

'The use of pollinators as disseminators of crop protection agents'

DIARP workshop 26-27 September 2005 in Wageningen, Netherlands

Program

Monday 26 September

9.00	Tjeerd Blacquièr	Welcome
9.15	Shula Manulis	Biological control of fire blight
9.45	Saul Burdman	Bacterial fruit blotch of cucurbits and potential of biological control
10.15	coffee break	
10.45	Arnon Dag	Introduction on using pollinators as vector for crop protection
11.15	Ygal Elad	Biological control of grey mould
11.45	Sharoni Shafir	Honey bees as disseminators of a biological agent to control grey mould in strawberry
12.15-13.30	Lunch	
13.30	Bettina Maccagnani	Development of devices for the use of bumblebees and mason bees as disseminators of biological control agents, and evaluation of insects' carrying efficiency
14.00	José M. Guerra Sanz	Pollinators as vectors against common pests and diseases of Almeria region greenhouses
14.30	Jürgen Köhl	Targeting and timing of <i>Ulocladium atrum</i> for bio-control of <i>Botrytis</i>
15.00	coffee break	
15.30	Jozef van der Steen	The use of honey bees as vectors for the dissemination of <i>Ulocladium atrum</i> against grey mould in strawberries
16.00	Bert Evenhuis	Application of <i>Ulocladium</i> to control grey mould

Tuesday 27 September

9.00	Jacoba Wassenberg	How to evaluate the risk for bees of plant protection products of natural origin
9.30		Discussion
10.00	coffee break	
10.30	Willem Wolters	Possibilities for EU funding
11.00		Discussion and conclusions + agreements
12.00	Lunch	
13.00-18.00	Excursion	

Evening 19.00: Diner together in Wageningen

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Meeting report

Summary

Pollinators visit flowers for pollen and nectar, but can also be used as transporters of antagonistic organisms to flowers, in order to control or compete plant pathogenic organisms in flowers. This dissemination by pollinators can become a good addition to biological control methods.

Several diseases and several crops may be treated this way, provided the disease enters the plant through flowers, or at least can (partly) be competed in the flowers. Much of the work discussed was about grey mould (*Botrytis cinerea*) in strawberry, but many other crop/pathogen combinations were and should be considered. These comprise fruit (tree) crops, vegetables and seed production crops, in greenhouses, net houses and open field. The pathogens comprise bacteria, viruses, fungi and insects.

To develop this 'flying doctors' concept several prerequisites have to be met, and many aspects have to be studied and developed:

- assess the relevant crops and diseases
- test several antagonists and develop inoculums
- test and develop adequate carrier substances for the inoculums
- find the appropriate pollinator (disseminator), and test and develop the application (combination of inoculum, carrier, dispenser, disseminator etc.)
- develop and improve the dissemination devices (dispensers)
- assess possible impacts of the antagonists on the pollinators and the environment (spread of antagonistic organisms)

Eventually registration of the agents and application method has to receive much attention, since it is very difficult and expensive to run a registration procedure. The procedures used so far for registration of crop protection agents are not suited for the pollinator disseminated biological control system.

Introduction

Many fungi and bacteria are involved as pests in agricultural production, causing important plant and crop diseases in a wide range of crops in agriculture, fruit growing and greenhouse cultivation, resulting in severe economic losses. Some of these pathogens may enter the plant through open flowers, which are weak spots in the plant's resistance barriers.

In addition to the protecting plant barriers like epidermis, cuticle and wax layers, some substances or organisms may protect against intruding pathogens. Several antagonistic organisms have been shown to be able to block or inhibit the establishment or development of a plant pathogen. Such antagonists or substances may be sprayed on the plant canopy. Some are used on a practical scale.

Pollinators, particularly insects are contracted by many plant species to fulfil a crucial role in their sexual reproduction, by taking care of the transmission of male gametes to the female organs. In return the insects are rewarded for their service with protein rich pollen and with nectar. These insects are equipped with structures that facilitate efficient transport and carrying of pollen. These structures make them also very suitable to carry other small particles. Combined with their behaviour this makes them very suitable to carry different kinds of material from and to flowers, and also to discard most of the pollen available in flowers. In addition to pollen, this may include spores, bacteria, and contaminants from the atmosphere, but it may also be possible to use their service to carry beneficial matters to flowers.

A number of crops in which flower-entering pathogens play an important role in the infestation process and which are regularly visited by pollinating insects are listed here:

- **Strawberry:** The foremost pest is *Botrytis*, causing grey mould. It enters through flowers, and its establishment can significantly be reduced by antagonistic fungi (*Trichoderma*, *Gliocladium*). It was shown that dissemination of the antagonists by pollinators is a good alternative for spraying it and a good substitution / addition to conventional chemical fungicides.
- **Pear (and other pome fruits):** Fire blight (*Erwinia amylovora*) is by far the most destructive disease, and today's way of controlling leads to the use and concomitant residues of antibiotics (streptomycin, oxolinic acid, tetracycline). It enters through flowers. Some antagonist bacteria have been reported.
- **Blueberry:** a disease called anthracnose (caused by the fungi *Colletotricum gloeosporides* and *C. acutatum*) results in serious losses of harvest. It enters through flowers, but also via fruits and

- peduncles.
- Eggplant: a disease called mucor flower wilting caused by *Rhizopus* can be inhibited by antagonistic fungi, which can be disseminated by honeybees or bumblebees in greenhouses.
- Sweet bell pepper: an important problem in pepper is the obscure presence of a fungal rot in apparently healthy ripe fruits. This appears to be a flower introduced disease, which does not widely spread inside the plant. *Fusarium* species are strongly suspected to be involved (many fusio's are present in obscure rotten fruits).
- Onion seed crop: *Botrytis aclada* may enter the plants of onion through the flowers, and infect by that way the seeds. An antagonist was effectively disseminated by honeybees, and it was transferred into the seeds.
- Watermelon and melon: *Acidovorax avenae* subsp. *citrulli* (Aac) is the causal agent of bacterial fruit blotch (BFB) disease of cucurbits that is specially threatening to watermelon and melon. Contaminated seeds represent the most important source of BFB inoculum, and it was shown that watermelon blossom inoculation with Aac can lead to seed infection. Recently, strains of *Pseudomonas fluorescens* and *A. avenae* subsp. *avenae* found to be able to protect blossoms from Aac and to prevent seed infection.

In some cases it may also be possible to disseminate a pathogen of insect predators by honeybees or other pollinators. However, since bees are insects themselves, care has to be taken that the pathogen is not pathogenic to bees or bee brood.

- In crimson clover (*Trifolium incarnatum*) bees were able to disseminate the Heliothis Nuclear Polyhedrosis Virus (HNPV), and cause reduced success of the *Helicoverpa* spp. larvae feeding on the flower heads of the crop [1].

Pathogens (diseases) and crops

Botrytis

Species of the genus *Botrytis* cause serious diseases in many fruit crops and ornamentals. In many cases the flowers are (one of) the main entrance (s) for the pathogen. Therewith botrytis is a good candidate to be tackled with the bee dissemination strategy with antagonists.

In strawberry (*Fragaria ananassa*) grey mould (*Botrytis cinerea*) causes fruit rot, the most important disease problem in strawberry. Antagonistic organisms can successfully be applied to (partly) control grey mould in strawberries. Most of the studies used preparations of *Trichoderma harzianum*, *Ulocladium atrum* or *Gliocladium roseum*. During the workshop several presentations dealt with this system (see abstracts). In some cases bee dissemination of an antagonist worked as good as spraying the antagonist (Bilu *et al.*, 2004) or even as the use of fungicide spraying, in other cases it worked almost as good as spraying the antagonist. Using *Trichoderma harzianum* 1295-22 it worked better than spraying [2].

In onion seed crop (*Allium cepa*) the infection with *Botrytis aclada* is a serious disease, which enters through flowers and can enter the newly formed seeds. Dissemination of *Ulocladium atrum* PBGY1 by honeybees resulted in seeds that were infected with the antagonist. Reduction of infestation rate by the pathogen was not yet proven [3].

In raspberry (*Rubus idaeus*) grey mould is an important fruit disease too, and it was successfully controlled with *Gliocladium roseum* disseminated by bees [4].

Erwinia amylovora

Erwinia causes fire blight in a number of fruit crops: apple, pear, and loquat. It is especially a very big problem in pear. Manulis gave a lecture about this and about the research and the situation in cultivation practice in Israel (abstract).

A number of antagonists have been reported (*Erwinia herbicola*, *Pseudomonas fluorescens* strains), and the use of honeybees to disseminate the antagonists has been studied [5].

Fusarium

Sweet pepper: an important problem in pepper is the obscure presence of a fungal rot in apparently healthy ripe fruits. This appears to be a flower introduced disease, which does not widely spread inside the plant. *Fusarium* species are strongly suspected to be involved (many fusio's are present in obscure rotten fruits).

Mango: the main site is the buds, which makes it unlikely to be useful to use pollinators as disseminators of a preventive antagonist. However, most of the inoculum of the *Fusarium* is disseminated from the inflorescence, so if flowers can be supplied with a competing biological control agent this might reduce the level of *Fusarium* inoculum.

Rhizopus

Aubergine /egg plant suffers from a disease called 'mucor', but caused by the fungus *Rhizopus*. The disease could be controlled by antagonistic agents by spraying, and dissemination of *Ulocladium* by bees was successful, although it not as yet resulted in control of the disease [6].

Acidovorax (bacterial fruit blotch of cucurbits)

This disease affects Courgette (Zucchini), cucumber, melon and water melon. Saul Burdman gave a lecture about this disease. A watermelon strain and a melon strain are distinguished. Biological control agents applied to the female flowers appear to be a promising approach to reduce the incidence of the disease spreading to seeds.

Other:

Sunflower head rot is caused by *Sclerotinia sclerotiorum*, and causes 10-20% loss of yield. It proved to be possible to reduce the incidence of the disease by applying different species and strains of *Trichoderma*, disseminated by honeybees [7].

Blue berry suffers from a disease called anthracnose (caused by the fungi *Colletotricum gloeosporides* and *C. acutatum*), which results in serious losses of harvest and damage to the plants. It enters through flowers, but only shows up later in the season. It can then also re-infect fruits, stems and peduncles. Bees (or bumble bees or osmia bees) are very important for pollination of blueberry crops and therefore always available in the blueberry orchards. There is not yet experience with an antagonist for anthracnose, and consequently neither with pollinator dissemination.

Insects

Although bees are also insects, they might nevertheless be used to disseminate control agents against some other insects, because many parasites are very host specific. None of these possibilities were covered in this workshop.

Induced resistance?

In some cases when an antagonistic organism is introduced to roots it may cause an Induced Resistance (IR) that is transported to the shoots. If the same works with antagonists that were introduced through flowers, dissemination by bees may also be helpful in controlling leaf introduced plagues and diseases.

How many visits needed and timing of application

It is important to know how long a flower is open, and susceptible for pollination, but also for the pathogen and for the antagonist. How many visits are needed to bring enough spores of the antagonist? Quite a lot is known regarding the number of visits needed for pollination, but for the antagonists it has to be determined. Some data became available in the lecture of Bettina Macagnani (see abstracts).

Antagonist inoculums

The first question for every potentially bio controllable disease is whether a bio control agent is available. Many are described in literature, but hardly any of these is commercially available up to now. *Trichoderma*, yeasts and one of the antagonists to fire blight were (some are still?) commercially available in some countries. The list consists of *Trichoderma* species and strains, *Ulocladium*, *Pseudomonas*, yeasts, *Bacillus subtilis*, *Erwinia herbicola*, *Gliocladium*. Important is whether these are as yet formulated or not.

Some of the agents proved to be effective, others were not. What causes these differences, is it the organism or the (not yet well tested and developed) formulations? What is their mode of action? We still lack a lot of knowledge about what happens with spores once they have been deposited onto the flowers, how is their survival and germination? Does quality decrease? How susceptible are these spores to light (ultraviolet)? Much of the knowledge until now is circumstantial and based on trial and error, and some antagonists may already have been discarded by error. Some developments may have been terminated before there was enough knowledge available to give a final well underpinned judgement. Therefore it is suggested that we should always start with preliminary testing, by applying the agents by hand, with different frequencies, and thus finding out how many spores survive, whether they germinate etcetera, before starting real field experiments.

It was concluded that quite some basic research still needs to be done before the concept will be made available to many crops and pathogens. Understanding the mode of action of the antagonists (competition, induction of resistance, toxicity etc.) is crucial on the way to their commercial application.

Carrier substances

A carrier substance has to carry the spores, but not negatively affect the spores and spore germination. The carrier should be inert, and there should be no effect of the carrier when used alone (without antagonist). If possible the carrier antagonist complex has a long shelf life, needs to have low hygroscopic capability and at best the carrier enhances the viability of the antagonist. The carrier should not be harmful for the insect. It should stick to the disseminator, but loosely since it must get off when arriving on the flower.

The carrier antagonist complex should be deposited at the site where the pathogen develops or attacks (petal, sepal, and stigma?).

Again much basic knowledge has to be gained: how many spores are deposited, how many on first, second, third visited flowers, how far from the hive do bees still deliver spores?

Which carrier and which disseminator are suited to specific flowers?

Finally, the carrier and the antagonist need to be safe for the disseminator (adults and brood), and this should always be part of the testing and implementation program.

Disseminator

The disseminators used so far are honeybees, bumblebees and osmia bees. Other insects can be used also, as has been practice in seed crops. Important are the hairiness of the disseminators, their behaviour (do they clean their bodies, do they reach and contact the proper spots in the flower), and their forage range. Do they display flower constancy, have crop preference.

It should be considered that the disseminator may also deposit the pathogen. In crops where pollination is not a need by itself, this may be a negative addition to the system. However, this might possibly also function as a kind of vaccination, caused by too low numbers of spores from the pathogen displaced to new flowers (?)

When pollination is essential for a crop under study, it is important to determine whether the effectiveness of the pollinator has been affected by its disseminating duties.

Residues of the antagonist and/or pathogen in the hive and honey have to be considered: how fast does it fade out.

Dispensers

It is now concluded that the dispensers should always be of the two-way type. These are available for bumblebees and osmia (Bettina Macagnani) and honeybees (Arnon Dag). A honeybee dispenser for the MiniBeute hive has been developed too (Sjef van der Steen).

We should now focus on improving the dispensers: it is important that we have a constant input of spores with carrier, there should be enough spores dispensed and the conditions in the dispenser need to be favourable for the spores, it needs to remain dry. As little as possible of the spores has to move into the hive. Loading should be easy carried out by growers, or it should be automatically dosed. It should not deposit the flour on eyes and antennae of the insects.

Again, it should be stressed that it needs to be tested in the field: how well is the material spread into the crop field.

Environment and legislation

Environmental conditions may be a limit to the success of the approach, especially the relative humidity may be a constraint very often (material gets wet, and is no longer adhering to bees and flowers).

In another way the environment may also restrict the possibilities: how much of the antagonist is brought to non-target crops and surroundings? For legal registration of a bio control agent it has to be determined and estimated how far and in which dilution the material reaches non-target fields. From many pollination studies and models this can be derived. The effect will fade out very quickly. Experiments with pollen dispensers showed that the effect was restricted to 50-200 meters. Also the effect on predators of the disseminators has to be considered, but is likely to be negligible. The problem of environment will be far less important for applications in confined environments (greenhouses and net houses). It should however be stressed that the dissemination of biological agents by pollinators represents a far more environmentally and consumer friendly approach than the conventional application of pesticides.

A specific problem is that for registration the same standards are set as for real pesticides, which makes it generally too expensive to make a registration financially worthwhile. A positive point is that data available from literature, and extrapolated by modelling, can be applied in the registration procedures. For instance many data are available from studies on pollen transfer from GM crops. This kind of use of literature data may reduce the costs of registration procedures.

References

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Tjeerd Blacquièrè, 12 December 2005

Abstracts

Bacterial Fruit Blotch of Cucurbits and Potential of Biological Control to Manage this Disease.

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Bacterial fruit blotch (BFB) of cucurbits is caused by the Gram-negative bacterium *Acidovorax avenae* subsp. *citrulli* (*Aac*). BFB gained special attention with severe outbreaks in the Mariana Islands and Florida in the late 80s'. In recent years, the pathogen was found to cause disease on other cucurbits such as melon, pumpkin and cucumber, and significant economic losses caused by the bacterium on several cultivated cucurbits have been reported in many parts of the world, including the USA, Nicaragua, Costa Rica, Brazil, Australia, Turkey, Japan, Korea, Thailand, Taiwan and China. In Israel, the bacterium was detected in imported seeds and in diseased seedlings grown from imported seeds during the 90s'. Although the occurrence of BFB in Israel has been sporadic, during 2000-2003, the disease caused yield losses in both melon and watermelon. Bacterial strains were isolated and confirmed as *Aac* using Koch postulates, followed by GC-FAME analysis and sole carbon substrate utilization profiles. Differences were found among strains for their pathogenicity on watermelon, melon and cucumber varieties, as well as for their carbon substrate utilization profiles, their DNA-fingerprinting profiles, and in additional features. *Aac* has the potential to be highly destructive. Under favorable conditions, bacteria spread rapidly throughout transplant houses and in the field, leading to seedling blight and/or fruit rot. Chemical control of BFB has been ineffective and complete/monogenic resistance against the disease is not currently available in commercial cultivars. *Aac* is transmitted by seeds, which usually serve as the primary source of inoculum for BFB outbreaks. The potential of biological control for BFB management is discussed.

Biocontrol of foliar pathogens: mechanisms and application

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Biocontrol offers attractive alternatives or supplements to the use of conventional methods for plant disease management. Vast experience has been gained in the biocontrol of plant diseases. Prevention of infection by biocontrol agents or suppression of disease is based on various modes of action. Resistance in the host plant may be induced locally or systemically by either live or dead cells of the biocontrol agent. As some pathogens are negatively affected by lack of nutrients in the infection court (e.g. *Botrytis cinerea*), competition for nutrients and space was long recognized as antagonism trait. Antibiosis and hyperparasitism affect pathogens of various groups (but that is not the case with our *Trichoderma harzianum* T39 BCA). Other valid mechanisms are reduction of the saprophytic ability and reducing conidia dissemination. Restraining of pathogenicity factors of the pathogens, i.e. host hydrolyzing proteins or reactive oxygen species takes place when biocontrol is used. An effect on rhizosphere and phyllosphere microbial populations and on active oxygen species and plant hormones were found to be involved with the activity of T39. It is likely that several modes of action concomitantly participate in pathogens suppression but the relative importance of each one of them is not clear. Examples of effective prevention of infection in the phyllosphere that rely on multiple modes of action will be demonstrated with *Trichoderma harzianum* T39 (TRICHODEX), *Bacillus mycoides* and *Pichia guilhermondii*, a filamentous fungus, bacterium and yeast biocontrol agents, respectively. Several commercial products based on microorganisms have been developed and are starting to penetrate the market. However, large-scale use is still limited because of variability and inconsistency of biocontrol activity. In some cases this may be caused by sensitivity of the biocontrol agents to environmental influences. Ways to overcome biocontrol limitations and to improve its efficacy are i. integration of biocontrol with chemical fungicides on a calendar basis or according to ecological requirements of the BCAs relying on the advice of a decision support system; ii. introduction of two or more BCAs in a mixture, assuming that each one of them has different ecological requirements and/or different modes of action; targeting the BCA to the pathogen infection court (via bees). Implementation of one (or more) of these approaches, using biocontrol preparations mentioned above lowered the variability and increased the consistency of disease suppression. The expected long-term result of the implementation of these suggested

strategies is reduced risk of uncontrolled epidemics and increase of confidence of growers in using this non-chemical control measure on a large scale.

Introduction on using pollinators as a vector for crop protection

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In addition to pollen, honey bees can transfer fungal spores and bacteria among flowers of different plant species. The ability of bees to vector microbes has been harnessed for biological control purposes by using bees to transfer biological agents to flowers. In practice, a dispenser is attached to the hive and loaded with a powder formulation of the desired agent. The intent is for foragers exiting the hive to become dusted with the agent and to deliver it to the target crop. The technique been applied to control fire blight (*Eriwinia amylovora*) in orchards and gray mold (*Botrytis cinerea*) in strawberry and raspberry. These two pathosystems are similar in that the flower serves as an infection site. The technique was also evaluated for the dissemination of viruses to control *Heliothis* in clover and for dissemination of insect-pathogenic fungus to control pollen beetles (*Meligethes aenus*) in oil seed rape. Despite the great potential for this technique (environmental- and consumer-friendly, inexpensive, and low negative effect on fertilization processes) it is hardly in commercial use. Further efforts of researchers in phytopathology, horticulture and apiculture must be invested in order to facilitate the application of the technique.

Application of *Ulocladium atrum* to control grey mould (*Botrytis cinerea*) in strawberries

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Botrytis cinerea is causing fruit rot of strawberry after infection of flowers and fruits by airborne conidia. Conidia of *B. cinerea* can be produced on necrotic tissues such as necrotic leaves of the strawberry crop but also on various other necrotic plant tissues inside and outside the crop. Spray application of *U. atrum* results in partial control of grey mould compared to chemical treatments. Optimization is necessary to implement the antagonist in horticultural practise.

Timing and targeting of *U. atrum* seems to be the biggest challenge. The results of field experiments demonstrated that intervals between antagonist applications during flowering must be short to guarantee that newly opened flowers are reached by the antagonist. When a regular application regime was applied, at least 2 applications per week were found to be necessary. In 2002 and 2003, field experiments were carried out using a decision support system (DSS) to optimise timing of antagonist applications and to avoid applications under conditions not favourable for disease development. In 2002, 8 applications of *U. atrum* at 3 to 4-day intervals resulted in a reduction of grey mould incidence by 50 %. When applications were timed according to DSS (at an infection chance >7.5 %), 4 applications were carried out resulting in a reduction of grey mould incidence by 30 %. In 2003, 7 applications of *U. atrum* at 3 to 4-day intervals resulted in a reduction of grey mould incidence by 24 %; 6 applications timed according to DSS resulted in a reduction of grey mould incidence by 39 %. Fungicide application timed following the same decision rules reduced grey mould incidence by 67 %.

Integration of the use of fungicides and antagonist in a control strategy based upon a DSS proved promising. Spraying a fungicide at high infection risk and *U. atrum* at moderate infection risk resulted in the same level of grey mould control than spraying with fungicides only in 2004. Integration of powdery mildew control has also to be considered. When the disease occurred in field experiments, fungicides aimed at grey mould control but not the antagonist achieved powdery mildew control.

To improve targeting of the antagonist a series of experiments was set up to vector the antagonist by bees or bumblebees to the flowers. Vectoring of *U. atrum* by pollinators from the hive to the strawberry flowers was shown. No disease control was observed in 2004. So to improve the technique a two-way dispenser was used in 2005, combined with a powdery formulation of *U. atrum* with a higher spore density and a more suitable

carrier substance. Deposition of the antagonist on the flowers was higher in 2005 compared to 2004. No data on disease control are available yet.

Risk assessment of plant protection products of natural origin (summary)

Jacoba Wassenberg, CTB, Wageningen

The Dutch Board for the authorisation of pesticides (CTB) has the duty of taking decisions on the authorisation of pesticides. The Board is an independent administrative body. The general framework and supervision is provided by the ministers involved, i.e. the Minister of Agriculture, Nature and Food quality, the Minister of Public Health, Welfare and Sports, the Minister of Housing, Spatial Planning and Environment, and the Minister for Social Services and Employment.

The CTB takes decisions on the authorisation of a pesticide on the basis of a dossier submitted by the applicant containing information about a number of aspects: efficacy, analytical methods, physical and chemical properties, risk for the public health, risk for the user, risk for the environment, risk for non-target organisms.

The Board takes decisions on the authorisation of pesticides within the framework of the rules and legislation concerned, being the Pesticide Directive 91/414/EC for pesticides. Data requirements and triggers are laid down in Annexes II and III, and VI, respectively, to this directive. Different versions of these Annexes are available for chemical substances and microbial plant protection products.

Within the environmental risk assessment, persistence in soil, leaching to the groundwater and effects on non-target organisms are looked at. As an example, the risk assessment for bees is explained: first, a simple Hazard Quotient is calculated by dividing the application rate by LD50-values from acute oral and contact toxicity tests. In the Pesticide Directive it is stated that the HQ must be lower than 50. If it exceeds the trigger of 50, a more realistic effect assessment must be done using cage and/or field tests. The risk for bee brood must always be assessed if the substance is an IGR.

The use of pollinators as vectors for microbial plant protection products has up till now not been taken into account in risk assessment of microbial plant protection products in the Netherlands or Europe. For glasshouse use, it seems that with current data a risk assessment could be done. For outdoor use however, many uncertainties are present, mainly to do with emission. Basic scientific research on this topic is needed.

Honey bees as disseminators of a biological agent to control grey mold in strawberry

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Grey mold, caused by *Botrytis cinerea*, is a major fungal disease of strawberries. Chemical control remains the most commonly employed method to control the disease. However, chemical control has undesirable environmental side effects, and may negatively affect pollination, seed set, and fruit formation. *Trichoderma* spp. isolates are known for their ability to control plant pathogens. The *T. harzianum* T39 isolate, in particular, has effectively controlled *B. cinerea* under laboratory and greenhouse conditions. However, frequent application is necessary. Frequent application of a biocontrol agent, especially in the field, is costly and labor intensive. An efficient alternative for continuous dissemination of biocontrol agents to strawberry in greenhouses and in the field may be the use of bees. We developed the two-way 'Triwaks' dispenser, which fits on a honey bee hive and can be loaded with inoculum. A comparison with three other dispenser types showed that the 'Triwaks' dispenser was most efficient at contaminating bees with high levels of inoculum over a whole day. Field tests in Israel, over two winter seasons in a commercial plot showed that honey bees disseminated adequate amounts of inoculum to strawberry flowers up to 200 m from the hive. The incidence of disease increased in untreated control plots over the season. Partial control of the disease was achieved in plots

treated every two weeks with fungicides according to commercial practice, alternating between 30% pyrimethanil, and 2.5% fludioxonil and 37.5% cyprodinil. The best control was achieved in plots open to visits by *Trichoderma*-carrying bees. Honey bee dispersal of *Trichoderma* seems a viable alternative to chemical control of grey mold on strawberry, and should be adopted on a commercial basis.

Biological Control of Fire blight

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Fire blight is one of the most destructive diseases limiting the production of pear, apple and other pome fruits in many part of the world. Flowers are the most common site of infection by the pathogen *Erwinia amylovora*. The disease is managed by application of antibiotics (streptomycin or oxolinic acid) to protect the blossoms from infection. However, antibiotics-resistant *E. amylovora* populations have been detected in several pear and apple-growing regions in Israel. Therefore alternative control measures against fire blight should be tested. The objectives of this study were to determine how effectively known biological control strain would colonize pear cultivars grown in Israel and to isolate more effective biocontrol agents from pear flowers. *Pseudomonas fluorescens* strain A506 is registered and commercialized for suppression of fire blight (BliteBan) in USA. This strain survived in inoculated flowers under Israeli conditions. Flowers that were open at the time of spray harbored from 10^5 to 10^7 cells per flower, while those that opened subsequent to spraying developed population sizes to about 10^5 cells per flower within 5 days. The incidence of fire blight infections were reduced about 3 fold in several trails in which moderate amounts of disease occurred in the plot areas.

Potentially antagonistic bacteria were isolated from healthy pear flowers collected from common cultivars grown in Israel. The colonies were screened by the immature pear fruit assay, by inoculating detached pear flowers and by spraying trees grown under net. Five strains reduced significantly the symptoms of fire blight. These strains will be further characterized and examined for control of fire blight under field conditions.

The use of honeybees and bumblebees as vectors for the dissemination of antagonists against grey mould in strawberries

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Antagonists can effectively be applied against *Botrytis cinerea* in strawberry. They can be administered by spraying, but since *Botrytis* enters the plant through the flowers, the use of pollinating insects, already present for their pollinating service, for the dissemination of antagonists seems promising. The research in 2004 showed that two different antagonists could be effectively distributed to the flowers by bumblebees and honeybees, although the number of spores recovered per flower was not yet sufficient. In 2004 cellulose and talcum were used as a carrier for the spores.

The research of 2005 focuses on improvement of the dispensers used on the hives of the bumblebees and honeybees, and on testing and comparing different carrier substances, for instance bentonite, cellulose, talcum, quartz. A good carrier has to adhere to the bees' body surface, but needs also to be transferred to the petals and adhere to them. The results will be presented and discussed.

Development of devices for the use of bumblebees and mason bees as disseminators of biocontrol agents, and evaluation of their carrying efficiency

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A dispenser for the dissemination of biocontrol agents by *Bombus terrestris* was developed. It was constituted by two partially overlapping passageways, communicating through a hole. Its efficacy in contaminating *Bombus terrestris* workers with fungal antagonists was compared with another hive mounted device, constituted by side-by-side passageways. Furthermore it was verified whether the amounts of inoculum (*Trichoderma harzianum* Rifai and *Gliocladium virens* Miller, Giddens, Foster) delivered onto tomato flowers by bumblebees were comparable to those from spray treatments. The overlapping passageways applicator was the most effective, with 100.0% of the captured bumblebees carrying mean inoculum loads of 4.3×10^4 CFU/bee; with the second dispenser only 12.5% of the captured bees carried a mean load of 1.4×10^2 CFU/bee. In both years, inoculum density on flowers collected from the bee-delivered treatment was significantly lower than on those treated with sprays.

Studies were carried out to evaluate the suitability of the highly efficient pollinator of pear *Osmia cornuta* (Hymenoptera Megachilidae) as a carrier of biocontrol agents (BCA), different strains of *Bacillus subtilis* against *Erwinia amylovora*, and its capacity was compared with that of *Apis mellifera*. A dispenser for the spread of bacteria by *O. cornuta* was successfully developed. Females exited and entered the dispenser through the proper pathways. The number of BCA reisolated from the body of *O. cornuta* exiting the dispenser ranged from 10^4 to 10^9 CFU/insect. Individuals of *A. mellifera* carried an average population of 10^6 CFU/insect. In net screened tunnel conditions, the efficiency of *O. cornuta* to collect the BCA from the dispenser (primary contamination) and deposit it on pear flowers during a single visit was higher than that of *A. mellifera*. The same result was obtained in a study on pollinators capacity of spreading the BCA from inoculated apple flowers to newly opened ones (secondary contamination). The contribute of pollinators in the secondary spread of BCA was also assed in field conditions (apple orchards) with encouraging results.

Further agreements

1. Compile the proceedings (this report) and send it around. Include Vaissiere and Fani in the mailing list.
2. Consider making a review: allocate tasks among the group. A review can be a good basis for a co-operative research proposal
3. Continue with own research with own funding, but keep in touch with each other.
4. Use the regular meetings in Europe for this aim, and try to arrange meetings around the theme in Europe
5. Market the approach by giving lectures whenever possible.
6. Make this report available through internet
7. If possible submit as a consortium research proposals to EU