A4034** | - | 15 | 4 | 45 | 72 | 58 CD |
| **Early Blush**® (2938) Rutbhart | - | 25 | 16 | 40 | 83 | 60 CD |
| **Canino** (1343) | - | 30 | 16 | 52 | 74 | 63 CD |
| **Vertige** (3845) | 6 | 30 | 28 | 53 | 84 | 68 D |
| **Orangered**® (2892) Bhart | 1 | 17 | 20 | 53 | 87 | 70 D |
| **Bergeron** (660) | 2 | 32 | 7 | 68 | 70 | 72 D |
| **Tardif deTain** (2490) | 1 | 31 | 16 | 65 | 78 | 72 D |
| **Bergarouge**® (2914) Avirine | 15 | 28 | 40 | 85 | 89 | 89 E |
| **Candide** (4025) | - | 21 | 19 | 93 | 95 | 96 E |
| **Frisson** (2821) | 1 | 36 | 31 | 91 | 95 | 100 E |

Leaf rust damages were observed in 2008 and 2009 in Gotheron site and in 2008, 2009 and 2010 in Torreilles site (table 2). A high variability to leaf rust was observed in both sites: mean scores range from 1.11 to 4.39 in Gotheron site and from 2.25 to 4.89 in Torreilles site. The ranking of cultivars susceptibility to leaf rust is similar between both sites.

Table 3: Mean severity score of leaf rust damages observed during 2008-2009 in Gotheron site and 2008-2010 in Torreilles site. Score scale: 0 = no symptom; 1 = 1-20% infected or fallen leaves 2 = 21-40%; 3 = 41-60%; 4 = 61-80% and 5 = 81-100%. Values are adjusted means after ANOVA. Values in the same column followed by different letters are significantly different ($P$<0.05) according to Newman-Keuls test.

| **Cultivars** | **Gotheron** | **Torreilles** |
|---|---|---|
| **Hargrand** (1814) | 1.11 A | 2.25 B |
| **Orangered**® Bhart (2892) | 1.35 AB | 1.43 A |
| **Early Blush**® Ruthbart (2928) | 1.58 B | 2.59 BC |
| **Vertige** (3845) | 2.00 C | 2.80 C |
| **Bergarouge**® Avirine (2914) | 2.05 CD | 2.48 BC |
| **Tom Cot**® Toyaco (2669) | 2.75 FG | 3.79 D |
| **Frisson** (2821) | 3.06 GH | 3.78 D |
| **Tardif de Tain** (2490) | 3.47 I | 4.52 E |
| **Bergeron** (660) | 4.39 J | 4.89 F |

## Discussion

Most of the common commercial cultivars assessed in this study (such as Bergeron) have an intermediate to high susceptibility to both diseases. A high susceptibility of Bergeron cultivar was also demonstrated in Hungary (Holb *et al.*, 2006). Some studies have investigated the susceptibility of apricot cultivars thanks to artificial infections of *Monilia laxa* (e.g. Trandafirescu & Teodorescu, 2006). However, studies conducted under natural conditions are still rare.

There is a need to further identify low disease-susceptible cultivars:

- to help growers in their choice of apricot cultivars. Because cultivars rarely combine low susceptibilities to all diseases, the cultivar choice will be motivated according to the most severe diseases observed in their production area.
- to provide reliable scientific data for breeding programs. One of the biggest challenge of apricot breeding concerns disease resistance (Bassi and Audergon, 2006). Genotype x Environment interaction makes the assessment of cultivars susceptibility a hard task.

The effect of the design (e.g. block *vs.* random) and the management (e.g. no disease control *vs.* disease control) of experimental plots on susceptibility assessment needs to be carefully considered.

## Acknowledgements

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# Compatibility of apricot cultivars with rootstocks Krymsk-1 and Krymsk-2

A. Skřivanová[1], R. Vávra[1], H. Drahošová[1], B. Krška[2], I. Ondrášek[2]

**Abstract**

*The objective of this study was evaluation of apricot genotypes compatibility to different rootstocks. Rootstocks Krymsk-1 (Prunus tomentosa x Prunus cerasifera) and Krymsk-2 (Prunus incana x Prunus tomentosa) were included in this study and compared with rootstocks MRS 2/5, St. Julien and apricot seedling MHL2. Apricot cultivars ´Betinka´, ´Darina´, ´Harcot´, ´Helena du Roussillon´, ´Lescora´, ´Kompakta´, ´Radka´ and hybrid ´LE-2927´ were evaluated in the trial. Total length of annual growth and trunk cross section area of tested cultivars were recorded in the nursery field at the end of growing season in the year 2010. Also the number of survival grafted plants was recorded on rootstocks Krymsk-1 and Krymsk-2 in this year of evaluation. In the year 2011 the behaviour of cultivars Betinka´, ´Darina´, ´Harcot´, ´Helena du Roussillon´, ´Lescora´, ´Kompakta´, ´Radka´ and hybrid ´LE-2927´ was evaluated on rootstocks Krymsk-1, Krymsk-2, MRS 2/5, St. Julien and apricot seedling MHL2. Survival rate of grafted genotypes ´Betinka´ and ´LE-2927´ was higher on the rootstock Krymsk-1 (84 %) in comparison with the rootstock Krymsk-2 (77 % respective 63 %).*

**Key words:** rootstocks, compatibility, Krymsk-1, Krymsk-2, *Prunus armeniaca*

## Introduction

The most significant characters which must be involved in selection of apricot rootstocks are ability to propagation, anchorage, growth rate, uniformity and compatibility, the environment adaptability, disposition for a formation of root suckers, the influence on precocity of bearing after planting and on yield. The extensive approach to rootstock breeding which mainly consisted in a selection from spontaneous natural resources (especially generatively propagated rootstocks) has been changed in purposeful rootstock breeding for different conditions. A remarkable amount of rootstocks with vegetative propagation has also been bred and introduced to practice in latest years (Loreti, 1994; Loreti, 1997; Reighard, 2000; Moreno, 2004; Layne, 1987; Okie, 1987). A lot of varieties of *Prunus* genus were utilized in the rootstock breeding. But the number of species which the most used rootstocks for apricots come from is not large. They are especially species *Prunus armeniaca* L., *Prunus cerasifera* Ehrh., *Prunus persica* L., *Prunus insititia* L. and *Prunus domestica* L. Rootstocks formed by interspecific hybridization whose application depends only on vegetative propagation are increasing (Vachun, 1995). The rootstock has a significant influence on plant decline percentage. The character of influence on a grafted variety is considerable with rootstock health, especially from the point of view of compatibility, growth-rate and apricot yield. Rootstocks Krymsk-1 (*Prunus tomentosa x Prunus cerasifera*) and Krymsk-2 (*Prunus incana x Prunus tomentosa*) are included in this study and compared with rootstocks MRS 2/5, St. Julien and apricot seedling MHL2.

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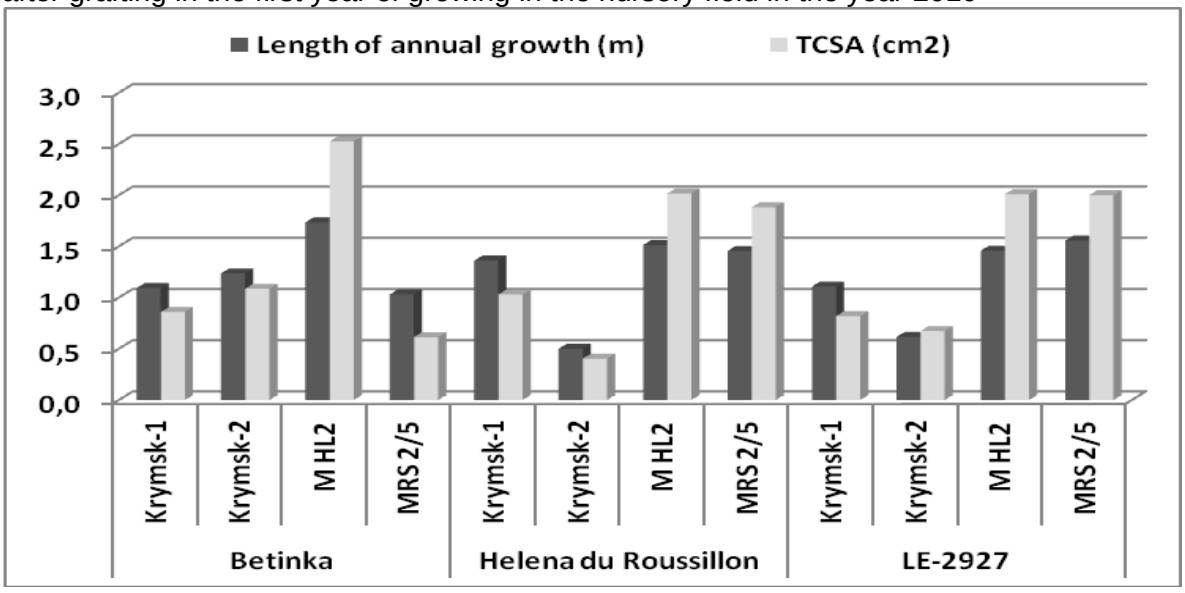
## Material and methods

Apricot cultivars ´Betinka´, ´Darina´, ´Harcot´, ´Helena du Roussillon´, ´Lescora´, ´Kompakta´, ´Radka´ and hybrid ´LE-2927´ were involved in the trial. Rootstocks Krymsk-1 (*Prunus tomentosa x Prunus cerasifera*), Krymsk-2 (*Prunus incana x Prunus tomentosa*), MRS 2/5, St. Julien and apricot seedling MHL2 were tested. Twenty buds of cultivar ´Betinka´ and hybrid ´LE-2927´ were grafted on tested rootstocks. Cultivar ´Helena du Roussillon´ was put into the trial as a cultivar that is characterized by bad compatibility with common cultivated apricot rootstocks. Total length of annual growth and trunk cross-section area (TCSA) of tested cultivars were evaluated in the nursery field at the end of growing season in the year 2010. Also the number of survival grafted plants was recorded on rootstocks Krymsk-1 and Krymsk-2 in this year of evaluation. In the year 2011 the behaviour of cultivars Betinka´, ´Darina´, ´Harcot´, ´Helena du Roussillon´, ´Lescora´, ´Kompakta´, ´Radka´ and hybrid ´LE-2927´ was evaluated on rootstocks Krymsk-1, Krymsk-2, MRS 2/5, St. Julien and apricot seedling MHL2 after planting on the permanent site. The increase of TCSA of apricot cultivars planted into randomized blocs (totally 12 pieces from each cultivar) on different rootstocks was recorded at the end of growing season. Total height of trees was not recorded due to tree trimming after planting.

## Results

Length of annual growth and trunk cross section area were recorded on evaluated cultivars on different rootstocks in the nursery field. Results are shown in the Figure 1. The most reduced growth and the higher increase of TCSA of the cultivar ´Betinka´ was recorded on the rootstock MRS 2/5 with 59.5 % and 49.3 % of TCSA in comparison with apricot seedling MHL2. In contrary the most reduced growth and TCSA of the hybrid ´LE-2927´ was recorded on rootstock Krymsk-2 with 42.3 % reduction of growth and 59.9 % of reduction of TCSA. Growth of the cultivar ´Helena du Roussillon´ was the most reduced on the rootstock Krymsk-2 (33.2 %) and TCSA was reduced to 81.2 % in comparison to growth on apricot seedling MHL2.

Figure 1: Length of annual growth and trunk cross-section area of one year old apricot cultivars after grafting in the first year of growing in the nursery field in the year 2010



Survival of grafted plants is shown in the Figure 2. From the records is evident higher survival rate on the rootstock Krymsk-1 of all three tested genotypes. The survival rate of the cultivar ´Helena du Roussillon´ that is characterized by bad compatibility with common cultivated apricot rootstocks was only 33.3 % on the rootstock Krymsk-2 and 43.5 % on the rootstock Krymsk-1.

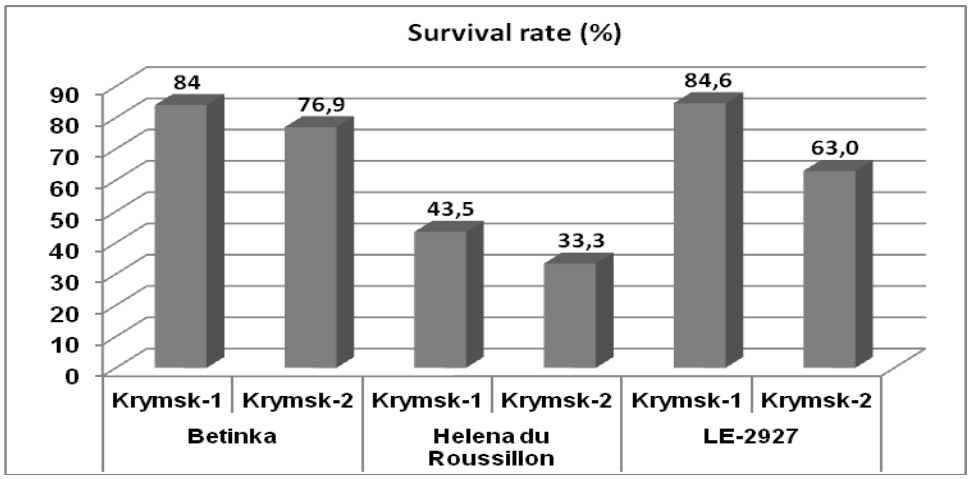



Figure 2: Survival of apricot cultivars after grafting on rootstocks Krymsk-1 and Krymsk-2 in the nursery field in the year 2010

Growth of apricot cultivars on different rootstocks after planting on the permanent site is shown in the Figure 3. On the contrary of the growth in the nursery field where was growth of cultivar Betinka the most reduced on the rootstock MRS 2/5 increasing of TCA on the permanent site in the first year of growing was on this rootstock the highest. Growth of tested cultivars was more vigour on the rootstock MRS 2/5 than on rootstocks St. Julien (none on cultivars ´Helena du Roussillon´and ´Darina´), Krymsk-1 and Krymsk-2. Growth of cultivars ´Darina´, ´Harcot´, ´Kompakta´, ´Lescora´ and ´Radka´ was evaluated only on rootstocks S. Julien and MRS 2/5.

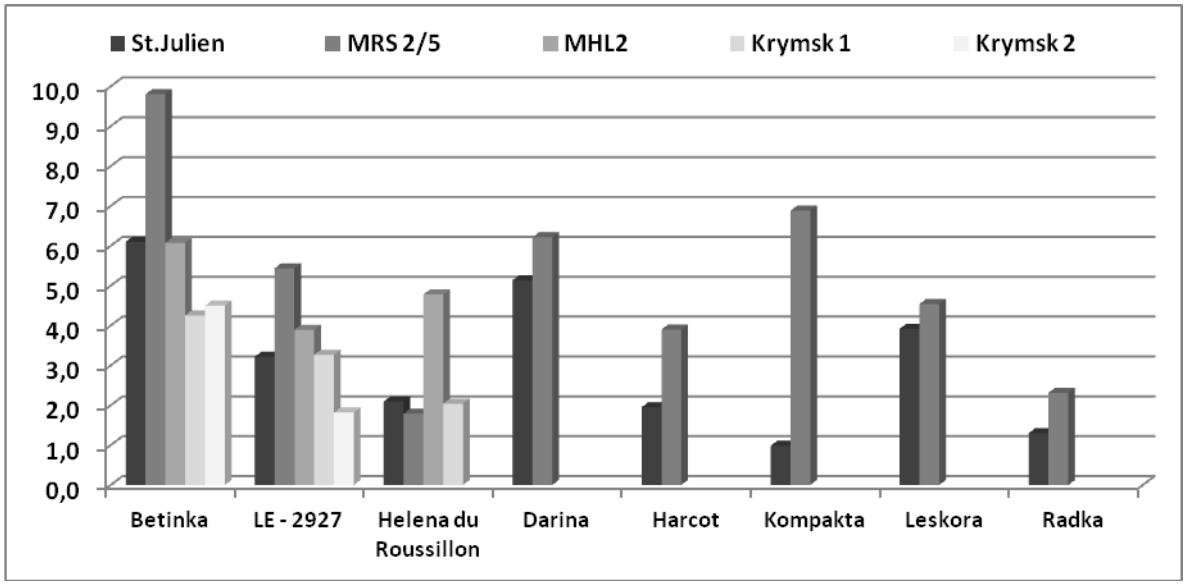



Figure 3: The increase of trunk cross-sectional area of apricot cultivars grafted on different rootstocks in the first year of growing on the permanent site (in $cm^2$)

## Discussion

The consumers request apricot fruits with high quality and nutritional value but also fruits from organic production without residues of chemicals. As the result of this situation the organic apricot production has been established and is flourishing. The healthy apricot trees on perspective rootstocks help to get successful results in this field. Tested rootstocks Krymsk-1 (*Prunus tomentosa x Prunus cerasifera*) and Krymsk-2 (*Prunus incana x Prunus tomentosa*) are challenge to increase health of apricot trees and to depress economic losses due to high tree decline also in organic orchard. The compatibility of tested cultivars shows different survival rate of grafted cultivars on those rootstocks. The growth of trees on rootstocks Krymsk-1 and Krymsk-2 is decreased and therefore these rootstocks are highly recommended into new-fashioned high tree density orchards that are suitable also for organic farming.

## Acknowledgements

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# Control of brown rot blossom blight (*Monilinia laxa*) on apricots – preliminary results

V. Psota[1], M. Bagar[1], P. Ackermann[2] and M. Schovánek[3]

**Abstract**

*During spring 2011, the effect of copper, sulphur, sodium bicarbonate, lime sulphur and plant resistance improver based on algae extract (Alginure) on brown rot blossom blight on apricots was studied. The small-plot trial was conducted in the commercial apricot orchard (variety Pincot) located in South Moravia (Czech Republic). Selected preparations were sprayed three times during flowering (April 7, 11 and 14). Evaluation was done on May 6 according to the EPPO 1/38(2) method. All treated variants were significantly different from the untreated control (F 6.3191, P 0.000264). The most effective was copper 66 % following by sodium bicarbonate 63 %, Alginure 63 %, sulphur 56 % and lime sulphur 38 %. Alginure achieved 65% efficacy in semi-field trial in the same location.*

**Keywords:** apricot, *Monilinia laxa*, brown rot blossom blight, organic agriculture

## Introduction

The causal agent of brown rot blossom blight in stone fruits is fungus *Monilinia laxa*. In spring it invades blossoms, mycelium then grows to the ovary and through the stalk to the wood (Hluchý *et al.,* 2008). Rainy and cold periods are ideal weather for this pathogen development (Holb *et al.,* 2006). Under these conditions *Monilinia laxa* can destroy a considerable part of blossoms (Gouramanis, 1999). Apricot is a thermophilic plant and the Czech Republic lies on the northern border of its growing. In spring the ideal conditions for the brown rot blossom blight development often occur here. Apricots in the Czech Republic are grown on 1200 ha, 426 ha of which are under the organic growing regime (Buchtová, 2011). For conventional and integrated production two effective substances, tebukonazol and bitertanol, are permitted (SRS, 2011). Control of brown rot blossom blight under the organic regime has not been studied yet in the Czech Republic. Therefore, in 2011, we started to test efficiency of the selected preparations permitted in organic agriculture.

## Material and Methods

In 2011 a small plot experiment was established in a commercial apricot orchard in the locality of Kobylí (south Moravia, Czech Republic) where the variety Pinkot has been grown. Totally five preparations were tested (Table 1). Each variant had 3 replications (4 trees). The preparations were applied with respect to the course of weather (Figure 1 and 2) and flowering on 7/4 (BBCH 64), 11/4 (BBCH 65) and 14/4 (BBCH 66).

In addition, a semi-field experiment was conducted in the same locality with the plant resistance improver Alginure in the dose of 5 l/ha. In case of semi-field trial Alginure was applied on 1 ha block (variety Pinkot).

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Both small plot and semi-field experiments were assessed according to the EPPO 1/38(2) method on May 5. Statistical evaluations were performed using the analysis of variance and Tukey's test (α 0.05). The efficacy was determined according to Abbott's formula.

Table 1: Selected fungicides and applied doses

| Preparation | Active ingredient | Dose |
|---|---|---|
| Alginure | algae extract 24%, plant aminoacids 7%, phosphates 20% | 5 l/ha |
| Kocide 2000 | Copper (53.8% of pure copper) | 0.35 kg/ha |
| Kumulus WG | Sulfur (80% of poor sulfur) | 3 kg/ha |
| VitiSan | Potassium hydrogen bicarbonate | 8 kg/ha |
| Polisenio | Lime sulphur (380 g/l) | 1% |

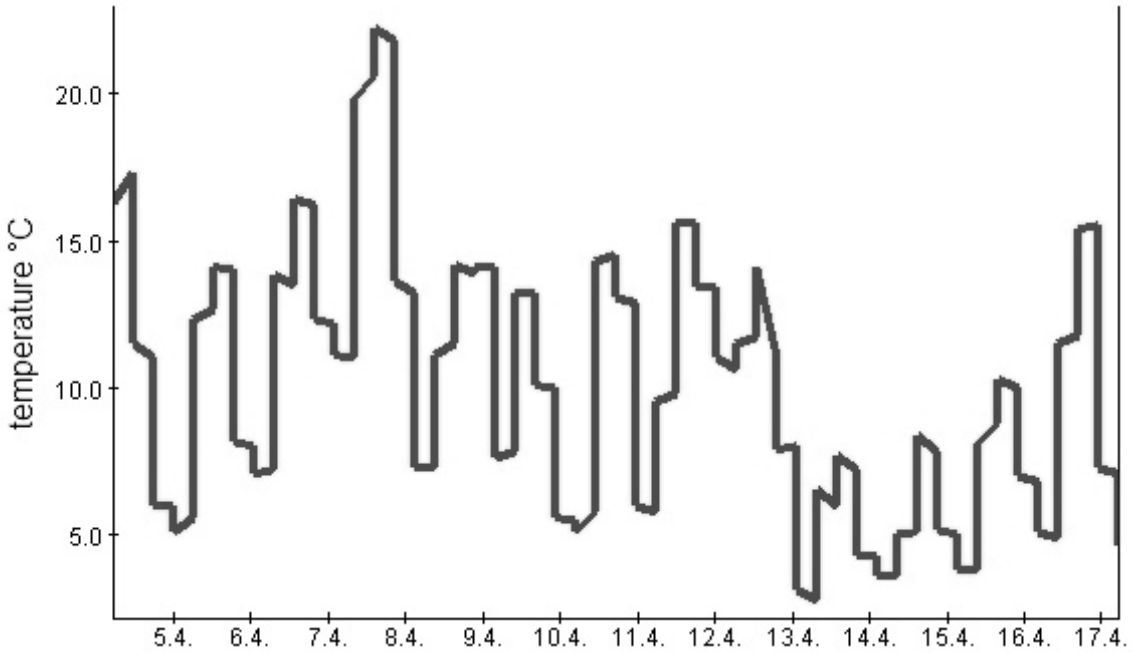


Figure 1: Course of temperatures in the selected locality of Kobylí from 4/4 to 17/4

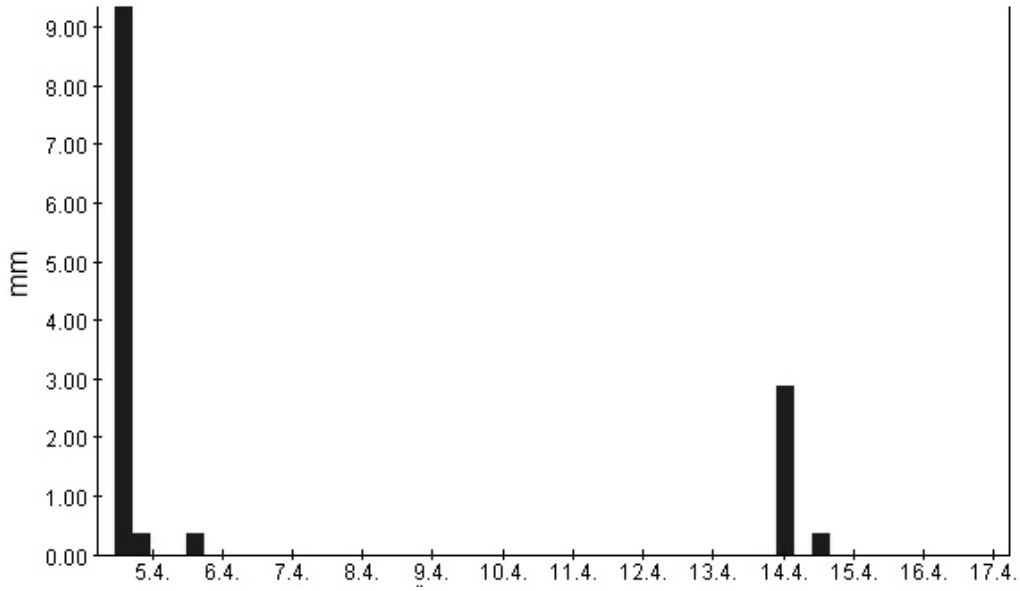

Figure 1: Rainfalls from 4/4 to 17/4 in the selected locality of Kobylí.

**Results**

In the small-plot trial, all treated variants differed significantly from the non-treated control (F 6.3191; P 0.000264). The infestation and efficacies determined in the individual variants are given in Table 2.

Table 2: The infestation and efficacies determined in the individual variants.

| Variant | Average number of shoots infested with brown rot blossom blight in one tree | Efficacy according to Abbott % |
|---|---|---|
| Alginure | 8.86A | 63.34 |
| Kocide 2000 | 8.14A | 65.82 |
| Kumulus WG | 10.57A | 56.21 |
| VitiSan | 8.86A | 63.34 |
| Polisenio | 14.86AB | 38.48 |
| Non-treated control | 24.14B | |

In the semi-field trial, the efficacy of the variant Alginure was 65 % according to Abbott. This variant also differed significantly from the non-treated control (F 4.3195, P 0.007767).

**Discussion**

All the tested preparations significantly lowered the infestation of apricots shoots with brown rot blossom blight. The highest efficiency was achieved with the preparations Alginure and Kocide 2000 (copper). While the efficiency of copper against this pathogen had been confirmed previously (Holb, 2006), application of Alginure against brown rot in apricots was tested for the first time.

The lower efficiency of the fungicide Kumulus WG (sulphur) was probably caused by lower temperatures during the experiment (Figure 1). Generally, the effectiveness of sulphur-based fungicides is lower at temperatures below 15 °C. In Hungary where the weather is warmer, the system against brown rot blossoms blight in cherries based on sulphur and lime sulphur was formed (Holb, 2005).

Results acquired in the scope of this experiment will be used for the further development of brown rot blossoms blight control in apricots in the Czech Republic.

**Acknowledgements**

The authors thank Dr. Tomáš Litschmann (www.amet.cz) for provided data about the course of weather.

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SRS, (2011): State Phytosanitary Administration – list of plant protection products. Available at: http://eagri.cz/public/app/eagriapp/POR/

# Responses of apricot genotypes to brown fruit rot (*Monilinia* spp.) to artificial and natural infections

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**Abstract**

*The objective of this study was evaluation of apricot genotypes resistance to brown fruit rot causing by fungi from Monilinia species. Totally 46 apricot cultivars and hybrids were evaluated in the three growing seasons from the year 2009 to the year 2011. In the year 2009 lower fruit brown rot injury was recorded after artificial infection on fruits of cultivars ´Goldrich´, ´Harcot´, ´Harogem´ and hybrid ´NPL 13/77´. In contrary high injury was recorded on fruits of genotypes ´Lou Tuo´, ´Sundrop´, ´NPL 14/35´ and ´NPL 13/151´. Low rots were recorded on fruits of genotypes ´Sundrop´ and ´NPL 13/70´ after natural infection, sensitivity to fruit rots was recorded on cultivars ´Velkopavlovická´ and ´Lou Tuo´ to this infection. In the year 2010 higher resistance to brown fruit rot after artificial infection by fungi from Monilinia ssp. showed cultivars ´Harogem´, ´Harlayne´, ´Harval´ and ´Darina´, in contrary lower showed cultivars ´Freda´, ´Veharda´ and ´Kompakta´. Cultivar ´Harlayne´ were evaluated as the most resistant, cultivar ´Freda´ as the most sensitive after natural infection. As resistant to brown fruit rot were in the year 2011 evaluated hybrid ´M 52´ and cultivar ´Betinka´, on the contrary the most sensitive were evaluated cultivars ´Candela´, ´Roxana´ and ´Veecot´ both after artificial and natural infection by fungi from Monilinia spp. Results showed differences among tested apricot cultivars and hybrids to infection by fungi from Monilinia spp. both after artificial inoculation and in conditions of natural infection.*

**Key words:** *Monilinia laxa*, *Monilinia fructigena*, brown fruit rot, *Monilinia* spp.

## Introduction

Apricot (*Prunus armeniaca*) can be damaged by a wide range of specific pathogens which can destroy both the yield and tree health or life. Therefore the study of apricot resistance to these pathogens is the major objective of the breeding program carried out at the research stations in lots of countries (Gutermuth et al., 2010). Brown fruit rot is serious fungal disease of apricot causing extensive economic losses in many growing areas in Europe (Wormald, 1954; Byrde & Willets, 1977; Batra, 1991; Holb, 2004a). Casual agent are fungi from *Monilinia* species: *Monilinia laxa* (Aderh. et Ruhl) Honey ex Dennis and *Monilinia fructigena* (Schröt. ex Aderh. et Ruhl) Honey ex Dennis (Batra, 1991). Potential casual agent of brown fruit rot could be another two closely related fungi *Monilinia fructicola* with origin in North America and *Monilinia polystroma* with origin in Asia. *M. laxa* tends to prevail upon vegetation organs therefore attacking twigs, leaves, shoots and flowers causing blossom blight, whilst *M. fructigena* is more common on fruits. Total amount of the pathogen spores and precipitation during blooming significantly influence infection of flowers (Luo and Michailides, 2001). Rainy and warm weather in harvesting time increase development of fruit rot whereas when weather conditions were dry and hot brown rot incidence of fruits is lower (Drén et al., 2007).

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*M. laxa* attacks flowers that dry and turn brown, the leaves wither and dry, remaining on the branches which, if infected, show brown concavities and then fissures from which comes rubber out. The fruits can be attacked both at the start of development and once mature. In both cases, they rot and become subsequently covered in mould. Infected mature fruits ‘mummify', i.e. they dry out, decrease in volume remaining on branches. Good preventive practice includes reducing potential sources of contagion, pruning the affected branches and removing the mummified fruits that remain on the plant. The collected material should be destroyed in early spring (Leeuwen et al., 2000, 2002). In hybrid offspring of parental combinations is possible evaluate resistant selections (Cociu et al., 1991; Nicotra et al., 2006). The potential apricot cultivars resistant to brown fruit rot were recorded in this trial. The results showed variation of the evaluated cultivars with resistance to infection by fungi from *Monilinia* spp. both after artificial inoculation and in conditions of natural infection.

## Material and methods

Totally 46 apricot cultivars and hybrids were evaluated in the three growing seasons from the year 2009 to the year 2011 to brown rot resistance both after artificial and nature infection by fungi from *Monilinia* spp. Tested cultivars and hybrids were collected in the experimental orchards of the Research and Breeding Institution of Pomology Holovousy Ltd. and the Mendel University in Brno. Infections by fungi from *Monilinia* spp. during blossom didn´t show any resistance differences among cultivars and hybrids. Artificial infections of blossom were conducted in a special moister chamber with high relative humidity (near 100 %). Very high blossom injury was recorded among all tested cultivars and hybrids (from 90 to 100 % of blossom). For this reason the evaluation of resistance was focused only to fruit brown rot after infection by fungi from *Monilinia* spp. Inoculum for artificial infection was prepared by washing of spores from mummified fruits and was applied by a micropipette to the surface of fruits. By the sterile needle a flat injury of the fruit peel were done at the place of infection. The number of spores was adjusted under the microscope in the Bürker´s chamber by dilution with water to $4x10^4$ spores in 1 ml. Samples of fruits were gathered from the experimental orchards which were every year treated by copper in early spring and by fungicide Sporgon during blooming to avoid blossom blight. In the years 2009 and 2010 two fungicides (Baycor, Talent) were applied against to brown fruit rot before harvesting due to high precipitations. In the sample 10 fruits of each cultivar respective hybrid were artificially infected and 10 fruits were observed with nature infection by fungi from *Monilinia* spp. Samples of apricots were placed in storage room with temperature 5 – 8°C. Every week was recorded number of healthy fruits and rotted fruits. The size of fruit rots were measured in diameter and the injury was evaluated according the scale 0 – 9 (0 – no visible symptoms, 1: up to 5 mm; 2: 5 – 10 mm; 3: 10 – 15 mm; 4: 15- 20 mm; 5: 20-25 mm; 6: 25 – 30 mm; 7: 30 – 35 mm; 8: 35 – 40 mm; 9: > 40 mm in diameter). Morphological variation of fungi was not distinguish, the injury was evaluated as rot infection by fungi from *Monilinia* spp. without detection whether causal agent is fungi *M. fructigena*, *M. laxa* or alternatively related fungi. Other fungal diseases and mould were not involved into the evaluation.

## Results

Year 2009:

Totally 21 samples of cultivars and hybrids collected in the experimental orchards in the Research and Breeding Institution of Pomology Holovousy Ltd. (RBIP Holovousy Ltd.) were involved into the evaluation this year. Samples of genotypes ´Goldrich´, ´Harcot´, ´Sundrop´ and ´NPL 14/172´ were collected from two locations of the experimental orchard

and marked as I respective II. Samples were harvested according fruit ripening from July 21st to August 1st and evaluated on subsequent five weeks. Results are shown in the Figure 1. Low fruit brown rot injury was recorded after artificial infection on fruits of cultivars ´Goldrich´ I, ´Harcot´ I, ´Harogem´ and hybrid ´NPL 13/77´. In contrary high injury was recorded on fruits of genotypes ´Lou Tuo´, ´Sundrop´, ´NPL 14/35´ and ´NPL 13/151´. Low rots were recorded on fruits of genotypes ´Sundrop´ and ´NPL 13/70´ after natural infection, sensitivity to fruit rot was recorded on cultivars ´Velkopavlovická´ and ´Lou Tuo´ to this infection.

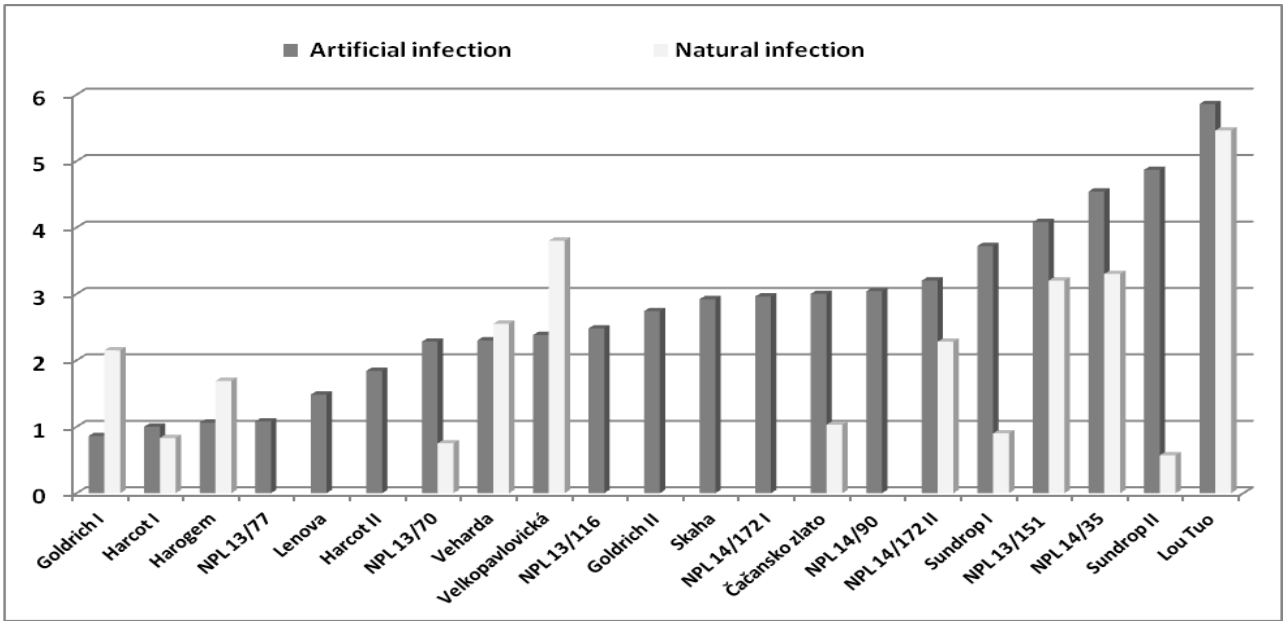


Figure 1: Response of apricot genotypes to brown fruit rot after infection by fungi from *Monilinia* spp. in the year 2009 (scale 0 – 9).

Year 2010:

Totally 16 samples of cultivars and hybrids from the experimental orchard in the RIBP Holovousy Ltd. were involved into the evaluation this year. Samples were harvested according fruit ripening from July 27th to August 11th and evaluated on subsequent five weeks. Cultivars ´Goldrich´, ´Harogem´ and ´Veharda´ were collected from different locations of the experimental orchard and samples were marked as I, II respective III. Results are summarized in the Figure 2.
Higher resistance to brown fruit rot after artificial infection by fungi from *Monilinia* spp. showed cultivars ´Harogem´, ´Harlayne´, ´Harval´ and ´Darina´ in contrary lower showed cultivars ´Freda´, ´Veharda´ and ´Kompakta´. The cultivar ´Harlayne´ were evaluated as the most resistant and on the contrary the cultivar ´Freda´ as the most sensitive after natural infection.

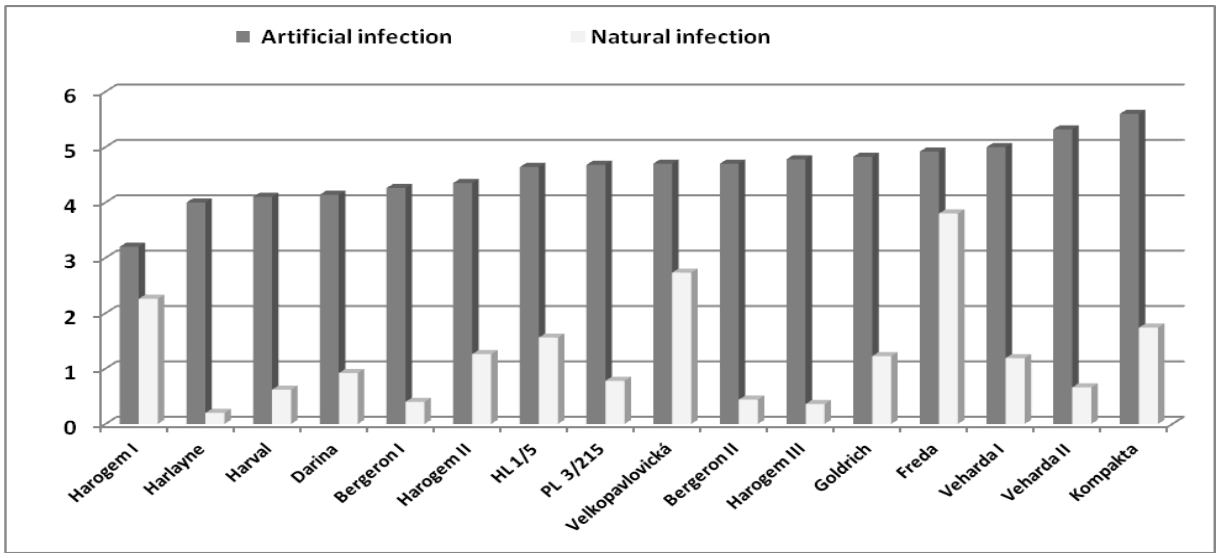


Figure 2: Response of apricot genotypes to brown fruit rot after infection by fungi from *Monilinia* spp. in the year 2010 (scale 0 – 9).

Year 2011:

Severe spring frost totally damaged crop of apricots in the orchards of RIBP Holovousy Ltd. in this year. Therefore cultivars and hybrids for testing were collected in the other place of the Czech Republic situated in southern Moravia. Fruits from experimental orchards in location Lednice of the Mendel University in Brno were transported and subsequently tested. Totally 26 samples were involved into the evaluation in this year that were harvested from July 14$^{th}$ to 27$^{th}$. The results are presented in the Figure 3. Hybrid ´M 52´ was harvested in two terms of maturity July 14$^{th}$ and 21$^{th}$. Early harvested sample marked as ´M 52´ (I) shown less susceptibility than the late harvested one marked as ´M 52´ (II). This proved that more matured and soft fruits are more susceptible to infection with fungi from *Monilinia* spp. (Michailides et al., 2000) than less matured. As tolerant to brown fruit rot were recorded hybrid ´M 52´ and cultivar ´Betinka´, on the contrary the most sensitive cultivars were evaluated cultivars ´Candela´, ´Roxana´ and ´Veecot´ both after artificial and natural infection by fungi from *Monilinia* spp.

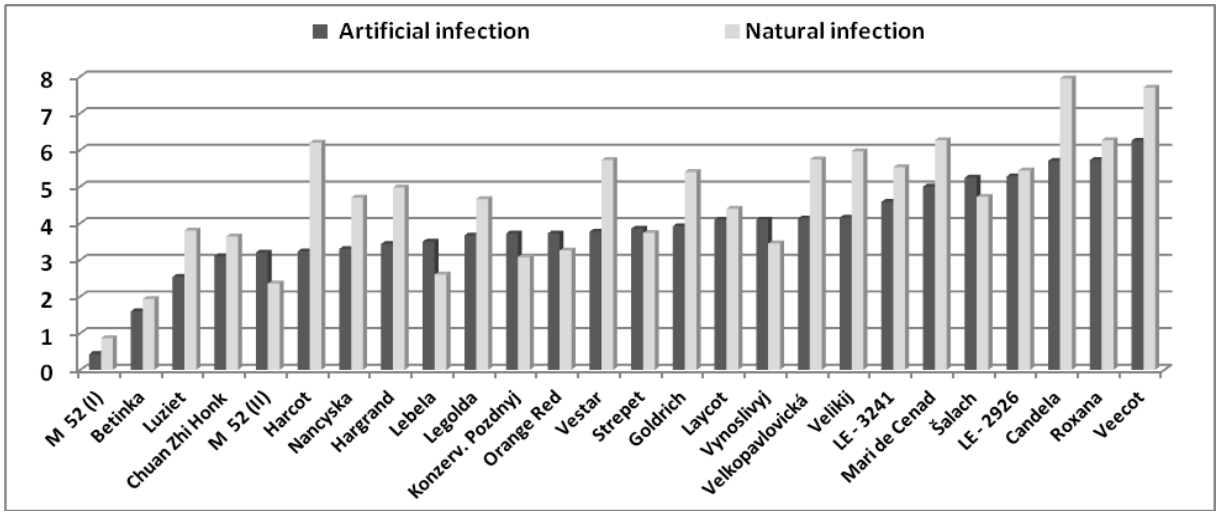


Figure 3: Response of apricot genotypes to brown fruit rot after infection by fungi from *Monilinia* spp. in the year 2011(scale 0 – 9).

## Discussion

Results showed differences among tested cultivars and hybrids to infection by fungi from *Monilinia* spp. both after artificial inoculation and in conditions of natural infection. This knowledge is very important especially for growers with organic apricot production due to impossibility to use chemical fungicides. From evaluations of rots in individual years are evident differences in fruit injury after natural infections by fungi *Monilinia* spp. In rainy season of the years 2009 a 2010 were two treatments of fungicides applied (May 4$^{th}$ and June 10$^{th}$ respective May 21$^{th}$ and June 4$^{th}$). These treatments depressed incidence of brown fruit rot in the years 2009 and 2010 compared to the year 2011 with untreated samples after blooming. Infection of flowers causing blossom blight has to be solved by treatment by copper (or chemical fungicides in IP) during tree blooming. The new cultivar ´Betinka´ and the hybrid marked ´M 52´ that show high tolerance to infection by fungi from *Monilinia* spp. rise to the challenge to depress economic losses of brown fruit rots in organic apricot production.

## Acknowledgements

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# New results from testing of different Sour cherry cultivars under organic cultivation

B. Pfeiffer[1], H. Rank [2] , Schmückle-Tränkle[1]

**Abstract**

*At the LVWO Weinsberg 30 different sour cherry cultivars were tested under organic cultivation methods. At Dresden-Pillnitz 11 similar cultivars have been evaluated since 2007, as part of a trial with more sites overall in Germany. Data about the phenology (bud breaking, duration and intensity of blossom), yield and loss of fruits were collected. Susceptibilities for infections by* Monilia sp*. and* Blumeriella jaapii *were assessed. Great differences between the cultivars under organic conditions were found from 2007 to 2011 depending on the weather in each year. 2009 rain during blossom caused in Weinsberg heavy damages by* Monilia sp.*, depending from the cultivar. 2010 still effects of this Monilia-infection influenced the yield, 2011 a very late frost at the beginning of May reduced the yield of the most cultivars. So in Weinsberg the summarized yield over all years was heavily influenced by the year 2011, best for 'Rubellit', 'Hartei', 'Safir', 'Jade', 'Ujfehertoi Fürtös' and 'Achat'. Not for all cultivars the estimation was the same in Dresden-Pillnitz and in Weinsberg, depending on the different weather conditions. This research work is part of the project "Evaluation and Optimizing of biological control methods of plum moth (*Cydia funeb*rana) and Monilia-disease in organic stone fruit production", granted by the Bundesprogramm Ökologischer Landbau und Nachhaltigkeit (FuE 06OE198).*

**Keywords:** Sour cherry, cultivars, *Monilia sp., Blumeriella jaapii,* organic cultivation

## Introduction

Infections by *Monilia laxa* H. during blossom cause great losses in yield in organic sour cherry orchards, where often still the cultivar 'Schattenmorelle' is grown. Experiments about blossom sprayings to prevent infections by *Monilia sp.* without use of copper showed often non-uniform results. Therefore it is necessary to proof newer cultivars under the conditions of organic fruit growing. Important characteristics are type of growing, susceptibilities for *Monilia sp.* and *Blumeriella jaapii* R., flowering behaviour, yield, taste and suitability for fresh market or production of juice.

## Material and Methods

Details about the tested cultivars, plant year and rootstock are described in table 1 for the organic grown trials in Weinsberg and Dresden-Pillnitz. The fertilization in Weinsberg e. g. was done with horn shavings in combination with foliar fertilizer Wuxal Aminoplant. In spring the trees were treated with Neemazal ®TS (if necessary) and preparations based on *Bacillus thuringiensis.* Beginning in 2007 in Weinsberg only few applications per year were done with wetting sulphur to prevent extreme infections by leaf spot, but not during the long time of harvest of the different cultivars. Data about the phenology (bud breaking, duration and intensity of blossom), yield and loss of fruits and growing type were collected.

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Susceptibilities for infections by *Monilia laxa* and *Blumeriella jaapii* and differences in the occurrence of aphids at end of June were assessed. In Dresden-Pillnitz the trees were planted with a different distance, the first branches are 80 cm high for mechanical harvesting. One part of the plot is not sprayed with organic plant protection agents. Average temperature per year in Dresden is about 0,8 °C lower than in Weinsberg resp. Heuchlingen, rainfall is comparable. In Dresden the climate is more continental, the winters are colder, vegetation period starts about 10 days later.

Table 1: Ripening groups of the tested cultivars (harvest data from Weinsberg)

| Site/Rootstock/plant year, distance, number of trees | early | middle | late |
|---|---|---|---|
| | last third of June | beginning of July | middle of July |
| Weinsberg, Piku 3<br>December 2004<br>5 m x 5 m<br>3 trees/cultivar | 'Ludwigs Frühe'<br>'Röhrigs Weichsel'<br>'Ciganymeggy 7'<br>'Erdi Nagygümölesü'<br>'Korai Pipacsmeggy'<br>'Favorit'<br>'Achat'<br>'Ciganymeggy 59' | 'Debreceni Bötermö'<br>'Oblacinska'<br>'Ujfehertoi Fürtös'<br>'Kantorjanosi', 'Jade'<br>'Schukowskaja'<br>'Morina', 'Topas'<br>'Malike emleke'<br>'Csengödi' | 'Hartei'<br>'Rubellit'<br>'Vowi'<br>'Schattenmorelle'<br><br>'Pitic de Jasi')<br>(end of July |
| Weinsberg, Colt, April 2006, 5 m x 5 m, 23 trees | | 'Safir' | |
| Weinsberg,*Prunus avium*<br>grafted in spring 2007,<br>5 m x 2.5 m,<br>6 resp. 15 trees/cultivar. | | 'Morina', 'Karneol'<br>'Pi-Sa 12,100'<br>'Pisa-13,1222' = Coralin | 'Vowi' |
| Dresden-Pillnitz, *Prunus avium,* autumn 2007,<br>4.5 m x 2.5 m, plot divided in unsprayed and organic treatments,<br>12 or 14 trees/cultivar | 'Achat' | 'Ujfehertoi Fürtös'<br>'Jade', 'Morina', 'Topas'<br>'Safir', 'Korund'<br>'Pi-Sa 12,100'<br>'Pisa-13,1222' = Coralin | 'Rubellit'<br>'Schattenmorelle' |

For the occurrence of *Monilia laxa* the following scheme was used: All infected twigs and single infected blossom clusters were counted at end of June and were removed from the trees. Every year at end of August the symptoms of *Blumeriella jaapii* were divided in classes from 0 (= no symptoms at all) to 9 (= leaves fell down, tree nearly bare). As a summary from the years 2005, 2006 and 2007-2011 the cultivars were graded into seven groups of susceptibility for *Blumeriella jaapii* (table 3). Similar four groups for susceptibility for *Monilia sp.* were formed (low / middle + good regeneration / middle-high susceptible + moderate regeneration/ high susceptible +bad regeneration) mainly based on the results from 2009. The potential of regeneration from heavier Monilia-infections in the following year (based on a consequent removing of infected twigs and mummies) was influenced the grading of susceptibility for Monilia. Every year the marketable yield and loss per tree were evaluated (Weinsberg), but no statistical tests were done because of the low number of trees per cultivar. Meanwhile only one tree is still alive of 'Schattenmorelle', the standard variety at the moment, the others were damaged by mechanical weed control. In Dresden-Pillnitz in 2011 the trees were harvested by machine for the first time. The contents of sugar and acid of the juice were analysed for selected varieties in 2009 and 2011.

## Results

In table 2 the most striking details about weather, infection risk of diseases and pests and level of yield are described. *Myzus cerasi* F. was watched only in 2011 on a low-middle level in Weinsberg, beneficial insects like ladybirds reduced the numbers of aphids soon.

Table 2: Level of infection-risk of different diseases and pests, frost, striking details from years 2007 to 2011 (Weinsberg)

| **year** | **2007** | **2008** | **2009** | **2010** | **2011** |
|---|---|---|---|---|---|
| frost? | no | no | no | **2 x slight frost at BBCH 54/55, -0.6 °C at beginning of flowering** | **strong late frost at beginning of May, about 2 weeks after blossom** |
| **risk for Monilia** | no | low | **very high** (rain during blossom) | **middle-high**, mainly late flowering varieties | low |
| **leaf spot** | **very high** | **middle-high** | **very high** | middle | **middle-high** |
| reasons for losses of yield | slight hail | strong hail, some *Monilia* on fruits | cracking of fruits, damages by wind and birds | fruit fall, European cherry fruit fly, damages by frictions, birds, *Monilia* on fruits | damages of the fruits by frost, frictions round stalk of cherries, birds, some *Monilia* on fruits |
| further striking details | strong fruitfall after blossom | | depending from variety bad fruit setting (bad weather during blossom) | long wet period in May, late effects from Monilia-infections in 2009, partly strong fruitfall, occasional cherry scab on leaves | strong fruitfall because of frost in May<br>occasional cherry scab on leaves, early leaf fall by leaf spot and frost at end of October |
| **level of yield** | low | low | **middle** | low | **high** |

Table 3: Susceptibilities for *Blumeriella jaapii* (Weinsberg, based on mean values 2007-2011, additional 2005+2006 for the cultivars planted in 2004)

| **Level** | **susceptibility** | **varieties** |
|---|---|---|
| 1-3 | Very low | 'Ludwigs Frühe', , 'Morina', 'Csengödi', 'Korai Pipacsmeggy', , 'Ujfehertoi Fürtös', 'Karneol' |
| 4 | Low-midle | 'Erdi Nagyügümölesü', 'Oblacinska', 'Ciganymeggy 59', 'Kantorjanosi', 'Debreceni Bötermö', 'Schukowskaja', Pi-Sa 12,100, Pi-Sa 13,122 (Coralin) |
| 5 | middle | 'Favorit', 'Rubellit', 'Ciganymeggy 7', 'Jade', 'Safir', 'Hartei', 'Schattenmorelle' |
| 6 | middle to high | 'Röhrigs Weichsel', 'Pitic de Jasi', 'Malike emleke', 'Topas' |
| 7 | high | 'Vowi' |
| 8 | High to very high | , 'Achat' |
| 9 | Nearly all leaves fallen down | |

Leaf spot was evaluated at Dresden-Pillnitz, too, the susceptibilities there were similar with differences of one class, in the part of the plot with more treatments with wetting sulphur the infection-level was about 2-3 classes lower in comparison to the unsprayed plot. If varieties had an assessment of 6 or more at end of August, leaves fell down to soil 2-3 weeks earlier than in conventionally grown experiments. In worst case the nutrition of flowering buds for the next season was reduced (e. g. ‘Achat‘ at Weinsberg showed 2009 severe infections with leaf spot and very early leaf fall (Pfeiffer, 2010), 2010 only low level of flowering and yield).

Table 4: Susceptibilities for *Monilia sp.* (Weinsberg, mainly based on assessments in 2009 and 2010) considering the potential of the trees for regeneration

| **Infection level of blossom clusters and twigs, potential of regeneration** | **varieties** |
|---|---|
| only few blossom clusters infected, seldom infected twigs | 'Achat', 'Jade', 'Favorit', 'Ujfehertoi Fürtös', 'Karneol', 'Pi-Sa 12,100', 'Csengödi' (here too less blossom-setting) |
| middle - high number of infected blossom-clusters, seldom whole twigs infected<br>**good regeneration** | 'Ludwigs Frühe', 'Röhrigs Weichsel', 'Topas', 'Morina', 'Debreceni Bötermö', 'Korai Pipacsmeggy', 'Hartei', 'Ciganymeggy 59', 'Pi-Sa 13,122 (Coralin)', 'Safir'<br>'Erdi Nagyügümölesü' 2010 middle regeneration after strong infection in 2009, 2011 well regenerated |
| blossom clusters and twigs infected middle to heavy, trees very good branching habitus,<br>**regeneration possible** | 'Ciganymeggy 7', 'Oblacinska', '<br>'Rubellit' (2009 only few infections, in 2010 middle-high because of latent infections in the wood) |
| middle to heavy infections on blossom clusters and twigs<br>less branches and twigs<br>**bad regeneration** | 'Schattenmorelle', 'Vowi', 'Pitic de Jasi',<br>'Malike emleke', 'Kantorjanosi', 'Schukowskaja' |

The last group of varieties should not be planted in organic orchards, because the risk of severe damages of wood and losses of yield by *Monilia* sp. is too high. In Dresden-Pillnitz *Monilia*-infections were observed seldom because of dry weather during blossom.

Table 5: Accumulated yield at site Dresden-Pillnitz 2009-2011 (kg/tree)

| **kg/tree** | **potential of yield** | **varieties** |
|---|---|---|
| < 6 | low | 'Rubellit' |
| 6 - 14 | middle | 'Topas', 'Pi-Sa 12,100',<br>'Morina', 'Pi-Sa 13,122 (Coralin), 'Ujfehertoi Fürtös' |
| 14 - 18 | high | 'Korund', 'Achat' |
| 18 - 23 | very high | 'Jade', 'Safir', 'Schattenmorelle' |

Figure 1 shows the accumulated yield (kg/tree) at site Weinsberg only for the cultivars with a sufficient yield (reasons for lower yield of the other cultivars: see also table 2!) For some cultivars differences to Dresden-Pillnitz could be seen, mainly influenced by the weather during blossom, especially for cultivar 'Rubellit', which had the highest yield in Weinsberg in spite of *Monilia*-infections in 2009 and 2010. At Dresden-Pillnitz the cultivars 'Coralin', 'Safir', and 'Morina' were harvested well by machine similar to 'Schattenmorelle', on the

other side 'Jade' and 'Achat' seemed to be less suitable for harvesting by machine. Both cultivars have not so high contents of acid and a pleasant taste, so they could be sold on fresh market, too, for that purpose the fruits should be harvested with stalk. 'Coralin' (Pi-Sa 13,122) had also an interesting flavour. The highest content of acid had 'Topaz' (2009 31.6 g Acid/l Weinsberg, 2011 26.0 g/l in Dresden-Pillnitz), so 'Topaz' could be used for mixed juices, but the trees have a weaker growing habitus, so yield was only on a middle level up to now. Altogether the cultivars ‘Safir’, 'Ujfehertoi Fürtös', ‘Morina’, ‘Coralin’ seem be suitable for organic fruit growers, ‘Achat’ and ‘Jade’ for fresh or subscription marketing.

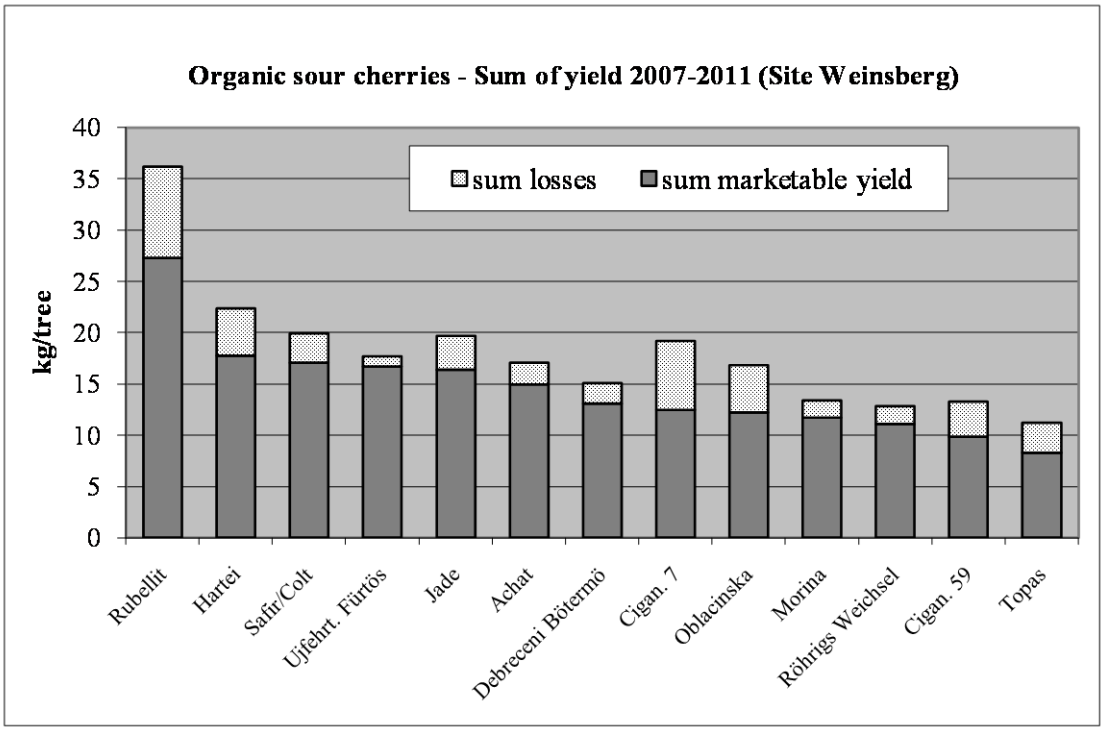


Figure 1: Accumulated yield 2007 to 2011, site Weinsberg, sorted on level of yield

## Discussion

For the most cultivars similar results were found at both sites, Dresden-Pillnitz and Weinsberg, but weather conditions have been very different over the seven years in Weinsberg, so cultivars should be tested for further years. Because wetting sulphur can be used in organic fruit growing, a middle to high susceptibility for *Blumeriella jaa*pii is not as critical as a high susceptibility for *Monilia sp*. When the use of copper-products will be limited heavily in future, there are at the moment not so much alternative and effective preparations available (like tested in *Monilia*-trials of Rank 2007, Brinkmann 2008, Rank, 2009 and 2010, Obenaus 2010, Joseph, 2011, all summarized in Rueß *et al.,* 2012). So only cultivars with a low to middle susceptibility to *Monilia sp.* (with good regeneration of the trees) should be chosen by organic fruit growers, but imperatively combined with consequent removing of infected twigs and fruit mummies in the orchard.

## Acknowledgements

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# The usefulness of stone fruit species and cultivars for organic fruit production in Poland

E. Rozpara[1], A. Głowacka[1]

**Abstract**

*In the spring of 2004, thanks to a grant received from the Ministry of Agriculture and Rural Development, Poland's first Ecological Experimental Orchard was founded at the Institute of Pomology and Floriculture (now the Institute of Horticulture). The orchard is located in Nowy Dwór near Skierniewice. It is certified annually and has the status of an organic farm. Right from the beginning the orchard has been used to conduct studies whose aim is, among other things, the selection of species and cultivars of stone fruit trees suitable for ecological cultivation. In the absence of the possibility of using mineral fertilizers and herbicides, and because of the limited use of plant protection products in ecological orchards, an extremely important role is played by a proper selection of cultivars. This is not an easy task because there are no cultivars resistant to pests and only a relatively small number of cultivars resistant to diseases. Consequently, the cultivars of stone fruit trees that were selected for research in ecological cultivation are those that for many years have been recommended for cultivation in commercial orchards in Poland, show tolerance or low susceptibility to pests and diseases, and produce fruit of high quality. In the case of sweet cherry, special attention was also paid to the time of fruit ripening because the fruit of early cultivars is not damaged by the cherry fruit fly. Trees of five species of stone fruit trees: sweet cherry, sour cherry, apricot,* Prunus domestica *L. and* Prunus salicina *Lindl. were planted in the spring of 2004. On the basis of several years of research, two apricot cultivars, 'Wczesna z Morden' and 'Harcot', were found to show high suitability for ecological cultivation because of the very low susceptibility of the trees and fruit to being affected by pests and diseases. Obtaining good quality fruit was also ensured by the early varieties of sweet cherry: 'Karesova' and 'Burlat', although in the conditions of the ecological orchard their trees were colonized by large numbers of aphids. Ecological cultivation of sour cherry proved to be more difficult than the cultivation of apricot and sweet cherry. Among more than a dozen of the tested cultivars the most suitable for an organic orchard were sour cherry trees selected at the Institute of Horticulture in Skierniewice, marked with the symbols: W2/02, W10/02 and W12/02. They showed relatively low susceptibility to infection by leaf spot and infestation by the cherry fruit fly. Ecological cultivation of plum trees was also difficult. Early varieties of* P. domestica *('Herman', 'Cacanska Rana') and* P. salicina *('Najdiena') proved to be better in the organic orchard than the late maturing varieties, mainly because of their lower susceptibility to infestation by the plum moth, which is a pest posing a serious problem in plum orchards*.

**Keywords**: stone fruits, cultivars, cherry, plum, apricot

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## Introduction

Owing to the increased interest in organic products, a comprehensive study was undertaken in Poland in 2004 on the development of effective methods of organic fruit production. The aim of the study is to determine, for example, which species and varieties of stone fruit trees are the most suitable for organic orchards. They should be resistant or at least have low susceptibility to the most dangerous pests and diseases because these characteristics play a vital role in reducing the extent to which fruit trees are affected by pathogens (Roen *et al.,* 2007). In ecological orchards, particularly dangerous is a group of pests that feed directly on or inside the fruit. When they occur *en masse*, they can substantially reduce fruit yields and impair fruit quality (Rozpara *et al*., 2010). The most dangerous pest of sweet cherry, and one that has recently become increasingly common also in sour cherry orchards, is the cherry fruit fly. In plum orchards, the plum moth is particularly dangerous. A lot of problems in the cultivation of stone fruit trees is also caused by the brown rot disease, and in the cultivation of sour cherry also bitter rot and leaf spot. The development of these diseases is favoured by prolonged periods of heavy rain. There are large differences between different species and varieties in the degree of their susceptibility to being affected by pests and diseases (Kahu & Klaas, 2007). It is thus very important to select those species and varieties of stone fruit trees that are the most suitable for cultivation in orchards managed with ecological methods, and this is the aim of this work.

## Methodology

In the spring of 2004, in the Ecological Experimental Station in Nowy Dwór-Parcela near Skierniewice, experiments were set up to assess the suitability of 5 species of stone fruit trees for organic cultivation. The experiments included: 3 sweet cherry cultivars (‘Karesova’, ‘Burlat’, ‘Summit’); 16 sour cherry varieties (‘Stevensbaer’, ‘Elmer’, ‘Pamiati Vavilova’, ‘Włodzimierska’, ‘Wanda’, ‘Naumburger’ ‘Słupia Nadbrzeżna’, ‘Oblacińska’, ‘Lucyna’ and 7 types of juicing sour cherry marked with the symbols: W1/02, W2/02, W7/02, W8/02, W9/02, W10/02, W12/02); 2 apricot cultivars (‘Wczesna z Morden’, ‘Harcot’); 4 cultivars of *Prunus domestica* L. (‘Herman’, ‘Cacanska Rana’, ‘Żółta Afaska’, ‘Valjevka’) and four cultivars of *Prunus salicina* Lindl. (‘Najdiena’, ‘Shiro’, ‘Vanier’, ‘Black Amber’). For the first two years, the soil in the orchard was kept in mechanical fallow. From the third year, the soil in the tree rows was still kept in mechanical fallow, while in the inter-rows grass cover was introduced. The trees were trained in the form of a spindle. Light sanitary and rejuvenation pruning was carried out every year. Irrigation of the trees began in 2007. Protection of the trees was based on biological preparations allowed for use in organic farming in Poland.

During the study, tree growth vigour, fruiting and fruit quality were assessed. In the first two years after planting, trunk diameter was measured, and from the third year – its circumference. Fruit yields were recorded annually, separately for each tree. In addition, the time of fruit ripening and fruit weight were recorded, with the latter parameter assessed on the basis of a sample of 50 fruits collected randomly from each replication. After harvesting, an assessment of the health status of the fruit was carried out, particularly in terms of the infestation of the sweet and sour cherry trees by the cherry fruit fly, and the plum trees – by the plum moth. The occurrence of these pests was assessed on the basis of 25 fruits taken from each replication of each tree variety. In total, 100 fruits were assessed for each variety. The same samples were used to assess the extent of infection with brown rot, and, in the case of sour cherry, also with bitter rot.

The results obtained during the study were evaluated statistically by the analysis of variance method, separately for each species. To assess the differences between means, Duncan's test was used at a significance level of 0.05.

## Results

### Sweet cherry

A serious problem in the ecological cultivation of sweet cherry were aphids, which fed in numerous colonies, causing the leaves and the tips of shoots to twist and, consequently, inhibited the growth of trees. The three varieties of sweet cherry, 'Karesova', 'Burlat' and 'Summit', did not differ in their degree of susceptibility to infestation by aphids, but differed in tree growth vigour, yielding, fruit quality and fruit ripening date. The largest cross-sectional area of the trunk after eight years of growth in the orchard was produced by the trees of the cultivar 'Burlat' (Table 1). Unfavourable weather conditions during flowering contributed to poor fruiting of sweet cherry trees in the years 2008-2011. Under those conditions, the most fruit was harvested from the trees of cv. 'Karesova', and the least – from the trees of cv. 'Summit'. Fruits of the cultivars 'Karesova' and 'Burlat' were free of larvae of the cherry fruit fly. Larvae of this pest were observed, however, in the fruits of the medium-early ripening cultivar 'Summit'. The fruits of this cultivar were also susceptible to decay during rainy years because of infection with the brown rot disease of stone fruit trees (Table 1).

Table 1. Tree growth and yielding, and fruit quality of three sweet cherry cultivars under ecological growth conditions

| Cultivar | TCSA* [cm²] 2011 | Cumulative yield 2008-2011 | Productivity index [kg/cm²] | Fruit ripening date | Fruit weight [g] | % damaged fruits | |
|---|---|---|---|---|---|---|---|
| | | | | | | cherry fruit fly | brown rot |
| 'Karesova' | 165.6 a | 9.2 c | 0.057 c | 15.06 | 6.2 a | 0 | 3.3 |
| 'Burlat' | 190.8 b | 7.9 b | 0.042 b | 17.06 | 6.8 b | 0 | 13.0 |
| 'Summit' | 163.3 a | 4.9 a | 0.031 a | 30.06 | 10.1 c | 14.3 | 28.5 |

*- trunk cross-sectional area

### Sour cherry

Ecological cultivation of sour cherry was not easy. The major problems in the cultivation of this species included: the leaf spot disease of stone fruit trees, pathogens causing fruit decay and the cherry fruit fly. Symptoms of the leaf spot disease appeared on the leaves of the evaluated sour cherry cultivars in all growing seasons. The degree of its severity was closely associated with the course of the weather and the cultivar. During rainy years, total defoliation of the most susceptible cultivars of sour cherry, which included 'Elmer', 'Stevensbaer', 'Naumburger' and W9/02, occurred as early as the end of July. The consequence of this was weak growth of trees of these cultivars and significantly lower yields produced by them in comparison with the cultivars less susceptible to the disease (Table 2). In the ecological orchard, the most strongly growing and best-yielding were the trees of the cultivar 'Lucy'. The largest and healthiest fruits were produced by the trees of sour cherry marked W12/02. Smaller, but of good quality, were also the fruits of the cultivars marked with the symbols W2/02 and W10/02. Obtaining good quality fruit from the trees of most of the sour cherry cultivars under evaluation was, however, difficult because of their being affected by the cherry fruit fly and brown and bitter rot. The degree of infestation of the fruits of the individual sour cherry cultivars by the cherry fruit fly

depended on their size. Fruits of a greater weight were significantly more damaged by this pest than smaller fruits (Table 2). The exception was the sour cherry W12/02, whose fruits were the largest among the fruits of all the evaluated cultivars, and the larvae of the cherry fruit fly fed in them only occasionally. This might have been affected by the fruit ripening date. It was observed that fruits of the later ripening cultivars were less infested than those of the early ripening cultivars. A similar relationship was found for the extent of infection of sour cherries with the brown rot disease (Table 2).

Table 2. Tree growth and yielding, and fruit quality of sixteen sour cherry cultivars under ecological growth conditions

| Cultivar | TCSA* [cm²] 2011 | Cumulative yield 2008-2011 | Productivity index [kg/cm²] | Fruit weight [g] | Fruit ripening date | % damaged fruit | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | cherry fruit fly | brown rot | bitter rot |
| 'Pamiati Vavilova' | 66.9 h | 6.7 cd | 0.101 a | 4.8 g | 02.07 | 18.7 f | 11.2 de | 2.3 abcd |
| 'Wanda' | 43.0 de | 18.0 i | 0.419 g | 3.7 d | 07.07 | 13.1 de | 10.8 d | 4.8 e |
| 'Oblacińska' | 35.1 bc | 17.2 i | 0.496 h | 3.4 c | 08.07 | 14.2 def | 9.6 cd | 0.0 a |
| 'Lucyna' | 99.5 i | 15.1 h | 0.154 bc | 4.3 ef | 10.07 | 24.3 g | 19.0 f | 0.0 a |
| 'Naumburger' | 38.7 cd | 6.1 c | 0.160 bc | 3.9 d | 12.07 | 16.8 ef | 13.1 e | 2.8 bcde |
| W 1/02 | 47.5 ef | 8.6 e | 0.185 cd | 4.5 ef | 12.07 | 7.5 bc | 7.5 c | 2.3 abcd |
| 'Elmer' | 21.9 a | 1.9 a | 0.087 a | 5.0 g | 13.07 | 28.1 g | 21.0 f | 7.0 f |
| 'Włodzimierska' | 56.2 g | 8.0 de | 0.142 b | 4.3 e | 13.07 | 14.7 def | 8.0 c | 2.8 bcde |
| W 8/02 | 48.8 ef | 8.5 e | 0.175 bc | 3.9 d | 13.07 | 10.2 cd | 4.8 b | 2.8 bcde |
| W 2/02 | 36.1 bc | 7.6 de | 0.214 de | 3.1 bc | 15.07 | 0.6 a | 1.6 a | 0.5 ab |
| W 9/02 | 30.1 b | 2.4 ab | 0.079 a | 3.0 b | 15.07 | 3.4 ab | 1.7 a | 3.0 cde |
| W 7/02 | 34.8 bc | 2.6 ab | 0.076 a | 4.5 f | 16.07 | 10.8 cd | 5.0 b | 1.8 abc |
| W 10/02 | 47.8 ef | 6.8 cd | 0.144 b | 2.8 a | 17.07 | 3.4 ab | 2.2 a | 1.3 abc |
| W 12/02 | 50.1 f | 11.7 f | 0.234 e | 5.6 h | 19.07 | 1.0 a | 0.0 a | 0.0 a |
| 'Stevensbaer' | 33.6 bc | 3.4 b | 0.102 a | 2.6 a | 20.07 | 1.2 a | 0.7 a | 2.5 bcde |
| 'Słupia Nadbrzeżna' | 36.9 c | 13.1 g | 0.360 f | 3.2 bc | 23.07 | 3.2 ab | 0.7 a | 4.5 de |

*- trunk cross-sectional area

## Apricot

The study showed a high suitability of apricot cultivars for ecological cultivation. The health condition of the trees of the evaluated cultivars was very good. Diseases and pests were not a serious problem in the cultivation of this species. Unfortunately, apricot flower buds were susceptible to damage from freezing during winter dormancy or in the spring during flowering, which was the reason why the apricot trees quite often failed to produce satisfactory yields. There were no significant differences in tree growth vigour between the cultivars 'Wczesna z Morden' and 'Harcot' in the ecological orchard, but they differed in terms of yield, fruit size, and fruit health status (Table 3). 'Wczesna z Morden' was a more productive cultivar and notable for healthier fruits than 'Harcot'. Fruits harvested from the trees of the two cultivars ripened in the last ten days of July, but not at the same time, so harvesting was carried out twice.

Table 3. Tree growth and yielding, and fruit quality of two apricot cultivars under ecological growth conditions

| Cultivar | TCSA* [cm²] 2010 | Cumulative yield 2008-2010** [kg/tree] | Productivity index [kg/cm²] | Fruit weight [g] | Fruit ripening date | % decaying fruit (brown rot) |
|---|---|---|---|---|---|---|
| 'Harcot' | 115.2 a | 15.9 a | 0.139 a | 55.7 b | 22-25.07 | 16.5 |
| 'Wczesna z Morden' | 128.2 a | 25.8 b | 0.203 b | 47.8 a | 28-31.07 | 0 |

*- trunk cross-sectional area

**- no fruits in 2011

*Prunus domestica* and *Prunus salicina*

Ecological cultivation of plum trees was difficult. After eight years of growth in the orchard, the largest cross-sectional area of the trunk among the *P. domestica* cultivars was achieved by 'Cacanska Rana' (Table 4), and among the *P. salicina* cultivars – trees of the cultivar 'Shiro' (Table 5). The cumulative yields obtained in 2008-2011 were not satisfactory for any of the plum cultivars. The poor yields in 2009 were partly attributed to late spring frosts, and in 2010 and 2011 pollination was adversely affected by windy and rainy weather during flowering. Under those conditions, the best-yielding among *P. salicina* trees was the cultivar 'Vanier' (Table 4), and among *P. domestica* trees – 'Herman' (Table 5). The largest fruits were produced by the cultivar 'Żółta Afaska' (*P. domestica* L.) and 'Black Amber' (*P. salicina* Lindl.). The largest numbers of the plum moth colonized the fruits of the *P. salicina* cultivar 'Vanier', but strongly infested by it were also fruits of the cultivar 'Shiro'. Among the *P. domestica* cultivars, the largest numbers of the plum moth were found on the fruits of 'Żółta Afaska'. Fruits of the late ripening cultivars were usually more strongly affected by the pest than the fruits of the early ripening cultivars. The highest percentage of decaying fruits was observed in the yield of the cultivars 'Żółta Afaska' (*P. domestica* L.) (Table 4) and 'Vanier' (*P. salicina* Lindl.) (Table 5).

Table 4. Tree growth and yielding, and fruit quality of four *Prunus domestica* cultivars under ecological growth conditions

| Cultivar | TCSA* [cm²] 2010 | Cumulative yield 2008-2010 | Productivity Index [kg/cm²] | Fruit weight [g] | Fruit ripening date | % damaged fruits | |
|---|---|---|---|---|---|---|---|
| | | | | | | plum moth | brown rot |
| 'Herman' | 70.0 a | 11.8 d | 0.173 d | 30.0 a | 23.07 | 2.7 ab | 3.2 a |
| 'Cacanska Rana' | 88.6 c | 3.3 a | 0.037 a | 48.4 c | 30.07 | 2.0 a | 3.3 a |
| 'Valjevka' | 82.4 bc | 5.1 b | 0.063 b | 33.8 b | 10.09 | 5.3 b | 2.0 a |
| 'Żółta Afaska' | 75.4 ab | 9.9 c | 0.136 c | 62.8 d | 10.09 | 17.3 c | 28.8 b |

*- trunk cross-sectional area

**- no fruits in 2011

Table 5. Tree growth and yielding, and fruit quality of four *Prunus salicina* cultivars under ecological growth conditions

| Cultivar | TCSA* [cm²] 2010 | Cumulative yield 2008-2010** | Productivity Index [kg/cm²] | Fruit weight [g] | Fruit ripening date | % damaged fruit | |
|---|---|---|---|---|---|---|---|
| | | | | | | plum moth | brown rot |
| 'Najdiena' | 71.5 b | 13.5 c | 0.191 c | 39.2 a | 30.07-06.08 | 1.3 a | 8.5 a |
| 'Shiro' | 112.7 d | 10.6 b | 0.095 b | 50.7 b | 07-10.08 | 16.7 b | 19.3 b |
| 'Vanier' | 50.6 a | 23.9 d | 0.488 d | 50.9 b | 21-25.08 | 44.5 c | 28.5 c |
| 'Black Amber' | 82.4 c | 4.3 a | 0.053 a | 64.7 c | 05.09 | 13.0 b | 11.1 a |

*- trunk cross-sectional area

**- no fruits in 2011

## Discussion

The growth and yield of each tree species is influenced by many factors, but the most important are climatic conditions and the cultivar (Turcu et al., 1998). It was mainly unfavorable weather conditions during the flowering of the stone fruit trees that adversely affected their yielding in the Ecological Experimental Orchard in Nowy Dwór-Parcela. Moreover, in the absence of the traditional protection the weather conditions contributed to the trees becoming more strongly affected by pests and diseases. In the organic cultivation of sweet cherry, the most problems were caused by the cherry fruit fly, although aphids were also a troublesome pest. This is confirmed by the observations of Badowska-Czubik et al. (2010). The cherry fruit fly, while laying its eggs, destroys the skin of the fruits, making them more susceptible to infection with brown rot. The fruit crops of those varieties of sweet and sour cherry that were more numerously inhabited by the larvae of the cherry fruit fly also contained a greater percentage of rotting fruit. These results are consistent with those obtained by Stamenkovic et al. (1996) in experiments carried out in former Yugoslavia.

In the case of plum trees, the quality of the fruits was determined by the extent to which they were infested by the plum moth and infected with brown rot. According to Rozpara et al. (2010), there is a chance of solving the plum moth problem in ecological orchards because the product SpinTor 240 SC used against this pest in the experiments in the Ecological Experimental Orchard in Nowy Dwór-Parcela has shown satisfactory performance. Spray treatments against the brown rot disease have been carried out with copper preparations registered for use in organic cultivation of stone fruit trees (Śliwa & Wiercińska, 2009). However, during the years favourable to the development of the disease they were not sufficiently effective in the cultivars susceptible to infection.

## Conclusions

The presented results indicate that:

1. Ecological cultivation of stone fruit trees in Poland is possible provided that the appropriate location of the orchard, the proper selection of cultivars and the use of preventive methods of protection against pests and diseases are all ensured.
2. Great usefulness for ecological cultivation is shown by apricot trees, which are relatively rarely affected by pests and diseases, and produce good quality fruit. Unfortunately, flower buds of this species are susceptible to damage from freezing during winter dormancy or during the flowering period in the spring, and fruit yields of apricot trees are therefore quite often unreliable.

3. A serious pest of sweet cherry, making ecological cultivation difficult, is the cherry fruit fly. It does not affect the fruit of early ripening cultivars such as ‘Karesova’ and ‘Burlat’, and until an effective method of protection against this pest is developed, only sweet cherry cultivars of this type should be selected for ecological orchards.
4. Ecological cultivation of sour cherry is difficult because of the lack of an effective method of preventing tree infection by leaf spot and fruit infection by decay-causing diseases, as well as infestation by the cherry fruit fly. Despite these difficulties, the varieties denoted as W2/02, W10/02 and W12/02 perform relatively well in an orchard maintained by ecological means.
5. Many problems in the cultivation of plum can be avoided by choosing the right cultivar. Among the *P. domestica* cultivars, ‘Herman’, ‘Cacanska Rana’ and ‘Valjevka’ proved to be better in organic cultivation than ‘Żółta Afaska’, whose fruits were significantly more strongly affected by the plum moth and the brown rot disease.
6. Ecological cultivation of the cultivar ‘Najdiena’ was easier than the cultivation of the other cultivars of *P. salicina* because of its lower susceptibility to being affected by pests and diseases.

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# Improving Organic Production of Plums (*Prunus domestica*) in Austria

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**Abstract**

*The aim of the still running project (2010-2012) has been to find out, what is necessary in order to establish an economically practicable organic plum production in Eastern Austria.*

*In terms of methodology, in 2011 a survey about farmers' experiences was done with a questionnaire and we have been conducting accompanying research on farms in order to assess important diseases and pests (e.g.* Cydia funebrana*) and other important production questions.*

*We want to discuss our first results with researchers from other countries.*

**Keywords:** plum, cultivars, *Cydia funebrana*

## Introduction

In Austria, organic production of plums (*Prunus domestica*) is still a niche with totally about 20 ha of production area (Ama, 2011). Nevertheless, there has been a rising demand on the market. The aim of our ongoing project (from 2010-2012) in cooperation with a wholesaler, farmers and research institutes has been to find out, what is necessary in order to establish an economically practicable organic plum production in Eastern Austria.

## Material and Methods

In January 2011, a questionnaire was sent to all Austrian farmers, who were producing organic plums on at least 0.04 hectares.

The questions were about production area, experience with rootstocks and cultivars, problems in plant protection, marketing and needs for research. In some questions the farmer also had the opportunity to assess (e.g. used cultivars, plant protection methods, demands for research) with a simple evaluation system (1=very good, 5=very bad experience).

In order to rate the importance of the evaluation of the used cultivars an index mark was calculated: index = ∑ [cultivars on the farm] + [total number of assessments for this cultivar]. The index was higher when a cultivar was assessed by more different farmers and also depended on how many cultivars a farmer was keeping.

From 2007 – 2011 the most important pests and diseases were monitored every year on an organic farm in Eastern Styria (farm A). The size of the more or less connected plum orchards on this farm is about 3 ha, and the method of mating disruption (Isomate OFM rosso) has been used since 2007 in the orchards. Every year during harvest season about 1000 plums of 4 different cultivars ('Katinka', 'Cacak´s Schöne', 'Valjevka', 'Top') were observed on damages by *C. funebrana*.

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In 2009, we also monitored the damage caused by *C. funebrana* in the orchards of smaller size (1 ha on farm B and 0.75 ha on farm C) of two other organic farmers who were using the mating disruption method (cultivars ‘Katinka‘, ‘Cacak´s Schöne‘) for the first time in that year.
In order to find out more about the control of *Monilia fructigena* we made some trials with two different products (Boni protect and Mycosin). Unfortunately we didn’t have enough fruits to get a comparable result. Also thinning methods with lime sulphur and Electroflor were discussed.

## Results

*Questionnaire*

18 questionnaires (32%) from a total of 57 were returned by farmers.

The average plum production area of the surveyed farmers is 0.51 ha (3% of their total farm area).

The most widely used rootstocks are ‘Jaspi Fereley’ (11 farmers), ‘Waxwa’ and ‘St. Julien GF 655/2’ (8) and ‘Wawit’ (5).

In the evaluations of cultivars by farmers the cultivars ‘Haganta’, ‘Top Hit’ and ‘Cacak’s Fruchtbare’ got very good assessments, and with still very little experience also the new cultivars ‘Top Taste’, ‘Top Giant’ and ‘Top Star Plus’, whereas the cultivars ‘Zimmers’, ‘Top Five’, ‘Jojo’ and ‘Hanita’ showed a poor performance (figure 1).

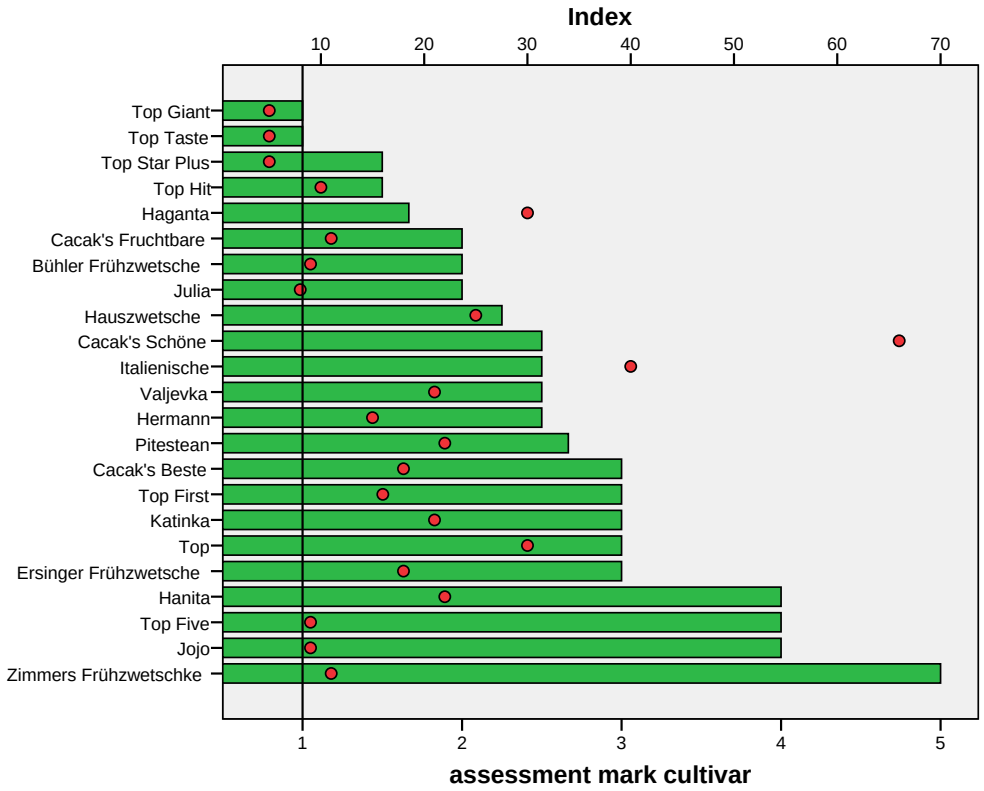


Fig. 1: Assessment of the used cultivars by the farmers (=balks) and calculated Index of the cv. (points; a high index means that the assessment is more reliable) (N=18 farmers, multiple mentions possible)

The farmers mentioned that the most important challenges in plant protection were fruit rot caused by *Monilinia fructigena*, plum sawfly (*Hoplocampa flava*) and *Cydia funebrana,* and

with some less importance also *Stigmina carpophila*, plum rust (*Tranzschelia prunispinosae,T. discolor*), voles (*Arvicola terrestris*), tree decline and plum pox virus.

40% of the produced plums are sold directly on the farm, 27% by grower organisations and 21% in the retail (figure 2). 21% of the plums are processed, on some farms most or even the entire harvest.

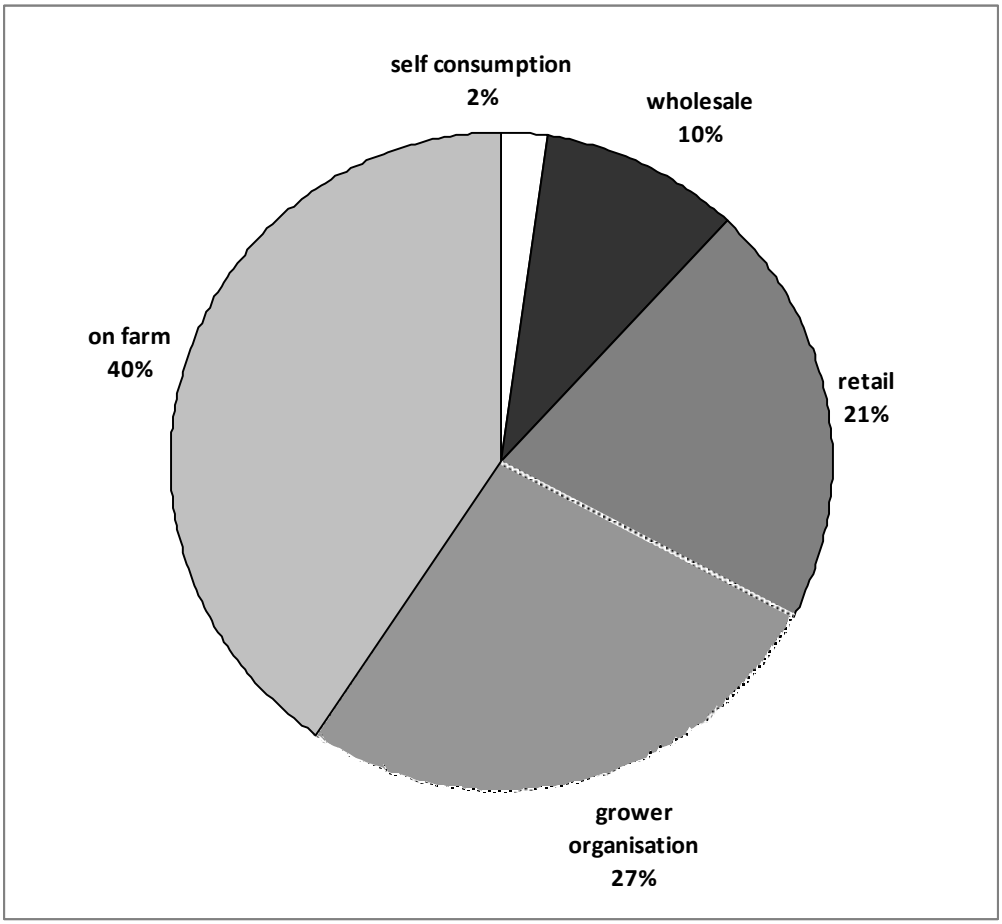


Figure 2: Ways of marketing of the produced plums (N=18 farmers; multiple mentions possible)

The main questions regarding the research for the organic growers are cultivars (13), plant protection in general (11), rootstocks (9) and plant material and nursery (7). And regarding special questions in plant protection: plum sawfly (*Hoplocampa flava*) and *Cydia funebrana* (both 9), Monilia fruit rot (7), tree decline (6), voles (*Arvicola terrestris*) (3) and plum pox virus (3) were mentioned most often.

On farm monitoring of *Cydia funebrana*

During the last 5 years the average damage through *Cydia funebrana* on farm A was between 0 to 2% which is an acceptable result for the market and the grower (table 1). Therefore on this farm with about 3 ha the mating disruption method was working very well.

In 2009, the damage through *C. funebrana* on the two other smaller organic orchards was about 1% infested fruits on farm B and more than 6% on farm C.

Table 1: Results of the assessment of *Cydia funebrana* on fruits during harvest from 2007-2011 on farm A

| cultivar | % damaged fruits by *Cydia funebrana* | | | | |
|---|---|---|---|---|---|
| | **2007** | **2008** | **2009** | **2010** | **2011** |
| Katinka | 0.00 | Hail | 0.99 | 0.00 | 0.00 |
| Cacak´s Schöne | 0.00 | Hail | 1.05 | 0.00 | 0.26 |
| Valjevka | 0.86 | Hail | No monitoring | 0.86 | No monitoring |
| Top Taste | No trees | No trees | 1st year | 2nd year | 0.00 |
| Top | 0.75 | 2.2 | 1.76 | 0.00 | 1.51 |

## Conclusions and outlook

As a first conclusion, we can state that the mating disruption, as the only effective method to regulate *C. funebrana* in organic production in Austria, has so far been working on the farm with about 3 ha of plum orchards.

For smaller or new orchards, the use of hail nets all around the orchard may be a good solution to control *C. funebrana*. At the moment there are two orchards where this method is tested.

In 2012 we will try to get more information about experiences with organic plum production from experts and producers from other countries through interviews. The monitoring and the accompanying research on farms will also continue.

## Acknowledgements

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# Studies on Biodiversity of *Fragaria vesca* L.

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**Abstract**

*Our cultivated strawberry* Fragaria ×ananassa Duch*. is a spontaneous hybrid between the wild American species* Fragaria virginiana Miller *and* Fragaria chiloensis (L.) Miller*. Due to funnel effects in a more than 250 years breeding history the search for important plant characteristics in wild species, for example* Fragaria vesca L*. is promising. There are some cultivars of* F. vesca *for example 'Rügen', 'Baron Solemacher' or 'Yellow Wonder'. Hybrids between the octoploid cultivated strawberry* Fragaria ×ananassa *and* F. vesca *are in the breeders focus since 1918 and resulted in the decaploid* Fragaria ×vescana R. et A. Bauer *with cultivars like 'Florika' cultivated as a meadow-type field.* F. vesca *is spread throughout Eurasia and North America with special ecological adaptations to the particular habitat.*

*In the present work we investigated the biodiversity of 25* F. vesca *accessions representing the whole geographic distribution for this species. Subspecies, formae (alba and semperflorens) as well a hybrid on the subspecies level (nothomorph) were included. The* F. ×ananassa *cultivar 'Elsanta' was used for comparison. Different vegetative and generative characteristics were documented throughout the growing season including fruit parameters. Altogether high diversity in* Fragaria vesca *accessions could be observed. Implications for breeding purposes are drawn in this work.*

**Keywords:** *Fragaria vesca*, wood strawberry, genetic resources, breeding, biodiversity

## Introduction

The genus *Fragaria* L. belong to the *Rosaceae* family and to the subfamily *Rosoidae*. Species of the genus *Fragaria* L. with different ploidy levels can be found all over the North Temperate Zone (Staudt, 1989). Some of the species have a small range of distribution while other are widely distributed. Wild strawberry species in Europe are the hexaploid *F. moschata* Duch. and the diploid *F. viridis* Duch., and *F. vesca* L. They all have edible fruits with an intensive flavour. One of the species with a wide distribution is *F. vesca* which is spread throughout Eurasia and North America with specific ecological adaptations to the particular habitat. Despite their small fruit sizes there are some cultivars developed for example 'Rügen', 'Baron Solemacher' or 'Yellow Wonder'. They are mainly cultivated for domestic use. Wild species are genetic resources which can be used to improve and widen the genetic background of crop plants. Therefore, the aim of this study was to investigate the biodiversity of different *F. vesca* accessions representing the whole geographic distribution of the species with a focus on possible hybridization with the octoploid cultivated strawberry *Fragaria* ×*ananassa*.

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## Material and Methods

For this study 25 *F. vesca* accessions of the collection of Hansabred representing the whole geographic distribution were available. Subspecies, formae (alba and semperflorens) as well a hybrid on the subspecies level (nothomorph) were included. The *F.* ×*ananassa* cultivar 'Elsanta' was used for comparison. Plants were one year old or older. Most of them were cultivated in open wooden boxes (size 0,85 x 07 x 0,15 m) and some accessions were planted in spring 2011 in the adjoining open field (Table 1) both with sandy loam. Different vegetative and generative characteristics were documented throughout the growing season. Phenological data were determined by using the BBCH-scale of Meier (2001) and as basis for description of all other characters the guidelines of the UPOV were used (Anonym, 2008).

Single fruit weight was calculated by counting and weighing all available fruit over the whole season. Fruit analysis was done with a mixed probe of all harvested fruit. For each parameter three repetitions per accession were used. From these results the mean values were calculated. Soluble solids (in °Brix) were determined as an indirect measurement for the sugar content (Quick Brix 60, Mettler Toledo, Germany). The acidity was determined as a citric equivalent by volumetric analysis with sodium hydroxid solution (0,1 molar) using an automatic titrator (METROHM 716 DMS Titrino, Germany).

## Results

The different *F. vesca* accessions had a wide range for showing the first flowers. The beginning of flowering was 19$^{th}$ April 2011 for *F. vesca* ssp. *californica* x *F. vesca* f. *semperflorens* 'Golden Gate' from California and 27$^{th}$ of May 2011 for *F. vesca* ssp *vesca* 'Oslo' from Norway. 'Elsanta' started to flower at 27$^{th}$ of April 2011.

The fruit ripening started between 02$^{nd}$ of June 2011 and 21$^{st}$ of June 2011 regarding all 25 *F. vesca* accessions and therefore later than 'Elsanta' (31$^{st}$ of May 2011). The earliest accessions were 'Korsika', 'Großolbersdorf', 'Micrantha', St. 98,04-4 and St.94,13. The harvest ended between 14$^{th}$ of June 2011 ('Wallis Schweiz') and 28$^{th}$ of July ('Bjørnestigen'), in comparison with 'Elsanta' at 7$^{th}$ of July 2011. The fruit of the remontant cultivars 'Red Wonder' and 'Yellow Wonder' were not harvested any longer past the 28$^{th}$ of July. The colour shades of the fruits varied in the genus but even in one accession depending on the position of the fruit (in shadow, upper fruit side etc.). Fruit shape varied with predominantly conical, round and oblate shapes, whereas 'Elsanta' showed mainly conical shaped fruit. Fruit size as diameter was in the mean per accession between 8mm ('R1' and 'Multiplex') and 21mm (St.08/101) compared to 35mm for 'Elsanta'. Other accessions with a comparable fruit size as St.08/101 were 'Golden Gate', 'Red Wonder', 'Yellow Wonder', 'Kaiserpfalz Tilleda', 'Weimar', 'Böhmen', 'Süd Öland 1' and 'Oslo'. Single fruit weight ranged between 0,34g ('Micrantha', 'Wallis Schweiz') and 1,04g for 'Red Wonder'. 'Golden Gate' and 'Queen Ferry' were with 0,96g respectively 0,95g only little lower. For 'Elsanta' single fruit weight was 11,34g. The *vesca*-typical weak adherence of the calyx could be observed for all accessions. The values of solible solids ranged between 9° Brix to 12,7° Brix including 17 accessions with more than 10° Brix. For 'Elsanta' the lowest value with 8,8° Brix was maesured (Figure 1). Also the acidity was higher for most of the *F. vesca* accessions with 1% ('Korsika') to 1,9% ('Bjørnestigen') than for 'Elsanta' with 0,9% (Figure 2).

### Discussion

Our cultivated strawberry *Fragaria* ×*ananassa* Duch. is a spontaneous hybrid between the wild American species *Fragaria virginiana* Miller and *Fragaria chiloensis* (L.) Miller. All three species are octoploid. During the breeding process genetic diversity is often reduced

resulting in so-called funnel effects with poor aroma patterns and lost resistances. Useful traits can possibly be found in other species of the genus *Fragaria* for example *F. vesca*. Hybrids between the octoploid cultivated strawberry *Fragaria ×ananassa* and *F. vesca* are in the breeders focus since 1918 (Kuckuck, 1980) and already resulted in the decaploid *Fragaria ×vescana* R. et A. Bauer with cultivars like 'Florika' cultivated as a meadow-type field (Bauer, 1993).

Regarding the different documented characteristics some of the accessions might be interesting for further use. The time of flowering and harvesting were altogether in the range of the cultivated strawberry. The remontant character of 'Yellow Wonder' and 'Red Wonder' is very interesting for breeding everbearers. The accessions 'Weimar' and 'Bjørnestigen' with a long harvesting period of about five weeks might also be interesting. A great diversity of forms of fruit could be observed between the accessions. Fruit size and fruit weight were also variable but never reached the size of the cultivated 'Elsanta'. Fruit sizes between 18mm and 21mm (St.08/101, 'Böhmen', 'Oslo' and 'Golden Gate') were the the highest for the *F. vesca*. They may be interesting for breeding for this aspect.

Table 1 *Fragaria vesca* accessions and *Fragaria xananassa* 'Elsanta' used in this study

| No. | Accession | Name | Source |
|---|---|---|---|
| **1** | *F.vesca f. alba* | St.08/101 | Gatersleben, Germany, via Staudt |
| **2** | F.vesca f. alba | 'S.Queen Ferry' | Scotland, South Queen Ferry, (Edinburgh), UK, via Staudt |
| **3** | *F.vesca ssp.vesca* | St.94,13 | Baikal-Region, Russia |
| **4** | *F.vesca ssp. bracteata* | St.98,04-4 | Nass River, British Columbia, Canada |
| **5** | *F.vesca ssp. americana* | St.14324 | Bot. Garden Montreal,Canada |
| **6** | *F.vesca f. semperflorens* | 'Red Wonder' | Cultivar |
| **7** | *F.vesca f. semperflorens* | 'Yellow Wonder' | Cultivar |
| **8** | *F.vesca ssp.vesca* | 'Island' | Bot. Garden Rejkavik, Iceland |
| **9** | *F.vesca ssp.vesca* | 'Kaiserpfalz Tilleda' | Saxony-Anhalt, Germany |
| **10** | *F.vesca ssp.vesca* | 'Korsika' | Corsica, St. Bonifatius,1800m, France |
| **11** | *F.vesca ssp.vesca* | 'Multiplex' | Pohlheim, Germany |
| **12** | *F.vesca ssp.vesca* | 'Weimar' | Garden of J.W.v. Goethe in Weimar, Germany |
| **13** | *F.vesca ssp.vesca* | 'Böhmen' | Bohemia, Stimmersdorf, near Prebischtor, Elbsandsteingebirge,Czech Republic |
| **14** | *F.vesca ssp.vesca* | 'Tüchersfeld' | Franken, Germany |
| **15** | *F.vesca ssp.vesca* | 'Süd-Öland 1' | Öland, Sweden |
| **16** | *F.vesca ssp.vesca* | 'R1' | Nesselwang, Germany |
| **17** | *F.vesca ssp, vesca* | 'Großolbersdorf' | Großolbersdorf (Ore Mountains), Germany |
| **18** | *F.vesca ssp, vesca* | 'Rübeland' | Rübeland (Harz), Germany |
| **19** | *F.vesca f. alba* | 'Moritzburg' | Moritzburg, near Dresden, Germany |
| **20** | *F.vesca ssp.vesca* | 'Wallis Schweiz' | Wallis, Switzerland |
| **21** | *F. vesca ssp vesca* | 'Bjørnestigen' | Bjørnestigen, Norway |
| **22** | *F. vesca ssp vesca* | 'Oslo' | Oslo, Norway |
| **23** | *F.vesca ssp.californica x vesca f. semperflorens* | 'Golden Gate' | Golden Gate, San Francisco, Californian, USA |
| **24** | *F. vesca ssp vesca* | 'Finnland' | Suur-Saimaa, north of Lapeenranta, Finland |
| **25** | *F.vesca ssp.vesca* | 'Micrantha' | Hamrafjället, Province Härjedalen, Sweden |
| **26** | *F. ×ananassa* | 'Elsanta' | cultivar, hybridization between 'Gorella' × 'Holiday'; IVT Wageningen, Netherlands 1975 |

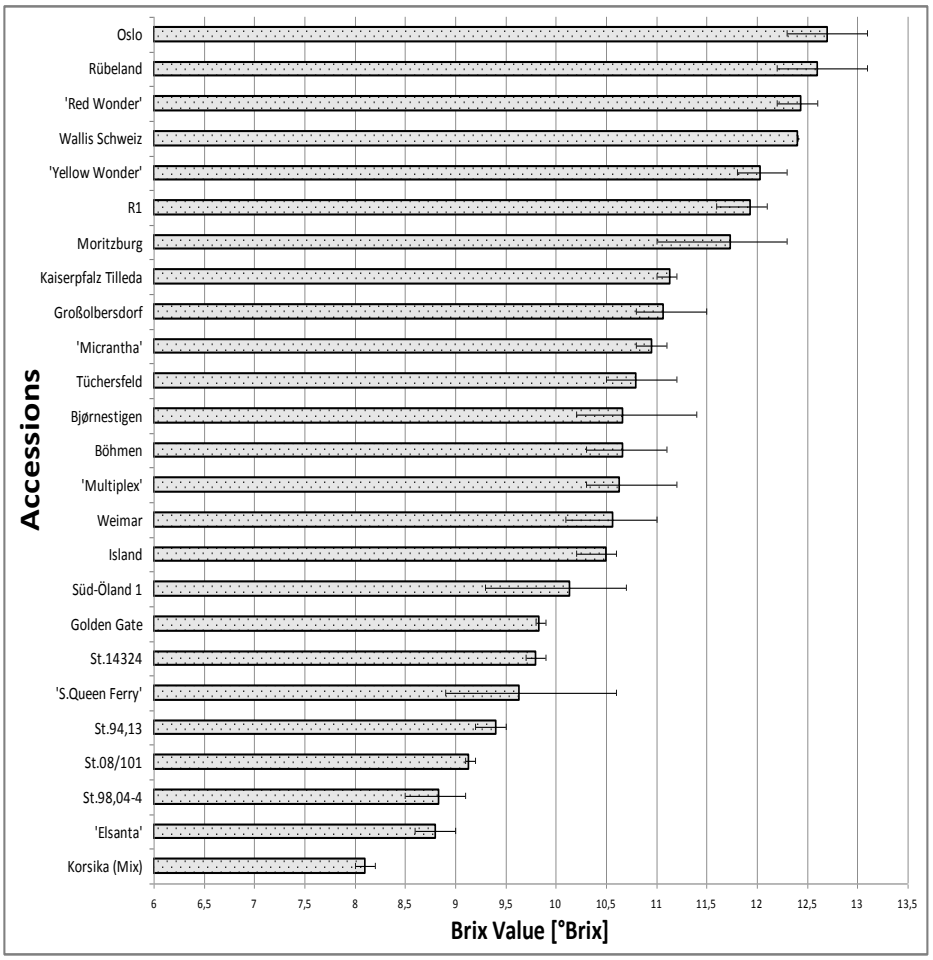


Figure 1: Mean value, max., min. of solible solids (°Brix) in strawberry fruit of 24 accessions of *F.vesca* and variety 'Elsanta'

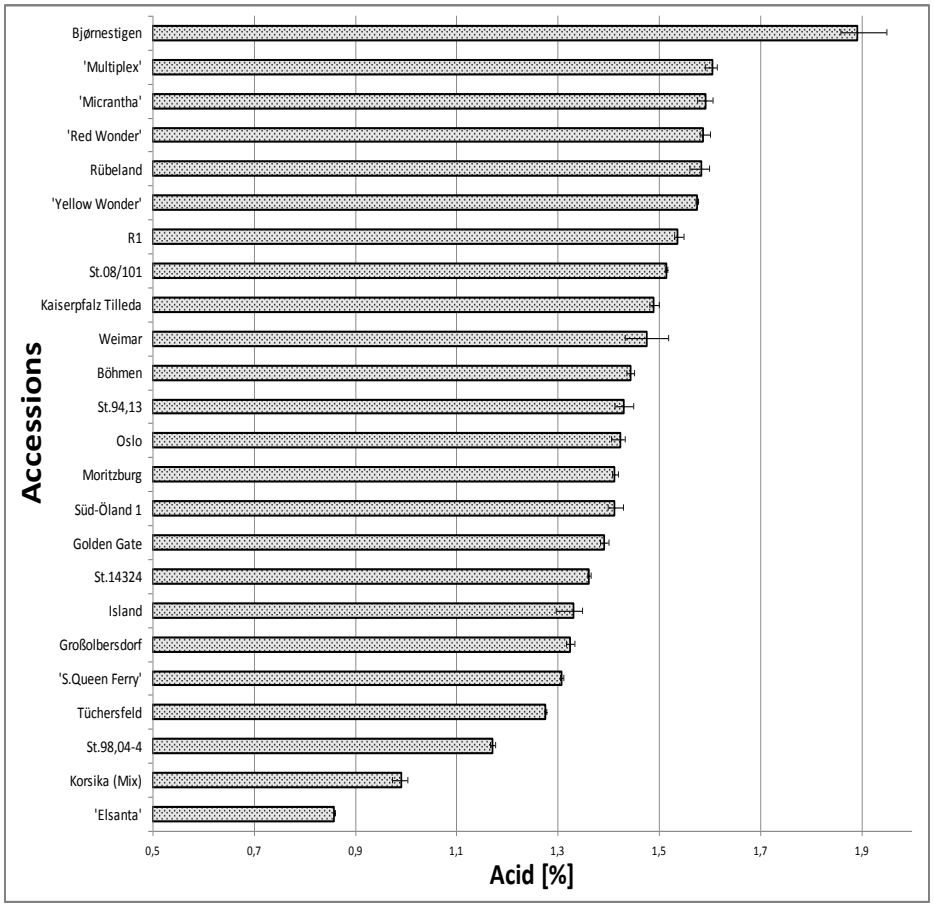


Figure 2: Mean value, maximum, minimum of percent acid in strawberry fruit of 23 accessions of *F.vesca* and variety 'Elsanta'

Important parameters for the flavour of a fruit are beside the aroma compounds and the mouth feeling the contents of sugar and acid and in particular the sugar/acid ratio. For sugar (measured as solible solids) and acid content the mean values were higher than the value for 'Elsanta' except the sugar content for 'Korsika'.

According to Duden (1992) sugar content should be more than 8°Brix for a good taste and more than 9° Brix for a very good taste. The latter was true for most of the *F. vesca* accessions. However for a good taste the sugar/acid ratio is also crucial.

*F.vesca* is known for exclusive aroma (Ulrich et al. 2007). Thus, it will be a good source for improving flavour of strawberry (Olbricht et al. 2009). The diversity of aroma patterns was measured additionally but is not topic of this paper. Two accessions are meanwhile included in breeding activities.

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# The assessment of allergenicity risk of selected strawberry cultivars on the guinea pig model.

M. Jasińska-Stroschein[1], J. Owczarek[1], P. Szcześniak[1], K. Rutkowski[2], J. Markowski[2], A. Miszczak[2] and D. Orszulak-Michalak[1]

**Abstract**

*The aim of the presented study was to assess the risk of any allergic reaction or food hypersensitivity resulting from topical application and chronic oral administration of fruit of selected strawberry cultivars – 'Elsanta' and 'Honeoye', coming from farms located in Central Poland. Plantations were managed according to organic (OR) or integrated production (IFP) systems. The experiments were performed on outbred young, adult, white albinotic guinea pigs (Dankin Hartley). Fruit characteristics included: the total soluble solids content (TSS), titratable acidity (TA), sugar content (sucrose, glucose, fructose, and sorbitol), polyphenols content (chlorogenic acid, p-coumaric acid, quercetin, kaempferol, procyanidins, anthocyanins, ellagic acid), and macro and micro nutrients (P, K, Mg, Ca, B, Cu, Fe, Mn, Zn, and S). Moreover in the tested fruits pesticide residues, heavy metals contamination, nitrate and nitrites content and microbiological contamination (fungi & bacteria) were also analyzed.*

*The observations of skin reactions resulting from topical application of 'Honeoye' strawberry fruit extracts according to Guinea-Pig Maximization Test (GPMT,) coming from plantation managed according to integrated or organic production, showed no significant changes in guinea pigs skin. Topical exposition to 'Elsanta' strawberry from plantation managed according to organic practice showed some changes (i.e. discrete, moderate or intense erythema and swelling), as compared to animals being exposed to 'Elsanta' from plantation managed according to integrated fruit production. Chronic oral administration of selected fruit extracts did not cause any skin reactions in groups receiving 'Elsanta' or 'Honeoye' from organic or integrated productions. Skin prick test did not show any immediate skin reactions as compared to exposure to 1% histamine hydrochloride solution. No impact of terms of production (organic vs integrated) on allergenicity risk was observed. The influence of any agronomical practices and environmental conditions on allergenicity risk from 'Elsanta' strawberry needs further investigations.*

*The quality of fruits depended not only on cultivars but also on production system.*

**Keywords:** strawberry, allergy, fruit quality, organic, integrated fruit production

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## Introduction

Fruits from the Rosaceae family (e.g. peach, almond, cherry, apple and strawberry) are widely consumed and have been increasingly reported as causes of allergic reactions. Strawberry has a reputation among the general population of being an allergenic fruit. Hypersensitivity to strawberry is commonly reported but poorly documented. Considering the growing interest in organic cultivation, assessment of hypo(hyper-) allergenic potential of these products requires further research, as well.

The aim of the study was to assess the risk of any allergic reaction or food hypersensitivity resulting from topical application and chronic oral administration of selected strawberry cultivars – 'Elsanta' and 'Honeoye', coming from organic or integrated plantations.

## Material and Methods

The strawberry fruits of 'Elsanta' and 'Honeoye' cvs were harvested at plantations located in Central Poland, managed according to organic or integrated fruit production systems. The assessment of the risk of any allergic reaction was performed on young, adult, white albinotic guinea pigs (Dankin Hartley). The performed procedures included: I. Guinea-Pig Maximization Test (GPMT) - OECD 406; II. chronic oral administration of selected fruit extracts; III. skin prick (Dreborg) test (Dreborg et al, 1989) and IV. Determination of total IgE antibodies (commercial Guinea Pig Immunoglobulin E, IgE ELISA Kit). Fruit characteristics included: the total soluble solids content (TSS), titratable acidity (TA), sugar content (sucrose, glucose, fructose, and sorbitol), polyphenols content (chlorogenic acid, p-coumaric acid, quercetin, kaempferol, procyanidins, anthocyanins, ellagic acid), macro and micro nutrients (P, K, Mg, Ca, B, Cu, Fe, Mn, Zn, and S). In the tested fruits pesticides residues, heavy metals contamination, nitrate and nitrites content and microbiological contamination (fungi and bacteria incl. Escherichia coli) were also analyzed. Pesticides residues in fruits were analyzed according QuEChERS method using gas chromatography with mass detector (GC/MS) and liquid chromatography tandem mass spectrometry (LC/MS-MS) for 170 pesticides.

Extraction and determination of nitrites from plant material were conducted according to method described in PN-EN 12014-2: „Foodstuffs – Determination of nitrate and/or nitrite content – Part 2: HPLC/IC method for the determination of nitrate content of vegetable and vegetable products".

Content of calcium, phosphorus, potassium, magnesium, boron, copper, iron, manganese, zinc and sulphur, was determined after drying fresh plant material and microwave mineralization using inductively coupled plasma optical emission spectrometry (ICP-OES). Soluble solids were determined by indirect refractometric method using ATAGO PR-101 refractometer (ATAGO, Japan). Titratable acidity was determined by standard titration methods with DL 50 Graphix titrator (Mettler Toledo, Switzerland), by titration with 0.1N NaOH to the end point at pH=8.1. The sugar and polyphenols content were determined using the HPLC methods.

## Results and Discussion

The validation process of Magnusson-Kligmann test, which was performed with the use of benzocaine – substance with mild-to-moderate skin sensitization properties – has confirmed the sensitivity and reliability of the above experimental technique. The observations of skin reactions resulting from topical tests according to Guinea-Pig Maximization Test (GPMT) showed no significant changes in guinea pigs being exposed to extracts from 'Honeoye' strawberry coming from integrated or organic production. Topical exposition to 'Elsanta' strawberry from plantation managed according to organic practice

showed some changes (i.e. discrete, moderate or intense erythema and swelling), as compared to animals being exposed to ‘Elsanta’ from plantation managed according to integrated production. Chronic oral administration of selected fruit extracts did not cause any skin reactions in groups receiving ‘Elsanta’ or ‘Honeoye’ from organic or integrated productions. Skin prick test did not show any immediate skin reactions as compared to exposure to 1% histamine hydrochloride solution. Data presented in Table 1 show that regardless of the production system ‘Honeoye’ fruits characterized by higher titratable acidity compared to ‘Elsanta’. For both strawberry cultivars fruits from organic plantation had higher total soluble solids content and lower titratable acidity compared to those from integrated production. However, the quality parameters of fruits more often depend on the season than crop management practise (Peck *et al.*, 2006).

Regardless of cultivar, higher content of Ca, B, and S were found in fruits from plantation cultivated according to organic farming than from plantation cultivated according to IFP. In contrast K, Mg and Mn content were higher in fruits from IFP than from OR. Reganold et al (2010) also showed lower potassium content in strawberries produced under organic practice. The sugar and polyphenols content depended both on cultivars and production system. Fruits from organic farming contained higher amount of ellagic acid. It confirms data obtained by Hakkinen and Torronen (2000).

All fruits (except ‘Elsanta’ from IFP) were free from pesticide residues. For ‘Elsanta’ two pesticides (boscalid and cyprodinil) were detected, but at very low concentrations: 0.08 mg*kg-1 and 0.02 mg*kg-1 respectively. The MRL values are 10 mg*kg-1 for boscalid and 5 mg*kg-1 for cyprodinil. There were no microbiological contaminations (fungi, bacteria including Escherichia coli) in the tested fruits.

## Conclusion

No impact of terms of production (organic vs integrated) on allergenicity risk was observed for ‘Honeoye’ cv. The influence of any agronomical practices and environmental conditions on allergenicity risk from ‘Elsanta’ strawberry needs further investigations. The quality of fruits depends on cultivars but also production system.

Table 1: The influence of production system on total soluble solid content and fruit acidity of ‘Elsanta’ and ‘Honeoye’ strawberry cultivars.

| Cultivars | Production system | Total soluble solid - TSS (%) | Titratable acidity - TA (%) | TSS / TA ratio |
|---|---|---|---|---|
| ‘Elsanta’ | OR | 9.6 | 0.63 | 15,2 |
| | IFP | 8.7 | 0.79 | 11,0 |
| ‘Honeoye’ | OR | 10.3 | 0.95 | 10,9 |
| | IFP | 7.7 | 1.02 | 7,5 |

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# *Anthonomus rubi* (Strawberry blossom weevil): Covering as a control possibility in the late strawberry cultivar *Malwina* in the first year

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**Abstract**

Anthonomus rubi *is one of the key pests in organic strawberry production. The adult female is able to reduce the yield of strawberries up to 60% by cutting of the flower stalks after laying the eggs in the unopened flower buds. These damaged buds ceased to develop and either fall to the ground or remain dangling from the partially severed stalk.*

*While strawberry cultivars with a higher number of flower buds tend to compensate these losses, cultivars with a smaller number of flower buds, like* Malwina*, don't. So that the strawberry blossom weevil can cause severe yield losses and an economical disaster for the grower. One control possibility in the first year to reduce the amount of damaged flower buds is covering the plants with fleece or net. But as several one year field trials in 2010 and 2011 showed, a careful control management is necessary to reduce the damages and increase the yield amount of first class fruits significantly at the same time. Therefore one important key is the timing of covering the plants before the weevil enters the field. The other key is to provide a less stressful climate for the strawberry plants and developing fruits under covering conditions. Otherwise the yield of the first class fruits could be significantly negatively affected. Additionally the amount of the second class fruits could significantly increase as also the amount of the decomposed fruits.*

**Keywords:** *Anthonomus rubi*, strawberry, covering

## Introduction

In organic strawberry cultivation *Anthonomus rubi* is one of the key pests by reducing the yield harvest up to 60% (Svensson, 2002). The adult weevil is brown-black, 3-3,5mm long (Höhn & Stäubli, 2010) and usually flies into the one year strawberry fields from nearby forest areas (Kovanci et *al*., 2005) when constant temperatures of 13°C-18°C are reached (Höhn & Stäubli, 2010). The major host plant of the weevil is the strawberry (*Fragaria*) but it also feeds on other *Rosacea* genera like raspberry and blackberry (Berglund et *al*., 2007).The damages are caused by the weevil by cutting the flower stalks after laying one egg into the unopened flower bud. After cutting the flower stalks, the buds either fall down to the ground or remain on the stalk and stay unopened. The whole development from egg to the adult weevil occurs in the unopened flower bud which is used for protection against predators, drying out through direct sunlight (Blümel, 1989) and as a food source (Höhn & Stäubli, 2010). To protect the strawberry fields in the first year from weevil damages regulation strategies with the covering types net and fleece has been observed.

## Material and Methods

For the trial season in 2011 one strawberry field in Remshalden-Rohrbronn (wider area of Stuttgart, Germany) near by a forest area with strawberries in their first year after planting has been chosen for field trials.

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Malwina was chosen as strawberry cultivar because as former field trials over the last three years showed, Malwina starts to bloom when the female weevil is already present and seeking for strawberry buds. Furthermore Malwina it is one of the latest cultivars, highly tolerant against *Verticillium*-Wilt (*Verticillium dahliae*), Powdery mildew (*Erysiphe necator*) and Leather rot (*Phytophtora cactorum*) and therefore highly valuable for organic growers (Hinzmann, 2011). The net and fleece trials where built as a completely randomised block design with four replications over two single rows and 48 plants per variable, which were labelled with numbered tags. White net (Rantai Type S48, 0,8mm*0,8mm) in combination with flexible steel rods and white fleece (23g) where chosen for covering. For the fleece method three different cover dates where chosen in dependence on the weevil occurrence in areas which were a) more than two fields far away ($F_{far}$), b) nearby the trial area ($F_{near}$) and c) already present in the trial area ($F_{present}$). In $F_{far}$ the plants were covered at the 12$^{nd}$ April, in $F_{near}$ at the 21$^{st}$ April and in $F_{present}$ at the 29$^{th}$ April while the control stayed uncovered. At the 13$^{th}$ Mai the fleece was removed. For the net trial two variables of fixing the net edges to the ground where chosen. One variable was to fix the edges completely by digging the edges into the ground ($N_{opt}$) while the other was to fix the edges just randomly to the ground with soil ($N_{easy}$) while the control stayed uncovered. The strawberry plants of both variables were covered at the 21$^{st}$ April and uncovered at the 13$^{th}$ May. To evaluate the damages of the strawberry blossom weevil the ratings criteria “Destroyed flower buds” and “Yield” were performed (Table 1).

Table 1: Fleece & Net: Rating criteria “Destroyed flower buds [%]” and “Yield [g/48 plants]”, 2011.

| Rating criteria | Counted data of amount of |
|---|---|
| Destroyed flower buds [%] | - destroyed flower buds, unopened flower buds, opened blossoms, early developed fruits |
| Yield [g/48 plants] | - first class fruits, second class fruits, unsalable fruits (fall) |

In fleece the data rating in regards to the “Destroyed flower buds” were performed at the 9$^{th}$June and in net at the 14$^{th}$ June. In regards to the “Yield” the rating in fleece and net started on the same day the destroyed flower buds were counted and were performed at six further dates (Table 2).

Table 2: Fleece & Net: Dates of yield rating, 2011.

| | Fleece | Net | | Fleece | Net | | Fleece | Net |
|---|---|---|---|---|---|---|---|---|
| 1. | 9$^{th}$ June | 14$^{th}$ June | 3. | 17$^{th}$ June | 21$^{st}$ June | 5. | 23$^{rd}$ June | 27$^{th}$ June |
| 2. | 14$^{th}$ June | 17$^{th}$ June | 4. | 21$^{st}$ June | 23$^{rd}$ June | 6. | 27$^{th}$ June | 30$^{th}$ June |

Statistic analysis of data was made with R 2.13.1, The R Project for Statistical Computing.

## Results

Fleece (F): The variable $F_{far}$ showed significant less destroyed flower buds (7,9%) than the Control (30,9%), $F_{near}$ (14,7%) and $F_{present}$ (20,6%). Furthermore the variables $F_{near}$ and $F_{present}$ showed significant less destroyed flower buds than the Control (Figure 1, left). In regards to the yield of the first class fruits (class I) $F_{near}$ showed significant more fruits (10.095g) than $F_{far}$ (7.728g) and the Control (7.704g). The yield of the second class fruits (class II) showed in $F_{far}$ significant more picked fruits (2.394g) than in the Control (925g) and $F_{present}$ (1.608g). Furthermore $F_{near}$ showed significant more second class fruits than the Control. The unsalable fruits (fall) showed yield amounts between 506 in the Control and 960g in $F_{far}$ but no significant differences (Figure 1, right).

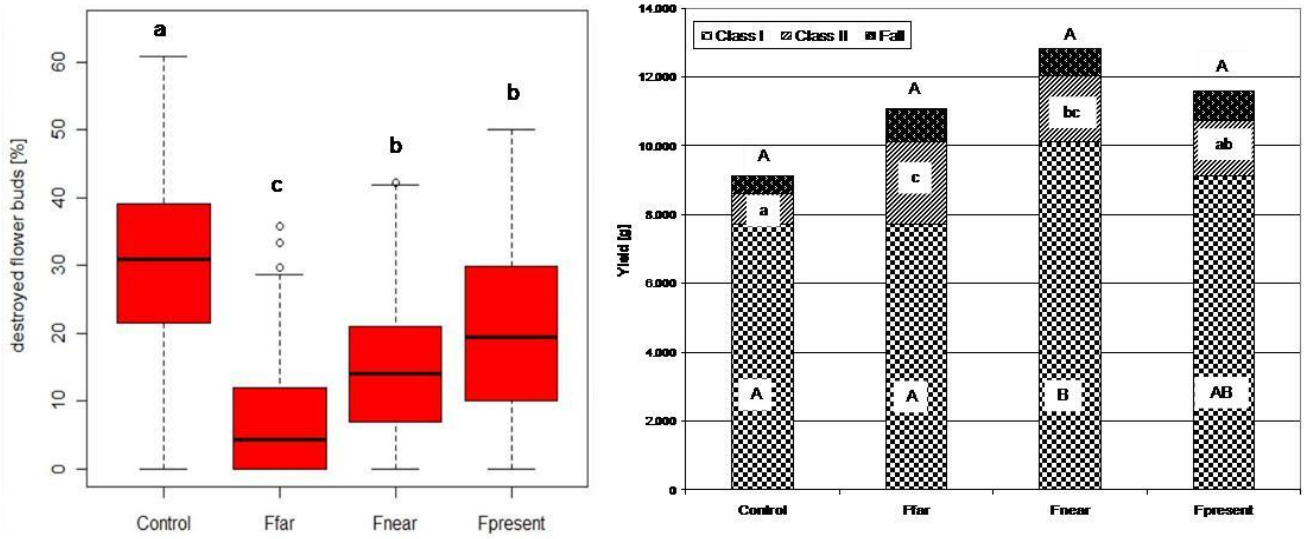


Figure 1: Fleece-Covering (2011) for $F_{far}$ (12.4), $F_{near}$ (21.4), $F_{present}$ (29.4) in the cultivar Malwina (n=48 plants/variable). Left: Destroyed flower buds [%] (pairwise CI, Harrell-Davis-Test, α=0,05). Right: Yield of first class fruits, class I (ANOVA, Tukey-Test, α=0,05), second class fruits, class II (ANOVA, Tukey-Test, α=0,05) and unsalable fruits, fall (pairwise CI, Hodges-Lehmann, α=0,05). Values with different letters differ significantly.

Net (N): $N_{opt}$ showed significant less destroyed flower buds (21%) than the Control (32%) (Figure 2, left). The yield of the first class fruits (class I) showed yield amounts between 6.381g in $N_{opt}$ and 8.056g in $N_{easy}$ and no significant differences between the variables. The yield of the second class fruits (class II) showed in $N_{opt}$ a significant higher yield amount (3.153g) than in the Control (2.292g). The yield of the unsalable fruits showed yield amounts between 800g in $N_{easy}$ and 1.102g in the Control and no significant differences (Figure 2, right).

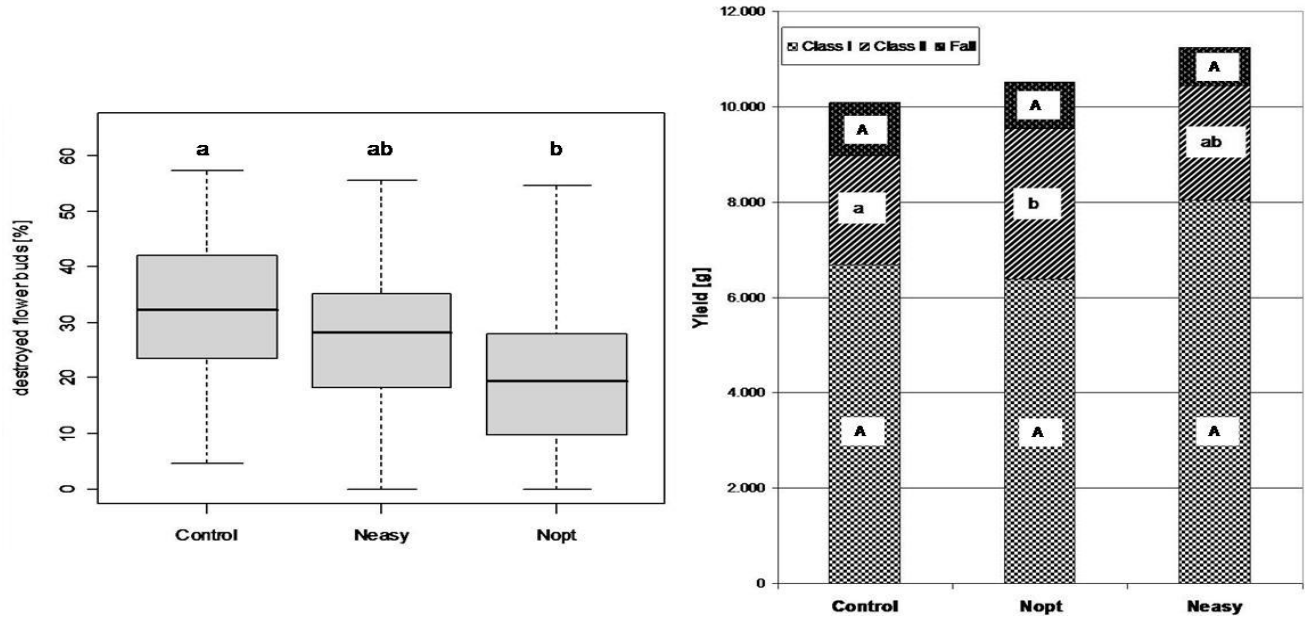


Figure 2: Net-Covering (2011) for $N_{easy}$ and $N_{opt}$ (21.4.2011) in the cultivar Malwina with n=48 plants/variable. Left: Destroyed flower buds [%] (ANOVA, Tukey-Test, α=0,05). Right: Yield of first class fruits, class I (ANOVA, Tukey-Test, α=0,05), second class fruits, class II (ANOVA, Tukey-Test, α=0,05) and unsalable fruits, fall (pairwise CI, Hodges-Lehmann, α=0,05). Values with different letters differ significantly.

## Discussion

At the present time it is quite difficult to control the strawberry blossom weevil in organic farming. For plants in the first year covering is a possibility but the covering date itself is highly important to reduce the destroyed buds significantly in comparison to the control. The early covering date ($F_{far}$) in **Fleece** showed the significant lowest percentages of destroyed flowers buds compared to the control and the variables with coverings dates of nine ($F_{near}$) and seventeen days ($F_{present}$) later than $F_{far}$. But $F_{far}$, the supposed most effective variable, did not reflect it in the yield of the first class fruits, because $F_{far}$ showed almost the same yield amount of first class fruits than the control. Furthermore $F_{near}$ and $F_{present}$ showed also significant less destroyed flower buds than the control and in contrast $F_{near}$ showed a significantly higher yield amount of first class fruits than the control and $F_{far}$. However $F_{far}$ showed a significantly higher yield amount of the second class fruits than the control and $F_{present}$. Meanwhile $F_{near}$ also showed significantly more second class fruits than the control. Even so the variables in **Net** where different compared to fleece they showed also that perfect covering as in $N_{opt}$, caused significantly less destroyed flower buds compared to the Control. But it showed also, that this effect did not reflect in the yield of first class fruits either. Therefore $N_{opt}$ showed also a significantly higher yield of second class fruits compared to the control. Due to the fact that covering causes higher temperatures and a microclimate with higher humidity (Wilcox & Seem, 1994) the plants and fruits had to grow and develop mainly under stressful conditions. So that even if the blossom weevil attack can be significantly reduced by covering the climate stress still influences negatively the yield of the more valuable first class fruits. Svensson (2002) and Berglund et *al*. (2007) showed for different cultivars the same effects and furthermore that the amount of decomposed fruits could also increase. The results for the cultivar Malwina showed furthermore that a) less effective methods like $N_{easy}$ and b) methods were the period of time for covering was shorter, like $F_{near}$ and $F_{present}$, can cause significantly higher yield amounts for first class fruits. More research has to be done with other types of covering to reduce the climate stress in combination with a monitoring method to optimize the period of covering.

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# Influence of antagonistic micro-organisms on the growth of strawberry plants in the presence of *Verticillium dahliae* and *Phytophthora cactorum*

I.L. Bisutti[1], J. Pelz[1] and D. Stephan[1]

**Abstract**

*Soil borne diseases are an increasing problem in organic strawberry production. In a national founded project, a strategy for biological control of* Verticillium *spp. and* Phytophthora *spp. was investigated. After an in vitro selection, four antagonistic micro-organisms (*Trichoderma harzianum*,* T. atroviride*,* Metarhizium anisopliae and Bacillus subtilis*) were tested in greenhouse and field trials (2010-2011) in the presence of* V. dahliae *and* P. cactorum*.*

*In greenhouse experiments the growth of strawberries in the presence of* V. dahliae *could be positively influenced by the antagonist. But these results were not confirmed by first field trials. In case of* P. cactorum *inoculation, neither in greenhouse nor in field trials differences could be observed.*

**Keywords:** Strawberry, soilborne diseases, *Trichoderma*, *Bacillus, Metarhizium*

## Introduction

Strawberry (*Fragaria* x *ananassa* Duchesne) is an important berry culture in Germany. In organic, but also in conventional production, soil-borne diseases (e.g. *Verticillium* spp., *Phytophthora* spp.) are an increasing problem because no efficient control systems are available.

*Verticillium dahliae*, distributed across the temperate and subtropical zones of the world, is a pathogen for a wide range of dicotyledonous plant species. (Barbara & Clewes, 2003; Fradin & Thomma, 2006). The pathogen invades the vascular system, with consequent wilting and eventually death of the plant (Barbara & Clewes, 2003; Dessimoz *et al*., 2011). In the soil, the fungus produces microsclerotia (MS) that can survive and infect plants up to 15 years (Fradin & Thomma, 2006; Dessimoz *et al*., 2011).

The pathogen *Phytophthora cactorum* has a wide host range and causes leather rot on fruits and crown rot in strawberry plants. The pathogen enters the plants through wounds (Maas, 1998); the plant wilts and browns at the base of the petiole and the upper part of the crown (Lilja *et al*. 2006).

Within an *in vitro* screening four micro-organism with antagonistic potential against the two pathogens were selected for further *ad planta* experiments. *Trichoderma* spp. and *Bacillus subtilis* are known to be antagonistic to different plant pathogens. *Metarhizium anisopliae*, an entomopathogenic fungus, is used for insect control. The aim of these experiments was to evaluate if/how the selected antagonists were able to influence the growth of the strawberry in the presence of soil-borne pathogens.

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## Material and Methods

Pathogen production

*V. dahliae* was produced as conidia in liquid Czapek-Dox (grown 7 days at 25 °C on a rotary shaker) and as MS following the description of Neumann (personal communication). *P. cactorum* was grown on rye agar with the supplementation of ß-sitosterol (25 °C for two weeks, afterwards 15 °C until use). Spores and mycelium were scraped from the agar plate with sterile water.

For both pathogens the inoculation concentration was $10^5$ conidia or spores $mL^{-1}$.

Antagonists production

*T. harzianum* T58 and *T. atroviride* P1 were grown for 14 days on boiled wheat at 25 °C in the dark. Afterwards the conidia were washed off with a 0.01% Tween® 80 solution. The concentration of the suspension used for the experiments was $10^5$ conidia $mL^{-1}$.

*M. anisopliae* Ma43 was grown under the same conditions as *Trichoderma* but on a rice-oat mix (4:1) as substrate. Conidia were washed off with 0.1% Tween® 80 solution and also adjusted to a concentration of $10^5$ conidia $mL^{-1}$.

*B. subtilis* FZB24® was provided as product and used as described on the package.

The Mixture was made using the four antagonist suspensions in the same proportion.

Greenhouse experiments

Tests were performed with Frigo strawberry plants. The cv. Honeoye was used in the trials with the pathogen *V. dahliae*, the cv. Sonata with the pathogen *P. cactorum*. For application of the antagonists the roots of the plants were dipped for 15 min in the antagonist suspension (or in water for the control) and then planted in pots containing 0-soil (Fruhstorfer Erde®). The pathogen inoculation was performed by pipetting 1 mL of conidial suspension directly on the roots before covering with soil. Afterwards, every two weeks (for four times) 250 mL of the antagonist suspension were added to the pot. The strawberries were kept at a soil temperature of 20 °C with a day/night photoperiod of 16/8 h. After four months the plants were harvested and the growth and yield parameters were determined. The trials were repeated three times with six plants each.

Field experiments

In 2010 field experiments were set up on fields at the JKI, Institute for Biological Control. For these trials the same cv. as described before were used. Again, the strawberry roots were dipped for 15 min in the antagonistic suspension and planted in the fields. Afterwards, every two weeks (for four times) the plants were watered with the antagonist suspension (5 L of $10^5$ conidia $mL^{-1}$ per row, 30 plants per row) In case of field 1 no pathogens were added (from analysis and history of the field it was know that both diseases were present.); for field 2 pathogens were added in form of conidial suspension for *P. cactorum* and of a MS/sand mix for *V. dahliae*. The visual rating followed after one year (field 1) and nine months (field 2) after planting (1. vital plant – 5. dead plant).

## Results and discussion

In greenhouse experiments two strawberry cultivars were inoculated with the soil-borne diseases and were treated with the four antagonists and a mixture of them. For both pathogens no clear infection symptoms were monitored. In the experiment with *V. dahliae* all antagonists show an increase of all growth parameters measured (tab. 1). When the four antagonists were compared no clear tendencies and no additive effects for the

mixture were observed. For *P. cactorum* no evident effects of the antagonists on the investigated growth parameters were seen (tab. 2).

Table 1: Results of greenhouse experiments cv. Honeoye inoculated with *V. dahliae*.

| Treatments | n* | % biomass increase | Leaves | | | Roots | |
|---|---|---|---|---|---|---|---|
| | | | Mean of plant height (cm) | Sum | DW (g) | Mean of length (cm) | DW (g) |
| Control | 15 | 54 (± 15) | 15.5 (± 4.6) | 144 | 59.62 | 22.0 (± 4.9) | 9.27 |
| *B. subtilis* FZB24 | 17 | 64 (± 10) | 18.3 (± 1.9) | 209 | 101.33 | 26.3 (± 2.8) | 19.07 |
| *T. atroviride* P1 | 16 | 63 (± 10) | 16.8 (± 2.8) | 170 | 78.56 | 24.5 (± 7.5) | 18.55 |
| *T. harzianum* T58 | 18 | 70 (± 7) | 19.1 (± 2.8) | 164 | 105.12 | 30.0 (± 4.3) | 16.68 |
| *M. anisopliae* Ma43 | 17 | 66 (± 9) | 19.3 (± 2.1) | 185 | 110.94 | 27.5 (± 6.6) | 14.79 |
| Mixture | 17 | 63 (± 6) | 18.8 (± 2.5) | 176 | 95.01 | 25.6 (± 4.7) | 10.87 |

DW=dry weight. *number of plants alive at the scoring day.

Table 2: Results of greenhouse experiments cv. Sonata inoculated with *P. cactorum*.

| Treatments | n* | % biomass increase | Leaves | | | Roots | |
|---|---|---|---|---|---|---|---|
| | | | Mean of plant height (cm) | Sum | DW (g) | Mean of length (cm) | DW (g) |
| Control | 18 | 68 (± 8) | 23.5 (± 2.7) | 158 | 104.46 | 27.9 (± 4.6) | 13.90 |
| *B. subtilis* FZB24 | 17 | 67 (± 10) | 23.8 (± 4.3) | 155 | 107.88 | 27.5 (± 3.5) | 14.17 |
| *T. atroviride* P1 | 17 | 66 (± 8) | 22.5 (± 3.3) | 168 | 108.34 | 27.4 (± 4.9) | 12.80 |
| *T. harzianum* T58 | 18 | 67 (± 9) | 23.1 (± 2.9) | 158 | 101.95 | 26.1 (± 4.1) | 13.48 |
| *M. anisopliae* Ma43 | 17 | 61 (± 12) | 19.9 (± 5.4) | 178 | 109.91 | 26.5 (± 5.7) | 21.97 |
| Mixture | 18 | 62 (± 9) | 22.5 (± 3.0) | 143 | 95.49 | 28.3 (± 4.3) | 13.84 |

DW=dry weight. *number of plants alive at the scoring day.

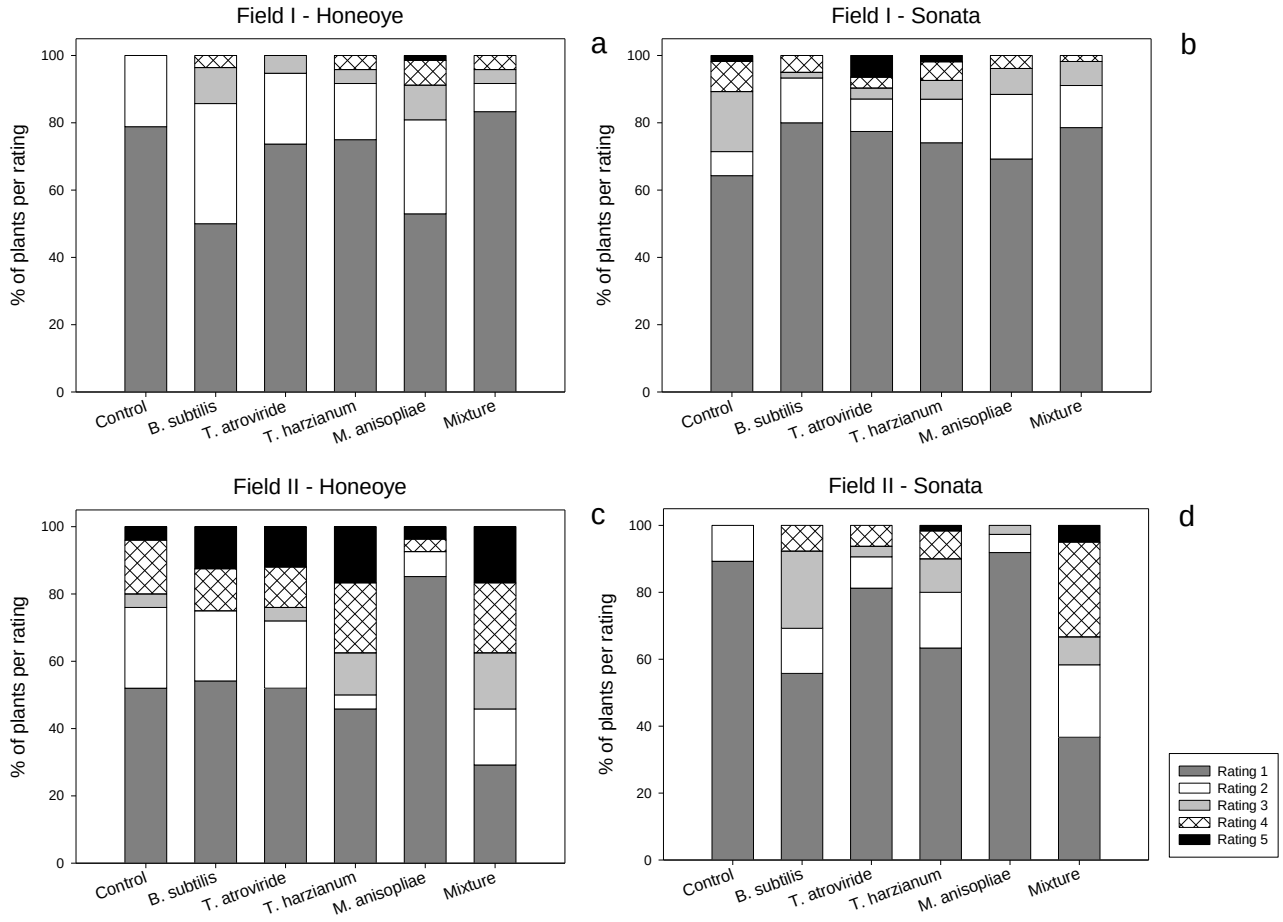


Figure 1: Visual rating of the two fields: Field 1 naturally infected; Field 2 artificially inoculated.

In the field 1 trials some differences between antagonists could be seen for the cv. Honeoye (figure 1a). For *B. subtilis* and *M. anisopliae* only ca. 50% of the plants were rated as vital (rating 1) whereas all other treatments, including control, show more than 70% of rating 1. After artificial inoculation (field 2, figure 1c) the number of plants with rating 1 was reduced for all treatments, except for *M. anisopliae*. The number of dead plants (rating 5) was increased in comparison to field 1.

For the cv. Sonata in field 1 (figure 1b) the percentage of plants with rating 1 was nearly similar. None of the treatments showed a positive effect. The artificial inoculation (field 2, figure 1d) did not show an increase of diseased plants in the control. It can be supposed that the weather conditions in 2010-2011 did not support the disease.

In field 2 (figure 1c and d) the negative effect of the Mixture and, on the other side, the positive effect of *M. anisopliae* can possibly be caused by other factors e.g. white grubs or different water capacity of the soil.

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# Etiology of the corymb wilting of Elderberry and its control in organic production

Dr. H.-J. Krauthausen[1], I. Toups[2], G. Hörner[1], B. Benduhn[3] , J. Zimmer[2], T. Schult[2]

**Abstract**

*The corymb wilting of elderberry (*Sambucus nigra*) is the major problem in organic elderberry growing. To investigate the possibilities to regulate the necrosis the first aim of the project was to determine the pathogen, which causes the corymb wilting, its timing of infection and overwinter spots. In all tested elderberry orchards* Colletotrichum acutatum *was the main cause of the necrosis (all Koch'sche Postulates were implemented). Other fungi pathogens such as* Fusarium sp., Phoma sp. *or* Marssonina sp*. were of subordinate importance. On samples collected in winter the pathogen could be found on not yet rotted berries, corymb parts and branches as well as on apical buts of the fruit wood of the next season. A lot of fungicides and plant strengtheners allowed in organic growing were tested of its potential to control corymb wilting in laboratory and in field trials, but showed no satisfying results in controlling the disease. Because the microclimate is of importance to the infection and the infestation level, a part of an elderberry orchard was roofed over a particular time of season in two years of trials. A roof from blossom to harvest lowers the infestation by almost a hundred percent, whereas roofing from blossom until July showed only slight decrease of the infestation level.*

**Keywords:** elderberry, corymb wilting, *Colletotrichum acutatum*

## Introduction

There are no treatment strategies to combat the corymb wilting in the organic elderberry growing by now. The aim of our project was to develop a relevant practice strategy to combat the necrosis with the combination of different methods and to establish them to the cultivation practice. To combat the necrosis, it is necessary to determine the timing of appearance of the pathogen and the pathogen itself, which is responsible for the necrosis. In Austria, *fusarium, alternaria* and *phoma* were found in fungus isolates, while *colletrotrichum* and *ascochyta* as the main cause of the necrosis were found in the LVG in Erfurt (Möhler, 2003, Steffek *et al*. 2001 and 2002).

The project "Etiology of the corymb wilting of Elderberry and its control in organic production" was funded for five years (2007-2011) by the Federal Agency for Agriculture and Food (Bundesanstalt für Landwirtschaft und Ernährung, BLE) within the "Bundesprogramm ökologischer Landbau und anderer Formen nachhaltiger Landwirschaft" (Project Number: 06OE327). The trials have been established in cooperation with several partners: At the "DLR Rheinpfalz, Kompetenzzentrum für Gartenbau" in Klein-Altendorf and the „DLR Rheinpfalz, Phytomedicine division" in Neustadt a.d.W. and in cooperation with the "Ökoobstbau Norddeutschland Versuchs-und Beratungsring e.V." (ÖON) in Jork. Selected laboratory and field trials results are shown in this paper.

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## Material and Methods

31 preparations, accredited in the organic fruit growing, were tested for their efficacy against *Colletotrichum acutatum* in a disk-diffusion-test on a PDA-Medium. Agar disks were spread with a suspension of spores. Five filter papers, dipped in sterile water, the reference preparation ‘Switch’ and three different organic fungicides, were placed on the disk and incubated for seven days at 20°C (five repetitions for each variant). After incubation visual interpretations of the inhibition of the mycelia growing were made.

During season, samples of corymbs in all stages from blossom to harvest were collected to validate the appearance of *Colletotrichum acutatum.* The samples were incubated for 7 days in wet chambers at 20°C. Afterwards fruit bodies and/or spores were visually interpreted under microscope.

To detect the overwinter spots of *Colletotrichum acutatum* samples of pruning remains, one year shoots and not yet rotted leaves, berries and corymbs were collected in an elderberry orchard in December and March/April. They were treated the same as the corymbs but incubated for 20 to 22 days.

To detect the influence of microclimate, in particular the humidity, on infection time and rate, parts of an elderberry orchard in West Germany were roofed over in the season 2010 and 2011 (Table 1).

Table 1: Roof trial variants in an organic elderberry orchard, West Germany, 2010/11

| variant | name |
|---|---|
| 1 | without roof |
| 2 | roofing from blossom to harvest |
| 3 | roofing from blossom to July |
| 4 | roofing from July to harvest |

## Laboratory results

Eight of 31 tested preparations in the disk-diffusion-test on PDA-nutrient solution showed a slight or middle inhibition on the mycelia growing of *Colletotrichum acutatum* (Table 2). But compared to the reference fungicide ‘Switch’, which causes a complete inhibition, it were only slight effects.

On the samples of corymbs in all stages from blossom to harvest, the pathogen appeared partially right from blossom on until harvest. In all orchards, the frequency of the pathogens increased dramatically at the start of ripening of the berries to a hundred percent infestation at harvest (Figure 1).

Table 2: results of the disk-diffusion-test

| No. | preperation | inhibition |
|---|---|---|
| 1 | B-End (Rutaceen-Plant-Extract) 1,5 l/ha | < 1mm |
| 2 | **B-End (Rutaceen-Plant-Extract) 3,0 l/ha** | **< 5mm** |
| 3 | Polyversum 100 g/ha | no |
| 4 | Polyversum 200 g/ha | < 1mm |
| 5 | B-End + Polyversum (1,5 l/ha / 100 g/ha) | < 1mm |
| 6 | Elderberryextract T1 | no |
| 7 | Black elderberry (powdered) | no |
| 8 | Wormwood (powdered) | no |
| 9 | Mistletoe (powdered) | no |
| 10 | Juniper (powdered) | no |
| 11 | Horse tail (powdered) | no |
| 12 | P1 (Trifolio) | < 1mm |
| 13 | Ventex | < 1mm |
| 14 | SPU 02700 (200 g Cu) | no |
| 15 | SPU 02700+ Polyversum (200 g Cu / 100 g/ha) | no |
| 16 | SPU 02700+ IBD B-10 (200 g Cu / 1,5 l/ha) | < 1mm |
| 17 | **Lime sulphur** | **5 mm** |
| 18 | sulphur | no |
| 19 | sulphur+ Polyversum | no |
| 20 | Vacciplant (Lamarin from brown alga) | no |
| 21 | Frutogard (phosphonate) | no |
| 22 | Amicarb (potassium-bicarbonate) | no |
| 23 | Armour-Zen (Chitosan) + Nufilm | no |
| 24 | Binab (Trichoderma) + sugar | no (no spores) |
| 25 | Folanx Ca29 (Calciumformiat) | no |
| 26 | Prev-AM (Orange-extract) | no |
| 27 | Cocana (Kali-soap) | no |
| 28 | Whey | no |
| 29 | Vitisan (potassium-hydrogencarbonate) | no |
| 30 | OmniProtect (potassium-carbonate) | no (no spores) |
| 31 | Steinhauers Mehltauschreck (sodium bicarbonate) | no |
| 32 | **Switch** (Fludioxonil + Cyprodinil) | **complete** |

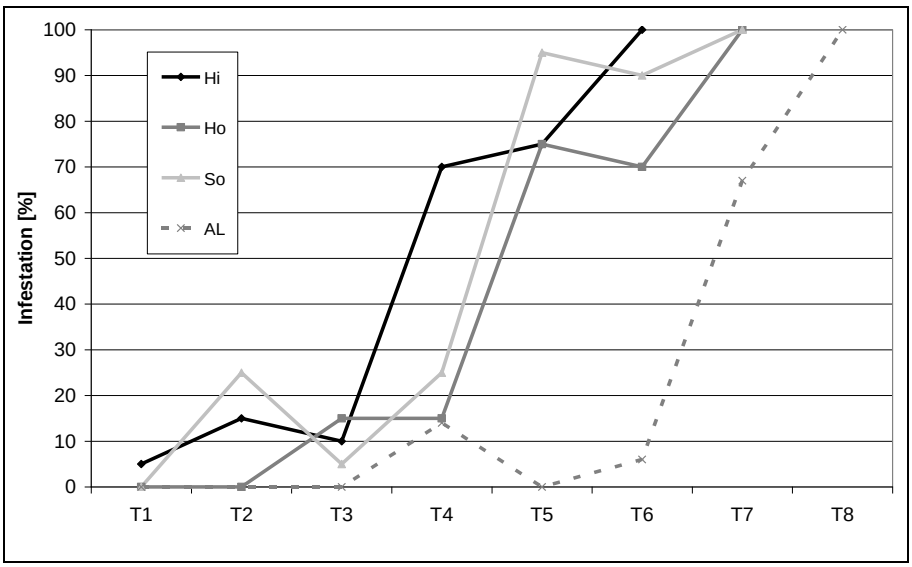


Figure 1: Percentage of infested corymbs in four different untreated orchards (Hi, Ho, So, AL) in the season 2008; T1…T8 = date of sample collection fortnightly from 29th of May to 11th of Sep

On the samples collected in an elderberry orchard in December, the pathogen could be found on not yet rotted berries and fruit stems with a frequency of 75 or 50 percent infestation. On samples collected in March and April the pathogen could be found on buts of one year shoots (fruit wood for the next season) mainly on apical spots. Also the pathogen appeared on not yet rotted branches and corymb parts of the previous season and on remaining branch parts on the tree after pruning the previous season's fruit wood.

**Field trials results**

With average levels near to 80 % corymb wilting in 2011, the infestation in the orchard was much higher than in the season of 2010. Still the roofing of parts of the orchard from blossom to harvest could decrease the infestation level by almost a hundred percent in both years (Figure 2). The roofing from blossom to July had no efficacy in 2010 and only 38.5 % in 2011. The roofing from July to harvest showed efficacies of 46.8 % in 2010 and 91.7 % in 2011.

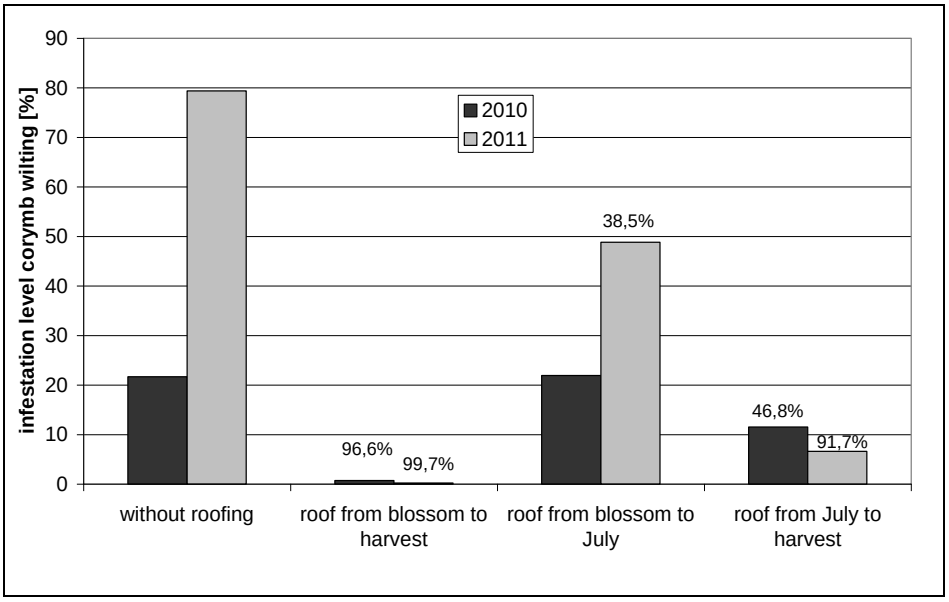


Figure 2: Infestation levels of corymb wilting at the time of first picking with and without a roof, 2010 and 2011 and efficacies of treatments

## Discussion

The phytomedical diagnostic detected *Colletotrichum acutatum* as the main cause of the corymb wilting in all tested elderberry orchards in West and North Germany (all Koch'sche Postulates were implemented). Other fungi pathogens such as *Fusarium* sp., *Phoma* sp. or *Marssonina* sp. were of subordinate importance. On samples collected in winter the pathogen could be found on not yet rotted berries, corymb parts and branches as well as on apical buts of the fruit wood of the next season. During the season, the infestation level of the corymb wilting increased with the start of ripening of the berries (turn of colour from green to red/black) and reaches often infestation frequencies of a hundred percent.

The control of the corymb wilting in organic elderberry growing turned out to be very difficult. The application of usual organic fungicides such as copper, sulphur and potassium-bicarbonates tested in various exact-trials in field, proved not to be of satisfying efficacies, especially in years of high infestation levels.

Because the microclimate is of importance to the infection and the infestation level, in two years of trials a part of an elderberry orchard was roofed over a particular time of season. A roof from blossom to harvest lowered the infestation by almost a hundred percent, whereas roofing from blossom until July showed only slight or no decrease on the infestation level. Supposedly, the time of blossom is important for the infection, but roofing from July to harvest reduced corymb wilting by nearly 92% in a season of high infestation levels. It can be concluded that blossom time is of lower importance for infection than previously expected. Nevertheless a roof is not realizable in practice, due to high effort and expense.

Because there is no effective control strategy by applications, further trials to control the corymb wilting by improving the orchard sanitation should be conducted. To decrease the pathogen potential, specific treatments should be applied to eliminate overwinter spots of the pathogen in the elderberry orchards.

**Acknowledgements**

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# Effect of some organic fertilizers and amendments on the quality of maiden trees of two apple cultivars

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**Abstract**

*Production of organic maiden trees is considered difficult due to the high needs of nutrients required to obtain trees of good horticultural quality. In the framework of the EU-funded research project 'Development of innovative products and technologies for organic fruit production a specific work package is devoted to the development of methods of nursery production. Maiden trees of apple cultivars 'Topaz' and 'Ariwa' grafted on M26 rootstocks were produced with the use of a* vegetal amino-acids *(BF Amin), a consortium of mycorrhizal fungi and plant growth promotion rhizobacteria (Micosat F12WP and Micosat FMS 200), and their quality was compared to plant grown with organic animal manure standard NPK or without fertilization.*

*The application of BF Amin resulted in the highest trunk thickness and tree height for both cultivars. The product induced also an increase of the branching of the maidens and of the length of lateral shoots. Micosat also positively influenced the branching of the maidens in comparison to control and NPK treatments, but to a lower extent with respect to BF Amin.*

*The two new biofertilizers seem to provide the plants with an adequate nutrient supply and could be utilized successfully for the application in organic apple nurseries.*

**Keywords:** organic nursery, bioproducts application, apple maidens quality

## Introduction

Fruit-growers have noticed that the quality of maiden trees has a decisive effect on their growth and fruiting during the first years in a newly-established orchard (Mika *et al*. 2003; Gudarowska and Szewczuk, 2004; Wociór and Kaplan 2005). It has been proven that each millimetre of increase in the trunk diameter of a maiden tree in the nursery results in a specific increase in the yield of the young tree in the orchard (Wociór and Kaplan 2005), which can result in an considerable increment of production per hectar. Therefore, fruit growers are looking for well developed maiden trees, with a suitably shaped, well branched crown (Shepherd 1979; Bielicki *et al*. 2002; Jaumień *et al*. 2004;).

Maiden trees that are not well fertilized in the nursery do not form lateral branches, or if they do, only very short ones. To stimulate the growth of maiden trees, many different mineral fertilizers with high nitrogen content can be used in conventional nurseries (Janisz *et al*. 2002; Wójcik 2003, 2009). In organically certified ones, however, the management of nitrogen nutrition is more difficult due to the restrictions deriving from either the EU legislation or International standards of organic production (Sas Paszt *et al*. 2010). Such difficulty is underlined by the possibility of planting organic fruit orchards with plants obtained in derogation to the EU regulations. In the framework of the EU-funded research project 'The development of innovative products and technologies for organic fruit production' a work package is devoted to the development of organic methods of fruit trees production. The aim of the research is to develop a nursery model suitable for commercial production of organic fruit trees.

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Conducting basic research with manual application of innovative products and determining their biological effectiveness (Grzyb *et al*. 2010).

The present paper reports the results of a trial established to evaluate the quality of maiden apple trees produced by applying various fertilizers and amendments.

## Materials and methods

The study was conducted during the seasons 2009-2010 in an experimental nursery located in Mokra Lewa near Skierniewice, Poland. The experiment was set up in a randomized block design with four replications of ten plants for each treatment. In the first year, M26 apple rootstocks were planted and treated with the different products (see below), and in the second year the same applications were made on maiden trees after grafting in first year, the rootstocks with buds of the apple cultivars 'Topaz' and 'Ariwa'. The following fertilization treatments were applied:

1 Micosat (CCS Aosta Srl), a microbial consortium formed of mycorrhizal fungi and plant growth promoting rhizobacteria; two formulations were utilized during each season: 'Micosat F12 WP', applied during the first application period to the soil in granular form at a dose of 10 g $m^2$, (100 kg $ha^{-1}$) and 'Micosat F MS 200' applied during the second period to the leaves at a dose of 1 g $m^2$ (10 kg $ha^{-1}$);
2 'BioFeed Amin' (Agro Bio Products B.V.), an vegetal amino-acids, applied to the soil at a concentration of 0.5% (0,05ml/$m^2$) (5 l $ha^{-1}$).

The treatments were compared to a control not receiving any fertilization (No fertilization) and to a standard fertilization with either an organic or a conventional fertilizer: 'Fertigo' (Ferm-O-Feed), granulated manure at a dose of 150 g $m^2$ (1500 kg $ha^{-1}$); NPK, at a dose of N – 60 kg $ha^{-1}$, P – 30 kg $ha^{-1}$, and K – 80 kg $ha^{-1}$ (it is 17,64 g/$m^2$ $NH_4NO_3$, 6,52 g/$m^2$ triple super phosphate, and 16,0 g/$m^2$ $K_2SO_4$).

The soil with the plants treated with Micosat and BF Amin, before the application of these products, was scattered on the surface with half dose of granulated manure (i.e. 75 g $m^2$) All the preparations were applied to the soil twice: in the rootstock nursery in mid-May and again in mid-June, and in the nursery of maiden trees – in late April / early May and the second time in the first 10 days of June. After the application of the preparations, the soil around the plants was always thoroughly mixed with hoes by hands.

In autumn, before digging up the trees, the following parameters were measured: trunk diameter at a height of 30 cm above ground, tree height, the number of branched trees, the length and number of lateral shoots longer than 5 cm.

The data were analyzed statistically by ANOVA. Means were compared with Tukey's test at a significance level of $p < 0.05$. The percentage of branched trees was standardized using Bliss' transformation. In the tables, the data that do not differ significantly are marked with the same letters.

## Results and discussion

The treatment of 'Topaz' maiden apple trees with Micosat and BF Amin significantly increased the trunk diameter in comparison to all controls (Zero fertilization, NPK and Manure) (Tab. 1A). No differences of the trees height between plants treated with Micosat and BF Amin and those receiving NPK and Manure were found (Tab. 1A). However, all the treatments induced a higher height in comparison to the zero fertilization treatment.

In view of what other authors have said about the effect of the quality of maiden trees on their subsequent fruiting in the orchard, the biopreparations BF Amin can be recognized as a product contributing to the production of high quality trees (Słowiński and Sadowski, 2000; Bielicki *et al*. 2002; Gudarowska and Szewczuk, 2004).

Maiden apple trees cv. ‘Topaz’ were in most cases branched, irrespective of the treatment applied (Tab. 1A). However, even though the analysis did not show statistical differences between treatments and the controls, the maidens treated with BF Amin and Micosat had a greater tendency towards better branching than the others. This also emerges from the analysis of the number of lateral shoots: trees treated with Micosat and BF Amin had almost twice the number of lateral shoots in comparison to zero fertilization and about 50% more than in trees fertilized with NPK or Manure. Furthermore, trees that had not been fertilized at all had only few, usually short lateral shoots, which resulted in a total length of about 30% in comparison to the fertilized trees. By contrast, Micosat, and especially BF Amin, significantly increased the number of shoots in the crown of the maidens in comparison to both kind of fertilizers (NPK and Manure) with a total length which was the same as of these two latter treatments (Tab 1A). Considering these two parameters together, even though we have not measured the diameter of the lateral shoots, we noticed that their size in trees receiving Micosat and BF Amin was higher than those of NPK and Manure treated plants. Considering the results obtained with the cultivar ‘Ariwa’, the type of fertilization did not have a major impact on the diameter of the maiden trees (Tab. 1B). Nevertheless, BF Amin induced an increase in their height. Similarly to ‘Topaz’, both Micosat and BF Amin induced a branching on a higher number of trees in comparison to the other treatments, though not statistically significant. BF Amin also significantly stimulated the branching and shoot development of the maiden trees of this apple cultivar. A similar effect, albeit on a slightly smaller scale, was also observed in the plants treated in the nursery with the preparation Micosat.

Several authors have studied the effect of the quality of maiden trees on their subsequent fruiting in the orchard (Słowiński and Sadowski 2000; Bielicki et al. 2002; Gudarowska and Szewczuk, 2004; Wociór and Kapłan, 2005). A well formed plant with branches of good size and length, as well as with an adequate diameter of the main shoot are assuring an early formation of the tree canopy and early yield. The results obtained with both the organic fertilizer (BF Amin) and the amendment (Micosat) used in this study, have shown that these products can allow to produce trees for both cultivars that meet the quality requirements for high-quality maiden trees, as specified by other authors involved in the production of trees in nurseries by conventional methods (Bielicki *et al*. 2002; Mika *et al*. 2003; Sadowski and Górski, 2005).

In assessing these results, it shall be considered that the amount of nutrients provided with the organic treatments is far below that applied with the chemical fertilizer (Tab 1). An vegetal amino-acids extracts are known to contain nutrients in organic forms that are much readily utilized by the plants (Sas *et al.* 2009), thus enhancing the efficiency of physiological processes and reducing the energetic need for the normal plant metabolism (Malusà *et al*. 2010). On the other hand, both arbuscular mycorrhizal fungi and PGPR are recognized to beneficially affect nutrient uptake of plants through several different mechanisms (Malusà *et al.* 2007; Bardi and Malusà 2012). However, the high nutrient requirements of tree nurseries are considered difficult to meet by using only organic fertilizers. For this reason, a specific derogation is foreseen in the EU Regulations that are ruling the organic farming system in the European Union, to allow the use of trees obtained with conventional methods also for establishing organic orchards. The preliminary results presented in the paper of ours are demonstrating that a fertilization management utilizing products with a different mechanism of action can allow a successful production of high quality plants. It shall also be mentioned that the health status of the plants after the application of the two organic products was particularly good, with no symptoms of deficiencies nor of diseases, likely due to their equilibrated growth.

Table 1. Effect of various fertilization treatments on plant growth parameters of apple maiden trees grown in an organic nursery (2010), means of 40 plants.

**A – apple cultivar 'Topaz'**

| Treatment | **Trunk diameter** [mm] | Tree height [cm] | **Number of branched trees** [%] | **Number of lateral shoots** | Total length of lateral shoots[#] [cm] |
|---|---|---|---|---|---|
| Zero fertilization | 11.8 a | 114.8 a | 70.0 a | 2.6 a | 37.5 a |
| NPK | 12.7 ab | 126.4 b | 90.0 a | 3.8 ab | 97.5 b |
| Manure | 12.6 ab | 125.3 b | 93.8 a | 3.7 a | 91.0 b |
| Micosat | 13.0 b | 127.7 b | 95.0 a | 4.9 bc | 85.7 b |
| BF Amin | 13.4 b | 125.1 b | 100.0 a | 5.3 c | 98.4 b |

Note: [#] shoots longer than 5 cm

**B – apple cultivar 'Ariwa'**

| Treatment | Trunk diameter [mm] | Tree height [cm] | Number of branched trees [%] | Number of lateral shoots | Total length of lateral shoots[#] [cm] |
|---|---|---|---|---|---|
| Zero fertilization | 11.0 a | 121.1 a | 76.7 a | 4.0 a | 139.0 a |
| NPK | 10.5 a | 126.7 ab | 66.9 a | 4.6 ab | 163.6 a |
| Manure | 11.3 a | 129.8 ab | 85.0 a | 4.7 ab | 149.5 a |
| Micosat | 11.3 a | 130.5 ab | 88.7 a | 5.4 b | 167.4 a |
| BF Amin | 11.5 a | 133.3 b | 100.0 a | 8.6 c | 253.9 b |

Note: [#] shoots longer than 5 cm

## Conclusions

From these preliminary data, the following conclusions could be drawn:

1. The liquid fertilizer BF Amin could be used successfully in organic nursery production of apple trees, since it improves the quality of maiden trees, increasing both the number of branched maidens and the number of shoots in the crown, and markedly stimulating the growth of shoots.
2. The microbial consortium Micosat has improved the growth of the trees by enhancing the plant uptake capacity of nutrients, since it does not contain any nutrient elements. Its effect on plant growth parameters was less evident than that of BF Amin, but still better than the common organic fertilizer.
3. The application of manure, which was considered the "organic control", confirmed that such product can well sustain the growth of the plants similarly to plants fertilized with chemical fertilizers.

These results, from a first two-year cycle of tree production, are under verification in parallel trials currently carried out in three different nurseries together with a larger range of fertilizers and amendments allowed for organic production.

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# Earwig Management Tool: a practical software application to predict and optimize the development of earwig populations in pip fruit orchards

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**Abstract**

*Earwigs* (Forficula auricularia L.) *are omnivorous insects that are considered as important beneficial insects in pip fruit orchards. They are, if abundant, capable of maintaining several pest species below economic thresholds. However, earwig abundance in orchards can greatly vary from location to location, and showed to be highly dependent on orchard management. Thorough knowledge of earwig population dynamics in the field is crucial in order to avoid negative effects of necessary orchard management, such as spray applications and soil tillage. A precise timing of these interventions taking into account the presence of vulnerable life stages of the earwig life cycle will enhance biocontrol in pip fruit orchards. To this end, we developed a day degree model capable of predicting the phenology of local earwig populations. This phenological model was integrated in a practical software application, called 'Earwig Management Tool', together with data concerning earwig sensitivities to distinct orchard management actions. Consultation of this user-friendly software enables fruit growers to organize their orchard management with respect for optimal development of earwig populations.*

**Keywords:** earwig, *Forficula auricularia* L.*,* day degree model, biocontrol, pip fruit orchards, advice software tool

## Introduction

The European earwig *Forficula auricularia* L., an important predator of several pests in pip fruit, e.g. woolly apple aphid *Eriosoma lanigerum* Hausmann (Mueller *et al*, 1988; Nicholas *et al*., 2005) and pear sucker *Cacopsylla* spp. (Lenfant *et al*., 1994; Phillips, 1981), has an univoltine life cycle including a leaf dwelling period from end of May until end of October (Gobin *et al*., 2006). During their life cycle, earwigs move in different strata of the orchards (soil, ground surface, tree), each with specific risks of side effects. Females lay eggs in an underground nest and provide broodcare to eggs and the first nymph stage. When maternal care ends, the nymphs live on the ground surface. These nymphs will disperse shortly thereafter and move into the trees. Considering earwig's population dynamics there actually exist two 'population types' in the field. Single-brood populations have one reproductive cycle a year and lay eggs before winter (November to December) (Moerkens *et al*., 2009). They are characterized by a prolonged maternal care by the female. Double-brood populations have two reproductive cycles a year and lay eggs after winter (January to February) and in summer (June to July) (Moerkens *et al*., 2009). In this second strategy, females abandon their young after the first moult, disperse and start a second nest. The free-foraging phase of nymphs starts from the third-instar stage in single-brood populations and second-instar stage in double-brood populations. The arboreal phase starts from the fourth-instar stage in single-brood populations and from the third-instar stage in double-brood populations (Moerkens *et al*., 2009).

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Their univoltine life cycle makes earwigs vulnerable to orchard management interventions and a single disastrous event has long-lasting repercussions (Gobin *et al*., 2006). An important mortality factor is the application of soil tillage in organic orchards. This is carried out for weed control, which is important for increasing nutrient uptake by the trees. It is often applied in autumn and/or in spring, when adult earwigs hibernate in their underground nests. Obviously, this application can cause nest destruction, hereby exposing the hibernating earwig to cold, deadly surface temperatures or loosing already laid eggs. In addition, the wrong use of spray applications in spring and summer can lead to severe reductions in earwig densities with long-term consequences (Peusens *et al*., 2010). Timing of soil tillage and spray applications is in practise very hard for pip fruit growers and often not realistic. To this end, we have developed a user-friendly software tool, based on an existing day degree model (Moerkens *et al*. 2011a) and detailed knowledge about orchard management causes of earwig mortality.

## Material and Methods

### Phenological day degree model

The phenological day degree model for earwigs was developed as described previously (Moerkens *et al*., 2011b), taking into account the existence of single-brood and double-brood populations. It consists of two components: (i) the prediction of the first appearance dates of all earwig's life stages from the nesting and free-foraging phase and (ii) the prediction of the variation in development time of earwig life stages in the trees. To calculate the lower and maximum developmental temperature thresholds and sum of day degrees we used development rates expressed as the reciprocation of development time between two developmental life stages (1/number of days). This was implemented in the model of Briére *et al*. (1999), which enables the simultaneous fitting of a nonlinear response to temperature and assessment of the lower developmental temperature threshold and the maximum developmental threshold. Detailed information about the development rates of earwigs in relation to many different temperatures; which were acquired by means of breeding experiments and published data, was used for the model. Development rates of the fastest individual to hatch and moult. were used to fit to the nonlinear model and parameters were estimated with the NLIN procedure of sas, version 9.1 (SAS Institute, 2002). This procedure performs a univariate nonlinear regression using the least squares method. Residuals were checked for normality. The phenological day degree model was programmed in sas (SAS Institute, 2002). This model was programmed with discrete time intervals of 1 day. Simulations started at the time of overwintering females in September and lasted until December of the next year. Detailed temperature data relevant for the nesting and free-foraging phase were used as input into the model.

### Determination of side effects of sprayings

Adult earwigs *Forficula auricularia* L. were collected in several orchards (apple and pear, integrated and organic) during the summer using cardboard shelters placed in trees. After sampling, test organisms were kept under controlled environmental conditions (temperature 16°C, humidity 60% RH, photoperiod 12/12 h) in separate plastic containers provided with water and food (crushed cat food) ad libitum. Prior to each test an equal number (50 % male/50 % female) of each population was chosen at random and mixed together to pool the test individuals.

Based upon the standard testing scheme of pesticides selectivity to non-target arthropods, residual laboratory tests on inert substrate and on natural substrate were conducted according to the recommended methods (Candolfi *et al*., 2000) with minor modifications.

We executed different laboratory tests in which spraying products were either directly sprayed on earwigs present on potted apple seedlings, or indirectly contaminated by residues on leaves or treated prey (woolly apple aphids, *Eriosoma lanigerum*). However, only the data generated by the indirect contact method in which earwigs were exposed to at beforehand treated leaves were used to feed the advice generation of the software tool. In these laboratory tests, exposure of residue was carried out in a test unit that consisted of a small petri dish (diameter 35mm). Test products were applied directly on the petri dish (initial toxicity on inert substrate) or on detached bean leaf disc mounted upside down on wet cotton wool in a petri dish (extended lab test on natural substrate) and closed with a ventilated lid. One hour after application when the residue had dried, earwigs were confined individually to a test unit for 24 hours after which the earwig was transferred to an untreated petri dish together with food and water. All compounds were sprayed using a handheld pressure sprayer until run-off under natural circumstances. Per test item 12 earwigs (6 males and 6 females) were tested. All test units and petri dishes were stored in an environmentally controlled growth chamber (temperature 14°C, relative humidity 60 % RH, photoperiod 12/12 h/h light/dark) during the entire test period. The condition of the earwigs was assessed and recorded as living, moribund (on their back, unable to right themselves up) or dead at 24, 48, 72, 96, 144 and 168 hours after treatment. Mortality (% moribund and dead earwigs) was calculated and corrected according to Abbott (1925) and rated according to the IOBC-classification for lab trials: 1 = harmless (<30% effect), 2 = slightly harmful (30-79% effect), 3 = moderately harmful (80-99% effect), 4 = harmful (>99% effect). All statistical analyses were performed using the Unistat Statistical Package, version 5.6 (Unistat Ltd. 2009, London, England). A non-parametric randomised block test of Friedman (two-way Anova) was conducted, in which the original data are ranked within each block. Multiple comparisons between treatments are based on ranking sums and differences are determined by t-distribution (Honestly Significant Difference)($p<0.05$).

Programming software Earwig Management Tool

The software tool was programmed in Visual Basic. The program automatically makes a connection with a mysql database on a secured webpage. In this mysql database temperature data (minimum and maximum temperature, air and soil (-5 cm)) are daily updated.

## Results and discussion

The Earwig Management software tool is an integration of the day degree model for earwigs with orchard management recommendations. The program has a user friendly graphical interface (Figure 1), easy-to-use options, and one-click features. The software can be downloaded at www.pcfruit.be, and installed on any personal computer with a windows operating system. While running the program, a connection to the internet is required for updating the temperature data in order to generate accurate predictions of the actual earwig phenology. In addition, a database with known side effects of soil tillage and spraying applications on the different life stages of earwigs is integrated in the system. The output gives the current status of the earwig population and management recommendations for activities critical for their survival. Hence, by consultation of this user-friendly software fruit growers can predict the earwig development in the field at any time, and organize the timing of orchard management actions taking into account the presence of (vulnerable) life stages of the earwig life cycle. Doing so, negative effects of specific

orchard management actions, such as badly timed spray applications and soil tillage, can be avoided.

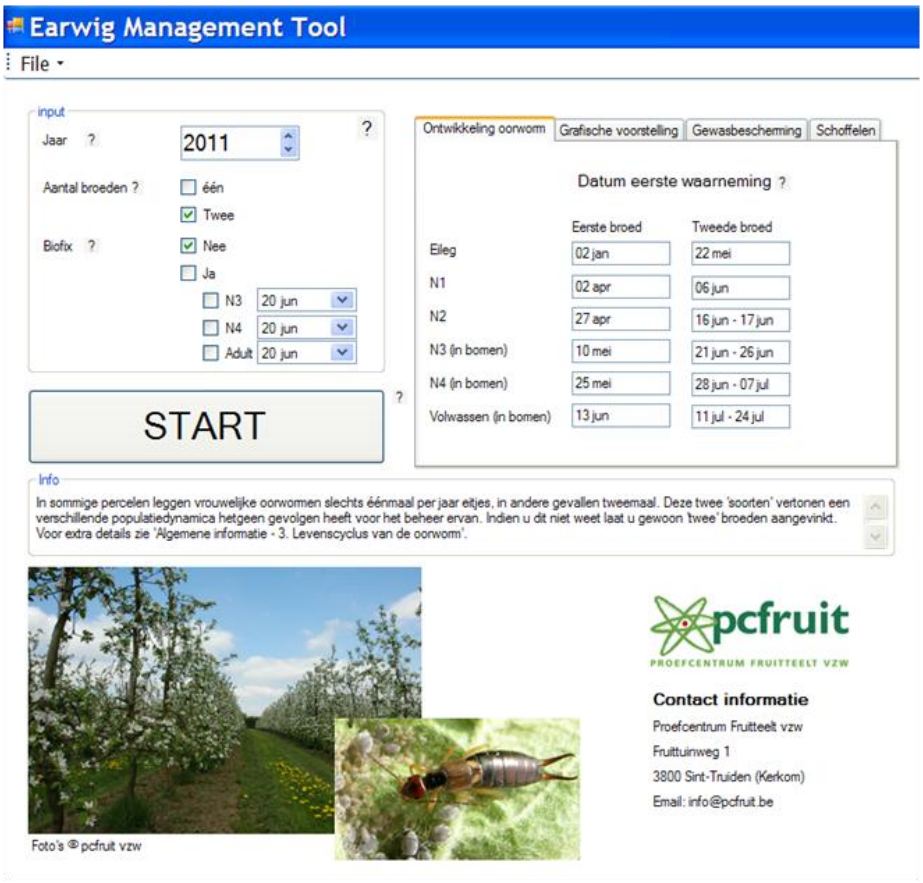


Figure 1: Graphical interface of the Earwig Management Tool program

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# Quality of organic and integrated ‘Topaz’ apples growing in selected orchards in Poland

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**Abstract**

*In 2009 and 2010 ten orchards in various regions of Poland were chosen for the experiment; three of them were organic. At harvest the following quality parameters were measured: fruit weight, percentage of blush, background and skin colour, fruit firmness, total soluble solids content (TSS), titratable acidity (TA), ascorbic acid and polyphenols content. The content of macro nutrients (N, Ca, Mg, P and K) in fruits was also determined. As maturity indicators internal ethylene concentrations, starch and Streif indices were used. Pesticides residues, nitrites and nitrates, heavy metals contamination in fruits were also analyzed to assess the risk to human health. The quality of ‘Topaz’ fruit depended more on orchard location and season than growing system. The pesticides residues were not detected in organic fruits (as expected), and also in some integrated orchards.*

**Keywords:** organic farming, fruit quality, pesticide residues, healthy components

## Introduction

Organic fruit production is among others characterized by the absence of synthetic herbicides and pesticides. Nowadays the choice of apple cultivars suitable for organic fruit production becomes very important. The breeding of apple cultivars resistant to scab started more than 70 years ago and the first scab-resistant cultivar was introduced in 1967 (Crosby *et al*., 1992). However, the taste of many of them was not widely accepted by the consumer. A modern scab resistant apple cultivar ‘Topaz’ is highly recommended for both organic and integrated fruit production.

The aim of the study was to compare the quality of ‘Topaz’ apples grown under organic and integrated conditions (IFP).

## Material and Methods

In 2009 and 2010 ten orchards in various regions of Poland were chosen for the experiment; three of them were organic. At harvest the following quality parameters were measured: fruit weight, percentage of blush, background and skin colour, fruit firmness, total soluble solids content (TSS), titratable acidity (TA), ascorbic acid and polyphenols content. The content of macro nutrients (N, Ca, Mg, P and K) in fruits was also determined. As maturity indicators internal ethylene concentrations, starch and Streif indices were used. Pesticide residues, nitrites and nitrates, heavy metals contamination in fruits were analyzed to assess the risk to human health. Background and skin colour were measured using MiniScan XE Plus (Hunter Lab, USA). Fruit firmness was measured on the opposite sides (blushed and unblushed surfaces) of fruit using an EPT-1R Pressure Tester (Kelowna, Canada), equipped with an 11-mm diameter tip. Soluble solids were determined using ATAGO PR-101 refractometer (ATAGO, Japan). Results were expressed in %.

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Titratable acidity was determined by standard titration method using automatic titration unit DL 50 Graphix (Mettler Toledo, Switzerland), by titration with 0.1N NaOH to the end point at pH=8.1. The results were expressed as malic acid in %. Malic and ascorbic acid contents were determined by an HPLC method. HP Agilent 1200 chromatograph was used, equipped with two Supelco LC-18 25cm columns connected in series. Detection was carried out at 210 nm for malic acid and 244 nm for ascorbic acid. Total phenolics were measured by modified Folin-Ciocialteau spectrophotometric method and were expressed as mg gallic acid equivalents per 100 g of fruits.

Pesticides residues in fruits were analyzed according QuEChERS method using gas chromatography with mass detector (GC/MS) and liquid chromatography tandem mass spectrometry (LC/MS-MS) for 170 pesticides. Extraction and determination of nitrites from plant material were conducted according to method described in PN-EN 12014-2: „Foodstuffs – Determination of nitrate and/or nitrite content – Part 2: HPLC/IC method for the determination of nitrate content of vegetable and vegetable products".

Content of calcium, phosphorus, potassium, magnesium, boron, copper, iron, manganese, zinc and sulphur, was determined after drying fresh plant material and microwave mineralization using inductively coupled plasma optical emission spectrometry (ICP-OES).

## Results and Discussion

The quality parameters of examined apples were not closely related to production system and ripening stage, but depended on orchard location. The percentage of blush varied from 40% to 90%, TSS from 11.2% to 14.8%, and titratable acidity from 1.29% to 0.76%. The content of macro nutrients also depended on orchard location but not on orchard management system. It confirms data presented by Peck *et al.* (2006).

Ascorbic acid content varied from 9 up to 20 mg/100 g. Total phenolic compounds content determined by Folin-Ciocialteau spectrophotometric method was from 80 to 110 mg/100 g. Health-promoting compounds content (ascorbic acid and phenolics) strongly depended on orchard location. It is opposite to the data presented by Wojdyło *et al*. (2010) and Worington (2001), who concluded that organic fruits contained significantly more vitamin C than fruits from conventional farming.

Residues of Captan and Trifloxystrobin were found in apples obtained from some (but not all) IFP orchards. However, the pesticide residues in all tested fruits were below Maximum Residue Level (MRL). Apples from organic production were free from the residues.

## Conclusion

The quality of 'Topaz' fruit depended more on orchard location and season than the growing system. The pesticides residues were not detected in organic fruits (as expected), and also in some IFP orchards.

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