

## Contributions of EU-project REPCO to apple scab control

Beitrag des EU-Projektes REPCO zur Bekämpfung von Apfelschorf

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### Abstract

The objective of REPCO is to contribute to the replacement of copper fungicides in organic agriculture by new measures for control of downy mildew (*Plasmopara viticola*) in grapevine and scab (*Venturia inaequalis*) in apple. Both major European organic crops strongly depend on copper fungicides. Permitted amounts will be reduced stepwise during the following years (Council Regulation (EEC) 2092/91, Annex II) to avoid environmental risks. In European countries where copper fungicides are already out of use, production of organic apples suffers severe economical problems because of insufficient scab control.

Potentiators of resistance, organically based fungicides and biocontrol agents are screened and evaluated in apple. Effects of crop management practices in organic agriculture on overwintering of *Venturia inaequalis* are assessed. Novel disease control measures and knowledge are integrated into organic management systems. 'Pipeline' products already under development elsewhere are included and where necessary optimised in their use.

**Keywords:** apple scab, copper, plant extracts, induced resistance, biological control, earthworms

### Introduction

Research on prevention and control of apple scab is carried out by seven partners focussing on the development of potentiators of resistance and organically based fungicides, of novel biocontrol agents and of other components for integrated management systems. Control measures are integrated and tested in field experiments. Details on the partnership and the distribution of tasks can be found on [www.rep-co.nl](http://www.rep-co.nl).

### Preliminary results

#### Development of potentiators of resistance and organically based fungicides

A list of more than 100 potential candidate materials for control of *V. inaequalis* was prepared. The list contains plant extracts and oils, products from micro-organisms, salts and other materials. Preliminary assessments of the materials covering availability and costs (economic feasibility), acceptability for organic growing and human and eco-toxicology were carried out. Eighty four compounds have been collected from several sources and screened in plant assays and conidium germination tests. Also seven compounds for use as stickers and surfactants were investigated. Several compounds showed promising control of scab on seedlings either as a preventive or as curative treatment, i.e. the effect was at least at the same level as elemental sulphur (standard treatment). The screening of these compounds, including fractions and different types of extracts of the most promising ones continues and work on improvement of compounds will be intensified. Useful collaboration with especially one commercial company has been strengthened.

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In 2005, 26 compounds were tested under orchard conditions in Denmark on cv. Jonagold for their effect to control apple scab. The treatments were carried out in the main primary ascospore discharge period in May using a small tunnel sprayer. Before and after the testing period the trees were treated identically with sulphur. Leaves and fruit were assessed for apple scab infections and phytotoxicity. Severity of apple scab on fruits was high with 97.5 in the untreated control. Treatments with copperoxychloride used as reference reduced apple scab severity to 65.7. Treatments with the best compound selected so far gave significantly better control with a scab severity of 34.1. Also treatments with some other compounds had an effect on apple scab.

#### Earthworms as components for integrated management systems

A literature review on (1) methods to monitor earthworms, (2) on methods to increase the palatability of apple leaves to earthworms and (3) on application of such measures in the field was carried out. A monitoring of earthworms was started in plots treated with different types of organic amendment with the aim to find methods that stimulate leaf consumption by earthworms. Substantial numbers of earthworms, including the major leaf eating earthworm *Lumbricus rubellus*, were found. Information resulting from the literature review was used to set up a cultivation of the earthworm species, *L. terrestris* and *L. rubellus*, with the aim to test methods to improve the palatability of apple leaves.

Three experiments were carried out on leaf decomposition by earthworms with the aim to increase palatability and consumption of apple leaves. There was neither a positive nor a negative effect of calcium hydroxide on *L. terrestris*. This is important since it is becoming a more and more common treatment in organic orchards in the Netherlands during the leaf fall period to control fruit tree canker, caused by the fungus *Nectria galligena*. Amino acids promoted leaf consumption by *L. terrestris*. Also beet pulp promoted leaf consumption by *L. terrestris*, especially when the dose was increased from 1 to 5 % or when it was freshly applied. However, there was little effect from the various treatments on fresh body weight for this species.

#### Development of novel biocontrol agents

Fungal endophytes isolated from apple leaves were screened under field conditions for their ability to reduce ascospore production of *V. inaequalis*. A pilot experiment was carried out in 2003/2004 to optimise methods used for antagonist screening under field conditions, e.g. spray application, assessment of colonisation and quantification of ascospore production. In autumn 2004, 25 candidate antagonists were applied to leaves in the orchard and their potential to reduce ascospore production was assessed in spring 2005. For ascospore production in leaf residues, a strong block effect and an unexpected high variation between replicates of treatments was observed. Three fungal isolates tended to reduce ascospore production by >70% in the two most reliable experimental blocks.

DNA was isolated from 252 apple leaf samples. PCR-DGGE analysis was performed on the bacterial community of the samples as well as on the fungal communities. Multivariate statistics was applied to species (DGGE) and environmental data. In 2003 no correlation was found between microbial community composition with number of ascospores and treatments. In 2004 a correlation was found of the community composition with the control treatments and one treatment. Treatments could not be correlated with reduced numbers of ascospores. Taking all fingerprint data and use the amount of ascospores as the only response variable, there is a possible relation of two fungal species and four bacterial species with reduced numbers of ascospores. This relation was confirmed with regression analysis. Sequencing of bands from fingerprints will give a general identification of the species which possibly counteract with the development of *V. inaequalis* in apple leaves.

A screening assay has been developed to test the possible effect of candidate antagonists on conidia production of *V. inaequalis* on leaves of apple seedlings under controlled conditions. A first set of fungal isolates could be selected which significantly reduced conidiation of *V. inaequalis* by more than 80% (Table 1).

Four selected fungal isolates were tested for their ability to produce conidia on different agar media and solid substrates. The fungal species showed big differences concerning their ability to produce conidia. Beside this the conidia production was dependent on the used medium. In the fermentation tests two isolates showed the best conidia production ability so far. It was possible to harvest more than 1E+9 conidia per gram culture substrate.

**Table 1.** Effect of candidate antagonists on conidiation of *Venturia inaequalis* on apple seedlings under controlled conditions.

Treatment	Number of conidia cm <sup>-2</sup> leaf surface		
	Ln-transformed	Backtrans- formed	Relative to control*
Control	7.20 ab**	1339	100
Fungus 1	5.20 c	181	14
Fungus 2	5.57 c	262	20
Fungus 3	6.67 bc	788	59
Fungus 4	6.01 bc	407	30
Fungus 5	5.57 c	262	20
Fungus 6	8.32 a	4105	306
Fungus 7	5.55 c	257	19

\* At backtransformed scale.

\*\* Values with a common letter do not differ statistically significant (LSD<sub>5%</sub> = 1.49).

### Field testing

In 2004, two experiments were carried out in the Netherlands to measure apple leaf decomposition and ascospore production of *V. inaequalis*. Treatments were applied in autumn by spraying just before leaf fall or by dipping. Leaf degradation was rather poor. Only urea (reference) enhanced leaf degradation. The numbers of ascospores in the remaining leaf tissue were very variable. No differences between treatments could be demonstrated except for urea.

During spring and summer, apple scab was controlled in an experiment according to EPPO-guidelines with natural compounds. Natural compounds were sprayed according the RimPro scab warning system from start of bud break until the mid of June. Severity and incidence was measured on the leaves and the fruit. Phytotoxicity and russetting was assessed. Schedules of natural compounds combined with sulphur were compared with the standard biological fungicides copper hydroxide and sulphur alone. Some of the treatments gave a level of control similar to copper in spite of a high infection pressure. However, some of the good-performing schedules produced substantial amount of russetting.

In an experiment with 14 treatments carried out in the Netherlands in 2005, summer epidemics of apple scab were very severe. Treatments with copper, sulphur or test compounds applied in combination with sulphur reduced scab on leaves. However, only one compound showed a significant reduction of scab in comparison with the adequate control treatment.