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 Executive Summary
Period 2

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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.

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 then 1E+9 conidia per gram culture substrate (dw input).

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 leafs.
Three experiments were carried out 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 an 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.

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. Phytotoxicitiy and russeting 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 russeting.

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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 fungicidial 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.

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.
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.