501452

REPCO

Replacement of Copper Fungicides in Organic Production of Grapevine and Apple in Europe

Specific Targeted Research Project

Priority 8.1 Policy-oriented research

Publishable Final Activity Report

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Project coordinator: Plant Research International
J. Köhl

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1. Project execution

Summary description of project objectives

The objective of REPCO was to contribute to the replacement of copper fungicides in organic agriculture by new measures for control of downy mildew (*Plasmopara viticola*) in grapevine (Figure 1) and scab (*Venturia inaequalis*) in apple (Figure 2). Both major European organic crops strongly depend on copper fungicides. Permitted amounts will be reduced stepwise in the years ahead (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 economic problems because of insufficient scab control.
Potentiators of resistance, organically based fungicides and biocontrol agents have been screened and evaluated in grapevine and apple. The risk of pathogen evolution during use of novel control measures has been estimated to enable the development of sustainable strategies. Effects of crop management practices in organic agriculture on overwintering of *Venturia inaequalis* were assessed. Novel disease control measures and knowledge have been integrated into organic management systems. 'Pipeline' products already under development elsewhere have been included and where necessary optimised in their use.
Implementation by end-users and industries qualified for commercialisation of project findings has strongly been emphasised. Small-medium enterprises (SME) as project partners ensured a strong link between end-users and research. At the end of the project several compounds and biocontrol agents can be delivered to qualified industries for development of products for use in organic agriculture. Additionally, knowledge of integrated use of control measures can be delivered to organic growers.
The project results thus strongly support EU policies to replace the use of copper fungicides in organic agriculture in the near future.

Figure 1. Damage of grapevine by downy mildew caused by Plasmopara viticola.

Figure 2. Damage of apple by scab caused by Venturia inaequalis.

Contractors involved

REPCO partner	Role in REPCO
Plant Research International B.V. (PRI) Dr. Jürgen Köhl Wageningen, the Netherlands Phone +31.317.476017; jurgen.kohl@wur.nl	Project coordination; development of novel biocontrol agents; effect of crop management on micro-organisms
Staatliches Weinbauinstitut (WBI) Dr. Hanns-Heinz Kassemeyer Freiburg im Breisgau, Germany Phone +49.761.4016530; hanns-heinz.kassemeyer@wbi.bwl.de	Screening of potentiators of resistance and fungicides for control of downy mildew of grapevine
Research Institute of Organic Agriculture (FiBL) Dr. Lucius Tamm Frick, Switzerland Phone +41.62.8657238; lucius.tamm@fibl.org	Screening of potentiators of resistance and fungicides for control of downy mildew of grapevine
Swiss Federal Institute of Technology Zürich (ETHZ) Dr. Cesare Gessler Zürich, Switzerland Phone +41.1.6323871; cesare.gessler@ipw.agrl.ethz.ch	Selection pressure of control measures and forced evolution in *Plasmopara viticola*
Istituto Agrario di San Michele all'Adige (IASMA) Dr. Ilaria Pertot S. Michele all"Adige, Italy Phone +39.0461.615222; Ilaria.pertot@ismaa.it	Integration of control measures in grapevine
Groupe de Recherche en Agriculture Biologique (GRAB) Vianney Le Pichon Avignon, France Phone +33.4.90840170; direction.grab@freesbee.fr	Integration of control measures in grapevine
Applied Plant Research (PPO) Dr. Bart Heijne Randwijk; the Netherlands Phone +31.488.473718; bart.heijne@wur.nl	Integration of control measures in apple; effect of crop management on earthworms
University of Copenhagen (LIFE) Dr. John Hockenhull Copenhagen, Denmark Phone +45.35333308; johoc@life.ku.dk	Screening of potentiators of resistance and fungicides for control of apple scab
University of Aarhus (UoA) Dr. Hanne Lindhard Pedersen Aarhus, Denmark Phone +45.63904171; hanne.lindhard@agrisci.dk	Screening of potentiators of resistance and fungicides for control of apple scab
ECOVIN: Bundesverband Ökologischer Weinbau (ECOVIN) Dipl. Ing. Paulin Köpfer Heitersheim, Germany Phone +49.7634.552818; ecovin@t-online.de	Communication with end-users and dissemination
Bio Fruit Advies (BioFruitAdvies) Marc Trapman Zoelmond, the Netherlands Phone +31.345.502627; m.trapman@wxs.nl	Communication with end-users and dissemination
Prophyta Biologischer Pflanzenschutz GmbH (Prophyta) Dipl. Ing. (FH) Ute Eiben Malchow/Poel, Germany Phone +49.384.252324; ueiben@prophyta.com	Development of novel biocontrol agents

Major achievements of REPCO

During REPCO the following scientific achievements have been obtained:

- Potential of more than 130 potentiators of resistance and fungicides for control of downy mildew assessed in screening experiments in grapevine.
- Potential of more than 100 potentiators of resistance and fungicides for control of scab assessed in screening experiments in apple.
- Potential of 40 potentiators of resistance and fungicides for control of downy mildew assessed in 14 field experiments in grapevine.
- Potential of 40 potentiators of resistance and fungicides for control of scab assessed in 18 field experiments in apple.
- Novel potentiators of resistance against *Plasmopara viticola* found in grapevine.
- Novel use of several plant extracts found highly effective against *Venturia inaequalis* in apple.
- More than 200 candidate antagonists assessed for their potential to control apple scab.
- One novel antagonist selected with high potential effectivity against *Venturia inaequalis* and with suitability for commercial product development.
- Stimulation of leaf degradation and reduction of *Venturia inaequalis* ascospore production by applications of vinasse demonstrated.
- Selection pressure of control measures and forced evolution in *Plasmopara viticola* in grapevine known.
- Complex microbial interaction in apple leaves characterised.

Collection of candidate compounds for control of *Plasmopara viticola* and *Venturia inaequalis* and risk assessment

Candidate compounds for control of *Plasmopara viticola* and *Venturia inaequalis* in organic grapevine and apple production under European conditions were collected and a preliminary risk assessment for each compound was made. An annotated list of candidate compounds (~100) for screening for control of *P. viticola* and *V. inaequalis* was compiled in the beginning of the project and a collection of potential candidate compounds was built up. The list contains plant protection products made from plant extracts and oils, materials made from micro-organisms and other materials. Materials were found through literature searches on the web, via personal contacts, early results from REPCO and other projects, and company contacts. Materials on the list were subjected to a preliminary database screening for toxicological risks using PubMed, economic feasibility and acceptability for use in organic growing. Only the materials meeting these criteria passed on to the next step in the screening process, i.e. screening against *P. viticola* and *V. inaequalis*, respectively. Additional compounds were added to the list during the remainder of the project period.
A preliminary risk assessment was also carried out for candidate antagonists tested for their potential in biological control of *V. inaequalis*.

Collection and assessment of compounds and candidate antagonists
Candidate compounds for control of *Plasmopara viticola* and *Venturia inaequalis* were searched for via literature, internet, personal and company contacts, and preliminary results from other projects. Candidate compounds considered were materials made from plants (extracts and oils), micro-organisms and other materials. Each material was assessed for acceptability in organic growing, economic feasibility and toxicological risks
Organic acceptability of materials (ORG) was assessed by FiBL. All screened and developed compounds and organisms will fulfil the requirements of Council Regulation (EEC) No 2092/91. Economic feasibility of materials was based on consideration of the market situation regarding quantity, quality and supply stability of the raw material and price (ECON). Plant extracts and oils were assessed by Nor-Natur ApS and products based on micro-organisms by Prophyta. Assessment of toxicological risks (TOX) was performed by LIFE, based on information retreived from the PubMed database, under the heading “species name and humans”. Materials with a total of 10 or fewer hits on PubMed were allowed to proceed, as well as materials with more than 10 hits where less than 25% of the first 60 hits reported toxicity and/or other adverse effects on humans.
An annotated list of all potential materials was made by LIFE; this list was organised in four parts:

- Part E: Candidate compounds based on plant extracts;
- Part O: Candidate compounds based on oils;
- Part M: Candidate compounds based on micro-organisms;
- Part S: Candidate compounds based on other materials.

All materials on the list were given a code number that was used by REPCO partners for dissemination outside the project. The list consists of two parts. The first part contains scientific and English vernacular names, sources and key information concerning use of the listed materials against other plant diseases/pathogens, key references etc. The second part contains summaries of a preliminary risk assessment for acceptability for use in organic farming (ORG), economic feasibility of the material (ECON) and toxicological risks (TOX) (see Figure 3, which shows sections of the first part and the risk assessment part of the list covering oils.) Initially, the list comprised 73 plant extracts, 59 oils, 8 micro-organisms and 23 other materials. During the project more than 40 additional materials were added to the list. The three risk assessments formed the basis for go/no-go decisions on each material for initiation of screening for efficacy against *Venturia inaequalis* and *Plasmopara viticola.*

Number (code)	Material	Product and source of availability	Origin	Treatment and Concentration	Type of assay	Efficacy	Assessment	Commodity	Pathogen	Reference
O1	*Aegle marmelos*, Bengal quince oil	Plant oil (seeds)	Not a commercial preparation	In vitro: 1000-3000 ppm	In vitro: PDA ammended with oil extract	Inhibited 40% fungal growth at 3000ppm	Fungal growth inhibition		*Aspergillus flavus*	Dwivedi SK, Dwivedi SK, Pandey VN & Dubey NK. 1991. Effect of essential oils of some higher plants on Aspergillus flavus Link. Infesting stored seeds of guar (Cyamopsis tetragonoloba L. (Taub.)). Flavour and Fragance Journal 6: 295-297
O2	*Allium sativa*; Garlic	Plant oil	Not a commercial preparation	In vitro: 9-900 ppm oil extract tested against 4 different isolates	In vitro: liquid test with oil extract at diff.conc and bacterial conc. of 10^6cfu/ml	Inhibited bacterial growth of all 4 isolates at 112,5 and 900 ppm	Bacterial growth inhibition (minimal inhibitory concentration)		*Erwinia amylovora*	Sortichini M. & Rossi MP. 1993. In vitro behaviour of *Erwinia amylovora* towards some natural products. Acta Horticulturae 338: 191-198
O3	*Artemisia vulgaris*; Mugwort oil	Plant oil (seeds)	Not a commercial preparation	In vitro: 1000-3000 ppm	In vitro: PDA ammended with oil extract	Inhibited 48.5% fungal growth at 3000ppm	Fungal growth inhibition		*Aspergillus flavus*	Dwivedi SK, Dwivedi SK, Pandey VN & Dubey NK. 1991. Effect of essential oils of some higher plants on Aspergillus flavus Link. Infesting stored seeds of guar (Cyamopsis tetragonoloba L. (Taub.)). Flavour and Fragance Journal 6: 295-297

Oils			Risk assessment[2]			Comments	Go/ no go[3]
Number (code)	Material	Category[1]	ECON (C-1)	TOX (C-2)	ORG (C-1)		
O1	*Aegle marmelos*; Bengal quince oil	1.2	Data Lacking	10(0)	low	ORG: Fulfills organic standards; Prerequisites for acceptance in EU Reg 2092/91: non contact clause removed; check side effects and additives	go
O2	*Allium sativa*; Garlic	1.2	Moderate	703	low	ORG: Fulfills organic standards; Prerequisites for acceptance in EU Reg 2092/91: non contact clause removed; check side effects and additives	go
O3	*Artemisia vulgaris*; Mugwort oil	1.2	Expensive	156	low	ORG: Fulfills organic standards; Prerequisites for acceptance in EU Reg 2092/91: non contact clause removed; check side effects and additives. ECON:Oil with low flash point	go

Figure 3. Examples from the annotated list (~ 100) in the category 'Oils' for the first three materials mentioned (coded O1-O3). The first part (above) contains the names, sources, key information and references, and the second part (below) contains the risk assessment and the go/no-go decisions for the same three materials.

For assessments of micro-organisms, the following guidelines have been used to exclude candidates with unacceptably high risks:

- Commission Directive 97/46/EC of 25.07.1997 amending Directive 95/44/EC containing the conditions under which certain harmful organisms, plants, plant products and objects should be listed in Annexes I to V,
- Directive 2000/54/EC on the protection of the workers toward risks related to exposure to biological agents at work (1.09.2000),
- the technical guidelines (BG Chemie, Germany – TRBA 450 and 460),
- Guidelines of the Federal Office 'Einstufung von Organismen – Pilze', Bundesamt für Umwelt, Bern, Switzerland, 2004.

Providing information on compound assessments
REPCO built up a huge collection of compounds that were tested for their efficacy against *Plasmopara viticola* or *Venturia inaequalis*. Most of these compounds were provided by industrial companies or research institutes. Results obtained with such compounds were communicated to the providers or are available for providers at request. The main results will be published as well. A total of four patent applications have been submitted by REPCO partners before publication to facilitate the exploitation of the obtained project results. Details can be found in Section 2 of this report.

Downy mildew of grapevine: Development of potentiators of resistance and fungicides for control of *Plasmopara viticola*

REPCO tested potentiators of resistance and organically based fungicides potentially compatible with organic standards with the objective to evaluate and develop compounds for *P. viticola* control in grapevine under controlled conditions and to assess the efficacy of selected potential plant protection products under field conditions. For selected compounds, formulation, timing and methods of application, and their integrated use in organic grapevine production, were optimised as well. The test products were obtained from companies as commercialised products or from companies and research labs as non-commercial test products or 'pipeline' products under development.
During the project, more than 130 substances or products were tested under controlled conditions for fungicidal and/or elicitor activity and/or as additives. Approximately 67% of the tested products showed no or only low efficacy in this test system. Of the remaining 33%, some only showed low efficacy when applied in a concentration ten times lower than recommended, indicating low efficacy under field conditions. The tests under controlled conditions resulted in 21 products being selected for screening under natural field conditions in the experimental vineyard. The field experiments also included strategies to improve the use of copper and/or to improve the current plant protection practice in organic vineyards, and these were compared with a reference copper treatment.
Only a few of the test products partially controlled downy mildew on grapevine plants. The efficacy as regards downy mildew protection was not sufficient from the producer/grower point of view. All these products need further research and development work before they can be recommended for use in grapevine production. For a complete replacement of copper in plant protection against downy mildew on grapevine, further new products with effective fungicidal impact are still needed.
Contacts with industries and SME partners qualified for commercialisation of project findings are ensured and several compounds can be delivered to these industries for further development into products for use in organic agriculture. A main achievement of REPCO is that four to seven products with partial field efficacy remain for further development. Although there is some potential for protection of grapevine plants against downy mildew with these newly developed or tested products, further research and development work with these products is necessary before they can be recommended for use in organic viticulture.

Product screening on leaf discs and in the laboratory
Leaf discs (16 mm diameter) of greenhouse-grown grapevine cultivar Müller-Thurgau were placed on water agar, upper surface down, in transparent square Petri dishes. A droplet of the test solution was applied to the leaf discs 24 hours before infection with downy mildew. The leaf discs were infected by applying a droplet containing sporangia of the pathogen and were incubated in a growth chamber under long-day conditions at 25 °C and high relative humidity. After six days the leaf discs were checked for sporulation of the pathogen and changes of the leaf discs structure such as lesions or necrosis.

Cell suspension cultures of grapevine cultivar Pinot noir were used to measure the induction of resistance by the test substances. In cell suspension cultures an early cellular reaction to pathogen attack is the transport of H+ ions through the plasmalemma into the cells resulting in alkalinisation of the culture medium. This alkalinisation was measured as an indication of increased plant defence (Figure 4).

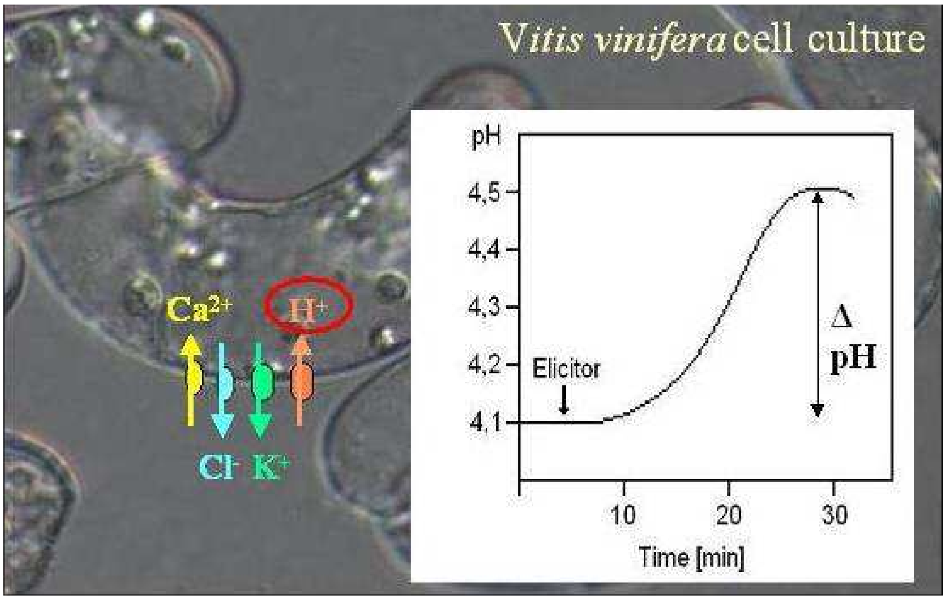


*Figure 4. pH changes measured in the grapevine cell suspension culture medium (*Vitis vinifera*) after addition of elicitor (HePC).*

The tested products were applied on leaf discs in aqueous solutions of various concentrations to establish the dosage range preventing the sporulation of the pathogen without harming plant tissue. Of the 134 substances tested in different concentrations and combinations in the leaf disc assay, 14 inhibited sporulation. Figure 5 shows the effect of HePC at different concentrations, a representative example of a substance developed within REPCO downy mildew control.

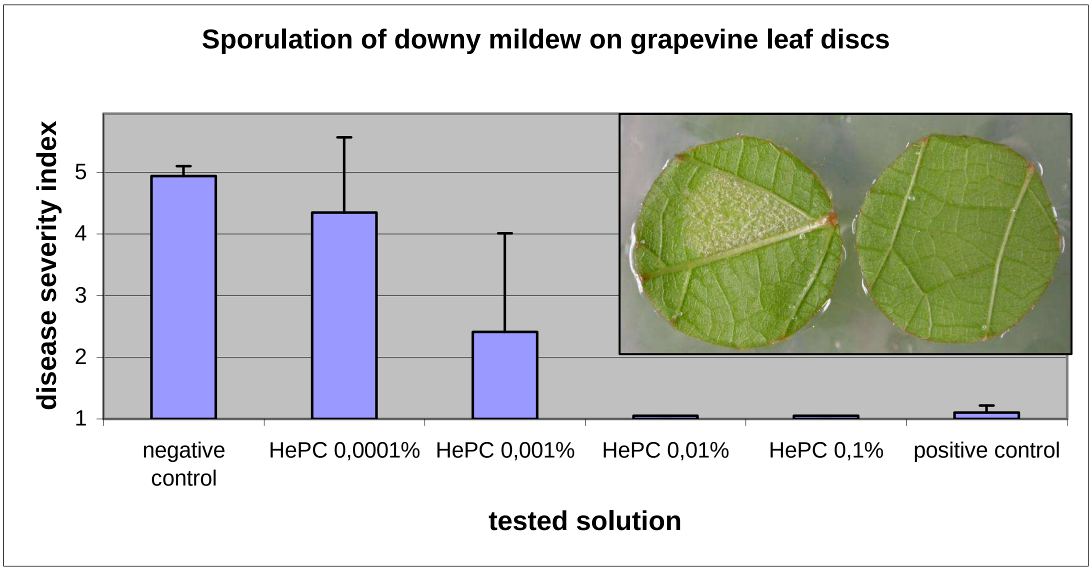


Figure 5. Disease severity on leaf discs pretreated with HePC at different concentrations prior to challenge by the pathogen downy mildew.

The influence of several additives on the leaf disc tissue and sporulation of the pathogen were also tested in the leaf disc assay alone or in combination with several compounds already screened positive for the control of downy mildew in grapes. Suitable compounds and combinations with additives were recommended for field trials.
Products studied and developed within REPCO were also tested for activators of the plant's innate defence response. Plants are able to defend themselves against the attack of fungi, oomycetes and bacteria with a set of defence mechanisms. This innate resistance is induced by substances from the surface of the pathogens and by breakdown products of the plants cell wall. REPCO products were screened for substances stimulating plant defence by simulating a pathogen attack, the so-called elicitors, using cell culture (Figure 1). Some of the fungicidal agents used in field application by REPCO partners enhanced defence mechanisms in the cell culture system; this means that there could be an additional beneficial effect of these agents as well.
The leaf disc assay showed that more than 80% of the tested substances were not worth further testing at plant level. Thus, this first screening of substances proved to be very useful to save costs. Products developed within REPCO, e.g. HePC, controlled downy mildew on plants and were provided to REPCO partners for field trials. Substances showing a resistance response in the cell culture system are worth testing for their potential as resistance inducers in plants.

Product screening on seedlings in the glasshouse

Efficacy testing for fungicidal and elicitor activity of products was performed on grapevine seedlings (cv. 'Chasselas') in greenhouses of FiBL. The seedlings were treated with test substances when 3-4 leaves were fully expanded. The tests substances were applied in an automatized spray cabinet. Each test substance was tested in at least two concentrations and was compared with reference substances. Plants were inoculated after application. Inoculation was performed by applying three individual 10 µl drops of sporangia suspension per leaf (elicitor screening) or spraying whole plants with sporangia suspension (fungicide screening). Subsequently, plants were incubated six days, following a given protocol. Standard disease assessment included disease incidence and disease severity and/or lesion diameter.
In general, the test systems are well suited to perform primary screenings of fungicidal activity. Generally, there was good to excellent protection of seedlings against *P. viticola* after application of copper hydroxide ('Kocide 2000') as fungicide reference. Copper reference treatments do sometimes not reach 100% control due to heterogeneous leaf coverage under experimental conditions. Disease incidence and disease severity in control treatments in various experimental sets generally were between 60-100% (incidence) and 80-100% (severity). In some sets, however, incidence and severity were lower, but still resulted in clear differences between treated and untreated variants.
Results of elicitor screening tend to vary considerably between experimental sets. 'BABA' was used as a reference elicitor throughout the experiments. Although BABA generally is working as well as an elicitor of resistance, there were experimental sets where BABA had only a very faint effect or even no effect. For these sets it is not clear if plants in general were not inducible. This means that it cannot be ruled out that the plants were not very well inducible and that some products tested as elicitors of resistance may have an effect.
A total of more than 100 substances were tested under controlled conditions for fungicidal and/or elicitor activity and/or as additives. Approximately 67% of all tested substances showed no effect on disease expression. Of the remaining 33%, some only showed slight effects at the recommended dosage. However, efficacy at lower dosages is necessary if any activity in the field is to be expected. Products showing promising results were confirmed in at least a second experimental set. The results obtained from these screenings led to the recommendations 'go', 'no go' or 're-test'.
Efficacy under field conditions is often limited by insufficient UV stability and rainfastness. To evaluate efficacy of fungicides after rain events, a rain simulation system was developed and used for screening under controlled conditions. Grapevine seedlings were treated with water (control)

and the copper reference product and were then submitted to simulated rain events. Rain events were simulated after drying of the fungicide on the test plants with a fixed duration of the simulated rain. The results show that there are large differences between test products for rainfastness and they show the necessity to do formulation work, e.g. with additives, to improve performance against rain events.
Elicitors are often relatively large molecules and consequently plant uptake may be a limiting factor. The efficacy and performance of candidate substances might therefore be considerably improved by optimum formulation of the active substance. Results of a series of tests where additives were combined with several test products (elicitors or fungicides) suggest that additives may not only increase but also decrease the efficacy of a test product. Formulations with stickers, wetting agents and adjuvants are crucial to improve the efficacy of a product.
The most promising materials showing a significant reduction of *P. viticola* were identified and recommended for field testing under natural conditions, either in the screening vineyard of FiBL or in vineyard experiments by REPCO partners IASMA (Italy) and GRAB (France). Priority was given to products with proven activity and high efficacy, as well as availability and formulation.

Product screening in the vineyard

The experiment was carried out in the screening vineyard of FiBL in Frick, Switzerland, at 390 meters ASL. The experimental vineyard consists of 576 plants of the susceptible grapevine varieties ‘Riesling-Sylvaner’ (‘Müller-Thurgau’) and ‘Chasselas’ (‘Gutedel’). The experimental setup allows for a Complete Randomized Block Design with twelve different treatments. An untreated control, a copper reference treatment (‘Kocide DF’) and a systemic fungicide reference treatment (‘Aliette’) were included as standards (Figure 6).
During the growing season the necessary maintenance work was done in the screening vineyard (one treatment against mites (*Colomerus vitis*) in April, mulching, untying saplings, hand weeding around the plants). The plants were treated weekly, or according to weather conditions and risk of infection, by means of air-assisted equipment, either a compressor-based spray system (spray pressure 2 bar) or a portable motor sprayer, until near run-off. The experiment was carried out following the relevant EPPO-guidelines.
Twenty one plant protection products that showed good activity against *P. viticola* in indoor screening experiments were tested outdoors in the screening vineyard and they were compared with ten strategies and three standards. Disease pressure due to *P. viticola* generally was high in the screening vineyard. Under such conditions, unprotected susceptible grapevine varieties are completely defoliated by *P. viticola* and grapes of susceptible varieties are completely destroyed.
Copper (reference) was highly effective in protecting plants from *P. viticola*. A low dose of copper also showed good efficacy in protecting leaves but it was not effective enough to protect grapes and bunches from the disease. Three of the strategies, one of them completely copper-free, protected leaves and grapes as effectively as the use of copper at regular concentrations.
Of the plant extracts or biologicals, Timorex partially controlled downy mildew severity on grapevine plants and the product has a potential for use in a combined strategy with other plant protection products. Teawet TQ liquid and Quiponin BS liquid showed an interesting potential but these products need further improvement in formulation (e.g. rainfastness). Another plant extract is thought to have a high potential as an organically based fungicide but needs further R&D work to improve extraction process, formulation and –finally- efficacy. The product ‘Saponin DeruNed’ showed at least partial efficacy in controlling the disease on leaves. There was a clear effect against *P. viticola* on grapes as well and the product showed interesting properties against Botrytis. Therefore, further evaluation of the product, possibly in combination with other products, is recommended.
Two other unformulated products with high efficacy under controlled conditions are thought to be worth further development as regards optimal formulation.

Figure 6. Effect of two test and two reference treatments in the screening vineyard in 2007.

Within the project, it was possible to test a wide range of different products and substances. Due to the well-suited test systems, results of the screening work allowed to efficiently select products and substances with a high potential for controlling *P. viticola* on grapevine. Selected products were further tested in vineyards in Italy and France by REPCO partners IASMA and GRAB.

Many of the new products showed at least partial efficacy in the field screening test. However, the efficacy was not sufficient for use in practice in vineyards and none of the tested new products and treatment combinations provided complete protection from downy mildew. Further new products with effective fungicidal impact are still needed for a complete replacement of copper in plant protection against downy mildew on grapevine.

Integration of control measures for downy mildew control in vineyards

Field trials on downy mildew control in Italian vineyards. The efficacy trials were conducted from 2004 to 2007 on the experimental organic farm of IASMA, in Navesel Rovereto (Italy), on the cultivar Cabernet Sauvignon.

During this period 32 new compounds, divided into the groups: plant extracts (15), clay (1), biocontrol agents (5) and other origins (11), with different dosages, times of application and strategies were tested in a total of 79 different treatments. Two strategies were evaluated. In the first strategy products were tested alone, in the second one they were alternated with copper hydroxide treatments (2005) or combined with low dosages of copper hydroxide (2006 and 2007). The solutions of experimental products and references were sprayed weekly (other timing if application time was under evaluation) with an experimental atomiser using a spray volume of 12 hl ha^{-1} (Figure 7). The statistical design was a completely randomized block design with four replicates.

Two plot sizes were used according to the available amount of product: large plots were about 200 m^2 in size with 83 plants (three rows) and small plots were about 17 m^2 with 7 plants. Large untreated plots were used as untreated control. Assessments were made after the incubation period of the main infections or, if no infection occurred, before the next treatment. For each replicate, 50 leaves and 50 bunches were scored to calculate disease severity (percentage of symptomatic leaf area or infected berries) and incidence (percentage of infected leaves or cluster). Phytotoxicity was assessed checking necrosis, discoloration and reticulated area on 50 leaves and on 50 bunches for each replicate.

Figure 7. Product application with experimental atomiser.

At the end of the growing season grapes were harvested and the average weight of clusters, sugar content (° BRIX, % sucrose w/w), acidity and pH were determined.
For statistical analysis collected data were normalised. ANOVA and Duncan or Tukey's test were run to compare severity %, incidence % and AUDPC of the products with copper hydroxide and untreated reference.
The evaluation of the products was difficult in most seasons due to the late appearance of the disease and its fast development. In three years (2004-2006-2007) the first oil spot appeared very late in the season and the epidemic was very fast thereafter.
Disease incidence on bunches was very low in 2005 but high in 2004, 2006 and 2007. Under these conditions full disease control was only achieved with the new low copper formulations (Labicuper and Naturam) or with K phosponate, not allowed in organic farming.
Among the 15 plant extracts tested, a weak control on leaves was achieved with Saponin and Elot-Vis with an efficacy about 50% lower than copper (Figure 8). Several plant-based compounds, two commercial products (Saponin and Novosil) and an experimental extract (SA) showed a very good control of the symptoms on bunches (Figure 9). The other plant-based compounds were not effective under conditions of high disease pressure.

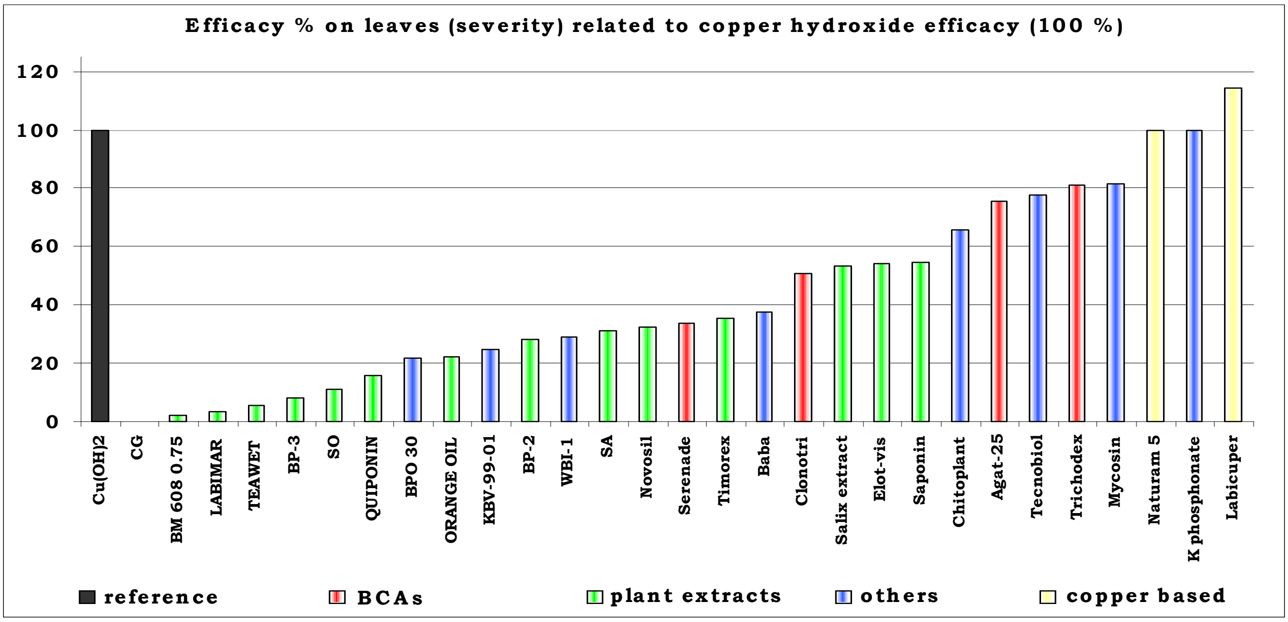


Figure 8. Efficacy of tested products on leaves. Efficacy was calculated on the normalised untreated control and related to copper hydroxide efficacy.

Of the other products, Chitoplant, Tecnobiol and Mycosin showed the best results, both on leaves and on bunches.

In the other strategies no difference was observed between products plus low copper dosage and the same low dose of copper used as reference. A statistical difference between a low dosage of copper and a normal copper dose used as reference was neither observed.

Phytotoxicity was observed on plants treated with Naturam (high damage) and with Mycosin (low damage). The laboratory analysis of harvested bunches revealed no significant difference between treatments in sugar content, acidity and pH.

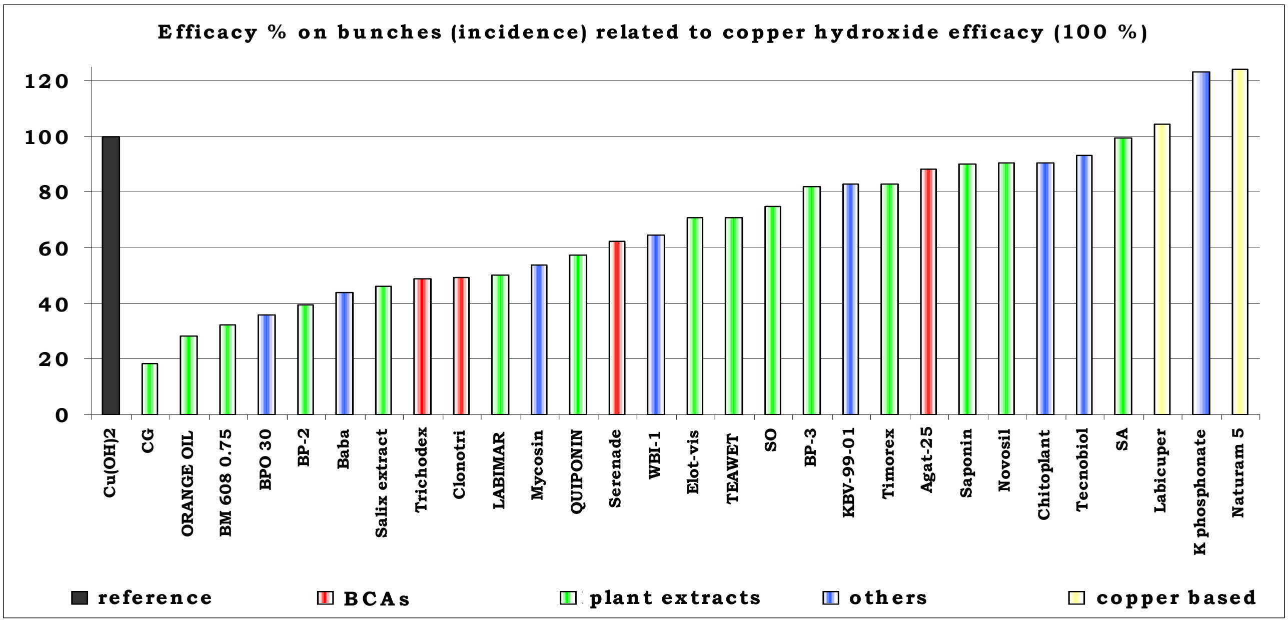


Figure 9. Efficacy of tested products on bunches. Efficacy was calculated on the normalised untreated control and related to copper hydroxide efficacy.

All treatments resulted in a certain disease control compared to the untreated control and some of them were equally effective as copper hydroxide. The best results on leaves were obtained with

copper-based fungicides, BCAs and compounds like clay, animal products, and fatty acid. Plant extracts had an activity up to half of that of copper.
The best results on bunches were again obtained with copper-based fungicides. One experimental plant extract and two commercial compounds showed an activity only 10% lower than that of copper.
The results suggest that at this moment a complete replacement of copper fungicides is not possible, but many new formulations containing low concentrations of copper are available and their integration and optimisation could allow an acceptable control of the disease with fewer treatments and less input of copper in the environment.
Many existing commercial and experimental plant extracts, BCAs and other compounds have potential as alternatives for replacing copper but significant improvement of dosages, formulations and applications is still necessary to obtain commercially acceptable results.

Field trials on downy mildew control in French vineyards. In the four field experiments conducted by GRAB at different locations in France in 2004 - 2006, climatic conditions were not favourable for downy mildew development and average severity never exceeded 10 % in the non-treated control. Copper as reference was more effective than the products under test. In an experiment at Montélimar, two compounds added to the lower dose of copper were more effective than the same dose of copper alone.
Two field trials in grapevine were conducted in 2007. At Barnave, Chitoplant provided a good protection of leaves and bunches, but the pressure on bunches was not very high. Saponin did not provide enough protection. BM 608 provided a moderate protection. It should be mixed with copper or an alternative application to increase the efficacy. Trapper did not increase the efficacy of BM 608. In disease control strategies with only three applications the tested products did not provide a good protection, except the copper strategy which showed the same result as copper reference modality with eight applications. At Chignin, Plasmopara outbreak appeared late, on June 20th, but then increased very quickly with more than 50% of the bunches diseased in three weeks. None of the tested products showed a sufficient efficacy. Even the copper reference provided a poor protection. This may be explained by the relatively long intervals between treatments of 12 days on average. Under the high pressure conditions, a low copper dose did not provide any additional efficacy to the tested products. Based on the trials in France, ChitoPlant (Chitosan) and tea tree oil (Timorex or BM-608) can be recommended for further research on optimisation of the applied concentration and timing of field applications.

Selection pressure of control measures and forced evolution in *Plasmopara viticola*

A method to detect the potential threat of selection for resistance to fungicides for use in organic farming in grapevine downy mildew populations was developed. The use of neutral markers (SSR) was effective for detecting selection in *P. viticola* populations exposed to fungicide treatments in the course of the epidemiological season. None of the nine organic products, one combination of products and one strategy tested was able to unmistakably select resistant *P. viticola* genotypes.
The use of cultivar mixtures was effective for reducing disease severity in many crops, including apple. In the *Vitis* spp. / *Plasmopara viticola* pathosystem cultivar mixtures contributed only to some extent to reducing downy mildew severity. Susceptible grapevine cultivars (as Müller-Thurgau) planted in a cultivar mix showed slightly less *P. viticola* symptoms than if they were planted in single cultivar plots. This trend is independent of the disease pressure. Tolerant (Isabella) and very tolerant (Solaris) grapevine cultivars planted in a cultivar mix show more disease symptoms than if planted in single cultivar plots if the disease pressure is low. In case of high disease pressure, tolerant cultivars behave as being susceptible. Planting grapevine cultivars in a

mixture helps in reducing disease severity by approximately 10%, but this is insufficient for implementation in commercial winegrowing.

No selection pressure by organically based fungicides found

P. viticola populations were treated with organically based fungicides. Samples were collected from infected grapevine leaves throughout the epidemiological season (55-145 samples/treatment) and were genotyped following microsatellite analysis (Gobbin *et al*., 2003). The Shannon-Wiener's indexes H and E_H, the evenness index E_5 and the rarefaction index were used as estimators of population diversity. A reduction of population diversity with respect to an untreated control indicates a potential population selection exerted by the organically based fungicide. The procedure was tested in 2004 under natural conditions with two products (azoxystrobin and Mycosan) and one untreated control. The procedure was then implemented in 2005 and 2006 with eight products (Chitoplant, Sonata, Tri-40, Yucca extract, Agat-25k, Novosil, Tecnobiol and Timorex), a combination of products (Chitoplant+Sonata+KBV 99-01), one copper-based strategy and five controls (2 x untreated, Aliette, 2x copper).

The method of analysis used was effective in detecting selection pressure exerted by fungicides on *P. viticola* populations (Matasci *et al*., 2008). The azoxystobin-treated population, used as a positive control, showed a reduction of genotypic diversity as compared to the untreated population. The population treated with Mycosan did not differ significantly in genotypic diversity from the untreated population. In the following years, 2005 and 2006, the analysis of 1325 *P. viticola* lesions further confirmed the preliminary study. According to the Shannon-Wiener's index H, the evenness index E_5 and the genotypic richness there is no indication of *P. viticola* population selection by organically based fungicides. Genotypic diversity was slightly but significantly reduced for the Aliette and Tecnobiol exposed populations according to the H estimator. We conclude that none of the organically based products gave a clear indication to be at risk of being overcome by a resistant strain. In the case of Aliette (control product), one of the indicators responded, indicating a significant possible risk but since other indicators did not respond, the question remains open.

***Plasmopara viticola* on vines grown in cultivar mixtures**

A vineyard consisting of 632 vines of tolerant (Bianca, Chambourcin, Isabella, Regent and Solaris) and susceptible cultivars (Gamaret, Merlot and Müller-Thurgau) was established in Cugnasco (Switzerland). Vines were planted in four cultivar mixture blocks (each consisting of eight rows with two plants of each cultivar planted nearby) and in one single cultivar block (monoblock, consisting of eight rows with 15 plants of each cultivar). *P. viticola* samples were collected in 2004, 2005 and 2006. Genotyping of the collected samples was performed according to Gobbin *et al*. (2003).

Disease severity was assessed visually according to a modified Horsfall-Barratt disease rating scale (Horsfall and Barratt, 1945). For each vine one central shoot was randomly chosen. Severity values were back-transformed to percentages using the midpoint rule (Campbell & Madden, 1990) and the obtained values were divided by the total number of leaves of the selected branch.

Disease severity was assessed three, five and four times in 2005, 2006 and 2007, respectively. The years 2005 and 2007 were characterised by a lower disease pressure than in 2006 (for instance, severity on Müller-Thurgau in the pure cultivar block: 2005: 80%; 2007: 65%; 2006: 90%). Bianca and Chambourcin showed the lowest disease severity in all survey years while Gamaret and Müller-Thurgau were highly susceptible (Figures 10 and 11). In 2005 and 2007 severity was lower in the mixed blocks for cultivars where higher severity was assessed (more susceptible cultivars: Merlot, Isabella, Müller-Thurgau and Gamaret), possibly depending on a dilution of inoculum, on a distance/barrier effect, or on both factors together. Severity was lower in the monoblock for cultivars where lower severity was assessed (more tolerant cultivars: Regent, Solaris, Bianca and Chambourcin) possibly depending on a reduction of the inoculum due to the presence of a larger

number of resistant plants. In 2006 for each cultivar disease severity in the single cultivar blocks was higher than in the mixed block, except for Isabella (Table 1).
Genetic analysis of the collected oomycete samples was performed and results are available as raw data.

Figure 10. Chambourcin (above) and Müller-Thurgau (below) on 23/07/06 and on 13/09/06 (note the complete defoliation of the Müller-Thurgau plant in front).

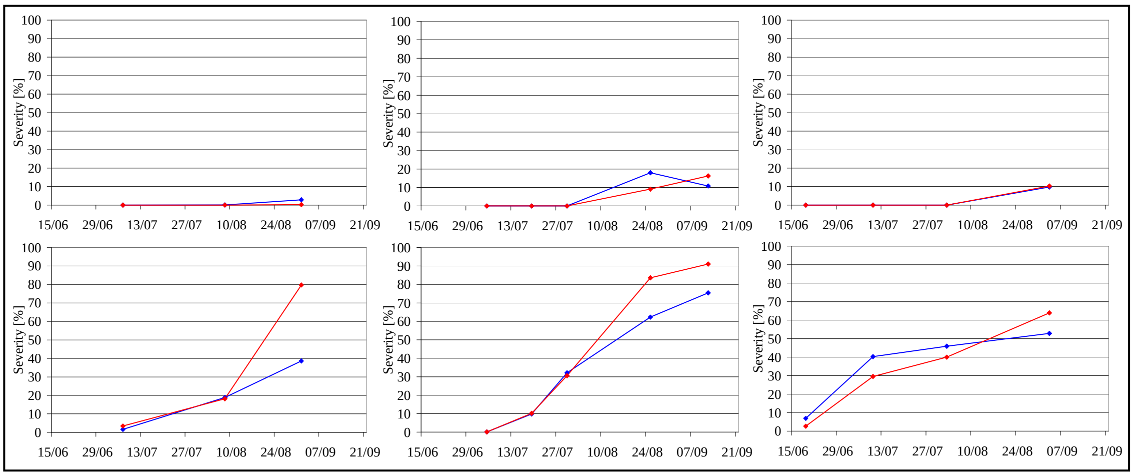
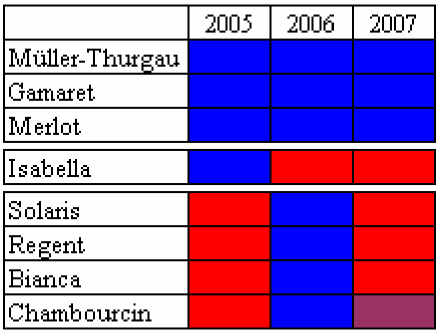


Figure 11. Disease severity recorded in the mixed cultivar block (blue) and in the single cultivar block (red) for the tolerant cultivar Chambourcin (above) and for the susceptible cultivar Müller-Thurgau (below) in 2005, 2006 and 2007 (first, second and third graph) in Cugnasco (Switzerland).

Table 1. Comparison between disease severity in the mixed cultivar block and in the single cultivar block in two years with low disease pressure (2005 and 2007) and in one year with high disease pressure (2006). Blue indicates that severity is higher in the single cultivar block than in the mixed cultivar block. Red indicates that severity is lower in the single cultivar block than in the mixed block. Violet indicates that severity in the single cultivar block is equal to the severity observed in the mixed cultivar block. Cultivars are ranked according to an increasing tolerance toward Plasmopara viticola.



Susceptible grapevine cultivars planted in a cultivar mix show slightly less *P. viticola* symptoms than if they were planted in single cultivar plots. This trend is independent of disease pressure. It is therefore always advantageous to plant susceptible varieties in cultivar mixes to reduce the disease. However, implementation into practice will depend on level of achieved reductions and additional costs. Tolerant and very tolerant grapevine cultivars planted in a cultivar mix show more disease symptoms than if planted in single cultivar plots if disease pressure is low. In case of high disease pressure tolerant cultivars behave as susceptible cultivars. Planting grapevine cultivars in a mixture helps in reducing disease severity under high disease pressure. Reductions range from 5 to 40%, with an average of 10%.

Development of potentiators of resistance and fungicides for control of *Venturia inaequalis* in apple

Over 100 potential candidate materials for apple scab control were screened under controlled conditions. Screening consisted of an *in vitro* assay to detect inhibition of *Venturia inaequalis* spore germination and a plant assay based on symptom development in apple seedlings artificially inoculated with *V. inaequalis*. Potential candidate materials consisted mainly of plant-based or non-plant based products obtained from companies and of crude plant extracts prepared as hot or cold water extractions of plant material and residues. Approximately 70 materials significantly reduced apple scab symptoms on seedling leaves at levels equal to or better than sulphur, when used preventively and/or curatively. Selected materials, e.g. yucca extract, that showed consistent, promising efficacies were further tested in different extraction types, in lower dosages, and in combinations, and the effect of timing of application of yucca extract was initiated.
Most of the materials with promising apple scab activity appeared to act mainly as fungicides. However, crude extracts from three plant species that effectively controlled the disease in the seedling test showed no fungicidal effect in the spore germination test, thus indicating that their mode of action was by resistance induction. Several approaches, including a biochemical assay and molecular methods, were used to study induced resistance in apple leaves.
Potential compounds were further screened during the main primary ascospore discharge periods in April to May in field experiments in 2005, 2006 and 2007. Significant reductions of apple scab on leaves and fruits were achieved with treatments of different yucca extracts, armicarb and some other plant extracts. Control levels reached those of reference treatments with copper oxychloride and

sulphur. As part of the dissemination activities of REPCO, a group of five commercial organic fruit growers developed and tested a strategy to apply a new material, potassium bicarbonate, as alternative to their standard control schedule.

Compound screening under controlled conditions

Potential candidate materials for apple scab control were screened in an *in vitro* spore germination test and in a plant assay. A single spore isolate of *V. inaequalis* was used for production of inoculum by the methods of Williams (1976) and Parker *et al.* (1995). Spore suspension concentration was adjusted to 1.5 $x10^5$ conidia ml^{-1} and the inoculum batches were stored at -18°C until use. Solutions of test materials were prepared according to protocols supplied by companies, from literature studies etc. Hot and cold water extractions of crude plant material and residues were prepared in standard concentrations of 5 and 2.5% (w/v). Further testing and optimisation of promising materials were carried out using solutions of lower concentrations, e.g. 1% and 0.5% (w/v or v/v), alone or in combination. For *in vitro* spore germination tests, conidia of *V. inaequalis* were mixed with the test solutions on glass slides, incubated for 24 and 48 h, and the percentage of germinated conidia assessed and compared with a water control. Assays with apple seedlings were carried out. Seeds of *Malus* x *domestica* 'Golden Delicious' were stratified at 4°C for 3-6 weeks and germinated seeds were sown in small pots with a peat-based substrate mixed with sand and these were grown in a growth chamber at 15-16°C for 4 weeks. Test materials were applied 1 day before inoculation (preventive treatments) and/or 1 day after (curative treatment) inoculation with *V. inaequalis*. Water and elemental sulphur were used as standard control treatments in all tests. Symptoms were assessed14 days after inoculation using a 0-7 scale derived from Croxall *et al*. (1952) and Parisi *et al.* (1993) (Figure 12). Disease severity score was determined from the average score of all susceptible leaves on the day of inoculation for each treatment and the data were statistically analysed and the effect was compared with the water and sulphur treatments. Biochemical and molecular methods were used for studying induced resistance in apple leaves treated with sulphur, yucca, and a synthetic resistance inducer. The specific activity of the enzyme β-1,3-glucanase, believed to be involved in the defence in apple leaves was studied following the methods of Bradford (1976) and Isaac & Gokhale (1982). Quantification of expression of some proteins generally believed to be involved in plant defence (PR-proteins) was studied by real-time QPCR.

Figure 12. Screening of materials for apple scab control on apple seedlings (left) and typical symptoms of apple scab on an apple seedling leaf (right).

Highly effective plant extracts selected on seedlings

Over 100 plant extracts and other materials were tested in the routine screening assays at LIFE and ~70 materials showed promising control of apple scab on seedlings, i.e., the effect was at least the same as the standard treatment with elemental sulphur, either as preventive or curative treatments, or both. The screening experiments also included different types of extracts and optimised use of materials, i.e. lower dosages, mixing of materials in different combinations, and timing of application. Examples of promising materials that consistently showed promising efficacies, especially when used preventively are extracts of yucca and quillaja (Figure 13A). The results shown in Figure 12B illustrate the problem finding materials with good curative action. As can be seen, sulphur and an extract of quillaja have only very low efficacies while an extract of yucca is more effective.

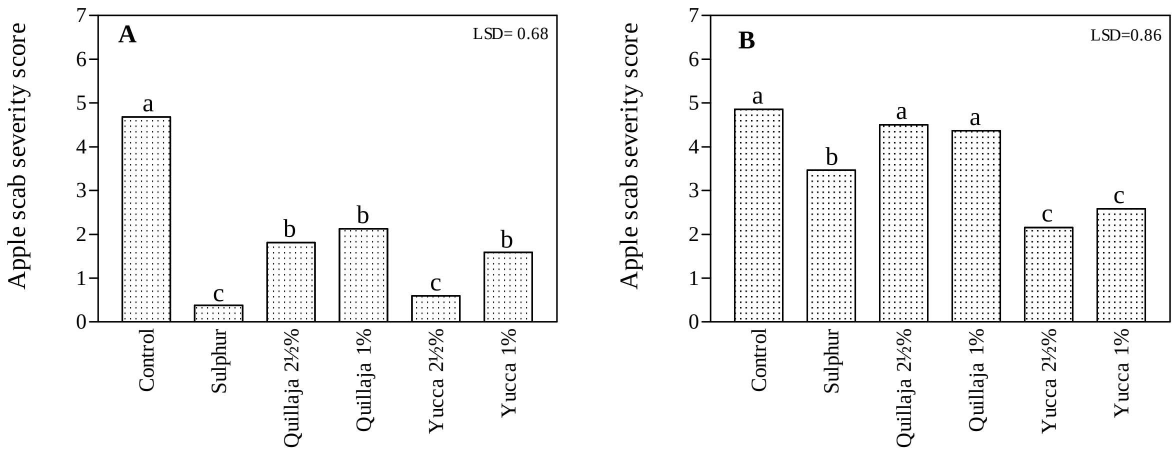


Figure 13A & B. Effect of sulphur, extracts of quillaja (2.5% and 1%, w/v) and extracts of yucca (2.5% and 1%, w/v) on scab symptoms on leaves of apple seedlings applied one day before inoculation (preventive, A) or one day after inoculation (curative, B) with conidia of Venturia inaequalis. *Disease assessment performed two weeks after inoculation. Treatment means with the same letter are not significantly different.*

Most of the plant extracts with effect in the plant assay showed a moderate to strong effect on conidium germination in the *in vitro* test (~60), thereby indicating that their primary modes of action were fungistatic or fungicidal (Figure 13). However, extracts from ~10 plant species appeared to act very differently as they exhibited no or only slight inhibition of spore germination on glass slides while reducing symptoms on apple seedlings (Figure 14), thus indicating their mode of action was wholly (3) or partly (~7) through resistance induction. Induced resistance in apple was studied in leaves treated with sulphur, yucca extract and a synthetic resistance inducer by using a biochemical assay and molecular methods. Measuring the specific activity of the enzyme β-1,3-glucanase, believed to be involved in defence in plants, showed no clear results while results from the molecular analysis of PR-proteins showed a tendency for up-regulation of some PR-proteins in leaves treated with yucca.

 Seedling assay	 Conidium test	Main mode of action	Number of materials
Disease reduction	Inhibition	**Fungicidal**	~60 materials
Disease reduction	No inhibition	**Potentiator of resistance**	~10 materials

Figure 14. Materials tested with efficacy against apple scab divided into two groups based on their mode of action.

The most promising materials of the novel organically based fungicides and potentiators of resistance were studied with regard to optimisation of application. The screening results formed the basis for the selection of suitable materials and know-how for screening in organic orchard trials in Denmark at UoA and trials on integration of the novel compounds into control strategies in the Netherlands at PPO.

The results and knowledge generated in this part of the project can be used for product development of promising materials by industries. From the beginning of the REPCO project, LIFE had a close collaboration with the company Nor-Natur ApS, Denmark, regarding several of the identified most promising compounds for apple scab control, which have succeeded in filing five utility model applications and two patent applications with Nor-Natur ApS. In addition, REPCO collaborated with several other companies on the screening of commercial and non-commercial products for apple scab control. Based on REPCO results with yucca extracts to control apple scab a patent application has been filed and the commercial exploitation of this invention is presently under investigation. Details can be found in Section 2 of this report.

Screening of compounds in the orchard

Field experiments were carried out in 2005, 2006 and 2007 at the University of Aarhus, Department of Horticulture, Denmark, during the main period of ascospore discharge. The experiments were done on Jonagold (*Malus x domestica* Borkh.) on M 9 rootstock, pruned as slender spindles. The trees were planted in a single row with every 11th tree as pollinator, Discovery, in 1999 at a planting distance of 3.5 x 1.3 m. Treatments were applied with a specially built tunnel sprayer mounted with 8 Tee-jet 110-02 nozzles, with a volume of 1000 litres ha^{-1} (Figure 15). Observations were made only on Jonagold, which is highly susceptible to apple scab. Experiments were done according to EPPO Guidelines. The best compounds from the plant assays were tested in a randomized block trial with 5 replicates. Each plot consisted of 5 trees. Observations were made on the three middle trees of each plot. The bordering trees served as a buffer between neighbouring treatments.

Treatments were carried out at 3–5 day intervals. A total of 10-11 applications were made, depending on the year. For all treatments 4 kg ha^{-1} Kumulus S (sulphur 80%) was applied weekly for apple scab control from green tip until the start of the trial period and again after the trial period and until the beginning of September. Treatments and dose rates are listed in Table 2. Copper oxychloride and Kumulus S were used as control treatments.

Figure 15. Tunnel sprayer with 8 nozzles, Tee-jet 110-02. Water volume: 1000 l ha^{-1}. Sprayer made special for this trial as alternative to knapsack spraying.

Apple scab infections on cluster leaves were assessed on all leaves on ten clusters at the end of the primary infection period and on 200 leaves on 20 extension shoots in July per plot. The number of leaves with and without infection and the number of scab spots per infected leaf were counted. At harvest apple scab infections on single fruits were assessed according to a 0 – 3 scale, where 0 = no scab, 1 = < 0.25 cm², 2 = < 1 cm², 3 = > 1 cm² of the surface covered with apple scab. Fruit russeting was evaluated according to a 1 – 3 scale, where 1 = 0 – 10%, 2 = 11 – 50%, 3 = 51 – 100% and cracking.

Compounds effective in scab control under orchard conditions

Apple scab infections were severe in 2005 and 2006. In 2007 the primary apple scab infections were low due to a warm, dry spring. The secondary infections were rather severe. The strategy of weekly applications of sulphur (4 kg ha^{-1} Kumulus S; sulphur 80%) to control apple scab in the period before and after testing of the new compounds resulted in severe infections on extension shoots in all years.

In 2005 Norponin BS liquid 2.5% resulted in the best apple scab control on leaves of spurs and extension shoots (Table 2). The other products also had and effect (Table 2). The positive achievements of Norponin for apple scab control resulted in a patent application. Apple scab infections on fruits were generally very severe. Untreated fruits were 100% infected. Only treatment with Norponin resulted in a significantly lower percentage of infected fruits compared to the control (Table 3). No russeting occurred.

Armicarb 1.33%, Norponin BS liquid 2.5%, Saponil NL 2.5%, Florina Proff 2.0%, Plant 4 5.0%, Plant 7 5.0%, Cocana 1.0% and Rapeseed oil 2.0% controlled apple scab on the cluster leaves at the same level as the control treatments in 2006 (Table 2). Fruit infections at harvest were very severe and russeting was generally low to medium (Table 3).

Apple scab incidence was very low at the time of cluster assessment in 2007. All treatments, except Plant 9, reduced apple scab infections. Later in July the incidence on untreated leaves of extension shoots was nearly 50%. The best effect was seen after treatments with copper oxychloride, Florina Proff, Saponil NL 2.5% and Plant 7 (Table 2). At harvest, apple scab spots were found on most fruits with incidence > 90%, except after treatment with copper oxychloride where only 77% of the fruits were infested. Copper oxychloride caused severe russeting (Table 3).

*Table 2. Infection of apple scab (*Venturia inaequalis*) on leaves, after control of infections in the primary infection period, 2005-2007.*

Treatment	Dose rate %	Leaves on clusters Incidence %[1)], primo June			Leaves on extension shoots Incidence %, beginning of July		
		2005	2006	2007	2005	2006	2007
Untreated		36.8	44.3	8.5	62.3	45.4	49.7
Copper oxychloride	0.02	10.0	-	-	24.1	-	-
	0.05	-	13.4	2.2	-	32.1	18.4
Kumulus S	0.4	5.9	6.1	0.7	32.0	30.0	35.8
Rapeseed oil	2.0	5.9	12.4	1.1	29.3	39.1	27.5
Norponin BS liquid	0.75	-	19.1	2.5	-	40.8	40.8
	2.5	2.2	6.2	-	12.3	30.8	-
Quiponin BS liquid	2.5	9.4	20.8	2.9	43.1	37.7	39.7
Florina Proff	2.0	-	9.3	2.6	-	30.3	20,4
Yucca filamentosa	2.5	-	17.1	2.5	-	38.7	37.6
Plant 4	5.0	-	9.8	3.6	-	34.1	32.4
Plant 7	5.0	-	10.6	3.3	-	32.3	24.0
Plant 9	5.0	-	24.0	5.6	-	39.5	29.2
Armicarb	1.33	-	3.2	2.8	-	32.4	29.8
Cocana	1.0	-	12.3	1.9	-	37.1	32.4
Saponil NL	0.75	-	22.0	2.6	-	40.4	35.1
(Deruned Saponin)	2.5	-	9.3	1.1	-	30.7	23.3

-: no data
[1)]: Incidence: % leaves with infection

*Table 3. Infections of apple scab (*Venturia inaequalis*) on fruits and russeting on fruits, after control of infections in the primary infection period for 2005-2007.*

Treatment	Dose rate %	Apple scab on fruits Incidence %[1], at harvest			Russeting[2]		
		2005	2006	2007	2005	2006	2007
Untreated		100	100	97.6	1.1	1.3	1.6
Copper oxychloride	0.02	89.3	-	-	1.1	-	-
	0.05	-	98.0	77.0	-	1.3	2.6
Kumulus S	0.4	86.9	98.0	90.2	1.0	1.5	1.8
Rapeseed oil	2.0	85.9	100	94.8	1.0	1.4	1.4
Norponin BS liquid	0.75	-	99.3	97.6	-	1.2	1.8
	2.5	69.1	98.0	-	1.0	1.4	-
Quiponin BS liquid	2.5	99.3	100	97.8	1.0	1.3	2.2
Florina Proff	2.0	-	100	93.4	-	1.0	1,2
Yucca filamentosa	2,5	-	99.0	96.4	-	1.3	2.5
Plant 4	5.0	-	95.8	97.0	-	1.5	2.0
Plant 7	5.0	-	100	95.4	-	1.5	2.2
Plant 9	5.0	-	99.3	97.2	-	1.3	2.2
Armicarb	1.33	-	98.7	98.8	-	1.8	2.3
Cocana	1.0	-	100	99.0	-	1.4	1.2
Saponil NL	0.75	-	99.3	95.2	-	1.2	1.6
(Deruned Saponin)	2.5	-	93.3	91.2	-	1.6	2.2

-: no data
Incidence [1]: % fruits with infection
Russeting [2]: average score, scale 1-3 where 1 = 0 - 10%, 2 = 11 - 50% and 3 = 51 - 100% fruits with russeting.

A major achievement of the series of experiments was to confirm that Norponin BS liquid 2.5% had an effect on apple scab in the primary infection period. A patent has been obtained. Florina Proff 2.0%, Plant 4 5.0%, Plant 7 5.0%, Armicarb 1.33%, Cocana 1.0% and Saponil NL 2.5% controlled apple scab at the same levels as the control treatments copper oxyclorid and Kumulus S in the primary infection periods in 2005-2007.

Integrated use of selected products for apple scab control in apple production
Natural compounds selected by REPCO were sprayed according the RIMpro scab warning system from start of bud break until mid June. Severity and incidence of apple scab were measured on leaves and fruits. Phytotoxicity and russeting were assessed. The natural compounds were compared with copper hydroxide and sulphur as standard fungicides permitted for use in organic farming.
Field experiments were carried out in 2004, 2005, 2006 and 2007 in the organic orchard of PPO at Randwijk, the Netherlands, during the summer epidemics. Similar experiments were done on a commercial organic farm in 2006 and 2007. The experiments were carried out on Jonagold pruned as slender spindles and planted in a single row. Apple scab infection periods were determined by

the Mety computer-based weather recorder and the RIMpro warning system throughout the ascospore infection period. The scab warning system calculated infection periods based on the hourly detected meteorological data, the modified Mills table, the simulation of ascospore release and the effect of previously used sprays. Experiments were carried out according to EPPO Guidelines.
The treatments were started from bud break until June. The interesting treatments were: copper hydroxide and sulphur as reference, yucca extract at 2.5, 5 and 7.5 l ha^{-1},either alone or with sulphur and potassium bicarbonate at 2.5, 5 and 10 kg ha^{-1}, either alone or with sulphur. Several other treatments were applied as well, such as herb extracts and commercially promising products. These, however, were less effective than the other treatments. Scab incidence and severity assessments were made on leaves and fruits. Phytotoxicity was measured and the percentage of class 1 apples was determined. All data were subjected to statistical analysis.

Efficacy of treatment schedules
Summarizing all years, it can be concluded that most treatments with copper, yucca extract + sulphur and potassium bicarbonate + sulphur gave the best scab control on both leaves and fruit (Figure 16). On leaves, sulphur alone was significantly less effective than copper. Yucca extract alone showed a significant effect against scab but the combination with sulphur was significantly better on both leaves as well as fruit. Potassium bicarbonate also showed significant control compared with the untreated plots; the combination with sulphur was significantly better on fruit than potassium bicarbonate alone. The combination was also significantly better than sulphur alone. Copper showed good scab control but also increased the amount of fruit russeting compared to the other treatments. This resulted in the percentage of class 1 fruit being the lowest of all treatments, including untreated (Figure 17). The combination of potassium bicarbonate with sulphur gave the highest percentage of class 1 fruit but this was not significantly different from the sulphur treatment. No effect on yield was found.

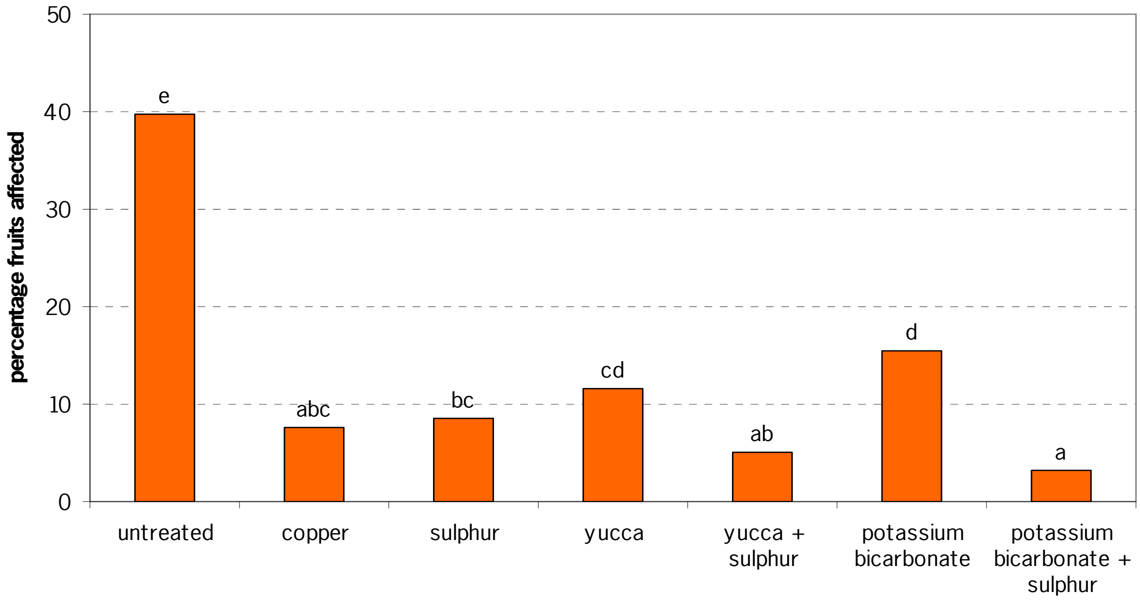


Figure 16. Percentage of fruits affected by apple scab at harvest 2006 on a commercial organic farm after different treatments during the primary apple scab season.

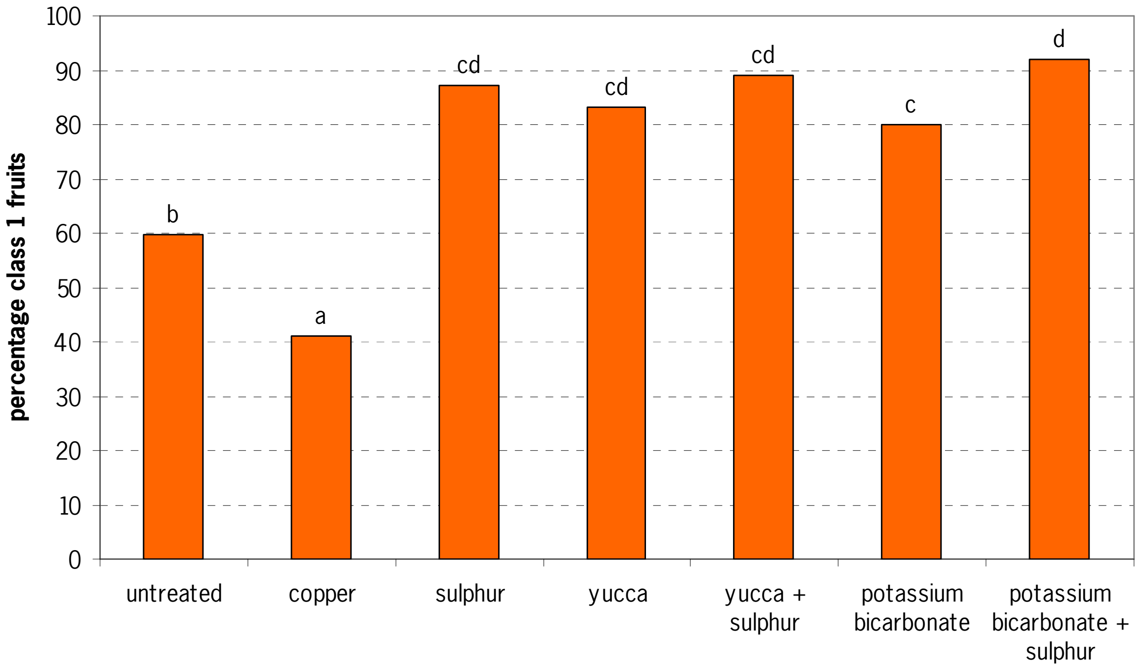


Figure 17. Percentage class 1 fruits at harvest 2006 on a commercial organic farm after different treatments during the primary apple scab season.

Experiments at the experimental organic orchard gave similar results as those on the commercial farm. Little dose response was found when applying yucca extract at 2.5, 5 or 10 l ha^{-1} and potassium bicarbonate at 2.5, 5 and 10 kg ha^{-1}, either alone or with sulphur for extension shoot leaf efficacy and on fruits. There was, however, a dose response effect for both yucca extract and potassium bicarbonate on cluster leaves.
It is concluded that yucca extract and potassium bicarbonate, both in combination with sulphur, have the potential to replace copper treatments during the primary apple scab season.

Disseminating knowledge: Using potassium bicarbonate in grower's trials
REPCO research has yielded scientific evidence that some compounds, especially potassium bicarbonate and Yucca formulations in combination with sulphur, have an effect on *Venturia inaequalis* infections. Control did, however, not always reach the level desired by commercial fruit growers and several practical issues need to be addressed before these materials can be considered as a useful alternative to copper.
As part of the dissemination activities of BioFruitAdvies in REPCO, in 2007 a group of five commercial organic fruit growers in the Netherlands was invited to form a working group that should develop and test a strategy to apply these new materials as alterative to their normal scab management strategy. On all five organic farms a block of full-grown Elstar trees was split into a 'Standard' part that was treated according the farm strategy, and a 0.5 to 0.75 ha large 'experimental Bicarbonate Strategy' block. Each grower had access to a weather station and used infection calculations according to RIMpro in his decision-making. During the growing season, growers made their own decisions on scab management, both on farm strategy and on the trial plot. In case of doubt they consulted their advisor. During the primary season, applications were mainly aimed at preventing ascosporic infections, during the secondary season at the prevention of conidial infections on fruits.
The weather pattern in 2007 caused an exceptional primary infection season for the Netherlands: March and April were exceptionally dry. No primary apple scab infections developed during these months. Rain events in the period from 6 to 12 May brought out extreme numbers of ascospores. During the dry periods in March and April, however, growers tended to use their overhead

irrigation frequently, sometimes causing infection or near infection events. In contrast to the warm and dry spring, the summer of 2007 was wet and clouded, but temperatures were above normal.
As the pattern of apple scab infections was comparable for the five trial orchards, all with the Elstar cultivar, differences in disease development reflect the efficacy of the scab management strategy. The scab lesions found on fruits at harvest were quite small, suggesting that these had been caused by infections in June-July rather than earlier. The low number of scab-infected fruits is remarkable when compared to the high number of infected leaves during summer in most orchards. For organic production the incidence and severity of scabbed fruits at harvest were low and the result quite acceptable. From the pinpoint lesions found at harvest, however, more scab lesions might develop during storage. This will be accessed on the stored fruits in a follow-up project.
The results of the trials confirm earlier observations that in organic orchards in the Netherlands an apple scab epidemic grows exponentially when not controlled properly, even on moderately susceptible apple cultivars like Elstar. Without control measures, the disease progress curve matches the rate of development of new leaves on the shoots, starting from any level of primary attack. This necessitates sanitary measures and effective disease control during the period of primary infections for early season disease control, but a low disease level at any given point in time during the growing season does not allow for interruption of the spraying plan when the management aim is not only to prevent infections on the fruits but also to keep inoculum levels at a low level.
In 2007 the growers were able to manage apple scab with potassium bicarbonate + sulphur as well as with their standard strategy, provided they could use lime sulphur in difficult situations. On average, the amount of scabbed fruits in the bicarbonate and in standard treated plots was equal (Figure 18).

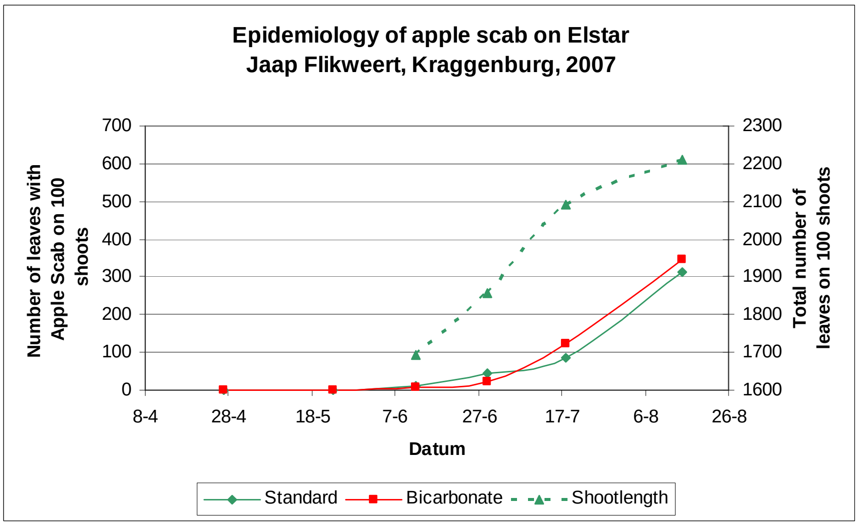


Figure 18. Apple scab development on Elstar. Jaap Flikweert, 2007.

No obvious phytotoxic effects were observed on leaves or fruits resulting from the use of potassium bicarbonate (5 kg ha^{-1}) and sulphur (5 kg ha^{-1}). The combination with a foliar fertilizer, however, resulted in severe leaf damage and leaf drop. This confirms the experience of other advisors who found that bicarbonate should not be tank-mixed with other materials to prevent physical problems in the spray solution and phototoxic effects.
Although the results of these trials are highly encouraging, many questions are still open. Knowledge on the mode of action and physical properties of potassium bicarbonate as fungicide are necessary to develop sound scab control strategies. The effect of the change of materials used for scab control on the complex of other orchard diseases such as mildew, sooty blotch, *Nectria*, black-rot and different storage diseases, need to be studied under practical conditions as well.
Finally, potassium bicarbonate can only be used by organic fruit growers in Europe as alternative to copper after being notified, positively reviewed, and listing in Annex 1 of 91/414 EC to be able to

be registered as crop protection substance in any European country, being positively reviewed and listed in Annex 2B of the EEC 2092/91 to be allowed for use as crop protection substance in organic farming in Europe, and having received local registration as plant protection product in the individual EU Member States.

Biological control of apple scab

Biocontrol strategies and sources of candidates

Apple scab epidemics are initiated by ascospores as primary inoculum produced in overwintering leaves. Micro-organisms present in apple leaves at leaf fall may interfere with the pathogen *V. inaequalis* during leaf colonisation in autumn, resulting in a reduced number of fruiting bodies and ascospores produced by the pathogen in spring. After primary infections caused by ascospores in spring, scab epidemics progress until autumn with multiple infection-sporulation cycles. Epidemics during summer are driven by conidia produced by biotrophic mycelium developing under the cuticle of apple leaves. In this situation, antagonists present in the phyllosphere may interfere with conidia of the pathogen during sporulation or infection.

The REPCO strategies were to evaluate whether fungal phyllosphere inhibitors suppress *V. inaequalis* ascospore production in spring and conidiation during summer epidemics. Scab infected apple leaves from organic orchards, old standard trees or abandoned orchards without any cropping management were collected at 216 locations in the Netherlands, Belgium, and northwest and central Germany. A collection of fungal cultures was built up with endophytic isolates obtained from surface-sterilised leaves and isolates obtained from sporulating colonies of *V. inaequalis.*

Since the aim of the study was to contribute to the development of commercial biocontrol products, only candidates were considered which fulfilled a range of criteria considering major constraints in development of biocontrol products. Candidates were tested on apple seedlings and selected isolates subsequently under orchards conditions during two seasons on fallen leaves during winter and in tree canopies during summer. For the superior antagonists, protocols for mass production, downstream processing and formulation were developed.

Screening protocols

A rapid throughput system was used for a first check of candidate antagonists regarding their potential risks and economic feasibility for development into a biocontrol product. Fungi belonging to the genera *Aspergillus*, *Penicillium* or *Fusarium* were discarded because of the potential of various species within these genera to produce mycotoxins. The remaining isolates of fungi were cultured on nutrient media and spore production was determined. Isolates producing less than 1 x 10^5 spores per plate were discarded because of a production of such isolates is considered not to be cost-effective. Subsequently, isolates growing at 5°C and at low water activity were selected because they were considered to be cold-tolerant and drought-tolerant. These characteristics are pre-requisites for successful colonisation of the phyllosphere. Isolates growing at 36°C were discarded because such isolates may demand special risk studies during a registration procedure. Less than 50% of the tested isolates passed these first screening steps.

The potential candidate antagonists to suppress the conidia production of *V. inaequalis* on infected leaves was tested on young apple seedlings. Seedlings were sprayed with conidial suspensions of *V. inaequalis* and placed in a moist chamber. After 5 days incubation antagonist suspensions were applied. After further incubation, the number of conidia of *V. inaequalis* produced on apple leaves was determined. A few antagonists were identified which reduced conidia production of *V. inaequalis* by more than 80%.

Promising isolates were selected and assessed in small-scale Solid-State Fermentation (SSF) for their suitability for large-scale biotechnological production processes. Several fermentation conditions regarding media and incubation conditions were evaluated. Only antagonists that passed this screening step were tested in subsequent experiments in the orchard.

The step to the orchard

Antagonists on overwintering leaves. In autumn of 2005, 25 fungal isolates belonging to different genera were applied on apple leaves from the organically managed orchard in Randwijk, the Netherlands. Treated leaves, fixed in nettings, were placed on the orchard floor until next spring. Soil coverage by leaves was estimated at the beginning of the experiment and again in early March 2006 to quantify possible treatment effects on leaf degradation. Six treatments with fungal isolates enhanced leaf degradation. Sub-samples of the treated overwintering leaves were collected at the end of January 2006, and the content of DNA of *V. inaequalis* was quantified by species-specific real-time PCR (TaqMan-PCR). Eight fungal isolates reduced the amount of *V. inaequalis* in residues of leaves treated in the preceding autumn by up to 72% (Figure 19). At the end of March, leaves were collected in the orchard to quantify the potential ascospore production by *V. inaequalis*. Only one antagonistic isolate, R406, significantly reduced ascospore production. After treatment of scabbed leaves with this antagonist in the preceding autumn, 70% less ascospores were found in spring.

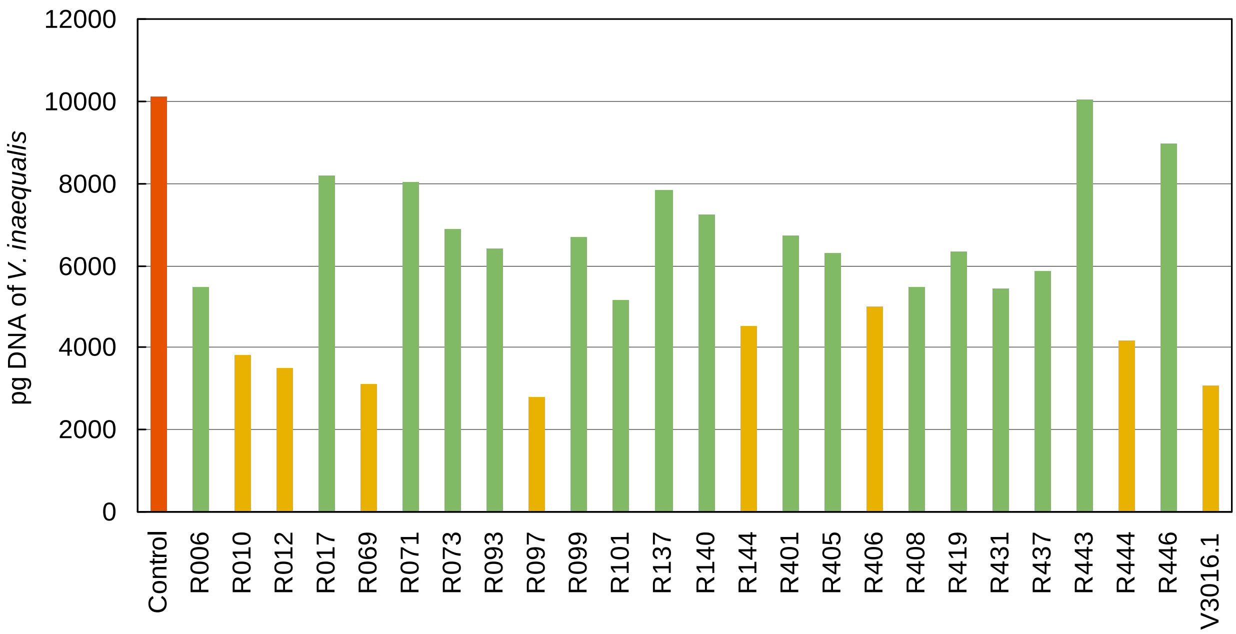


Figure 19. Effect of antagonist applications on the amount of DNA of Venturia inaequalis *in the residues of 80 treated scabbed apple leaves. Leaves treated in October 2005 overwintered on the orchard floor and were sampled at the end of January 2007. Yellow bars represent values significantly different from the untreated control.*

Antagonists during summer epidemics. A series of eight experiments was carried out in 2006, each on a different set of trees of the apple variety Jonagold in an organically managed orchard at Randwijk, the Netherlands. Trees were pruned during spring and summer so that trees produced new shoots with young leaves highly susceptible for *V. inaequalis*. Four different candidate antagonists were applied to leaves and the production of conidia by *V. inaequalis* was quantified on treated leaves. From the four tested antagonists, only the first formulated spore product of the antagonist H39 significantly reduced pathogen sporulation by 35 to 55%. In 2007, apple trees were treated with formulated H39 with similar results. A significant reduction by 51 to 69% was found for two assessment dates (Figure 20). However, at a later assessment date no difference between treated and untreated trees was found. This may be due to a reduced quality of the available antagonist product which had an insufficient shelf life. A second antagonist, R406, which had shown high efficacy against ascospore production on overwintering leaves in the earlier experiment, had been included in the experiment and also showed partial antagonistic effect against conidia production of *V. inaequalis*.

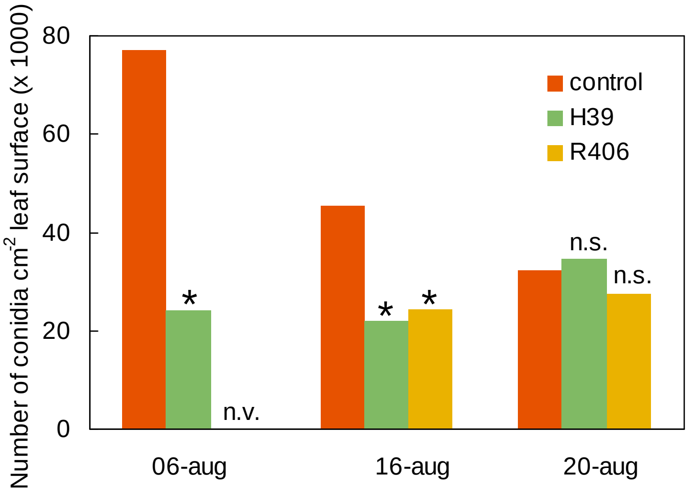


Figure 20. Effect of antagonists H39 and R406 on conidia production by the apple scab fungus Venturia inaequalis *under orchard conditions.*
** significantly different from untreated control; n.s. no significant difference; n.v. no value.*

Development of prototype biocontrol products

Final experiments were carried out to develop protocols for mass production, down-streaming and formulation of the two selected antagonists R406 and H39. Fermentation was based on the Prophyta laboratory scaled SSF system. Spore production in Solid-State Fermentation was investigated cultivating isolates on substrate mixtures consisting of oats and rolled oats. For the strain H39 it was possible to harvest more than 1.5 x 10^9 conidia per gram culture substrate (dw input). For R406, spore production depended on substrate composition and duration of incubation. However, the overall quantities and qualities of produced spore were not satisfactory (Figure 21).

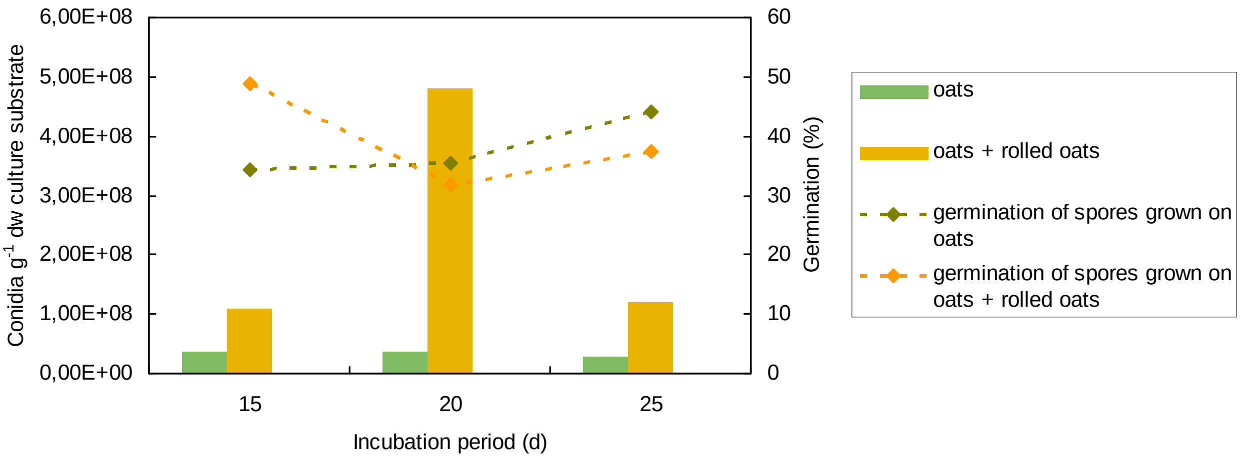


Figure 21. Effect of growth substrate and incubation period on spore quantity and quality of R406.

Unfortunately, most samples of the produced final products of both isolates showed a limited shelf life. The only exception was a formulation of spores of isolate H39 as a wettable powder. Even after 8 months of storage at 4°C or -20°C, sufficient spores were viable and able to germinate on nutrient agar (Figure 22). Since spore products were developed for application on leaf surfaces, UV light may affect spore survival after application. Spores produced and formulated under various conditions were tested for their susceptibility to UV light. Spores of H39 formulated as wettable powder showed a lower susceptibility to exposure to UV light than spores produced and formulated under other conditions. Unfortunately, the final formulated product regarding spore production, shelf life and UV stability could not be tested for its potential in apple scab control in orchards during the project period.

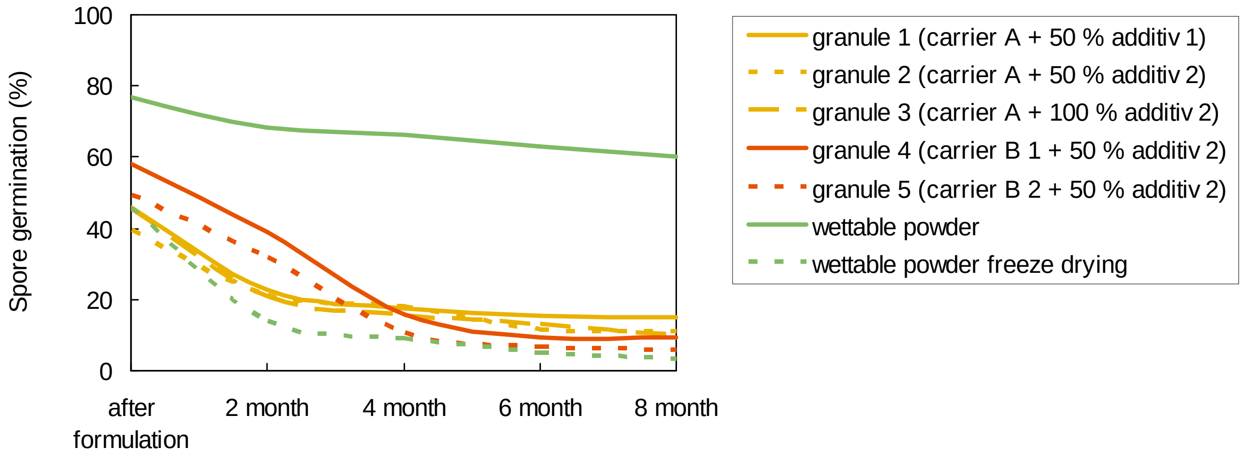


Figure 22. Effect of different formulation techniques on shelf life of spores of H39 mass-produced in Solid-State Fermentation and stored at +4 °C.

The next research steps require testing of the effect of H39 during a whole scab epidemic at orchard level. In these studies, sufficient product quantities of formulated product of H39 as wettable powder are needed. Furthermore, the potential of formulated H39 to reduce ascospore production in overwintering apple leaves should be assessed. Based on these data, a decision on the development of a commercial biocontrol agent containing H39 will be possible.

Enhancement of degradation of overwintering apple leaves to reduce the ascospore potential of scab (*Venturia inaequalis*)

Venturia inaequalis, the scab fungus, infects both fruit and leaves, resulting in a lower production of quality apples. The life cycle of *V. inaequalis* consists of a sexual and an asexual phase. During the sexual phase the fungus lives on fallen leaves. On these leaves ascospores are formed in fruiting bodies during winter. These ascospores are the primary inoculum in spring and will infect the newly formed leaves and fruit. It has been demonstrated that a lower number of fungicide applications were needed to control scab in low-inoculum orchards.

In REPCO, various treatments of fallen leaves were carried out to enhance leaf degradation, resulting in reduced production of ascospores on such leaves. Micro-organisms play an important role in leaf degradation. The effect of several leaf treatments on microbial communities was studied. Members of the communities were identified which dominated in overwintering leaves with lower ascospore production of *V. inaequalis* and thus may have an antagonistic effect on the pathogen. Furthermore, the role of earthworms in leaf degradation was studied. Treatments to increase consumption of leaves by earthworms and to increase earthworm populations in orchard soils were assessed.

Leaf treatments stimulating degradation

There are several methods to reduce *V. inaequalis* inoculum in orchards. One of the most effective methods is an application of 5% urea at leaf fall. Urea stimulates the decomposition of leaves and reduces the amount of ascospores. However, urea is produced synthetically and is not approved in

organic culture guidelines. The objective is to find alternatives for urea that simulate the decomposition of apple leaves and reduce ascospore production.
Experiments were carried out in the period 2004 - 2007 by PPO. Leaves were picked and dipped in different substances (Figure 23). After dipping, the leaves were placed in iron nettings and placed on the orchard floor (Figure 24). Leaf area was measured at the beginning of the decomposition experiment. Leaf degradation was measured twice between January and April.

Figure 24. Leaves placed in iron nettings on the orchard floor.

Figure 23. Dipping of leaves.

A sub-sample of the leaf residues of each sample was incubated in moist chambers for 21 days to allow maturation of asci. After incubation, leaf residues were transferred into bottles containing water. Air was bubbled through the water for 2 h, resulting in heavy turbulence. Thereafter, the resulting suspension was passed through a sieve to remove leaf debris. Ascospore concentration in the suspensions was determined microscopically. The products that were tested were: urea, *Coniothyrium minitans* (Contans), beet residues (Vinasse and Melasse), Compost tea, amino acids (Aminosol), calcium hydroxide, difenoconazole and potassium phosphonate.
A significant effect was found compared with the control when leaves were dipped in urea (Figure 24). Adding extra earthworms increased leaf degradation even more than urea. This shows the importance of earthworms to reduce the amount of inoculum source during winter and spring. Dipping the leaves in *Coniothyrium minitans* (8 kg/ha) decreased leaf degradation. On 18 April no significant difference in effect was found between the control, urea and the treatment with extra earthworms. *Coniothyrium minitans* showed significantly lower leaf degradation at both concentrations. Beet residues (Vinasse; 600 l ha^{-1}) gave the lowest leaf degradation. In two other years, beet residues actually stimulated leaf degradation in different concentrations.

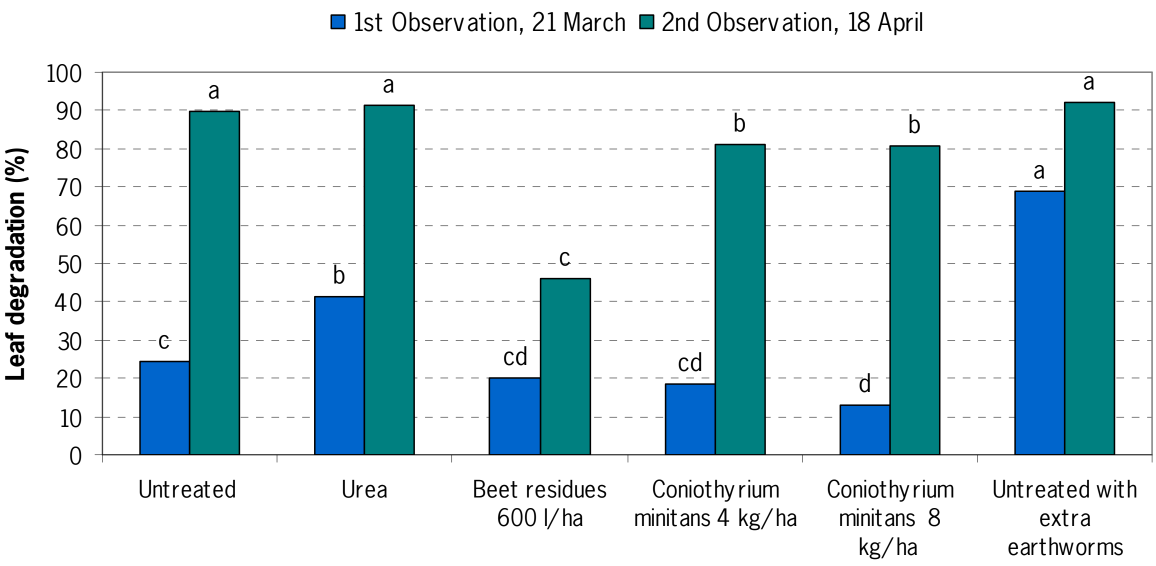


Figure 24. Leaf degradation in the dipping experiment on 21 March and 18 April 2005.

Significant differences were found in the ascospore production after dipping the leaves in different treatments (Figure 25). Urea showed a significantly lower ascospore production per 100 leaves and per gramme leaf. The treatment with beet residues (600 l ha^{-1}) also reduced ascospore production. In two other experiments beet residues reduced the number of ascospores in different concentrations and for some concentrations showed no significant difference from urea. In 2006, all concentrations of beet residues reduced the number of ascospores compared to untreated. The concentration of 200 and 400 litres per hectare tended to give the highest reduction of ascospores. In one experiment the amount of ascospores was reduced by 92% (per 100 leaves) when potassium phosphonate was dipped together with amino acids (data not shown).

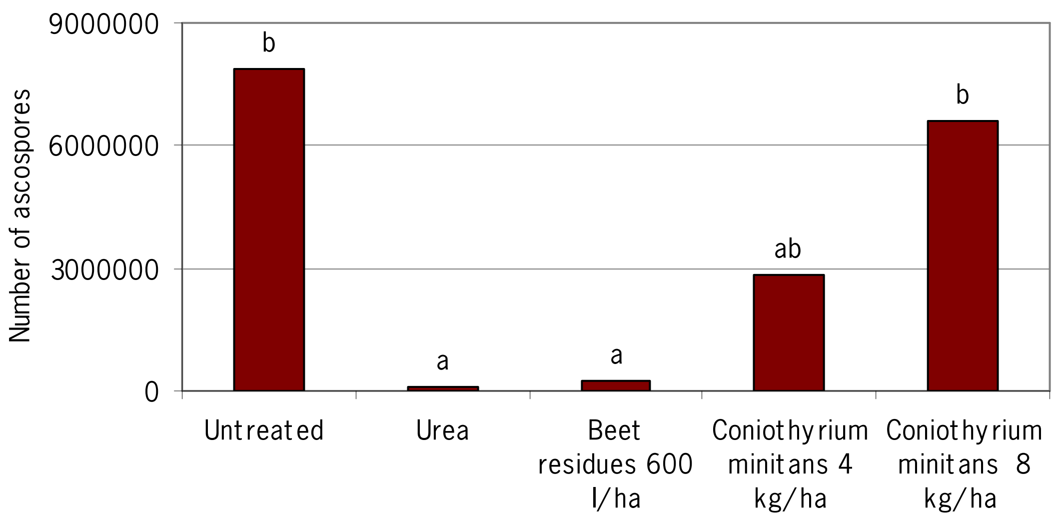


Figure 25. Ascospore production of 100 leaves per treatment in the dipping experiment.

It was concluded that in most experiments urea gave a significant stimulation of leaf degradation. Significant reductions of ascospores by urea were found as well. Compost tea and calcium hydroxide showed no stimulation of leaf degradation and gave no reduction of ascospores. Only a dipping treatment combined with potassium phosphonate significantly increased leaf degradation and reduced the amount of ascospores compared with untreated by 92% and was not significantly

different from the treatment with urea. No differences were found in leaf degradation and reduction of ascospores of amino acids from plants or animals.
The *C. minitans* treatments showed a significantly lower decomposition compared with untreated. *C. minitans* had no significant effect on the ascospore production compared with untreated in both years. A higher concentration of 8 kg/ha neither resulted in a lower amount of ascospores.
The dipped treatment of beet residues of 600 litre per hectare showed a reduction in number of ascospores and leaf degradation in 2005. In 2006 and 2007 beet residues stimulated leaf degradation and decreased the number of ascospores. A series of concentrations was tested in 2005-2006. The optimum concentration of beet residues found in this experiment was 400 litres per hectare. No stimulation of leaf degradation was found after applying another type of beet residue (Melasse). Melasse showed no effect on ascospore production.

Role of micro-organisms in overwintering apple leaves
The effects of cultural practises on micro-organisms naturally present on or in green and senescing apple leaves and their interaction with *V. inaequalis* were evaluated by PRI. Leaf samples were obtained from the field experiments described above. DNA was isolated from several hundreds of leaf samples. PCR-DGGE analysis was performed on the bacterial and fungal communities of samples collected in autumn. From corresponding sub-samples of leaves, ascospore production of *V. inaequalis* was assessed in spring. After real-time PCR analysis (TaqMan-PCR) became available during REPCO, also the amount of DNA of *V. inaequalis* was quantified in leaf samples collected in spring. Treatments, especially with urea, had a strong effect of presence or absence of certain bands. Correlations were found between certain band positions present in DGGE gels with reduced amounts of DNA or ascospores of *V. inaequalis* present in leaves in spring. The presence of several bands representing certain bacterial or fungal populations was correlated with low amounts of DNA or ascospores of *V. inaequalis* in leaves. Sequencing of bands from fingerprints gave first insights in fungal species that possibly counteract the development of *V. inaequalis* in apple leaves.

DGGE fingerprinting
Apple leaves fixed in nettings were treated with different products such as urea (reference), Vinasse, Melasse and amino acids in autumn (see above). Treated leaves were subsequently placed on the orchard floor in the organically managed orchard of PPO at Randwijk, the Netherlands. Decomposition and ascospore formation in spring was determined. Sub-samples of treated leaves for DGGE fingerprinting were collected in autumn. Sub-samples of treated leaves for TaqMan-PCR were collected end of winter. DNA was isolated from leaf samples and PCR amplification of fungal ITS sequences and of bacterial 16S rDNA was performed. PCR products were analysed by DGGE. After electrophoresis, the gels were stained and photographed. Banding pattern analysis and comparison of gels was processed by GelcomparII software. Experimental data were included in this database containing band positions and relative band intensities. Bands from DGGE patterns were excised, sequenced and the obtained sequences were blasted against all sequences available in the NCBI genome sequence database. The most closely related species was identified.
For quantification of *V. inaequalis* in leaf samples, specific primers and a probe were designed and used for amplification and detection in real-time using an ABI Prism 7500 Sequence Detector System. Calibration curves for the quantification of *V. inaequalis* were prepared with a dilution series of genomic DNA. A fixed and known amount of internal extraterrestrial DNA control was used to exclude DNA extracts showing PCR inhibition.
DNA samples extracted from leaves representing different replicated treatments were analysed by DGGE after PCR amplification of fungal ITS sequences or direct or nested PCR amplification of bacterial 16S rDNA genes. The total number of different bands detected was approximately 25 for fungi, 34 for bacteria after direct PCR amplification before DGGE analysis and 37 for bacteria after nested PCR amplification before DGGE analysis. An example is shown in Figure 26. From the

various leaf treatments assessed, only the reference treatment with urea caused significant shifts within both bacterial and fungal communities.

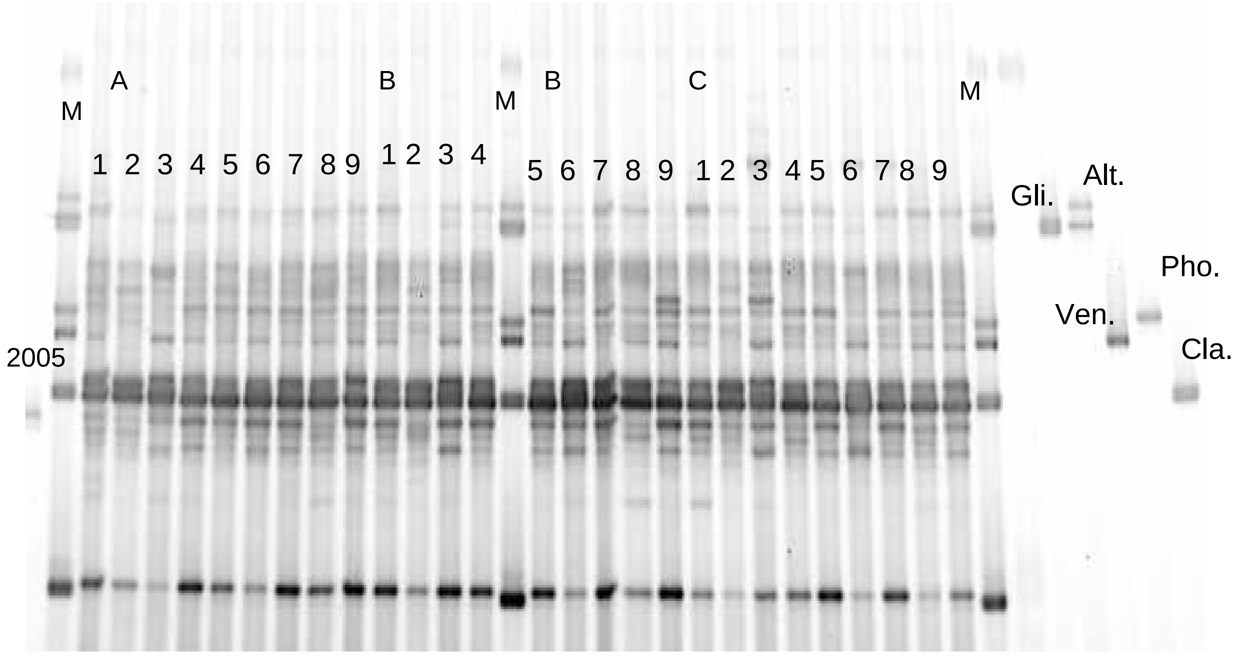


Figure 26. Fungi ITSDGGE of leaf samples from blocks A-C, treatments 1-9, field experiment 2007. M = marker; No 1-9 = different treatments; ABCDEF = blocks; Alt = Alternaria alternata; Ven = Venturia inaequalis; Pho = Phoma fimeti; Cla = Cladosporium cladosporioides; *2005 = interesting bands from field experiments Randwijk 2003/2004 and 2004/2005* Mycosphaerella punctiformis / Ramularia *sp.*
Note the different band patterns representing fungal communities for leaves treated with urea coded A2, B2 and C2.

For each band position, absence or presence in the individual samples was determined using GelcomparII. The concentration of *V. inaequalis* DNA measured by TaqMan-PCR and the number of ascospores produced in the leaf residues of the individual samples with presence or absence of a certain band were statistically analysed using t-tests ($\alpha = 0.05$). Bands present in all samples or in less than three samples were not analysed.
In 2005/2006, two band positions, coded F31_8 and F59_4, were identified which correlated with significantly lower amounts of ascospores produced mg^{-1} of leaf residue in samples with presence of the respective band. For the experiment 2006/2007, the number of such band positions was 1 for the concentration of *V. inaequalis*-DNA and 4 for the amount of ascospores produced on leaf residues (Fcar41_9, Fcar67_3, Fcar71_7, and Fcar96_6). In both experiments urea had been applied as reference treatment. In experiment 2005/2006 the fungicide difenoconazole had been applied as additional reference treatment. Both compounds have a known direct effect on *V. inaequalis* and possibly also have a direct effect on other microbial populations. Since both treatments may interfere directly with microbial populations, data analysis was also conducted without such treatments. Results obtained for bands F31_8 and F59_4 (experiment 2005/2006) and Fcar41_9, Fcar67_4, Fcar71_7, and Fcar96_6 (experiment 2006/2007) were similar when treatments with urea or difenoconazole were excluded from the analysis. In the presence of the identified bands, the amounts of ascospores produced g^{-1} leaf residue was 66% (F31_8), 86% (F59_4), 63% (Fcar41_9), 70% (Fcar67_4), 63% (Fcar71_7), and 90% (Fcar96_6) lower compared to samples that did not show the respective band. Examples are shown in Figures 27 and 28.
In most cases, the presence of band positions did not correlate with enhanced leaf decomposition. Only the presence of band position Fcar31_8 (experiment 2005/2006) was correlated with a 50%

reduced leaf weight after overwintering compared to samples without this particular band. Data on the production of ascospores totally produced on the leaf residues of 80 leaves gave the same results as the analysis of ascospore production g^{-1} of leaf residues.

With a similar analysis for DGGE bands representing bacterial communities after direct or nested PCR bands were identified that correlated with significantly lower concentrations of *V. inaequalis*-DNA (TaqMan-PCR) or with significantly lower amounts of ascospores produced mg^{-1} of leaf residue in samples with presence of the respective bands.

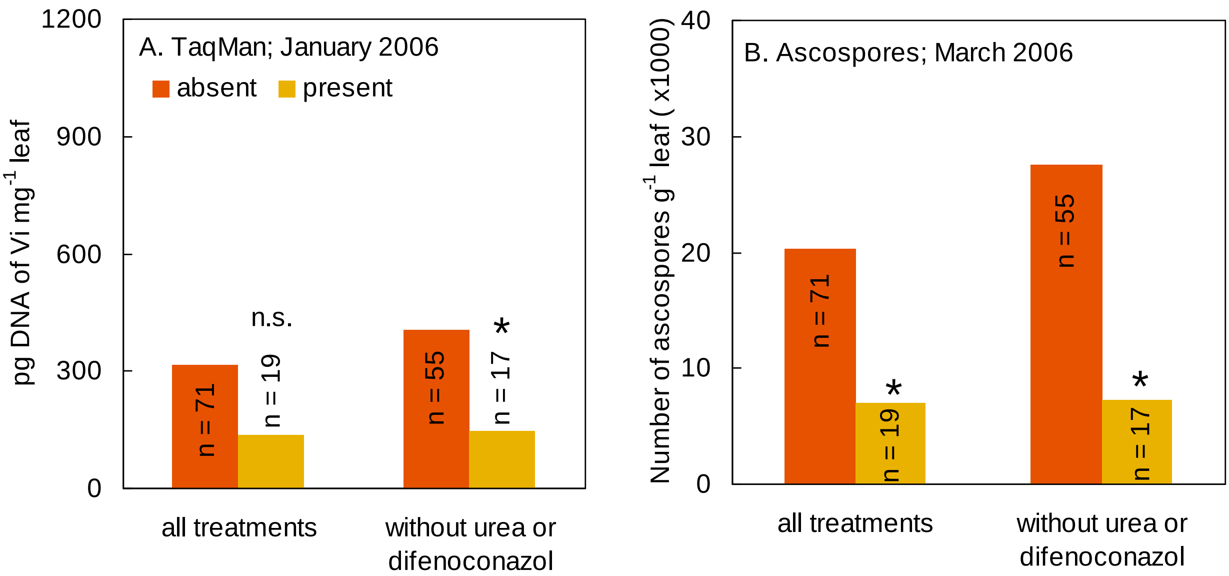


Figure 27. Concentration of V. inaequalis *DNA* mg^{-1} *leaf and amount of ascospores in leaf residues in samples with presence or absence of band position F31_8 on DGGE for fungal community fingerprinting. Leaves for DGGE fingerprinting were sampled on 10 November 2005.*

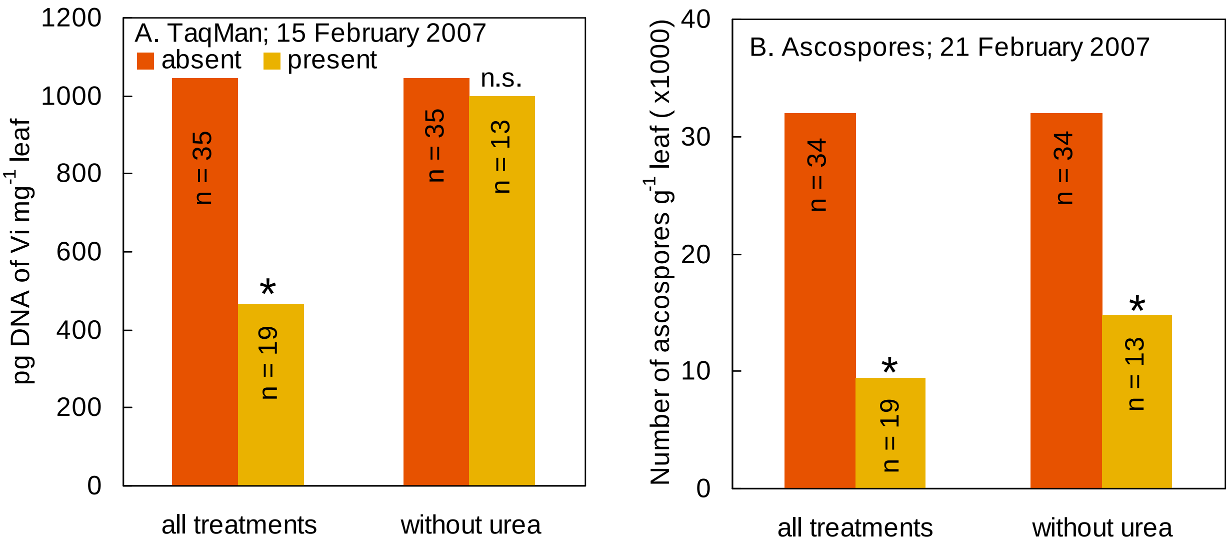


Figure 28. Concentration of V. inaequalis *DNA* mg^{-1} *leaf and amount of ascospores in leaf residues in samples with presence or absence of band position Fcar67_3 on DGGE for fungal community fingerprinting. Leaves for DGGE fingerprinting were sampled on 4 January 2007.*

Sequences of interesting bands have been obtained and analysed. Final validation steps are ongoing. DGGE in combination with sequence analysis of obtained bands was applied in REPCO to identify new fungal and bacterial antagonists against a plant pathogen. The obtained results show that it is possible to relate band patterns to environmental data on pathogen development and to identify relevant bands. This technology can be used in the future in screening programme to identify antagonistic organisms within complex microbial populations as a novel screening tool.

Stimulating earthworm activity
Some earthworm species specifically feed on fallen leaves. Examples are *Lumbricus terrestris Lumbricus rubellus* and *Aporrectodea longa.* It is assumed that promoting these earthworm populations might contribute to enhanced leaf degradation and thereby reduce ascospore inoculum of *V. inaequalis* in early spring. Therefore, in this part of REPCO experiments were carried out to stimulate earthworm populations with the aim to increase leaf degradation and to reduce scab inoculum during winter.
A field experiment was initiated in autumn 2003 and continued until winter 2006-2007. Plots were treated with different organic materials to increase earthworm populations and their ability to decompose apple leaves. Treatments were applied on the same plots during three consecutive years. The treatments were chicken manure as a standard treatment, mulch, spent mushroom substrate and cattle manure. Quantities of organic material were adjusted to standard annual nitrogen amounts under commercial organic apple production conditions. Earthworm numbers and their weight were determined in sub-plots, and leaf degradation was measured. In addition, a combination experiment was done where treated leaves were placed in the fertilization treatments during the winter 2005-2006. The experiment was located at an organically maintained apple orchard of 6 ha. Trees were pruned to modern spindle shape. The experiment took place on cultivar Jonagold.
Subplots were established in the orchard for fertilization treatments. They were maintained from autumn 2003 till spring 2007. Each plot consisted of one row of fruit trees with a length of 8 trees (Figure 29). Five earthworm sampling plots were established within the fertilization plots. Each earthworm sampling plot was used only once during the total experimental period. Hence, five earthworm samples were taken, i.e. in October 2003, April 2004, October 2004, November 2005 and November 2006.

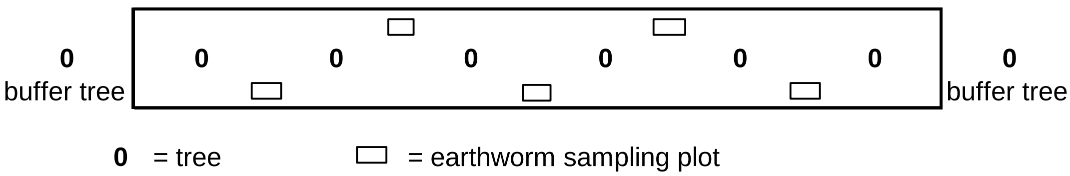


Figure 29. Lay-out of the plots.

Number and fresh weight of earthworms and leaf decomposition were recorded. The procedure for measuring number and biomass of earthworms was a combination of using the repellent allyl isothiocyanate (AITC), a component of mustard oil, with hand sorting.

Efficacy of treatments on earthworms
The earthworm samples taken in October 2003 were used to determine which species of earthworms were present in the orchard. A limited number of species were found in larger quantities. These were *Lumbricus rubellus*, *Allobophora chlorotica*, *Eisenia fetida*, *Apporectodea caligenosa* and *Aporrectodea longa*. From these *L. rubellus* and *A. longa* are leaf-eating species. Small numbers of other species were found, i.e. *Lumbricus terrestris*, *Lumbricus castaneus*, *Apporectodea trapezoides*, *Octolasion cyaneum* and *Octolasion tyrtaeum*. It was noted that burrows

of *L. terrestris* were found in relatively high numbers, but limited numbers of earthworms were caught with the combined allyl isothiocyanate sampling method and hand sorting.
The measurement of April 2004 was meant to establish a picture of the numbers and fresh weight of the most important earthworms before fertilization treatments started. It was found that *L. rubellus* was by far the most abundant leaf-eating earthworm species in the orchard samples. Further research focused on *L. rubellus.* The category of "others adults" mainly consisted of *Apporrectodea caliginosa*. A high variation in both numbers and weight of *L. rubellus* over different plots was noted.
In 2005 numbers and weight of *L. rubellus* adults were increased by the cattle manure treatment. Although there was a similar tendency for *L. rubellus* juveniles, this was only significant for fresh weight. Adults from other earthworm species increased in number and weight in the mushroom substrate and cattle manure treatments.
No increase in numbers and weight of *L. rubellus* adults and juveniles was found. Numbers, and especially weight, of *L. rubellus* adults and juveniles were lower than in 2004. Adults from other earthworm species increased in number and weight in the cattle manure treatment.
In 2006, numbers and weight of *L. rubellus* adults were increased by the mushroom substrate treatment. Numbers and weight of adult other earthworm species were increased as well, not only in the mushroom substrate treatments, but also in the cattle manure and mulch treatments and similar tendencies were found in juveniles of other earthworm species.
Earthworm numbers and weight found in 2006 are the result of tree years consecutive treatments. Differences were found between years.
Both, numbers and weight of *L. rubellus* adults and juveniles and also other earthworm species were found to be significantly lower in 2005 than in 2004 and 2006. Both, numbers and weight of *L. rubellus* adults and adults of other earthworm species increased in the mushroom substrate and cattle manure treatments when all years were analysed together. No significant effects were seen for the juveniles.

Efficacy of treatments on leaf degradation
L. terrestris is the largest earthworm in our experimental orchards which mainly feeds on fallen leaves. Only low numbers of *L. terrestris* were caught with the combined iso allylthiocyanate sampling method and hand sorting, while many burrows were found that were associated with the presence of this earthworm. Because of the low numbers collected of *L. terrestris* we were not able to detect an effect of fertilization treatments on numbers of weight of this major leaf-eating species.
Numbers and biomass of earthworms was considerably lower in 2005 than in the other years. This was most probably due to early frost in autumn and postponing further sampling till after the cold spell. It is known that optimum temperature lies between 10 and 20°C for most earthworm species and below 0°C they do generally not survive. At that the time of sampling, from 29 November till 15 December, earthworms probably retracted deeper into the soil and were less efficiently caught with the method used.
Adults of *L. rubellus* nearly doubled in numbers and biomass when soil was fertilized with cattle manure compared to the standard fertilization with chicken manure. In most situations food is limiting growth and size of the earthworm populations as demonstrated by the general increase in population in response to amendments. Also, the quality of organic amendments is as important as quantity. This was also demonstrated for apple orchards. Nonetheless, no effect was measured on leaf degradation as a result of the fertilization treatments. This was surprising, especially because a clear stimulation of microflora on fallen leaves as a consequence of urea spraying and an increased leaf consumption have been reported. Doubling the numbers of earthworms may not be enough to reach a measurable increase of leaf degradation. Leaves dipped in 30 % or 60 % Vinasse solutions promoted leaf degradation in the combination experiment, independent of the fertilization treatment.

The following conclusions can be drawn. Mushroom substrate and cattle manure stimulated population development of *L. rubellus* and other earthworm species. The population increase of the leaf-eating earthworm *L. rubellus* was not enough to increase leaf degradation. No effect of the fertilization treatments through earthworms was demonstrated on reduction of *Venturia inaequalis* inoculum during winter. Vinasse treatments of leaves can contribute to increased leaf degradation.

2. Dissemination and use of knowledge

Major dissemination activities of REPCO

Results of the dissemination and exploitation activities of the scientific achievements in REPCO:

- Information on results of screening experiments with potentiators of resistance and fungicides for control of downy mildew of grapevine provided to or available for 60 industrial companies and 10 scientific institutes.
- Information on results of screening experiments with potentiators of resistance and fungicides for control of apple scab provided to or available for 30 industrial companies and 2 scientific institutes.
- Information on results of 14 field experiments with 40 potentiators of resistance and fungicides for control of downy mildew of grapevine provided to or available for industrial companies and scientific institutes.
- Information on results of 12 field experiments with 40 potentiators of resistance and fungicides for control of apple scab provided to or available for industrial companies and scientific institutes.
- 6 dissemination trials for apple growers in the Netherlands and Denmark.
- 45 presentations of REPCO results at international scientific conferences.
- Various presentations of REPCO activities and results to growers, advisors and the general public during meetings and open days.
- Close collaboration with potential producers of biocontrol agents within REPCO.
- Application of one patent on the use of potentiators of resistance and fungicides for control of downy mildew in grapevine.
- Application of five utility models and five patents on the use of potentiators of resistance and fungicides for control of apple scab.
- Information on REPCO objectives, achievements and publications available on www.rep-co.nl

After the project period, further scientific publications, conference contributions and two patent applications are foreseen.

Exploitable knowledge and its use

REPCO produced exploitable knowledge on the use of several potentiators of resistance or organically based fungicides in organic production of grapevine or apple. Such novel products showed promising activity against downy mildew of grapevine (*Plasmopara viticola*) and/or apple scab (*Venturia inaequalis*) and may have a potential for the control of other plant diseases. An antagonistic isolate for effective control of apple scab has been found as well. In part, exploitable knowledge has been or will be protected by patent applications to facilitate the commercial development of plant protection products by industries. General information on REPCO patent applications is given in Table 4.

REPCO evaluated a substantial number of formulated or non-formulated products obtained by various industries or scientific institutes (Table 5). REPCO partners have agreements with such industries or scientific institutes and knowledge on test products has, on the basis of agreements, been exchanged directly between REPCO partners and the respective industries or scientific institutes so that this knowledge can be exploited. In other cases, knowledge is available for industries on request and/or will be published and thus be available for exploitation by industries.

It is planned to publish results from the whole REPCO screening programme. These publications will include details on all materials tested, i.e. both materials that were effective and those that were not. Publication of negative results is of importance to all who are planning similar research in the future, as it will guide them as to which materials to select and which to avoid.

Table 4. General information on patent applications by REPCO partners.

Exploitable knowledge	Exploitable product(s) or measures	Sector(s) of application	Time-table for commercial use	Patents or other IPR protection	Owners & other partner(s) involved	Plans for exploitation
A new material for control of scab in organic apple growing	Formulated product based on extract (s) from *Yucca* spp.	Plant protection industry, especially organic apple growing	~ 5-10 years for development	Patent application submitted: "New composition for control of foliar diseases"	PPO (lead owner), UoA, LIFE	Contacts established with several industries
A new material for control of scab in organic apple growing	Formulated products based on extract (s) from *Camellia* spp. *Trigonella foenum-graceum Rosmarinus officinalis*, *Thymus* spp., *Origanum* spp., *Salvia* spp. and/or *Mentha* spp.	Plant protection industry, especially organic apple growing	~ 5-10 years for development	Patent application submitted: "A natural product having a fungus inhibiting effect on specific fungal pathogens and a growth promoting effect for improving plant production"	LIFE & Nor-Natur ApS (lead owner)	Under negotiation
A new material for control of scab in organic apple growing	Formulated products based on extracts of *Quillaja saponaria*, *Chenopodium quinoa*, *Camellia* spp., and a saponin-containing plant material	Plant protection industry, especially organic apple growing	~ 5-10 years for development	Patent application submitted: "A natural product containing fenugreek, tea and/or rosemary having fungus inhibiting and growth promoting effect for improving plant production and its	LIFE & Nor-Natur ApS (lead owner)	Under negotiation

Exploitable knowledge	Exploitable product(s) or measures	Sector(s) of application	Time-table for commercial use	Patents or other IPR protection	Owners & other partner(s) involved	Plans for exploitation
				use”		
A new material for control of downy mildew in grapevine growing	Formulated products based on alkylphospho-lipid and lyso-phospholipid	Plant protection industry, especially organic grapevine growing	~ 5-10 years for development	Patent application submitted: Alkylphospho-lipid and lyso-phospholipid for plant protection	WBI and Klinik für Tumorbiologie, Freiburg, Germany	Contacts established with several industries
Fungicidal activity of a plant extract, extraction procedure	Organic fungicide based on plant extract	Plant protection industry, especially organic agriculture	2010-2012	Patent application considered: Fungicidal activity of a plant extract; extraction procedure	FiBL	
A new material for control of scab in organic apple growing	Product based on spores of an antagonistic fungal isolate	Plant protection industry, especially organic apple growing	~ 5-10 years for development	Patent application considered: Use of fungal isolate for scab control in apple	PRI	Contacts established with industry

Table 5. List of knowledge exploitable by industries or in other research activities.

Industry / Institute	**Materials**	**Information**	**Knowledge owner**
ChiPro GmbH Bremen D	5 Chitosanes	Efficacy against Pv in vitro or on seedlings	WBI
DEGUSSA GmbH, Düsseldorf, D	7 Adhesives and detergents	Efficacy against Pv in vitro or on seedlings	WBI
Madora GmbH, Lörrach, D	1 Plant extract	Efficacy against Pv in vitro or on seedlings	WBI
Novosil, Novosibirsk RUS	1 Extracts from spruce leaves	Efficacy against Pv in vitro or on seedlings	WBI
Oro Agri Int. Ltd. Hoppe, CH	1 Extract from orange skins	Efficacy against Pv in vitro or on seedlings	WBI
Culture Collection of Algae and Protozoa, Argyll UK	3 Algae	Efficacy against Pv in vitro or on seedlings	WBI
Elicityl, SA Crolles, F	5 Elicitors	Efficacy against Pv in vitro or on seedlings	WBI
AkzoNobel, Deventer, NL	6 Peroxides	Efficacy against Pv in vitro or on seedlings	WBI
CEVA Centre d'Etude et de Valorisation des Algues, F	3 Algae extracts	Efficacy against Pv in vitro or on seedlings	WBI
Nor-Natur ApS, DK	8 Plant extracts	Efficacy against Pv in vitro or on seedlings	WBI
Syngenta Crop Protection AG, Basel, CH	2 Bion and DCINA	Efficacy against Pv in vitro or on seedlings	WBI
Spiess-Urania Chemicals GmbH, Hamburg, D	1 Sticker	Efficacy against Pv in vitro or on seedlings	WBI
Lebosol Dünger GmbH, Deideshem, D	2 Phosphonate and aminoacids	Efficacy against Pv in vitro or on seedlings	WBI
Wacker Chemie , Burghausen, D	1 Polysaccharide	Efficacy against Pv in vitro or on seedlings	WBI
Proagro GmbH, Abenberg, D	1 Adhesive	Efficacy against Pv in vitro or on seedlings	WBI
Univ. of Haifa, Israel	1 BABA	Efficacy against Pv in vitro or on seedlings	WBI
Univ. of Freiburg	3 Bacteria and plant extracts	Efficacy against Pv in vitro or on seedlings	WBI
Klinik für Tumorbiologie Freiburg, D	20 Phospholipids and 2 Liposomes	Efficacy against Pv in vitro or on seedlings	WBI
Univ. of Göttingen, D	3 Algae	Efficacy against Pv in vitro or on seedlings	WBI
Univ . of Plodviv, Bulgaria	3 Plant extracts	Efficacy against Pv in vitro or on seedlings	WBI
Nor-Natur ApS, DK	6 test products (plant extracts)	Efficacy against Pv on seedlings and in field experiments	FiBL
Biomor Israel Ltd, Ramat Gan 52521, Israel	2 test products	Efficacy against Pv on seedlings and in field experiments	FiBL

Industry / Institute	**Materials**	**Information**	**Knowledge owner**
BIOCURA Productions Co. Ltd, Bangkok, Thailand	3 test products (plant extracts)	Efficacy against Pv on seedlings	FiBL
Oro Agri Int. Ltd. Hoppe, CH	1 Extract from orange skins	Efficacy against Pv on seedlings	FiBL
Novosil, Novosibirsk RUS	1 Extract from pine tree	Efficacy against Pv on seedlings and in field experiments	FiBL
Bio-Biz Company, Moscow, Russia	2 test products	Efficacy against Pv on seedlings and in field experiments	FiBL
Unipoint AG, Langenmoos 9, CH-8475 Ossingen	1 product (Klinoptilolith, Zeolith)	Efficacy against Pv on seedlings	FiBL
Swiss Food Tech Management AG, Switzerland	1 treatment technique (PhytO3)	Efficacy against Pv on seedlings and in field experiments	FiBL
Protexx	1 test product	Efficacy against Pv on seedlings	FiBL
Gen-Rame	1 test product	Efficacy against Pv on seedlings	FiBL
Tulum Consulting, Switzerland	Moringa leaf powder	Efficacy against Pv on seedlings	FiBL
HKI 07745 Jena, Germany	1 experimental substance	Efficacy against Pv on seedlings	FiBL
Manda Fermentation Co., Ltd. Japan	1 test product	Efficacy against Pv on seedlings	FiBL
Grünkraft	1 test product	Efficacy against Pv on seedlings	FiBL
ChiPro GmbH Bremen D	1 test product	Efficacy against Pv on seedlings and in field experiments	FiBL
Stähler Suisse SA, Zofingen, Switzerland	1 test product: Agricure	Efficacy against Pv on seedlings and in field experiments	FiBL
Prof. Y. Cohen, Ramat Gan, Israel	1 series of test products	Efficacy against Pv on seedlings and in field experiments	FiBL
Global Biobased Asia, Hong Kong	1 test product	Efficacy against Pv on seedlings	FiBL
Procédés Roland Pigeon SA, Avenches Switzerland	1 test product (PRP Blauwasser)	Efficacy against Pv on seedlings	FiBL
IASMA, San Michele Italy	1 test product: Tecnobiol	Efficacy against Pv on seedlings and in field experiments	FiBL
Koppert Biological Systems, NL	1 test product	Efficacy against Pv on seedlings and in field experiments	FiBL
Hédel Sàrl, 1700 Fribourg, Switzerland	3 test products	Efficacy against Pv on seedlings	FiBL
AJE GmbH, 8832 Wollerau, Switzerland	1 test product (‘Targanic’ fulvic acids)	Efficacy against Pv on seedlings	FiBL
Andermatt Biocontrol AG, 6146 Grossdietwil, Switzerland	4 test products and 6 batches of Mycosin	Efficacy against Pv on seedlings	FiBL
Koppert Biological Systems (NL)	1 test product	Efficacy against Pv on seedlings and in field experiments	FiBL

Industry / Institute	**Materials**	**Information**	**Knowledge owner**
Omya Agro (CH)	1 test product	Efficacy against Pv on seedlings and in field experiments	FiBL
BBA (D)	2 test products	Efficacy against Pv on seedlings	FiBL
Trifolio-M GmbH; 35633 Lahnau; Germany	3 test products	Efficacy against Pv on seedlings and in field experiments	FiBL
Bernard Paul; Institut Jules Guyot ; Université de Bourgogne 21078 Dijon, France	1 test product	Efficacy against Pv on seedlings and in field experiments	FiBL
Melaxa s.r.l (I)	2 test products	Efficacy against Pv in crops	IASMA
IntachemBio (I)	4 test products	Efficacy against Pv in crops	IASMA
Sicit 2000 s.p.a. (I)	5 test products	Efficacy against Pv in crops	IASMA
ELEP s.p.a. (I)	1 test product	Efficacy against Pv in crops	IASMA
Vend (UA)	1 test product	Efficacy against Pv in crops	IASMA
Ofakim (IL)	1 test product	Efficacy against Pv in crops	IASMA
Deruned (NL)	1 test product	Efficacy against Pv in crops	IASMA
Prophyta GmbH (D)	2 test products	Efficacy against Pv in crops	IASMA
Biomor (IL)	2 test products	Efficacy against Pv in crops	IASMA
Ghielmini e associati (CH)	1 test product	Efficacy against Pv in crops	IASMA
Nor-Natur Aps (DK)	2 test products	Efficacy against Pv in crops	IASMA
Agrimport s.p.a. (I)	1 test product	Efficacy against Pv in crops	IASMA
ChiPro GmbH (D)	1 test product	Efficacy against Pv in crops	IASMA
Andermatt Biocontrol (CH)	3 test products	Efficacy against Pv in crops	IASMA
Syngenta (CH)	1 test product	Efficacy against Pv in crops	IASMA
Koppert (NL)	1 test product	Efficacy against Pv in crops	IASMA
Tecnotrea (I)	1 test product	Efficacy against Pv in crops	IASMA
Jeneil Biosurfactant Company (US)	1 test product	Efficacy against Pv in crops	IASMA
Montanwerke brixlegg (A)	1 test product	Efficacy against Pv in crops	IASMA
Newagri s.r.l. (I)	1 test product	Efficacy against Pv in crops	IASMA
Valagro (I)	1 test product	Efficacy against Pv in crops	IASMA
Agricenter (I)	1 test product	Efficacy against Pv in crops	IASMA
Biofert s.p.a (I)	2 test products	Efficacy against Pv in crops	IASMA
Eden Bioscience (US)	1 test product	Efficacy against Pv in crops	IASMA
Ecosfera (CH)	3 test products	Efficacy against Pv in crops	IASMA
Shinetsu (US)	1 test product	Efficacy against Pv in crops	IASMA
Omya Agro (CH)	1 test product	Efficacy against Pv in crops	IASMA
Sigma-Aldrich (US)	1 test product	Efficacy against Pv in crops	IASMA
Safecrop centre (I)	18 test products	Efficacy against Pv in crops	IASMA
BBA (D)	4 test products	Efficacy against Pv in crops	IASMA
GRAB (F)	1 test product	Efficacy against Pv in crops	GRAB
Biomor (IL)	2 test products	Efficacy against Pv in crops	GRAB
ChiPro GmbH (D)	1 test product	Efficacy against Pv in crops	GRAB
Deruned (NL)	1 test product	Efficacy against Pv in crops	GRAB
Prof. Y. Cohen, Ramat Gan, Israel	1 test product	Efficacy against Pv in crops	GRAB

Industry / Institute	Materials	Information	Knowledge owner
Inobio (F)	1 test product	Efficacy against Pv in crops	GRAB
UFAB (F)	1 test product	Efficacy against Pv in crops	GRAB
Helena Chemical Company (US)	1 test product	Efficacy against Pv in crops	GRAB
Maktehteshim Chemical works (IL)	1 test product	Efficacy against Pv in crops	GRAB
Agrimport s.p.a.(I)	1 test product	Efficacy against Pv in crops	GRAB
Omya Agro (CH)	1 test product	Efficacy against Pv in crops	GRAB
Nor-Natur Aps	~5 formulated, ~50 non-formulated test products	Efficacy against Vi on seedlings	LIFE (Joint)
Natur-Drogeriet A/S	~50 non-formulated test product	Efficacy against Vi on seedlings	LIFE
Duxon Aps	4 formulated test product	Efficacy against Vi on seedlings	LIFE (Joint)
Koppert Biological Systems	1 formulated test product	Efficacy against Vi on seedlings	LIFE (Joint)
Biomor Israel Ltd	1 formulated test product	Efficacy against Vi on seedlings	LIFE (Joint)
UoA (Bayer, Karlhamns Binol)	~3 formulated test products	Efficacy against Vi on seedlings	LIFE
Prophyta	9 formulated, 1 non-formulated test products	Efficacy against Vi on seedlings	LIFE (Joint)
PPO (DeruNed, Biofa and others)	~5 formulated, 2 non-formulated test products	Efficacy against Vi on seedlings	LIFE (Joint)
Codena, CA	1 formulated test product	Efficacy against Vi on seedlings	LIFE (Joint)
Citrus Europe, N	1 formulated test product	Efficacy against Vi on seedlings	LIFE (Joint)
Cintamani, PL	1 formulated test product	Efficacy against Vi on seedlings	LIFE
Biosa Denmark, DK	1 formulated test product	Efficacy against Vi on seedlings	LIFE (Joint)
A. Nikolov, Bulgaria	3 formulated test products	Efficacy against Vi on seedlings	LIFE (Joint)
UoA, DK	2 formulated products	Efficacy against Vi in crops	UoA
Jean Pierre Laffranque, F	1 formulated product	Efficacy against Vi in crops	UoA
PPO, NL	1 formulated product	Efficacy against Vi in crops	UoA
Bestebreurtje BV, NL	1 formulated product	Efficacy against Vi in crops	UoA
Natur-Drogeriet, DK	17 non-formulated products	Efficacy against Vi in crops	UoA

Industry / Institute	**Materials**	**Information**	**Knowledge owner**
Nor-Natur, Dk	10 formulated products	Efficacy against Vi in crops	UoA
Biodexa, F	1 formulated product	Efficacy against Vi in crops	UoA
Chipro Company, G	1 formulated product	Efficacy against Vi in crops	UoA
Deruned (NL)	1 test product	Efficacy against Vi in crops	PPO
Helena Chemical Company (USA)	1 test product	Efficacy against Vi in crops	PPO
Paques BV (NL)	1 test product	Efficacy against Vi in crops	PPO
Goëmar (F)	1 test product	Efficacy against Vi in crops	PPO
Koppert B.V. (NL)	1 test product	Efficacy against Vi in crops	PPO
Asepta BV (NL)	standard test product copper hydroxide	Efficacy against Vi in crops	PPO
Syngenta Crop Protection (NL)	standard test product Thiovit Jet	Efficacy against Vi in crops	PPO
ChiPro GmbH (D)	1 test product	Efficacy against Vi in crops	PPO
Xeda international (F)	1 test product	Efficacy against Vi in crops	PPO
Nor-natur ApS (DK)	several test products	Efficacy against Vi in crops	PPO
Natur-drogeriet A/S (DK)	several test products	Efficacy against Vi in crops	PPO
Agro-Centrum BV	1 test product	Efficacy against Vi in crops	PPO
Carmeuse (NL)	1 test product	Efficacy against Vi in crops	PPO
Deruned (NL)	1 test product	Efficacy against Vi in crops	PPO
Schuurmans en van Ginneken B.V.	Melasse and Vinasse	Promote leaf degradation	PPO
Vlamings, Mortel	Melasse and Vinasse	Promote leaf degradation	PPO
Agro-Centrum BV (NL)	Melasse and Vinasse	Promote leaf degradation	PPO
Lebosol Dünger (D)	3 test products	Promote leaf degradation	PPO
Hydro Agri Benelux (NL)	standard test product Urea	Promote leaf degradation	PPO
Prophyta (D)	biocontrol agent	Promote leaf degradation	PPO
Compara (NL)	1 test product	Promote leaf degradation	PPO

Dissemination of knowledge

An overview of the dissemination activities of REPCO partners during and after the project period is presented in Table 6. Detailed information on publications, oral conference contributions and poster conference contributions are listed below. Details of planned activities are listed as well.
Note: Publications have been produced wholly or in part through support by REPCO. Conference participation has been supported only in part by REPCO.

Table 6. Overview table: Dissemination activities of REPCO partners during and after the project period.

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audi-ence	Partner responsible /involved	Reference
Project Period 1						
12 Dec 2003	Newsletter	Growers	NL, B		BioFruitAdvies	Announcement of REPCO in EKO Bericht 44 (2003) (newsletter to organic fruit growers in the Netherlands and Belgium)
3-5 Jan 2004	Meeting	Advisors, Research	International		BioFruitAdvies	Discussion with 11th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit Growing, Weinsberg, Germany
16-18 Jan 2004	Conference	Research	International		PPO	11th Meeting on Apple Scab, Gleisdorf, Austria
02 Feb 2004	Press release	Growers	D		WBI, ECOVIN	Der Badische Winzer, Der Deutsche Weinbau, Weinmagazin
10 Feb 2004	Meeting	Growers/Research	NL	8	PPO/BioFruitAdvies/PRI	Discussion with Dutch working group Organic Fruit Growing
09 Mar 2004 11 Mar 2004	Meeting	Growers	NL, B		BioFruitAdvies	Discussion during two meetings with growers on crop protection in organic fruit production in NL and B
24-27 Mar 2004	Poster	Research	International	150	PRI/all	General Info REPCO Poster; Disease Biocontrol, Seville
Mar 2004	Publication	Research	International		PPO	The role of earthworms in strategies against scab (*Venturia inaequalis*) in apple orchards; Literature study
Apr 2004	Meeting	Growers/Research	NL		PPO/BioFruitAdvies	Discussion with Dutch working group Organic Fruit Growing
04-09 May 2004	Conference	Research	International		WBI	Poster on Resistance Inducer, IOBC Meeting Induced Resistance, Helsingör Denmark
12 May 2004	Congress	Grower, Extension service	D, AU, CH	250	WBI, ECOVIN	INTERVITIS INTERFRUCTA 2004 1. Internationales Symposium für

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audi-ence	Partner responsible /involved	Reference
						ökologischen Obst- und Weinbau
May 2004	Project web-site	General public	International		PRI/all	www.rep-co.nl
9-13 Jun 2004	Poster	Research	International	150	PRI/all	General Info REPCO Poster; IOBC, S. Michele
Jun 2004	Flyer	General public	NL		PRI	Biologisch onderzoeksbericht 11
Jun 2004	Publication	Research/Growers	DK		LIFE	DARCOFenews, June, No. 2. Control of apple scab by use of the plants own defence mechanisms (www.darcof.dk/enews/june04/scab.html)
Jun 2004	Publication	Research/Growers	DK		LIFE	FØJOnyt, juni, Nr. 3. Bekæmpelse af æbleskurv ved brug af plantens egne forsvarsmekanismer (www.foejo.dk/enyt2/enyt/juni04/skurv.html)
Jun 2004	Meeting	Growers	D		WBI, ECOVIN	Presentation of project to ECOVIN members
Jun 2004	Meeting	Growers	D		WBI	Presentation of project to growers
Jul 2005	Press release	General public	D		WBI	Project presentation as general info
05 Aug 2004	Exhibition / Demonstration	Growers	DK		UoA	Open house arrangement for organic fruit and vegetable growers at DARCOF, 5th August.
21-22 Aug 2004	Exhibition / Demonstration	General public/ Growers	CH		FiBL	Open door at FiBL
24 Aug 2004	Meeting	Growers	D		Ecovin	Discussion of Ecovin members on Plant Protection in organic grapevine
26 Aug 2004	Exhibition / Demonstration	Government	NL	3	PPO	Ministry of Agriculture
Aug 2004	Exhibition / Demonstration	Growers	DK		UoA	Open day for organic fruit growers at UoA, AArslev
15 Sep 2004	Field demonstration	Extension service	D	60	WBI	8. Freiburger Versuchsführung
22 Sep 2004	Conference	Research	D		WBI	Presentation of results on Induction of

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audi-ence	Partner responsible /involved	Reference
						resistance in grapevine by different Inducers, Deutsche Pflanzenschutztagung
Sep 2004	Exhibition / Demonstration	Higher education	DK		LIFE	Presentation of the project & demonstration of laboratory screening procedures for 2nd year students at LIFE
Sep 2004	Exhibition / Demonstration	General public/ Growers	DK		UoA	Open day arrangement for general public at UoA, AArslev
Sep 2004	Meeting	Growers/Research	NL		PPO/BioFruitA dvies	Discussion with Dutch working group Organic Fruit Growing
Sep 2004	Publication	Research/Growers	DK		LIFE & UoA	DARCOFenews, September, No. 3. New fungicides for apple scab control in organic growing. (www.darcof.dk/enews/sep04/scab.html)
07 Oct 2004	Meeting	Experts	D	25	WBI	Fachbeirat Nachhaltiger Pflanzenbau, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit
13 Oct 2004	Meeting	Experts	D	50	WBI	AK Pflanzenschutz beim Forschungsring des Deutschen Weinbaus Workshop Pflanzenstärkungsmittel
15 Oct 2004	Exhibition / Demonstration	General public	DK		LIFE	Information on REPCO distributed at the NORDIC Council of Ministers´ open day: “Store Nordiske Æbleday” on biodiversity & food security, Copenhagen
Oct 2004	Exhibition / Demonstration	Higher education	DK		LIFE	Project presented in lecture for students attending Biological Control course at LIFE
Oct 2004	Publication	Research/Growers	DK		LIFE / UoA	FØJOnyt, Nye midler til bekæmpelse af skurv I økologisk øbledyrkning.
Project Period 2						
01 Nov 2004	Newsletter	Growers	NL, B		BioFruitAdvies	Announcement of REPCO in EKO Bericht 44 (2003) (newsletter to organic fruit growers in the Netherlands and Belgium)
24 Nov 2004	Meeting	Extension service	D	70	WBI	Annual meeting of the extension service for

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audi-ence	Partner responsible /involved	Reference
						viticulture, Baden-Württemberg
25 Nov 2004	General Assembly	Grower	D	50	Ecovin WBI	Ecovin Baden Generalversammlung
Nov 2004	Course	Growers	DK	40	UoA	Talk at course for Organic apple growers
08-09 Dec 2004	Meeting	Growers	F	150	Grab	Presentation of RECPO in meeting of ITAB
Dec 2004	Meeting	Growers	D		WBI	Presentation of project to members of 'Badischer Weinbauverband'
12 Jan 2005	Meeting	Growers	F	10	Grab	National meeting on organic viticulture
21-22 Jan 2005	Conference	Research	International	20	LIFE	Presentation and discussion at the 12th meeting on apple scab, Sweden
26 Jan 2005	Meeting	Growers	NL		BioFruitAdvies	"Studiedag Biologische fruitteelt". The Dutch organic growers were informed on the effectiveness of potassium phosphate on apple scab.
10 Feb 2005	Meeting	Growers	international		BioFruitAdvies	International Meeting to discuss effectiveness, and possible negative impacts of the use of potassium phosphate in organic fruit growing. Organised by the Fördergemeinschaft Ökologischer Obstbau in Weinsberg.
24 Feb 2005	Exhibition / Demonstration	Growers	DK	10	LIFE	Presentation and demonstration of the project for the board of fruit and ornamental tree club.
04 Mar 2005	Course	Growers	CH	20	FiBL	Course for growers to improve organic vine production
09 Mar 2005	Meeting	Growers	CH	60	FiBL	Information day on plant protection news for farmers
01-02 Apr 2005	Conference	Growers, advisors	E	50	FiBL	Oral contribution
18-22 Apr 2005	Conference	Research	International		PRI	Oral contribution at 1st International Conference on Plant-Microbe Interactions – Endophytes and Biocontrol Agents, Saariselkä, Finland.
21 Apr 2005	Meeting	University students	DK	20	LIFE	Presentation of the project for 2nd year

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audi-ence	Partner responsible /involved	Reference
						students at LIFE
11 May 2005	Conference	Research	DK	25	LIFE & UoA	Presentation at meeting of the Danish Society of Plant Pathology
12-13 May 2005	Conference	Research and advisors	International	100	FiBL	Oral contribution and paper
30 May 2005	Exhibition / Demonstration	General public / High School students	DK	>100	LIFE	Demonstration of the project at “Svampefestivalen” at Botanical Garden, Copenhagen
May 2005	Conference	Research, advisors	D		WBI	Presentation of results at Annual Meeting of the “Forschungsring des Deutschen Weinbaus”
May-Sept 2005	Exhibition / Demonstration	Growers, general public, advisors, researcher	CH and International	5 - 50	FiBL	Presentations of FiBL-reseach and advisory facilities and activities
02 Jun 2005	Meeting	LIFE’s leadership	DK	3	LIFE / PPO	Presentation of the project for the Prorector for research in connection with patent enquiry
Jul 2005	Press release	General public	D		WBI	Project presentation as general info
31 Aug-04 Sep 2005	Conference	Research	International	40	PRI	Oral contribution at 7th International IOBC/wprs workshop on pome fruit diseases, Italy/September, 2005
31 Aug-04 Sep 2005	Conference	Research	International	40	LIFE	Contribution at 7th International IOBC/wprs workshop on pome fruit diseases, Italy/September, 2005
31 Aug-04 Sep 2005	Conference	Research	International	40	PPO	Oral contribution at 7th International IOBC/wprs workshop on pome fruit diseases, Italy/September, 2005
Aug 2005	Course/Open day	Growers	Dk	20	UoA	Talk and field day for organic apple growers at UoA
Aug 2005	Meeting	Scientists/growers	European	50	UoA	Field trip with visitors joining the annual Fruit prognosis meeting. Denmark was host in 2005.
Aug 2005	Meeting	Growers	Dk	30	UoA	Field day for Danish apple and pear growers

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audi-ence	Partner responsible /involved	Reference
14 Sep 2005	Field demonstration	Extension service	D	60	WBI	9. Freiburger Versuchsführung
21-23 Sep 2005	Flyer/Poster	General public	International		PRI	Distributed at ISOFAR Conference, Adelaide, Australia
22 Sep 2005	Meeting	University students	DK & SE	23	LIFE	Presentation of the project for bachelor students at LIFE
Sep 2005	Meeting	Growers	D		WBI	Presentation of first results to growers
Sep 2005	Meeting	Growers	D		WBI, ECOVIN	Presentation of first results to members of ECOVIN
03 Oct 2005	Exhibition / Demonstration	Lower secondary school students	DK	25	LIFE	Presentation of the project & demonstration of laboratory screening procedures
04 Oct 2005	Exhibition / Demonstration	University students	DK & SE	23	LIFE	Presentation of the project & demonstration of laboratory screening procedures for bachelor students at LIFE
20-21 Oct 2005	Conference	Research	International	100	ETHZ	Meeting of the IOBC/WPRS Working Group "Integrated Protection and Production in Viticulture", Boario Terme, Italy
20-21 Oct 2005	Conference	Research	International	100	Grab	Poster at meeting of the IOBC/WPRS Working Group "Integrated Protection and Production in Viticulture", Boario Terme, Italy
20-21 Oct 2005	Conference	Research	International	100	IASMA	Meeting of the IOBC/WPRS Working Group "Integrated Protection and Production in Viticulture", Boario Terme, Italy
21 Oct 2005	Poster	University students	DK & SE	23	LIFE	Student group present project result on apple scab including use of botanical fungicides
Project Period 3						
Nov 2005 – Oct 2006	Exhibition, Demonstration	Growers, General Public, Researcher	CH and International	2-40	FiBL	Several groups of visitors at FiBL: Presentation and demonstration of activities

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audi-ence	Partner responsible /involved	Reference
						within REPCO
01-03 Nov 2005	Conference	Research	International		PRI	International Workshop on Implementation of biocontrol in practice, Flakkebjerg, Dk
09 Nov 2005	Conference	Growers, advisers	I	100	FiBL	Oral contribution, short communication follows
14-15 Nov 2005	Flyer	General public	International		PRI	Distributed at CER2005, Brussels
15 Nov 2005	Conference	Students	F	20	Grab	Plant protection in organic viticulture
24 Nov 2005	Meeting	Extension service	D	70	WBI	Presentation of first results on the Annual Meeting of the extension service for V viticulture in Baden-Württemberg
Dec 2005	Meeting	Growers	D		WBI	Presentation of project to members of 'Badischer Weinbauverband'
Dec 2005	Meeting	Growers, Advisors	D		WBI, ECOVIN	Presentation of first results on the Annual Meeting of ECOVIN
22-23 Jan 2006	Meeting	Growers, Advisors	A	100	FiBL	Oral contribution
31 Jan – 01 Feb 2006	Conference	Advisers, research	Europe	50	PRI	12th Int. conference Organic Fruit Growing, Weinsberg, D
16 Feb 2006	Meeting	Research	F	10	Grab	Workshop on replacement of copper in organic viticulture
08 Mar 2006	Meeting	Growers, Advisors	CH	30	FiBL	Information day on plant protection news and others (Presentation, Oral contribution)
08 Mar 2006	Meeting	Growers, Advisors	CH	60	ETHZ	Biorebbautagung, Olten, CH (Oral contribution)
25-26 Mar 2006	Exhibition / Demonstration	General public	DK	>1000	LIFE	Presentation of the project & demonstration of laboratory screening procedures at open house arrangement, LIFE, Frederiksberg, Dk
27 Mar 2006	Meeting	Research / growers	F	30	Grab	Information day on plant protection news
27-29 Mar 2006	Conference	Research, growers	I		IASMA	Presentation at Giornate fitopatologiche Italy
29-30 Apr 2006	Conference	Research	D		PRI	Workshop DPG-Biocontrol, Geisenheim, Germany
29 May 2006	Press release	General public	International		PPO/DIAS/	Press release by PPO on patent application

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audi-ence	Partner responsible /involved	Reference
					KVL	
29 May 2006	Publication				LIFE	Utility model applications published in: Økologisk Jordbrug nr. 366: “Planteudtræk virksomt mod skurv”
30 May 2006	Congress	Experts	International	1500	WBI, ECOVIN	International Congress for Biocontrol
30-31 May 2006	Publications	Research	Europe		All partners	14 contributions in: Proceedings of the European Joint Organic Congress, Odense, Dk;
30-31 May 2006	Conference	Growers, consumers, research	Europe	1000	All partners, 10 contributions	6 oral and 8 poster presentations; Joint Organic Congress in Odense, Dk
01 Jun 2006	Exhibition / Demonstration	Growers	DK, S	60	UoA	Field day for fruit growers
02 Jun 2006	Press release	Grower	Germany		WBI	Der Badische Winzer, Der Deutsche Weinbau, Weinmagazin
11-15 Jun 2006	Conference	Research	International		IASMA	12th Mediterranean Phytopathological Congress, Greece
18-23 Jun 2006	Conference	Research	International	200	IASMA	Presentation at 5th International Workshop on Grapevine Downy and Powdery Mildew, S. Michele all’Adige, Italy
18-23 Jun 2006	Conference	Research	International	200	WBI	Presentation at 5th International Workshop on Grapevine Downy and Powdery Mildew, S. Michele all’Adige, Italy
18-23 Jun 2006	Conference	Research	International	200	ETHZ	Presentation at 5th International Workshop on Grapevine Downy and Powdery Mildew, S. Michele all’Adige, Italy.
20 Jun 2006	Exhibition / Demonstration	Advisors	NL		PPO	Excursion to field experiments for Dutch extension service DLV
21 Jun 2006	Protection of knowledge	Patent	International		WBI	Application of a patent: Alkylphospholipid and lyso-Phospholipid for plant protection
Jun 2006	Course	Students	International		PPO	Instruction and excursion to field experiments for International Students from IFP-course

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audi-ence	Partner responsible /involved	Reference
Jun 2006	Press release	General public	International		PPO, LIFE, UoA	Efficacy of products in organic apple scab control
Jun 2006	Press release	General public	NL		PPO	Efficacy of products in organic apple scab control
Jun 2006	Conference	Research	International		PRI	Congress of the International Academy for Microbial Ecology (ISME-11), Vienna, Austria
Jun 2006	Meeting	Research	D		WBI	Common workshop wit colleagues from the “Klinik für Tumorbiologie Freiburg
10 Jul 2006	Press release	General public	DK		UoA	Yucca-planter til bekæmpelse af svampesygdomme I æbler. http://www.agrsci.dk/djf/nyheder/yucca_planter_til_bekaempelse_af_svampesygdomme_i_aebler
12 Jul 2006	Press release	General public	DK		LIFE	LIFE press release in Denmark on yucca patent application
Jul 2006	Meeting	Growers	D		WBI/ECOVIN	Presentation of results at members of ECOVIN
01 Aug 2006	Meeting	Advisers/scientists	DK	10	UoA	Information Research
13-17 Aug 2006	Conference	Research	DK	>80	LIFE + all apple scab partners	Oral contribution and Poster presentation at 8th Conference of the European Foundation for Plant Pathology & British Society of Plant Pathology Presidential Meeting LIFE, Copenhagen, Dk
17 Aug 2006	Exhibition / Demonstration	General public, growers, advisers	NL	270	PPO	Stand about PPO research results at Open Day
23 Aug 2006	Publication	General public	NL		PPO	Article in Agricultural Newspaper "Agrarisch Dagblad"
25 Aug 2006	Publication	Growers + General public	DK		LIFE	Økologisk Jordbrug nr. 366: “Planteudtræk virksomt mod skurv”
25 Aug 2006	Exhibition / Demonstration	Growers	DK	50	UoA	Field day for Danish apple and pear growers
13 Sep 2006	Field	Extension service	D	60	WBI	10. Freiburger Versuchsführung

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audi-ence	Partner responsible /involved	Reference
	demonstration					
23-24 Sep 2006	Exhibition / Demonstration	General public	DK	>3000	LIFE	Presentation of the project & demonstration of laboratory screening procedures at open house arrangement, Pometet, LIFE, Taastrup
25-28 Sep 2006	Conference	Research, advisers	D		PRI	Pflanzenschutztagung, Göttingen, Germany
Sep 2006	Publication	General public	NL		PPO	Article in Fruit growers Journal "Fruitteelt"
Sep 2006	Meeting	Growers	D		WBI	Presentation of results to growers
13 Oct 2006	Exhibition / Demonstration	General public	DK	>2000	LIFE	Presentation of the project & demonstration of laboratory screening procedures at open house arrangement, LIFE, Frederiksberg, Dk
23 Oct 2006	Conference	Students	F	20	Grab	Plant protection in organic viticulture
	Project web-site	General public	International		PRI/all	Regular updates of www.rep-co.nl
Project Period 4						
06 Nov 2006	Meeting	Growers	D		ECOVIN, WBI	Ecovin, General assembly of Ecovin Baden in Grunern, D
19-20 Nov 2006	Congress	Research	DK		LIFE	Poster presentation at COST Action 864 meeting, AGES, Vienna, Austria
21 Nov 2006	Meeting	Extension service	D	70	WBI	Fachtagung Weinbau des Ministeriums Ländlicher Raum und Ernährung Baden-Württemberg
06 Dec 2006	Meeting	Experts, Extension service	D	50	WBI	10. Freiburger Rebschutztag
08 Dec 2006	Seminar	Research	D	20	PRI	Seminar BBA, Institut für Biologische Bekämpfung, Darmstadt.
Dec 2006	Meeting	Extension service	D		WBI	Presentation of results on the Annual Meeting of the extension service for viticulture in Baden-Württemberg
Dec 2006	Meeting	Growers	D		WBI	Presentation of the results at the annual

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audi-ence	Partner responsible /involved	Reference
						meeting of the “Absolventen der Weinbaufachschule Freiburg”
Dec 2006	Publication	Growers	D		LIFE	Bioland 12.: “Bitterstoffe gegen Apfelschorf“
During 2007	Newsletter	Growers	NL, BE		BioFruitAdvies	Various reports on REPCO dissemination trials in EKO Bericht (newsletter to organic fruit growers in the Netherlands and Belgium)
18 Jan 2007	Congress	Growers	I		(Nor-Natur ApS)	Oral contribution by Bea Nielsen, Nor-Natur ApS at Bioland growers meeting in Italy
19 Jan 2007	Seminar /Congress	Research	DK	60	LIFE	Oral contribution at the research seminar “Approaches to alternative disease control: today and tomorrow” 19 January 2007, LIFE, Frederiksberg, Denmark
19 Jan 2007	Meeting	Scientists	DK	50	UoA	Information, research
19-20 Jan 2007	Meeting	Research	International		BioFruitAdvies	International Apple Scab Working Group; presentation of REPCO results
26 Jan 2007	Meeting	Fruit growers		50	BioFruitAdvies	Seminar on Organic Fruit Growing, Gleisdorf, Austria; presentation of REPCO results
02-03 Feb 2007	Conference	Advisors	International		BioFruitAdvies	Obstbautagung, Weinsberg, Germany; presentation of REPCO results
2007	Meeting	Growers, advisors	NL		BioFruitAdvies	Studiedag biologische fruitteelt, Driebergen, the Netherlands
14-15 Feb 2007	Meeting	Growers	I	30	BioFruitAdvies	Seminar on organic fruit growing, S’ Michele, Italy
6 Mar 2007	Meeting	Growers, Advisors	CH	30	FiBL	Information day on plant protection news and others (Presentation, Oral contribution)
7 Mar 2007	Meeting	Growers	F	20	Grab	Information day on plant protection in organic viticulture
24-25 Mar 2007	Exhibition / Demonstration	General public	DK	>1000	LIFE	Presentation of the project & demonstration of laboratory screening procedures at open

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audi-ence	Partner responsible /involved	Reference
						house arrangement in the green houses, LIFE , Frederiksberg, Denmark
27 Mar 2007	Conference	Research / growers	F	200	Grab	Annual meeting of Rhône valley producers
Mar 2007	Publication	Growers + research	Nordic countries		LIFE	Forskningsnytt om økologisk landbruk I Norden nr. 1: "Samarbejde mellem universitet og industri fremmer udviklingen af alternative produkter til bekæmpelse af plantesygdomme i økologiske dyrkningssystemer"
05 Apr 2007	Meeting	Research / growers	F	50	Grab	Annual meeting of Grab
16 Apr 2007	Meeting	Growers	F	10	Grab	Information day on plant protection in organic viticulture
May 2007	Patent filing				PPO/ LIFE / UoA	Filing updated patent application covering yucca
May 2007	Patents filing				LIFE	Filing updated two patent applications covering materials from utility model applications
10 Jun 2007	Workshop	Growers	F/E/(I)	22	FiBL	Workshop/seminar on organic viticulture
25 Jun 2007	Field day	Growers	DK, PL	40	UoA	Field day for fruit growers
26 Jun 2007	Meeting	Growers	CH	28	FiBL	Course on organic viticulture: Demonstration of screening activities indoor and outdoor
02-06 Jul 2007	Conference	Research	International	100	ETHZ	Oster presentation The Downy Mildews 2nd International Symposium Olomouc (CZ)
10 Jul 2007	Meeting/Visitors	Growers	F	9	FiBL	Visit at FiBL: Fédération des producteurs de fruits du Haut-Rhin. Demonstration of REPCO-activities for copper replacement
06 Aug 2007	Field day	Growers	DK	30	UoA	Field day for fruit growers
09 Aug 2007	Exhibition / Demonstration	General public/ Growers	I	>100	IASMA	Open day for vine growers at IASMA, Italy, 9 August 2007
19 Aug 2007	Exhibition / Demonstration	General public/ Growers	CH	aprox. 2000	FiBL	Day of open door at FiBL

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audi-ence	Partner responsible /involved	Reference
				(200 interested in copper replacement)		
21 Aug 2007	Meeting	Advisers/scientists/ growers	Czech republic	50	UoA	Information Research
07-08 Sep 2007	Meeting	Growers	F	>1000	Grab	National meting on organic farming
12 Sep 2007	Press	General public	F, D		Grab	Recording a report on ARTE Channel about European programs research in organic viticulture
14 Sep 2007	Publication	Interested public	DK		LIFE	Utility model applications published by the Danish Patent and Trademark Office in “Dansk Brugsmodeltidende” (DK 2006 00153 U3, DK 2006 00154 U3, DK 2006 00155 U3, DK 2006 00156 U3, DK 2006 00157 U3)
26-28 Sep 2007	Exhibition / Demonstration	Secondary school pupils & general public	DK	>200	LIFE	Presentation of the project & demonstration of laboratory screening procedures at the annual “Life science village”
12 Oct 2007	Exhibition / Demonstration	General public	DK	>2000	LIFE	Presentation of the project & demonstration of laboratory screening procedures at open house arrangement, LIFE, Frederiksberg, Denmark
25-27 Oct 2007	Conference	Research	International	100	ETHZ	Oral and poster presentation at IOBC/WPRS Working Group on “Integrated Control in Viticulture” Marsala (Sicily)
25-27 Oct 2007	Conference	Research	International	100	IASMA	Presentation at IOBC/WPRS Working Group on “Integrated Control in Viticulture” Marsala (Sicily)
Nov 2006 – Oct	Exhibition,	Growers, General	CH and	2-40	FiBL	Several groups of visitors at FiBL:

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audi-ence	Partner responsible /involved	Reference
2007	Demonstration	Public, Researcher	International			Presentation and demonstration of activities within REPCO
Planned activities						
06 Nov 2007	Meeting	Vine growers	A	12	FiBL	Information day with Austrian Demeter vine growers
08 Nov 2007	Conference	Students	F	20	Grab	Plant protection in organic viticulture
28-29 Nov 2007	Meeting	Growers, Researcher	F	400	Grab	EUROVITI Meeting
04 Dec 2007	Conference	General public/ Growers	I	Exp. 200	IASMA	Technical workshop in viticulture for growers, 4 December 2007
2007	Publication	Organic community	DK		LIFE	Publication of screening and research results in FØJOenyt.
2007	Publication	Fruit growers	DK		LIFE	Publication of screening and research results in Frugt & Grønt
Feb – Mar 2008	Meeting	Growers	F	10	Grab	Information day on plant protection in organic viticulture
05 Mar 2008	Meeting	Growers	CH	60	FiBL	Information day on plant protection news for farmers
Nov 2007 – Oct 2008	Exhibition, Demonstration	Growers, General Public, Researcher	CH and International	2-40	FiBL	Several groups of visitors at FiBL: Presentation and demonstration of activities within REPCO
20008	Press release	General public	NL		PRI	General achievements of REPCO
after 2007	Approximately 13 publications in scientific journals are planned by REPCO partners (details see below)					
after 2007	Approximately 6 contributions at scientific conferences are planned by REPCO partners (details see below)					

Publications produced wholly or in part through support by REPCO

Bengtsson, M., H.J.L. Jørgensen, A. Pham, E. Wulff & J. Hockenhull (2006). Screening of organically based fungicides for apple scab (*Venturia inaequalis*) control and a histopathological study of the mode of action of a resistance inducer. IOBC/wprs Bulletin Vol. 29 (1): 123-128.

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Speksnijder, A.G.C.L., C.H. Lombaers-van der Plas, & J. Köhl (2006). Screening of micro-organisms for *Venturia inaequalis* control by means of DGGE. European Joint Organic Congress, 30-31 May 2006, Odense, Denmark.

Conference contributions - Poster presentations

Bengtsson, M., B. Heijne, P.F. de Jong, H.J.L. Jørgensen, K. Paaske, H.L. Pedersen, M. Trapman, E. Wulff & J. Hockenhull (2006). Prospecting for new fungicides to control apple scab (*Venturia inaequalis*) in organic fruit growing. COST Action 864: Pome Fruit Health Research in Europe - Current Status 2006, 20-21 November 2006, AGES, Vienna, Austria.

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Speksnijder, A., C.H. Lombaers-van der Plas & J. Köhl (2006). Screening for antagonistic microorganisms for *Venturia inaequalis* control by means of DGGE community analysis. Congres of the International Academy for Microbial Ecology (ISME–11), 20–25 Augustus 2006, Vienna.

Speksnijder, A., C.H. Lombaers-van der Plas & J. Köhl (2006). Selektion antagonistischer Mikroorganismen zur biologischen Bekämpfung von *Venturia inaequalis* durch Analyse von DGGE-Fingerprints mikrobieller Gemeinschaften. 55. Deutsche Pflanzenschutztagung, 25–28 September 2006, Göttingen, Germany.

Scientific publications in preparation

Bengtsson, M., J. Hockenhull, A. Pham, E. Wulff & H.J.L. Jørgensen. Inhibition of *Venturia inaequalis* in apple leaves following pre-treatment with a yucca extract and acibenzolar-S-methyl (ASM).

Bengtsson, M., E. Wulff & J. Hockenhull. Prospecting for potential new botanical fungicides for control of apple scab (*Venturia inaequalis*) in organic fruit growing.

Köhl *et al*. Selection and field testing of antagonists suppressing conidia production of the apple scab pathogen *Venturia inaequalis.*

Köhl *et al.* Role of microbial apple leaf communities in ascospore production of the apple scab pathogen *Venturia inaequalis* under orchard conditions.

Köhl *et al.* Selection and field testing of antagonists suppressing ascospore production of the apple scab pathogen *Venturia inaequalis* under orchard conditions.

Matasci, C. L., D. Gobbin, H.-J. Schärer, Ch. Stutz & C. Gessler (2008). Detecting selection for resistance in *Plasmopara viticola* populations treated with organically based fungicides

Matasci, C. L. *et al.* (2008). Effect of grapevine cultivar mixtures onto downy mildew – Epidemiology (Provisional title).

Matasci, C. L. *et al.* (2008). Provisional title: Effect of grapevine cultivar mixtures onto downy mildew – Population genetics. (Provisional title).

Matasci, C. L. *et al.* (2008). Spatial patterns of *Plasmopara viticola* populations at single vine scale. (Provisional title).

Matasci, C. L. *et al.* (2008). Minimize risks of forced evolution of *Plasmopara viticola* in response to novel control measures and resistant cultivars in grapevine. (PhD thesis; Provisional title).

Pertot, I., S. Dagostin & T. Formolo. Efficay of formulated tea tree oil against downy mildew.

Schweikert, C., H. J.. Schärer, H. H. Kassemeyer & L. Tamm. Mode of action and efficacy of organically based fungicides against *Plasmopara viticola*.

Tamm, L., S. D'Agostin, H.J. Schaerer & I. Pertot (2008). Screening and testing of products for copper replacement in European viticulture.

Joint publication LIFE & UoA. Screening potential new botanical fungicides for control of apple scab (*Venturia inaequalis*) in organic fruit orchards.

Conference contributions after project period

Chovelon, M. (2008). Integration of different control measures to maximise disease control of *Plasmopara viticola* in organic grapevine French field trial in Repco project. 16th IFOAM Organic World Congress: Cultivate the Future, June 16-20, 2008, Modena Italy.

Heijne, B., P.F. de Jong, M. Trapman, H. Lindhard Pedersen, K. Paaske, M. Bengtsson, J. Hockenhull, J. Köhl & U. Eiben (2008). EU-project replacement of copper fungicides in organic production of apple (REPCO): Strategy against apple scab and results. Organic Fruit Conference, 16-17 June 2008, Vignola, Italy.

Köhl, J. (2008). Screening of biocontrol agents for control of foliar diseases. 9th International Congress of Plant Pathology, 24-29 August 2008, Turiono, Italy.

Köhl, J., W.M.L. Molhoek, B.H. Groenenboom-de Haas & H.M. Goossen-van de Geijn (2008). Development of novel biocontrol agents for apple scab control in organic farming. Organic Fruit Conference, 16-17 June 2008, Vignola, Italy.

Köhl, J., W.M.L. Molhoek, B.H. Groenenboom-de Haas, H.M. Goossen-van de Geijn (2008). New approaches in biological control of apple scab. Ecofruit-Conference, 18-20 February 2008, Weinsberg, Germany.

Tamm, L., T. Amsler, B. Thürig & H.-J. Schärer (2008). Efficacy testing of novel organic fungicides and elicitors for control of *Plasmopara viticola* in grapevine: from the lab to the field. 9th International Congress of Plant Pathology, 24-29 August 2008, Torino, Italy.

Tamm, L., T. Amsler, B. Thürig & H.-J. Schärer (2008). Efficacy testing of novel organic fungicides and elicitors for control of *Plasmopara viticola* in grapevine: from the lab to the field. 16th IFOAM Organic World Congress: Cultivate the Future, June 16-20, 2008, Modena Italy.

Publishable results

During the course of REPCO four patents and five utility model applications have been submitted. Four REPCO partners and companies or institutes outside REPCO were involved. Furthermore, two partners consider a possible patent application of two different patents after the project period.
Although the appropriate measures have been taken to protect IPR, patents have not been fully published at the date of project reporting so that details cannot be presented as publishable results at this stage.
Partners as IPR owners of the different findings will use CORDIS marketplace http://www.cordis.lu/marketplace/about.htm#summ after patent information has fully been publicised for promotion of the use of their exploitable knowledge.