Investigations on alternative substances for control of apple scab - Results from Conidia germinating tests and experiments with plant extracts[1]

Untersuchungen zum Einsatz alternativer Stoffe zur Regulierung des Apfelschorfes - Ergebnisse aus Konidienkeimtests und Versuchen zu Pflanzenextrakten[1]

Pfeiffer B.[2], Alt S.[3], Hein B.[2], Schulz C.[3], Kollar, A.[3]

Abstract

The intention of this research project, which was supported within the "Bundesprogramm Ökologischer Landbau", was to find alternatives for the control of *Venturia inaequalis* for the organic fruit-grower. Beside the investigations on reducing the ascospore potential on fallen leaves, experiments were conducted in laboratory, greenhouses and in orchard to test direct control of scab with different plant extracts, concentrations and methods of extraction. Extracts from *Inula viscosa*, *Quillaja saponaria*-bark, *citrus*-species (AGROMIL) and *Saponaria officinalis* revealed a distinct efficacy against apple scab in greenhouse studies on apple seedlings. ELOT-VIS, CHITOPLANT, COMCAT, MOOSEXTRAKT, SILIOPLANT und FZB 24 did not show sufficient efficacy with the application schedule used for control of scab. Mixtures of *Quillaja*-saponine and sulphur reduced effectively apple scab incidence. In an experiment concerning rain stability *Citrus*-extract and *Quillaja*-saponine showed a lower efficacy against scab after a simulated rain of 5 mm. The screening of different supplements to *Citrus*-extract as surfactants and adhesives revealed GREEMAX and BIOPLUSS as promising additives. Both combinations showed an efficacy comparable to copperoxychloride corresponding to 400 g elementary copper per ha.

Keywords: Apple, organic fruit-growing, *Venturia inaequalis,* plant extracts, saponins

Introduction

At the moment organic apple growers in Germany can use the registered preparations based on copper-oxychloride for scab control. The registration will end on December 31st 2004. Products with low copper quantities (copper-octanat CUEVA) are still in the development stage. The aim of this project was to look for alternatives for scab control. Based on earlier results of Späth (2000), Siegrist (2000) and Pfeiffer (2002) plant extracts were produced from different medicinal plants. Plant extracts which had shown efficacy on *Plasmopora viticola* were also tested (Cohen, 2002 and Kast 2002).

Material and Methods

Extracts from medicinal plants normally were produced on two ways: 10 g dried plant material was mixed with 100 ml ethanol (99 %), then it was extracted in a water bath at 60 °C for 2 hours. The closed jars were cooled down to room temperature and the extracts were filtered. Water extracts were produced like tea from herbs: 100 ml boiling hot water was poured on 10 g dried plant material, 10 minutes later the extract was filtered. Different types of experiments were used depending on the questions that should be answered.

Conidia were washed from scab infected leaves and a suspension of 10 000 conidia/ml was produced. Different concentrations of plant extracts were added to that suspension. 1 ml of the mixture was filled in sterile Cellstar-Multiplates and incubated at 20 °C for 24 hours. With the binocular the **germinated conidia** were differentiated into three groups: "not germinated", "with a short germinat-

[1] supported by the BMVEL within the "Bundesprogramm Ökologischer Landbau"

[2] BBA Dossenheim, Schwabenheimer Str. 101, D-69 221 Dossenheim/LVWO Weinsberg, Traubenplatz 5, D-74189 Weinsberg

[3] BBA Dossenheim, Schwabenheimer Str. 101, D-69 221 Dossenheim

ing tube", "with a long germinating tube". It was classified as "short", if the length of the germinating tube was not longer than twice the conidia itself. The proportions of not germinated conidia of plant extract and control (control = 100 %) were compared and the efficacy according to ABOTT was calculated.

In the **greenhouse-experiments** 12 seedlings from 'Golden Delicious' (apples from an orchard, where no resistant varieties were grown) per treatment were sprayed in a preventive schedule with a small hand-sprayer. The tip of the youngest fully expanded leaf was removed and the number and the size of the younger susceptible leaves were registered. About 12 hours after the preventive application the artificial inoculation was done with a suspension of 100 000 conidia/ml. The seedlings were put into plastic bags for 24 hours to maintain a high relative humidity of the air. About 14 days after the inoculation the first symptoms appeared. For each leaf the scab infection was classified from 0 (no symptoms visible) to 4 (big expansion of the symptoms). Since not all lesions can be detected by the naked eye all leaves, that were susceptible for scab at the day of inoculation, were removed and the conidia were rinsed out with aqua dest. The titer of conidia was determined with a Kolkwitz-Plancton-cytometer (0,5 ml, Hydro-Bios, Germany) to estimate the reproducibility of fungus.

For the testing of **rain-stability** of extracts from *Citrus sp*. (AGROMIL) the seedlings were sprayed in the morning of 21.10.2002. Rain (5 and 15 mm) was simulated in the early afternoon, in the morning of October 22nd the plants were inoculated with conidia.

Another type of experiment was chosen to test the efficacy of combinations of **plant extracts with surfactants and adhesives** for one infection period under natural conditions. Seedlings were sprayed preventively. After the treatments had dried, the seedlings were distributed in a randomised pattern under heavily scab infected trees of the variety 'Jonagold' (untreated control of a scab trial in quarter 617 of Heuchlingen, the experimental station of the LVWO Weinsberg). In this way the conidia in the rainwater running down from scabbed leaves caused the natural infection. Two or three days later the seedlings were returned into the greenhouse until scab symptoms appeared.

Especially two experiments with AGROMIL (as powder and fluid formulation) were done like this. In July 2003 AGROMIL (powder, 0,25 %) was combined with additives like ADHÄSIT (0,01%), PROFITAL FL. (0,01%), GREEMAX (0,01%) or BIOPLUSS (0,8%). These treatments were compared to untreated control and copperoxychloride (according to 400 g Cu per ha). The plants were treated on July 2nd in the morning and distributed in the orchard in the afternoon. There were two leaf-wetness-periods: 17 hours at 16 to 17 °C and 86 % relative humidity of the air (weak to medium infection) and at the next day 16 hours at 14 to 15 °C and 93 % relative humidity of the air (medium to severe infection). On July 4th the plants were put back into the greenhouse in the early afternoon. During the exposition in the orchard 11,6 mm rain fell.

In a similar trial AGROMIL (powder, 0,25 %) was compared with AGROMIL + GREEMAX (powder, 0,25 % + GREEMAX 0,01%), AGRO NEU FL. 1(fluid, 0,5%), AGRO NEU FL. 2(fluid, 0,5 %), untreated control, copperoxychloride (according to 400 g Cu per ha) and CUEVA (according to 180 g Cu per ha). The higher concentrations of AGRO NEU FL. 1 and 2 were chosen because of the fluid formulation. The seedlings were treated in the morning of October 2nd, exposed in the orchard on October 3rd in the morning and returned to greenhouse on October 5th in the morning. Two weak infections occurred: 10 hours leaf-wetness at 13 to 14 °C and 92 % relative humidity of the air and 11 hours leaf-wetness at 8 to 9°C and 95 % relative humidity of the air. During the exposition in the orchard 10,6 mm rain fell.

Results

31 different extracts from medicinal and other native plants (water based or ethanolic extracts), formulation agents and plant strengthening preparations were tested in varying concentrations. Normally the extracts were compared at concentrations of 2 %, 4 % and 6 %. Ethanol (99 vol.%) itself had a efficacy between -49 % and 40 %, the average was 10 %. The adhesive preparation GREEMAX had no inhibiting effect on the germination. **Table 1** shows the plant extracts, that in most tests had efficicacy rates of 85 to 100 %. The concentrations of the extracts are listed as volumen % normally.

Table 1: Testing of plant extracts on inhibition of germination of apple scab - Extracts with efficiency rates (WG after ABOTT) 85 to 100 %.

Botanical name, method of extraction / plant strengthening preparation, concentration	Part of the plant	WG	n*
Rhamnus frangula ethan. 2 - 6 %	bark	2 to 100 %	10
Inula viscosa. Aceton 0,4 %, ethan. 2 - 6 %	leaves	88 to 100 %	8
Primula veris aqueous 2 - 6 %	roots	- 43 to 100 %	17
Primula veris ethan. 2 - 6 %	roots	18 to 100 %	31
Saponin from *Quillaja saponaria* aqueous 0,5 - 2 % (w/v)	bark	68 to 99 %	10
Saponaria officinalis ethan. 2 - 6 %	roots	22 to 100 %	6
LEBERMOOSER 0,5 - 2 % (extract from moss)		22 to 100 %	6
AGROMIL (powder, extract from *Citrus sp.*) 0,25 % - 0,5 % (w/v)	fruits	100 %	9
BIOPLUSS 0,4 - 1,2 vol. % (= 0,64 - 1,92 %, w/v)		100 %	4
Salviathymol 0,2 - 1,2 %	leaves	100 %	9

*n: number of tested concentrations (with replications) in few conidia-germinating-tests

The conidia germinating tests were used for screening different plant extracts and for optimising the method of extraction and the concentrations. For the production of extracts from *Primula veris* it seemed to be important, to mix the dried roots with cold water and to boil the water slowly. High efficacy under laboratory conditions could be detected, if 10 g dry roots were extracted with 100 ml water and this extracts were used with concentrations between 4 and 6 %. By ethanolic extracts (10 per 100 ml, 99 % ethanol) a concentration of 6 % was necessary for high inhibition of germination of conidia.

In a greenhouse experiment with seedling of 'Golden Delicious' aqueous extracts from *Rhamnus frangula*, *Sambucus nigra*, *Rheum officinale*, *Polypodium vulgare* and *Saponaria officinalis* were compared to copperoxychloride (500 g Cu/ha) and control. The results (scab lesions on the leaves and production of conidia) are listed in **table 2**. the lowest conidia production was evaluated in the treatments with *Rhamnus frangula* and with *Saponaria officinalis*. In some greenhouse experiments there are differences between data from visual assessment of scab lesions on the leaves and results for the production of conidia, that were shaken from the leaves with water.

In the last tests in November and December 2002 different plant strengthening preparations like CHITOPLANT, ELOT-VIS, SILIOPLANT, COMCAT and antagonists based on yeasts or *trichoderma sp*. were tested in a preventive spraying schedule. These preparations had no inhibiting effects on scab.

Table 2: Efficacy of a preventive application of different plant extracts on scab lesions on the leaves and production of conidia of V. inaequalis (greenhouse May 2002)

Extract / Preparation	Concentration	Lesions Leaf [(1)]	Conidia/ml
Control		2,07	204.800
Copper 500 g Cu/ha (Oxychlorid)	1,1 %	1,64	185.762
Bark of *Rhamnus frangula* (aqueous extract)	5 %	1,89	88.458
Tea of flowers of *Sambucus nigra*	5 %	2,02	408.037
Roots of *Rheum officinale* (aqueous extract)	5 %	2,76	784.037
Rhizoms of *Polypodium vulgare* (aqueous extract)	5 %	2,92	425.704
Roots of Saponaria officinalis (aqueous extract)	5 %	1,47	115.380

[(1)] Lesions divided in classes from 0 to 4

An obvious reduction of scab was visible in a greenhouse-experiment in July 2002 with extracts from *Saponaria officinalis* (2 % and 6 %), *Inula viscosa* (1 % and 2 %) and *Quillaja*-saponin (0,5 % and 1,0 %). Extracts from *Inula* showed an efficacy of 50 to 74 %, *Saponaria* 80 to 90 % and *Quillaja*-saponin 87 to 94 %. Based on this test sulphur was added in different concentrations to increase the effect of *Quillaja*-saponin. Combinations of 0,75 % *Quillaja*-saponin with 0,4 or 0,6 % sulphur (according to 2 or 3 kg per ha, sprayed with 500 l/ha) inhibited infections by scab nearly completely. But some questions remained wether the high efficacy was caused by a physical reaction of the inoculation suspension or if the applicated droplets did not stay in the same shape on the leaves as in the control.

In the experiments done by Rudolph (2003) AGROMIL, an extract of citrus fruits from Brasil, showed a distinct inhibition of scab in conidia-germinating-test in laboratory as well as in the in greenhouse. In order to proof the rain stability of AGROMIL - being an essential problem in 2002 during an orchard-experiment with plant extracts - 5 and 15 mm of rainfall were simulated by using a watering device (water sprinkler). The most important results are summarized in **table 3**.

Table 3: Effect of different amount of simulated rainfall on the infection with *V. inaequalis* on seedlings of 'Golden Delicious' after a preventive application of different plant extracts (greenhouse, October 2002)

Amount of rain simulated	0 mm	5 mm	15 mm	0 mm	5 mm	15 mm
	Scab lesions leaves [(1)]			Production of conidia/ml[(2)]		
Untreated Control	1,94	2,24	2,28	752.000	1.262.000	1.449.000
FUNGURAN (750 g Cu/ha)	0,95	1,84	2,09	224.000	985.000	854.000
AGROMIL (0,25 %)	0,55	1,73	2,13	100.000	688.000	892.000
Quillaja-Saponin (1,0 %)	0,13	1,33	1,9	5.961	325 949	545 651

(1) Lesions divided in classes from 0 to 4, (2) Conidia per ml; average from 3 replications a 10 Pflanzen, *Quillaja*-saponin only 4 plants per replication

Without artificial rain AGROMIL showed a clear reduction of scab in comparison to control. With increasing amount of rain the effect decreased severely. The same observation was made at *Quillaja*-saponin, which had the lowest incidence of scab. One reason for that could be that the plants were exposed under the sprinkling device two hours later to have equal conditions at the distribution of the simulated rain, so that probably the preventive treatment dried for a longer time.

Based on this experiment in 2003 the main intention in the trials was to find combination either of AGROMIL with surfactants or adhesives either of *Quillaja*-saponin with adhesives. Meanwhile two fluid formulations of AGROMIL were available. **Figure 1** shows the effect of combination of AGROMIL with different adhesives (see **Material and methods**).

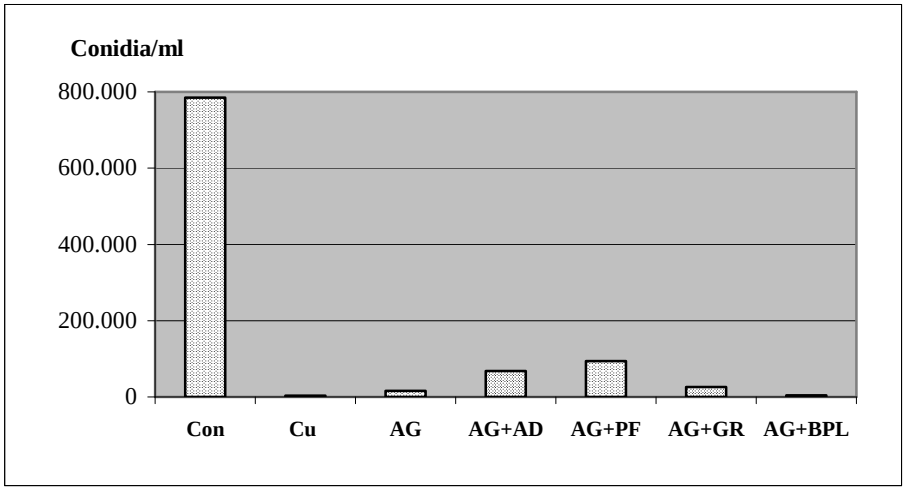

Figure 1: Efficacy of combinations of AGROMIL with different adhesives on natural infections with *V. inaequalis* (AG = AGROMIL powder, AD=ADHÄSIT, PF=PROFITAL FLÜSSIG, GR=GREEMAX, BPL= BIOPLUSS).

The adhesives ADHÄSIT and PROFITAL FLÜSSIG did not enhance the scab reduction effect of AGROMIL. The addition of GREEMAX or BIOPLUSS did not show increased effect of AGROMIL, the amount of rain during the deposition in orchard was about 11 mm, which fell regularly.

In October 2003 both fluid formulations were compared with AGROMIL powder and with AGROMIL (powder)+GREEMAX. During that natural infection period the addition of GREEMAX was advantageous, the production of conidia was about 10 % lower than with AGROMIL alone. Both fluid formulations were not as effective as AGROMIL powder.

Discussion:

The initial results of this research project, that ended on December 31st 2003, show that there is a promising potential in some plant extracts like AGROMIL, *Inula viscosa* or *Quillaja*-saponin for the use against apple scab. The step from greenhouse into practice in the orchards is always lengthy, especially because there are only few adhesives or formulations that can be applied in organic fruitgrowing. GREEMAX and BIOPLUSS are encouraging preparations for enhancing the effect of extracts and should be tested in the next season in orchards. Before that the question of residues of conventional plant protection agents in the extracts has to be answered. Furthermore the extraction has to be optimised to lower the costs for the extracts.

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