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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 Executive Summary
Period 3**

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Project coordinator: Dr. J. Köhl

Project coordinator: Plant Research International

Publishable Executive Summary

Summary description of project objectives

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

Potentiators of resistance, organically based fungicides and biocontrol agents will be screened and evaluated in grapevine and apple. The risk of pathogen evolution during use of novel control measures will be estimated to allow the development of sustainable strategies. Effects of crop management practices in organic agriculture on overwintering of *Venturia inaequalis* will be assessed. Novel disease control measures and knowledge will be integrated into organic management systems. 'Pipeline' products already under development elsewhere will be included and where necessary optimised in their use.

Implementation by end-users and industries qualified for commercialisation of project findings will be strongly emphasised. SME partners will ensure a strong link between end-users and research. At the end of the project several compounds and biocontrol agents will be delivered to qualified industries for development of products for use in organic agriculture. Additionally, knowledge of integrated use of control measures will be delivered to organic growers.

The project will thus strongly support EU policies to replace the use of copper fungicides in organic agriculture in the nearby future.

Contractors involved

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Work performed and results achieved so far

Development of potentiators of resistance and organically based fungicides

Objectives during the **first reporting period** were to collect candidate compounds for control of *Plasmopara viticola* (Pv) and *Venturia inaequalis* (Vi), to conduct a preliminary risk assessment for each compound and to select potentiators of resistance and organically based fungicides for Pv control in grapevine and Vi control in apple in a first series of screening experiments.

A list of more than 100 potential candidate materials for control of Vi and Pv was prepared. The list contains plant extracts and oils, products from micro-organisms, salts and other materials. Preliminary assessments of the materials covering availability and costs (economic feasibility), acceptability for organic growing and human and eco-toxicology were carried out.

A first series of agents for Pv control was tested on grapevine leaf discs (**WBI**). Seven compounds were indicated for further supporting tests so far. **FiBL** screened several compounds against Pv in indoors experiments and in a field experiment. **KVL** carried out a preliminary screening of over 20 materials using an in vitro assay designed to detect inhibition of Vi spore germination and a plant assay based on symptom development in apple seedlings artificially inoculated with Vi. Several promising materials showing significant reduction of Vi were identified. Screening work continues.

Objectives during the **second reporting period** were to select potentiators of resistance and organically based fungicides for Pv control in grapevine and Vi control in apple in a series of screening experiments and to further collect candidate compounds for control of Pv and Vi.

Compounds were screened by **WBI** for Pv control on grapevine in a leaf discs test system. To improve the uptake of compounds into the leaf tissue and the rain fastness surfactants and stickers were used. Their action on the plant tissue (phytotoxic) and their role in improving Pv control were tested using several compounds already screened positive for Pv control in 2004. Suitable compounds and combinations with additives were recommended for field trials 2005. Another 21 new compounds were tested. Six substances showed an effect on disease severity of Pv. The substances efficient in a preliminary screen were tested in further trials in order to quantify the efficiency additionally. Suitable substances have been passed over to FiBL for further investigation. Furthermore, four extracts from plants and fungi showed an effect on Pv control which is yet to slight so that further investigations are necessary. Extracts from cyanobacteria and fungi were scanned for potentiators of plant resistance using a cell culture system. Four extracts from plant and fungi caused resistance induction which should be further investigated.

A total of 39 substances was tested by **FiBL** under controlled conditions for fungicidal and/or elicitor activity against Pv. The test products were obtained from companies as commercialized products or from companies and research labs as non-commercial test products. Four test products were selected based on results obtained by project partners KVL and WBI in year 1 of the project.

Four plant protection products that showed good activity against Pv in indoor-screening experiments of FiBL were tested outdoors in the screening vineyard and they were compared to five

strategies and two standards. All of the new products showed at least partial efficacy in the field test. However, the efficacy was not sufficient for what a producer would expect. Three of the strategies, one of them completely copper free, protected leaves and grapes as efficiently as does the use of copper at high concentrations. For a complete replacement of copper in plant protection against downy mildew on grapevine, further new products are definitely needed.

A list of compounds with promising activity against Vi was made and compounds were recommended by **KVL** for testing under orchard conditions at DIAS and PPO in spring/summer 2005. Eighty four compounds have been collected from several sources and screened in plant assays and conidium germination tests. Seven compounds for use as stickers and surfactants were obtained from Prophyta and investigations with these materials were initiated. Several compounds showed promising control of scab on seedlings either as a preventive or as curative treatment, i.e. the effect was at least at the same level as elemental sulphur (standard treatment). The screening of these compounds, including fractions and different types of extracts of the most promising ones continues and work on improvement of compounds will be intensified. Useful collaboration with especially one commercial company has been strengthened.

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

Objective during the **third reporting period** was to screen potentiators of resistance and organically based fungicides for Pv control in grapevine (WBI) and Vi control in apple and to improve timing and application and formulation methods for potentiators of resistance and organically based fungicides.

During the reporting period, the objective was to continue the screening of potentiators of resistance and organically based fungicides for Pv control in grapevine and to improve their efficacy. Another 21 promising new products have been tested by **WBI** for Pv control on grapevine in a leaf discs test system to quantify the efficient concentration. Ten of them showed an effect on disease severity in the leaf disc assay. In the 2006 field trials (WP7.1), 6 of them were involved. Three products were tested for the enhancement of the activity of an already working agent. Furthermore, two extracts from algae and fungi showed an effect on Pv control which is yet too slight so that we try to extract purer. Products studied and developed within REPCO have been tested for potentiators capacity of resistance not only in *Petroselinum* but also in *Vitis* cell culture systems. The products causing resistance induction should be further tested for a potential priming effect on greenhouse plants.

FiBL tested further 36 substances or products in 14 independent experimental sets under controlled conditions for fungicidal and/or elicitor activity and/or as additives. The test products were obtained from companies as commercialized products or from companies and research labs as non-commercial test products. Several test products were selected based on results obtained by project partners in the two previous years of the project. From these tests under controlled conditions, six products were selected for screening under field conditions in the third season of the project (2006). These treatments were compared to strategies and standards. Three of the new products showed at least partial efficacy in the field test. However, efficacy in protection from downy mildew was only sufficient for one test product. Three of the strategies, one of them completely copper free, protected leaves and grapes as efficiently as does the use of copper at high concentrations. For a complete replacement of copper in plant protection against downy mildew on grapevine, further new products with effective fungicidal impact are still needed.

The objective for **KVL** was to screen and optimise potentiators of resistance and organically based fungicides for control of *Venturia inaequalis*. A list of compounds with promising activity against Vi was made and compounds were recommended for testing under orchard conditions at DIAS (WP4.4) and PPO (WP7.2) in spring/summer 2006 (Deliverable 12a). A total of 61 materials have been tested at KVL. Of these 27 completely new compounds were collected and screened in plant assays and conidium germination tests. An additional 39 materials were re-tested in seedling assays, either at lower dosages, as different formulations or as different types of extraction (water/ethanol). Seventeen new combinations of promising compounds have been tested in the plant assays, and 3 compounds have been tested as curative treatments 2 days after inoculation. Several compounds showed promising control of scab on seedlings either as a preventive or as curative treatment, i.e. the effect was at least at the same level as the standard treatment, elemental sulphur.

Work on improvement of a few highly promising compounds will be intensified in period 4, a number of stickers, wetting agents and adjuvants will be screened and the best combinations tested with the promising control compounds.

Appropriate methods will be used to study disease resistance induction by certain botanical extracts that appear to work via this type of mechanism.

DIAS tested 26 compounds in 2006 for their effect to control apple scab in apples cv. Jonagold. The treatments were carried out in the main primary ascospore discharge period in May using a small tunnel sprayer. Before and after the testing period the trees were treated identically with sulphur. Leaves and fruit were assessed for apple scab infections and phytotoxicity. Some Yucca extracts, Florina Proff, Coconut soap and potassium bicarbonate had an efficacy similar to sulphur on leaves. This confirms results from earlier years. A dose increase resulted in a better efficacy for the Yucca treatments.

Development of novel biocontrol agents

Objectives during the **first reporting period** were to build up collections of micro-organisms isolated from grapevine or apple leaves as candidates for antagonist screenings and to conduct a first selection round.

A protocol for a preliminary assessment of production costs and possible risks for candidate micro-organisms has been developed. More than 100 epi- or endophytic micro-organisms isolated from apple leaves were assessed (**PRI**). Twenty five candidates were applied in the orchard just before leaf fall for screening against Vi ascospore production. Possible hyperparasites were isolated from Vi infected leaf areas (>200 leaf samples; >400 isolates). Prophyta investigated the mass production of various fungal isolates to assess the economics of production of candidate antagonists. The economically sound production of various isolates seems to be feasible.

Objectives during the **second reporting period** were to build up collections of micro-organisms isolated from grapevine or apple leaves as candidates for antagonist screenings, to conduct a first selection round considering economic production and potential risks for each candidate micro-organism, to screen Vi antagonists for suppression of ascospore production on overwintering leaves (**PRI**), to screen Vi antagonists for suppression of Vi conidia production in summer (**PRI**); and to assess mass production for groups of potential antagonists (Prophyta).

Fungal endophytes isolated from apple leaves were screened by **PRI** under field conditions for their ability to reduce ascospore production of Vi. A pilot experiment was carried out in 2003/2004 to optimise methods used for antagonist screening under field conditions, e.g. spray application, assessment of colonisation and quantification of ascospore production. In autumn 2004, 25 candidate antagonists were applied to leaves in the orchard and their potential to reduce Vi ascospore production was assessed in spring 2005. For ascospore production in leaf residues, a strong block effect and an unexpected high variation between replicates of treatments was observed. Three fungal isolates tended to reduce ascospore production by >70% in the 2 most reliable experimental blocks.

A screening assay has been developed by PRI in collaboration with KVL to test the possible effect of candidate antagonists on conidia production of Vi on leaves of apple seedlings under controlled conditions. A first set of fungal isolates could be selected which significantly reduced Vi conidiation by more than 80%.

Four selected fungal isolates were provided by PRI to **Prophyta** for testing their ability to produce conidia on different agar media and solid substrates using Prophyta's laboratory scale SSF system. The fungal species showed big differences concerning their ability to produce conidia. Beside this the conidia production was dependent on the used medium. In the fermentation tests two isolates showed the best conidia production ability so far. It was possible to harvest more than 1E+9 conidia per gram culture substrate (dw input).

Objectives during the **third reporting period** for **PRI** were to screen antagonists against oospores of Pv in crop residues of grapevine (WP5.2; PRI), to screen Vi antagonists for suppression of ascospore production on overwintering leaves (WP 5.3; PRI), to screen Vi antagonists for suppression of Vi conidia production in summer (WP5.4; PRI); and to assess mass production for groups of potential antagonists (WP5.6; **Prophyta**).

For the development of biocontrol agents directed at oospores of Pv (WP5.2), samples of grapevine leaves infested with downy mildew were provided by WBI, FiBL, IASMA, GRAB and ECOVIN. The number of viable oospores in the leaf tissue was established by staining. Isolates of bacteria and fungi were obtained from those samples which showed a (slight) decline in viability during incubation in 2003. In 2004, such declines were not observed but occasionally an increase of oospore numbers during incubation. The antagonistic capacity of the obtained 56 isolates microorganisms has not been screened. Go/no go decisions were foreseen in WP5 for the different biocontrol strategies addressed in WP5.2, WP5.3 and WP5.4. Because progress and perspectives in WP5.2 were less than expected it was decided by the Project Management Team at 9 November 2006 not to continue WP5.3 but to put more emphasis on WP5.3 and WP5.4.

For the development of biocontrol agents reducing ascospore production of Vi in apple leaves during the winter season (WP5.3), fungal endophytes isolated from apple leaves were screened under field conditions for their ability to reduce ascospore production of Vi. A pilot experiment was carried out in 2003/2004 to optimise methods used for antagonist screening under field conditions, e.g. spray application, assessment of colonisation and quantification of ascospore production. In autumn 2004, 25 candidate antagonists were applied to leaves in the orchard and their potential to reduce Vi ascospore production was assessed in spring 2005 (Reporting Period 2). For ascospore production in leaf residues, a strong block effect and an unexpected high variation between replicates of treatments was observed. The experiment was repeated with the same treatments in autumn 2005 (Reporting Period 3) with slight modifications. Treatment of apple leaves in autumn with 8 out of 25 isolates resulted in statistically significant reductions of Vi-DNA (quantified by TaqMan-PCR) by 50–70 % in leaf residues sampled end of January. Treatment of apple leaves in autumn with one of the isolates significantly reduced the number of ascospores produced on treated leaves in the subsequent spring by 70 %. All other treatments had no effect on ascospore production.

During the reporting period, the objectives in WP5.4 were to screen Vi antagonists for suppression of Vi conidia production in summer under controlled conditions and to test selected antagonists under orchard conditions. A screening assay has been developed in collaboration with KVL to test the possible effect of candidate antagonists on conidia production of Vi on leaves of apple seedlings under controlled conditions. Screenings were carried on seedlings under controlled conditions. A set of 13 fungal isolates could be selected which significantly reduced Vi conidiation by more than 80%. The best 4 antagonists out of 72 isolates tested were chosen based on results of the screening experiments under controlled conditions and experiments of mass production and formulation of candidate antagonists (WP5.6). These 4 isolates were tested under orchard conditions in series of 9 experiments. Freshly produced inoculum or formulated inoculum (WP5.6) was used for spray

applications in the orchard. Treatments with the formulated isolate H39 reduced Vi conidiation by 30 to 55 % in repeated experiments. Other treatments did not result in reduced Vi sporulation.

Objective of WP5.6 for **Prophyta** was to find those antagonistic isolates with a good suitability for large scaled biotechnological production processes based on Solid State Fermentation (SSF). Furthermore, the condition to produce the highest possible fermenter yield had to be optimized, and the shelf life of the fungus produced and formulated under different conditions had to be investigated. Eleven isolates were tested concerning their ability to produce conidia on different agar media. In summer 2006 the first formulated isolates have been shipped to PRI for the field trials. Unfortunately, most of the final products showed a limited shelf life and improvements are needed.

Components for Integrated Management Systems

Objectives during the **first reporting period** were to (1) quantify the possible selection pressure of potentiators of resistance and organically based fungicides on Pv under controlled conditions; (2) to set up broad and group-specific PCR-DGGE systems applicable for green / senescent apple leaves and to quantify microbial populations in apple leaves after various treatments; and (3) to set up experiments on decomposition of apple leaves by earth worms.

ETHZ studied the change in ratio of genotypes (e.g. disappearance/dominance) under controlled conditions by inoculating mixes of sporangia suspensions from different genotypes of Pv. DNA was extracted from each generated lesion and analysis was performed with the SSR markers ISA, CES and GOB. Preliminary test for the adjusting of the methodology and test if the available molecular markers are sensitive enough to recover selection pressure were conducted at Fibl in Frick. Sampling and epidemiological observations in an experimental parcel in Cugnasco (Ticino, Switzerland) were performed.

PRI adapted protocols for DNA extraction, PCR and DGGE-tools for analysing fungal and bacterial communities in and on apple leaves. Applying these molecular tools will include evaluation of the not yet cultured or unculturable micro-organisms which are expected to be present in apple leaves. Effects of cultural practises on micro-organisms naturally present on or in green and senescing leaves and their interaction with Vi ascospore production can now be evaluated. Samples from field experiments aimed at stimulation of leaf decomposition were obtained. Total microbial populations were determined by plating endo- and epiphytic bacteria and fungi. None of the treatments stimulated microbial colonisation on or in leaves. Several treatments reduced the size of microbial populations.

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

Objectives during the **second reporting period** were to (1) quantify the selection pressure of potentiators of resistance and organically based fungicides and forced evolution on Pv under controlled conditions and under field conditions; (2) to quantify effects of cultural practises on micro-organisms naturally present on or in green and senescing leaves and their interaction with Vi ascospore production; (3) to set up experiments on leaf decomposition by earth worms with the aim to increase palatability and consumption of apple leaves.

Isolates of Pv originating from cultivars RxS, Merlot, Gamaret and Isabella were inoculated in mixtures and singularly on treated (RxS) and untreated (RxS, Merlot, Cabernet Sauvignon, Gamaret and Chasselas) leaf disks by **ETHZ**. From the resulting lesions, sporangia were collected and

reapplied to leaf disks of the same type three times. The highest number of sporulating leaf disks was assessed for cultivar RxS and for the treatments Chitoplant, and Sonata. Genetic analysis is in progress.

Field samplings were performed in 2004 in the experimental vineyard at Fibl in Frick (Aargau, Switzerland) on untreated and treated vines of cultivars Chasselas and RxS. The mutated allele conferring resistance to strobilurins was determined in 26.67%, 84.55% and 49.70% (samples from 16.08.2004) of the analyzed samples collected on vines treated with two test compounds and untreated ones, respectively. In 2005, 1882 oilspots were collected on untreated and treated vines (8 different treatments) on dates following important infective events. Samplings were performed at Fibl instead at IASMA, due to the late beginning of the epidemic in S. Michele.

4244 lesions were collected on 7 dates, following important infective events in a mixed cultivar vineyard in Cugnasco (Ticino, Switzerland). Severity was lower in the mixed blocks for cultivars where higher severity was assessed (more susceptible cultivars: Merlot, Isabella, RxS and Gamaret), possibly depending from a dilution of inoculum, from a distance/barrier effect or both factors together. Severity was lower in the monoblock for cultivars where lower severity was assessed (more resistant cultivars: Regent, Solaris, Bianca and Chambourcin) possibly depending from a reduction of the inoculum due to the presence of a larger number of resistant plants.

The populations of endophytic bacteria and fungi of apple leaves treated by various treatments were quantified by plating by **PRI**. Different from the results obtained in 2004, no treatment effect on total bacterial or fungal populations could be detected.

DNA was isolated from 252 apple leaf samples. PCR-DGGE analysis was performed on the bacterial community of the samples. A new and better reproducible PCR- DGGE system was applied for the analysis of fungal communities. Multivariate statistics was applied to species (DGGE) and environmental data. In 2003 no correlation was found between microbial community composition with number of ascospores and treatments. In 2004 a correlation was found of the community composition with the control treatments and one treatment. Treatments could not be correlated with reduced numbers of ascospores. Taking all fingerprint data and use the amount of ascospores as the only response variable, there is a possible relation of 2 fungal species and 4 bacterial species with reduced numbers of ascospores. This relation was confirmed with regression analysis. Sequencing of bands from fingerprints will give a general identification of the species which possibly counteract with the development of *Venturia* in apple leaves.

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

Objective of the WP6.2 during the **third reporting period** was to analyse forced evolution of *P. viticola* exerted by potentiators of resistance or organically based fungicides in field trials of IASMA (2005) and FiBL (2006). 1112 oilspots were collected by **ETHZ** from untreated vines and vines treated with 5 different test compounds at beginning and at the end of the epidemic. Samplings were performed at Fibl instead of at IASMA, due to the very late beginning of the epidemic in S. Michele. Analysis of samples is ongoing. Objective of WP 6.3 was to quantify the selection pressure of grapevine cultivars and hybrids planted in mixtures and forced evolution on *Plasmopara viticola* under field conditions. 4063 lesions were sampled by ETHZ on 6 collection dates, following important infective events in a mixed cultivar vineyard in Cugnasco (Ticino, Switzerland). Disease severity and incidence were assessed on six dates. Analysis of samples is ongoing.

Objective of WP 6.4 was to identify the effects of cultural practises on micro-organisms naturally present on or inside green and senescing leaves and their interaction with Vi ascospore production. **PRI** applied several broad and group-specific PCR-DGGE systems to characterise the microbial (fungal and bacterial) communities on and in apple leaves which are, to different extents, infested with Vi. DNA was isolated from 90 leaf samples. The community structure and the dynamics of a range of leaf-inhabiting fungal and bacterial micro flora constituents were assessed by PCR-DGGE analysis. In addition to ascospore counts, *Venturia* DNA was quantified by real-time PCR analysis (TaqMan-PCR). Univariate statistics was applied to species (DGGE) and environmental data. A correlation was found of the community composition with reduced *Venturia* measured by DNA quantification and ascospore counting. Treatments could be correlated with reduced ascospores. Treatments can also stimulate specific species. Sequencing of bands from fingerprints will give a general identification of the species which possibly counteract with the development of *Venturia* in apple leaves.

In WP6.5 the objective for **PPO** was to develop methods to increase earthworm populations and their ability to decompose leaves as ascospore source of Vi. The objectives are directed, first, to earthworm populations them selves and secondly to make apple leaves more attractive to earthworms. In the first case, the objective is to promote population growth of earthworms by fertilisation methods under orchard conditions. In the second case, the objective is to find products for addition to leaves to stimulate earthworms for increased consumption of apple leaves.

Four different fertilisation treatments were applied each year during 2004 to 2006. They were chicken manure (standard), mulch, mushroom manure and cattle manure. Numbers and weight of earthworm *Lumbricus rubellus* and other earthworms were measured in October to November each year. Cattle manure stimulated populations of *L. rubellus* in 2004, but there was only a trend in 2005. Analysing data together for 2004 and 2005 demonstrated a significant year effect. Non-the-less, cattle manure fertilisation significantly increased both numbers and weight of *L. rubellus* adults.

Integration of control measures

Objectives during the **first reporting period** were to carry out field experiments in Italy (IASMA) and France (GRAB) in grapevine on Pv control combining known control measures incl. 'pipeline' products. Furthermore, two field experiments in apple on Vi control were scheduled in The Netherlands, one on combined applications of control measures directed on the summer epidemics and one on applications in autumn directed at reduction of ascospore production (PPO).

Field experiments were carried out in Italy and France in grapevine on Pv control combining natural compounds. In Italy (**IASMA**), all treatments, if compared with the untreated control, gave a certain control of the disease, but only treatments with copper based compounds (copper hydroxide and copper pepidate) and one compound not containing copper resulted in a commercially satisfying control of the disease. Other compounds tested may only be acceptable in low disease pressure seasons in organic vineyards. Several resistance inducers were totally ineffective in controlling the disease in the field.

In the two field experiments of **GRAB** (France), climatic conditions were not favourable for downy mildew development and average severity never exceeded 10 % in the non-treated control. Copper (reference) was more effective than other products tested. In the experiment at Montélimar, two compounds added to the lower dose of copper were more effective than the same dose of copper alone.

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

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

Objectives during the **second reporting period** were to carry out field experiments in Italy (IASMA) and France (GRAB) in grapevine on Pv control combining known control measures incl. 'pipeline' products. Furthermore, two field experiments in apple on Vi control were scheduled in The Netherlands, one on combined applications of control measures directed on the summer epidemics and one on applications in autumn directed at reduction of ascospore production (PPO). In 2005, the disease pressure was very low in the field experiment of **IASMA**. Conditions were not very suitable for efficacy testing of compounds on leaves and bunches because of low presence of symptoms. Among 4 tested resistance inducers, only Bion[®] 50 WG (reference treatment) guaranteed a reduction in downy mildew severity compared to copper. One fungicidal compound gave acceptable results under the low disease pressure in the organic vineyard, confirming last year results. However, plants treated with this compound showed some phytotoxicity symptoms. Application of other compounds tested did not result in lower disease incidence or severity than found for the adequate control treatment.

A field experiments with 10 treatments was carried out by **GRAB** in the Rhône Valley. Climatic conditions were not favourable for development of downy mildew since spring and summer were dry and hot. No downy mildew has been observed on the plot. It was therefore impossible to assess efficacy of the tested products against Pv.

In additional experiments with potted plants, none of the tested products showed any effect on Pv on young leaves. On old leaves, only treatments with copper (reference treatment) showed an efficacy to protect vines against Pv. Selected compounds had no effect when applied alone and had no additional effect when associated with copper lower dose (compared to copper lower dose alone).

An experiment with 15 treatments was carried out by **PPO** aimed at the stimulation of decomposition of fallen apple leaves during winter and spring. Different from the field experiment from 2003/2004, no increased decomposition was found after treatments with amino acids, but also not after application of urea used as reference. In a second experiment urea treated leaves showed a higher rate of leaf degradation and also a reduction in the amount of spores. Also beet pulp gave a reduction in the number of ascospores when applied at high concentration of 600 l/ha. Applying extra earthworms increased the decomposition with 44 % compared with the control.

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

Objectives during the **third reporting period** were to carry out field experiments in Italy (IASMA) and France (GRAB) in grapevine on Pv control combining known control measures incl. 'pipeline' products. Furthermore, three field experiments in apple on Vi control were scheduled in The Netherlands, two on combined applications of control measures directed on the summer epidemics and one on applications in autumn directed at reduction of ascospore production (PPO).

In grapevine, two experiments on grafted plants of susceptible cultivars were carried out by **IASMA** in a pathogen-free greenhouse and in total the effects of 10 compounds were compared to an untreated control and a copper reference. In the first trial, a partial control of *P. viticola* was

achieved with all tested products. The highest level of control of *P. viticola* was achieved with 3 different of which one controlled the disease even better than copper hydroxide. In the second greenhouse trial none of the tested products was able to substantially reduce disease severity or incidence compared to the untreated control.

Based on the results of WP4 (WBI, FiBL) and the additional greenhouse experiments of IASMA, a field trial was performed from May to September 2006 in Northern Italy, in the experimental organic farm of IASMA, Navicello (Rovereto). In total, 14 different treatments with compounds were compared to an untreated control and 2 different reference treatments with copper. All compounds were tested also in combination with copper treatments at low dosage.

Due to weather conditions, the Pv epidemic started late in the season, but subsequently progressed extremely rapidly. On leaves disease incidence reached 100% at the end of the season and all products that are not based on copper failed to control downy under field conditions.

Only one plant extract partially controlled downy mildew severity on leaves. On bunches another plant extract allowed the control of pathogen development with a substantial reduction of downy mildew severity and incidence compared to copper. Four other formulations showed at least a partial disease control performing better compared to the untreated, but probably at an unacceptable level under high disease pressure. No symptoms of phytotoxicity was detected on bunches and on leaves. In the second strategy all products plus copper decreased the disease severity and incidence if compared with the untreated. Anyway there was no difference between plants treated with the tested compounds plus copper and plants treated only with the same low dose of copper used as reference. This suggests that the disease was totally controlled by copper and that no effect of the test compounds was considerable. The laboratory analysis of weight, acidity and pH reveal not significant difference between the treatments.

GRAB carried out a field experiment in the Rhône Valley at Barnave. A high downy mildew pressure is common at this location and Muscat petits grains as a variety susceptible to downy mildew was used. However, downy mildew outbreak was not very important in the 2006 season due to the dry and hot conditions which were not favourable for downy mildew development on bunches. No lesion on bunches was observed. Downy mildew could only be observed on leaves but severity for the non treated control at the end of the observations was below 10%. Four compounds were tested without and with additional copper treatment at low dosage in comparison with an untreated control and a copper reference. Two plant extracts did not provide any protection. Modalities containing copper have provided correct protection. Protection for copper at low dose or high dose was equivalent. Two other test compounds seemed to provide an intermediary protection. Remanence for these products is lower than for copper products. Treatments with these products should therefore be realised more frequently.

In apple, **PPO** applied treatments to foliage of apple trees or leaves by dipping in the autumn of 2005 to study effects on stimulation of decomposition. Leaf samples were taken at the leaf fall period to follow leaf degradation. These leaves were put between iron nettings and placed on bare soil under the trees. Leaf degradation was measured on different moments until spring. The number of ascospores was counted from the remaining leaves. The objective was to find alternatives for urea that simulate the decomposition of apple leaves and reduces the ascospore production. Leaves were treated with urea, difenoconazol, beet residues (in different concentrations), amino acids and a combination of amino acids with potassium phosphonate. The leaf area was measured at the beginning of the decomposition experiment between 8 and 16 November 2005. On 4 and 5 January 2006 and at the end of February 2006 leaf area was measured. Leaf residues of 2 x 50 leaves per replicate were collected for ascospore counting. Urea stimulates the leaf degradation and reduces the inoculum of scab. None of the treatments reduced the number of ascospores more than urea. Treatments with beet residues, amino acids + potassium phosphonate and difenoconazol showed comparable reductions of ascospores. Beet residues and amino acids + potassium phosphonate can be used for organic farming. An optimum concentration of beet residues was found in this experiment of 400 litres per hectare.

Furthermore, PPO carried out 2 experiments aimed at controlling Vi during the summer epidemic. Natural compounds were sprayed according the RimPro scab warning system from start of bud break until the mid of June. Severity and incidence was measured on the leaves and the fruit. Phytotoxicity and russet was assessed. The natural compounds were compared with the standard biological fungicides copper hydroxide and sulfur. The objective was to find an alternative to copper against apple scab in a modern orchard system by using natural compounds, resistance inducers or plant extracts compared with sulfur applications. A field experiment was carried out in 2006 in the organic orchard of PPO in Randwijk, The Netherlands, and in Tuil, the Netherlands during the summer epidemics. At PPO different concentrations of Yucca extract (2.5, 5 and 7.5 l/ha) and potassium bicarbonate (2.5, 5 and 10 kg/ha) were tested. The same concentrations were tested in a combination with sulfur. At the grower only one concentration of Yucca extract (7.5 l/ha) and potassium bicarbonate (5 kg/ha) was tested together or without sulfur. Treatments were compared with copper and sulfur in both experiments. Both Yucca extract and potassium bicarbonate gave significant control of scab on leaves and fruit at PPO and at the grower. There was a tendency that the highest concentration of Yucca extract and potassium bicarbonate gave more control than the lower concentrations. The combination of Yucca extract and potassium bicarbonate with sulfur was significant more effective on leaves but not on fruit compared with sulfur alone at the PPO experiment. At the grower only the combination potassium bicarbonate with sulfur was significant lower scab incidence on both leaves and fruit than sulfur alone and was not significant different from copper. The combination of Yucca extract and potassium bicarbonate with sulfur gave a lower severity on fruit and leaves compared with copper. Copper was found very effective against scab but also caused more russet of the fruit. Therefore the percentage of class 1 fruit was reduced because of the russet.

Expected end results

Novel potentiators of resistance, novel organically based fungicides and novel biocontrol agents for promising control strategies will be systematically screened and developed for use to control *Plasmopara viticola* in grapevine and *Venturia inaequalis* in apple. All screened and developed compounds and organisms will fulfil the requirements of Council Regulation (EEC) No 2092/91.

Novel integrated management systems will be evaluated, including exploitation of 'pipeline' products for disease control, such as already known, but not registered, potentiators of resistance, organically based fungicides and biocontrol agents.

The sustainability of novel control measures in respect to evolution of the pathogen population will be monitored.

Intentions for use and impact

The policy of the European Commission aims at the promotion of sustainable, quality-based agriculture production systems and the implementation of Council Regulation (EEC) No 2092/91 Appendix II Part B-IV on (reduced) use of copper fungicides. Derogation of the use of copper fungicides because of their specific environmental risks especially to soils, as scheduled for the period after 2006 (Council Regulation (EEC) No 2092/91), is threatening organic grapevine and apple production if alternatives for copper fungicides will not become available. REPCO will strongly support EU policy of promotion of sustainable, quality-based agriculture production systems by developing new organically based fungicides and potentiators of resistance, new biocontrol agents and new integrated management systems for disease control of *Plasmopara viticola* in organic grapevine and *Venturia inaequalis* in organically grown apple.

Scientific information obtained in the project will be published in research reports, scientific papers and contributions to international workshops and congresses. Partners ECOVIN and BioFruitAdvies form a platform to reach organic growers as the end-users of the developed technology. The regularly updated Website (www.rep-co.nl) contains information on partners, workpackages and project progress.

Results relevant for commercial exploitation will be scientific and technological knowledge on the use of potentiators of resistance, organically based fungicides and biocontrol agents as well as filed data relevant for registration on field experiments, production and formulation of such compounds and organisms. Commercial exploitation of results is foreseen by industrial partners.

Before publication of any information on knowledge and technology produced in the project, it will be evaluated whether specific details crucial for patent filing will not be made public before being protected by a patent. This will guarantee optimal exploitation of the results in respect to future commercialisation by interested industries.

During the **third reporting period** patent applications were filed for a number of compounds; a patent application on the use of yucca extracts for apple scab control was filed by **PPO, DIAS** and **KVL**; based on the promising results from screening assays with other botanical compounds, 5 utility model applications were filed on the same date by KVL and Nor-Natur Aps, DK.

Knowledge produced by the project which can directly be introduced in organic agriculture without the need of commercial product development by industrial partners, such as integration of existing control measures in management systems, will be communicated to extension services and end-users thereby facilitating a fast implementation in organic agriculture.