Mid-term report

for the CORE Organic II funded project

"BICOPOLL - Targeted precision biocontrol and pollination enhancement in organic cropping systems"

Period covered: **01.12.2011 – 31.05.2013**

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<tr><td>Project acronym:</td><td colspan="4">BICOPOLL</td></tr>
<tr><td>Title:</td><td colspan="4">Targeted precision biocontrol and pollination enhancement in organic cropping systems</td></tr>
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Project partners and contact persons:

Partner no.	**Country**	**Organisation name:**	**Functions*):**	**Involved in WP's:**	**Contact person with e-mail address:**
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2	**Germany**	**Lower Saxony State Institute for Consumer Protection and Food Safety, Institute for Bee Research and Apiculture**	**WPM**	**2,6,7,8**	**Otto Boecking** **Otto.Boecking@laves.niedersachsen.de**
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***)** PC: Project coordinator, WPM: Work package manager, WPCM: Work package co-manager, P: Participant

Projects website: http://www.bicopoll.net

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Project summaries, pre- and post/mid-project

Mid-term project summary suitable for web publication

The BICOPOLL project has progressed according to the original plan, and in many instances, much more has been achieved than what was originally anticipated. On the research and demonstration side BICOPOLL has progressed very well. The consortium has identified several aspects of the technique, which could be (and some have been already) improved, and the consortium is looking forward to the coming field season to study these in reality. During 2012 field demonstration trials on strawberry cultivations were carried out in four countries: Finland, Estonia, Italy and the UK, while demo trial sites have been established in several other countries for trials during 2013 (e.g., Norway, Åland, Turkey, Belgium, Germany, Slovenia). Demonstration trials in Sweden and Denmark are being prepared, but may not be available yet in summer 2013.

A conservative estimate for Finland is that the uptake of the entomovector technique by growers is rapid, and in 2012 close to 500 ha of strawberry cultivation was using the technique (over 10% of growers and of the growing area). The excellent control results from the Finnish case have been presented at several international and national conferences. Further field results from BICOPOLL partner trials in summer 2012 showed that in Italy – despite extremely difficult weather conditions – significant reduction over untreated control (mean 39% mould) by biocontrol alone (13%) was achieved, and similarly by the combined treatment (11%), while chemical control (26%) did not differ significantly from the untreated control. In the UK trial entomovectoring by bumble bees resulted in control of the grey mould, which was as good as by the chemical control. In Estonia, field studies at very low pathogen pressure nevertheless showed significantly less grey mould, and higher marketable berry yields in plots entomovectored either by honey bees, or by bumble bees (separate field studies). So far, all field tests using entomovectoring and *Gliocladium catenulatum* (Prestop Mix) have shown excellent control results, and we intend to broaden these trials to include still other BICOPOLL partner countries in 2013 and 2014.

Several project partners have participated in applications for further funding on the topic (e.g., from the last calls in FP7). Active dissemination of project results in scientific, professional, and general media has been pursued. A significant development is the plan to publish a scientific book on the BICOPOLL topic as an outcome for the project; the publishing contract has already been signed (with Springer, to be included in their series "Progress in Biological Control"). A series of practical "how-to-do" handbooks/pamphlets for use by farmers in each of the participating countries (in their national languages) is also being planned.

Pre-project summary

Organic berry and fruit production suffers heavily from the lack of effective disease and pest management tools, and from inadequate insect pollination at times. As a consequence, the expanding demand on organic berries cannot be filled today. BICOPOLL expects to change this, and to significantly improve the yield and quality of organic fruit and berry production and thus, farm economics. We will use bees to (i) target deliver biological control agents to the flowers of the target crops to provide control of problem diseases (or pests), and to (ii) improve the pollination of organic horticultural crops. BICOPOLL will provide a pan-European case study on protecting organic strawberry from its most important disease, the grey mould. In addition we will improve the efficiency of the entomovector technology via innovative research on bee management, manipulation of bee behavior, components of the cropping system, and on the plant-pathogen-vector-antagonist –system, and will investigate possibilities of expanding the use of the concept into other organic berry and fruit growing systems. This is a highly innovative approach to solving some of the most difficult disease and pest problems in organic berry and fruit production, offering solutions in areas where no solutions as yet exist. The entomovector approach represents the only significant breakthrough in sight for improving plant protection in organic cropping systems, particularly in high-value crops. BICOPOLL brings together for the first time the fragmented research in the area, where small groups have worked on their own. We investigate, exploit, and support the natural ecological functions of biocontrol and pollination, and enhance these via innovative management. The entomovector technology contributes to improved resource use and efficiency in production, and enhances local biodiversity unlike most other plant protection systems. The main target groups of the BICOPOLL project are organic strawberry growers, other organic berry and fruit growers, as well as beekeepers and their organizations, to whom technology and knowledge transfer will be implemented during the project via direct contacts.

1. Main results, conclusions and fulfilment of objectives

1.1 Summary of main results and conclusions

Steering of the bees to the target crop

The most important results:

- Although strawberries are obviously not as attractive to honey bees if compared with other crops, honey bees were frequently foraging in the strawberry field where we placed them.
- We could hardly detect any strawberry pollen in the bees' honey stomach. From these data we can conclude, that obviously the honey bees search mainly for pollen and not for nectar, if they forage in strawberry blossoms depending on strawberry varieties.
- Concerning our scientific hypothesis we gained only nonspecific results. From this first investigation period we could not find differences in the number of strawberry foragers, if we compare small with larger bee colonies, although all queens of these experimental colonies were genetically "sister" queens.
- The public awareness about this ongoing CORE organic II project, the interest of strawberry farmers and beekeepers in this approach could be raised with some PR activities.
- Efficacy of commercial bee attractants, and of sugar syrup feeding with fresh strawberry blossom scents, in steering honeybee (and other pollinator) foraging to the target crop (strawberry) was tested at two locations. No significant steering effects were observed, although honey bees visited the strawberry frequently regardless of the treatment. Some attractant effect in increasing bumble bee visits on apple flowers was observed.

Other bees as vectors

- The data provides strong evidence that bumblebees can effectively vector a MCA to reduce significantly *B. cinerea* incidence not only in greenhouse strawberries but also in open field conditions where the landscape is heterogeneous with many competing crops and wild flowers.
- Corbicular pollen gathered from homing bumblebees contained 25-40% of strawberry pollen and 1/3 of the foragers visited mostly or only strawberry during a foraging trip. This allows suggesting that bumble bees are quite effective strawberry pollinators and MCA disseminators.
- Beside strawberry the competing pollen in corbiculas originated from plant families *Rosaceae, Laminaceae* and *Ranunculacea.* The strawberry fields where the samples were gathered were surrounded by orchards and gardens which provided plentiful alternative food resources.
- The data provides indirect proof that bumble bees are able to disseminate the Prestop Mix evenly to strawberry flowers at least within 100 m radius from the hive. There were no significant decrease in disseminated CFUs between first, second and third flowers visited.
- The first experiments with *Osmia cornuta* show that they can be used to collect powdery preparation at very high extent – 106 CFUs for each body part. This means that potentially each osmia-bee can transport several millions of potential inoculum cells to the visited flowers, even when the charge is reduced to the half of the initial one.
- The first experiments with *Osmia cornuta* show that they can be used to collect powdery preparation at the exit of a shelter hosting the nesting materials, on which a dispenser is mounted.

- The dispenser was loaded with 5 ml and 2.5 ml of Amylo-X® (Intrachem-Italia), a powdery biopreparation based on *Bacillus amyloliquefaciens* strain D747, containing $5x10^{10}$ CFU/g, an efficient antagonist of *Erwinia amylovora*, the causal agent of the pear fire blight.
- *Osmia cornuta* females exiting through the dispenser and crawling on the powdery preparation loaded up the biocontrol agent at a very high extent – 10^6 CFUs for each body part (head, thorax, abdomen), as showed by means of bacteriological analyses in Petri dishes on selective medium.
- This means that each *O. cornuta* female can transport several millions of potential inoculum cells to the visited flowers, either at full charge or when the charge is reduced to the half of the initial one.

Carriers and dispensers

- The impact of different carriers on the acquisition of Prestop-Mix on the bumblebee body and the dissemination into the strawberry flower was assessed. This by blending Prestop-mix and Maizena-Plus, which is a corn starch based sauce thickener. The different mixtures had concentration ratios (w/w) of 100/0; 75/25; 50/50; 40/60; 25/75 and 10/90 Prestop-mix/Maizena-Plus. When the acquisition directly after the dispenser was tested, concentrations on the bumblebee body dropped together with decreasing amounts of Prestop-Mix added. However, after a flight of 60 seconds, the number of spores per bumblebee body showed no significant difference between the ratios 70/25, 50/50 and 40/60. Subsequently, the numbers of spores deposited by the bumblebee in the strawberry flower were examined for a 50/50 (w/w) ratio and for Prestop-Mix alone. Here a ten times higher deposit was measured when Maizena-Plus was added compared with Prestop-mix alone.
- Using laser diffraction it was seen that Prestop-mix has a wide size distribution, this due to the fact that this product contains spores, mycelium and other types of fungal cell structures which have a varying size range. Also other unknown formulation components cause different wide peaks in the laser diffraction pattern. Using microscopy, an attachment from the fungal spore to the Maizena-Plus granule was observed.
- The safety of the bumblebee nest for a combined use with Prestop-mix was determined using a miniature dispenser test. Here, a miniature hive with five female bumblebees was used. The bumblebee workers were forced to forage for sugar water through a 20cm long miniature dispenser. Acute parameters as worker mortality and chronic parameters as drone production were determined to assess the powder formulation safety for the vector. This approach reflects the same exposure circumstances as a real dispenser. Using this test, no negative impact on the bumblebees was observed by Prestop-mix.
- The first experiments were conducted with the existing side-by-side passageway (SSP) dispenser. Dispensers like these have different compartments and this seemed too difficult for the bumblebees to pass through. In search of a better dispenser, the optimal length was established by allowing the bumblebees to walk through dispensers with a varying length from 5 to 40 cm. It was shown that a dispenser length of 20cm gave an optimal loading of microbial control agent on the bumblebee body. Second, a funnel (F) dispenser was developed with a different entrance and exit route. In order to prevent the bumblebees from using the exit holes as entrance, bumblebee-in-closers were mounted. The F-dispenser gave a ten times higher loading of the bumblebees than the SSP-dispenser. To easily use this in the field, a built-in dispenser was developed. Here, the dispenser is mounted on top of the lid of the bumblebee hive lid.
- For the dispersion of Prestop-mix with the honeybee, the BeeTreat dispenser was used. To see what de influence of the mounting of the dispenser on the bee colony is, cameras were installed inside the dispenser. With an empty dispenser, it was clear that the dispenser

space was used by workers for other functions such as ventilation of the hive and protection. When the dispenser was filled with powder, bees had problems passing the large amount of powder and fights between bees were observed. This behaviour was probably caused by the physical presence of the powder. Also, it was seen that honeybees had problems getting up the landing strip, a less steep angle and grooves on the landing strip can solve this problem.

- For *Osmia cornuta*, a low cost dispenser was developed. Therefore, per nest, 50 females and 100 males in their cocoons were released. After the mating and when the females started nesting, a metal grid was installed in front of the nest to oblige the females to get out passing through the dispenser. A free space above the dispenser gave female bees the possibility of returning to their nest without crossing the dispenser. Although some time was needed to learn how to enter the nest using the free space above the dispenser, it ended up being successful. Exiting and passing the dispenser did not result in any hindrance. This experiment shows that successful nesting activity and a good dispersion of microbial control agent can be obtained with the use of solitary bees.
- For *Osmia cornuta*, a low cost dispenser was developed. Being *O. cornuta* a solitary nesting bee species, showing gregarious nesting behaviour, the dispenser was inserted in the shelter hosting the nesting material (wooden blocks with cavities measuring 9 mm diameter x 15 cm length in which paper tubes were inserted).
- Two polystyrene made honeybee hives (37x24x48 cm, height, width depth) were adapted as a shelter, by removing the cover and positioning the box vertically. For each nest, 50 females and 100 males in their cocoons were released.
- The nesting material was placed inside, and a very simple dispenser was inserted in the upper part of the shelter box, leaving a free space of around 8 cm between the dispenser and the top of the shelter box.
- The dispenser consisted in a plastic ramp housed in an wooden box (6x24x7 cm, height, width, depth) opened in the back and in the front parts
- After the mating, females started foraging freely flying from and to their nests. When most of them have initiated nesting activities, a metal grid was installed in front of the nesting material ending exactly at the bottom of the box housing the dispenser ramp.
- In this way, females were obliged to get out passing through the dispenser. Coming from their nest, they crawled on the powder at the base of the ramp, rose on the ramp and exited the dispenser box to go foraging. The free space between the dispenser and the top of the shelter gave female bees the possibility of returning to their nest without crossing the dispenser.
- Some time was needed to learn how to enter the nest using the free space above the dispenser, it ended up being successful. Exiting and passing the dispenser did not cause any hindrance.
- This experiment shows that successful nesting activity and a good dispersion of microbial control agent can be obtained with the use of solitary bees.

Suppression of grey mould by antagonists

- The biofungicide Prestop-Mix, based on *Gliocladium catenulatum* J1446 Gilman &Abbott (now *Clonostachys rosea* f. *catenulate* J1446 (Gilman &Abbott) Schroers), *Clonostachys rosea* f. *rosea* (Link: Fr.) Schroers, Samuels, Siefert and W. Gams (formerly *Gliocladium roseum* Bainier), *Trichoderma asperellum* Samuels, Lieckf. &Nirenberg and *Botrytis cinerea* Pers.: Fr. S-TR-20 (an infected strawberry at Erzincan, Turkey) were used for an agar plate assays.
- One flower was placed on 15 ml of sterile 1.5 % water agar in 10-cm-diameter petri dishes.

Each flower was inoculated at one point at the base of the petals with 10 µl drops of spore suspension containing 10^3, 10^4or10^5conidia/ml of *B. cinerea*, alone ore mixed with 10^3, 10^4or10^5conidia/ml of *Clonostachys rosea* f. *catenulate* J1446, *Clonostachys rosea* f. *rosea* or *Trichoderma asperellum.*

- As a result; There were no significant differences between disease severity (%) of spore suspensions (10^3, 10^4,10^5 conidia/ml) of *B. cinerea*. The *C.catenulata*10^5, *C.rosea*10^5 and *T. asperellum*10^5 exhibited a remarkably higher disease control activity on petioles, whereas disease control activity on strawberry petioles was not significantly different between 10^3 and 10^4 treatments.
- Pot Experiments; Strawberry (cv. Aromas) seedling were planted in 25-cm diameter plastic pots (1 seedling per pot) containing a soil mix. The plants were grown in a climate-controlled greenhouse where air temperature averaged 18° to 24°C during the day and 13° to 17°C at night. Pot experiments will start soon.
- FieldTrials; The experiment was located in Erzincan, Turkey. The frigo plants of 'Aromas' strawberry cultivars were used. Frigo plants were planted in raised and black polyethylene mulched beds of June in 2012 at distances of 25 ×30 cm. The strawberry plants were grown in the field using routine cultivation practices. No chemical fungicides were used.
- The field contained 7564 plants and consisted of twenty one rows on raised beds. The beds were provided with trickle irrigation. One month, flowers and stolons were cut down for homogenous and strong growing was taken place. The experimental design was completely randomized with 4 repetitions. Each plot (no cage and cage) consisted of 70 strawberry plants planted in an area of 3.30 m × 4 m. The total yield of each plot was recorded and then divided by the total number of plants from each plot and expressed in g/plant. During the season strawberries were harvested 5 times. Yield was very low for 2012.

Landscape management for BCA dissemination

- In 2012 ITACAA started the research for **Task 6.1** - Role of ecological infrastructures in supporting *Osmia* establishment (ITACAA), and **Task 6.3** - Impact of ecological infrastructures on wild pollinators in orchards
- One trial was planned in 2012 in order to maximize the foraging activity of *Osmia cornuta* on pear (*Pyrus communis*) by supporting its establishment in the orchard thanks to the presence of ecological infrastructures, whose period of flowering occurs before pear flowering.
- Due to the adverse weather conditions it was not possible to carry on the research. After a very cold February (a lot of snow, with temperatures down to -13°C), suddenly the weather turned towards very warm temperatures.
- Cocoons were immediately brought to the pear fields, but the flowering of the crops progressed much faster than emergence of the adults from cocoons, and the flowering of the two crops overlapped almost entirely.
- This meant for us that it was not possible to use the peach flowering as the period necessary for *O. cornuta* females to emerge from cocoon, feed themselves and start with the nesting activity before pear flowering, and these behaviors were largely performed while pear had already started blooming.
- This prevented the possibility to compare the behavior of *O. cornuta* females in the two ecological situations, and we decided not to go on with the collection of the data.
- In September 2012 we started with the preparation of the fields in which the trials will be carried on in 2013.
- Along one side of a pear field surrounded by extensive cultivations, a row of 50 m of *Brassica napus* and *Brassica oleracea* with different ripening times from 30 to 120 days were sowed.

- We expect that *Brassica* will survive the winter at the stage of 6-7 leaves, and in this condition it will be flowering at the beginning of march, before pear bloom starts.
- *Osmia cornuta* population was rearing was carried on by opening the nesting tubes, extracting and sexing the cocoons. Checks were made for parasites. *Osmia cornuta* populations undergo overwintering process following a scheme of gradual reduction of the temperature down to 4°C, They will be kept in the refrigerator for at least 120 days.

Safety to bees and consumers

- Detection of biocontrol agent *Gliocladium catenulatum:*
  Efficient and specific qPCR assay for detection and quantification of *G. catenulatum* J1446 was developed (additional strains for specificity testing are needed). DNA extractions from difficult matrices (bees, BCA with carrier substance) were optimized.
- Do honey bees transfer the BCA successfully to the field?
  The behaviour of forager bees in the dispenser without and with Prestop Mix (5 g) was observed by two video cameras. Observations show that Prestop Mix changes bee behaviour and results in waste of product and some loss of bees. Most of Prestop Mix was spent first hour after application. We suggest using less Prestop Mix at a time and improvement of the dispenser.
- Learning ability of honey bees exposed to BCA:
  The effect of exposure to Prestop Mix on learning and memory formation was tested in the laboratory on harnessed foragers. The olfactory conditioning of PER (proboscis extension reflex) was used to test differences between treated and untreated bees. Before learning responsiveness to sucrose solution was tested. Bees were conditioned to the olfactory stimulus once and tested for PER response by presenting odour alone one or 12 min after conditioning. No statistically significant differences were found between treated and untreated bees.
- Life span of honey bees exposed to BCA:
  Affect of Prestop Mix on mortality of honey bees in cages in incubator (34°C, 96 hours) was tested. Bees were exposed to high amounts of Prestop Mix for 15 s only first day (acute exposure) or every day (chronic). Control group was not exposed to Prestop Mix. Each day number of dead bees was counted. Exposure to high amounts of Prestop Mix (in laboratory conditions) increased mortality of honeybees.

Field applications

- In the BICOPOLL-project we aim to demonstrate Europe-wide the applicability of the entomo-vectoring approach, using as a case study the control of strawberry grey mould *Botrytis cinerea*, with the biocontrol fungus *Gliocladium catenulatum*, vectored by honey bees or bumble bees. The joint trial targets strawberry cultivations in the open field, and ideally includes four treatments: untreated control, chemical fungicide, entomovectored biocontrol, and chemical + biocontrol combined. In organic fields, no pesticide treatments are included. The proportion of mouldy berries, and/or the marketable yield of healthy berries is recorded from each treatment, along with other parameters of local interest. In 2012 such joint field trial was carried out in Estonia, Italy and in the UK – besides Finland, where large areas commercially already use entomovectoring (see below).

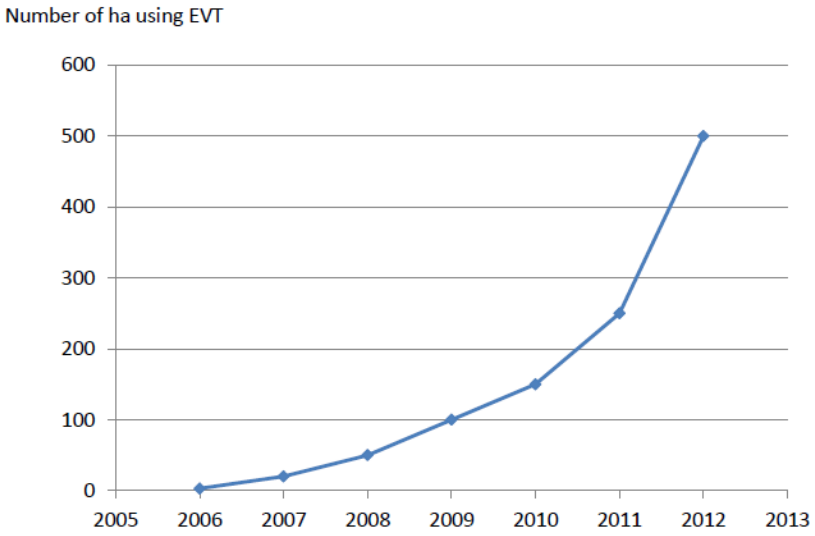


Fig. 1. Commercial uptake of honey bee vectored precision biocontrol on strawberry in Finland. The method has been approved for general use in 2008.

- A conservative estimate for Finland is that close to 500 ha of strawberry cultivation was using the technique in 2012. The excellent control results have been presented earlier e.g. at the 63rd ISCP in Ghent in 2011. Field results in summer 2012 in Italy showed – despite extremely difficult weather conditions – significant reduction over untreated control (mean 39% mould) by biocontrol alone (13%), and by the combined treatment (11%), while chemical control (26%) did not differ significantly from the untreated control. In the UK trial entomovectoring by bumble bees resulted in control of the grey mould, which was as good as by the chemical control. In Estonia, field studies at very low pathogen pressure nevertheless showed significantly less grey mould, and higher marketable berry yields in plots entomovectored either by honey bees, or by bumble bees (separate field studies). So far, all field tests using entomovectoring and *Gliocladium catenulatum* (Prestop Mix) have shown excellent control results, and we intend to broaden these trials to include still other BICOPOLL partner countries in 2013 and 2014.

1.2 Fulfillment of objectives

To what extend did the project achieve its objectives?

BICOPOLL achieved and in most cases, exceeded practically all its scientific and technical objectives during the reporting period. For project dissemination activities much more was carried out than planned, while for some envisaged parts there still is scope for improvement (e.g., number of scientific and popular articles by some partners).

2. Milestones and deliverables status

Milestones:

No[1]	Milestone name	Planned delivery month[2]	Actual delivery month[2]	Means of verification
M3.3	Use of solitary bees as vectors and pollinators	36	18	Report available
M4.2	Improved dispenser models	18	18	Prototypes avalable
M 8.1	Quantitative PCR-method for detection of *Gliocladium catenulatum*	14	16	Method validated

Deliverables:

No[1]	Deliverable name and language	Nature[3]	Dissemination level[4] and link to the document	Planned delivery month[2]	Actual delivery month[2]
D3.3	Use of solitary bees for primary and secondary BCA vectoring	Report, protocol	PU	36	18
D4.2	Improved dispenser models	Prototypes	CO	18	18
D6.1	Impact of key ecological infrastructures on foraging by managed and wild bees	Report	PU	24	18
D7.1	Technical and ecological feasibility of entomovector technology in strawberry production in Europe	Report, paper	PU	30	18
D 8.1	Quantitative PCR-method for detection of *Gliocladium catenulatum*	Protocol report	PU	14	16

Additional comments (in case of major changes or deviation from the original list)

[1] Please use the numbering convention <WP number>.<number of milestone/deliverable within that WP>. For example, deliverable 4.2 would be the second deliverable from work package 4.
[2] Measured in months from the project start date (month 1).
[3] Please indicate the nature of the deliverable. For example Report, Paper, Book, Protocol, Prototype, Website, Database, Demonstrator, Meeting, Workshop…
[4] Please indicate the dissemination level using one of the following codes: PU = Public; INT= Internal (Restricted to other project participants); RE = Restricted to a group specified by the consortium; CO = Confidential, only for members of the consortium.

3. Work package description and results:

WP 1	Project management
Responsible partner: P1, UHEL, WP manager Heikki Hokkanen	

Original description of work:

WP1 aims to ensure that the activities of the project are successfully carried out. This is achieved by:

- Implementation of the organizational structure for the consortium
- Ensuring timely initiation of the project according to the contract and consortium agreement
- Ensuring that the individual contracts are synchronised both in time and content
- Ensuring administrative coordination and effective interaction with the CORE Organic II secretariat
- Coordination of work packages and project activities, and ensuring that deliverables are obtained and milestones are met as planned
- Solving problems raised during the project according to the consortium agreement

Task 1.1. Overall coordination and communication (UHEL)
The overall coordination and management of the consortium is the responsibility of the co-ordinator, UHEL. The co-ordinator manages the project's ultimate decision making body, the annual General Assembly (GA), consisting of representatives from all partners. The co-ordinator with support of the GA and the Work-Package Steering Committee coordinates, focuses, and monitors all aspects of research. Bi-monthly management meetings with all WP leaders via teleconferencing (Skype) are held throughout the project. The co-ordinator ensures effective communication between the partners and with the CORE Organic II secretariat. With support from all project partners, the dissemination activities as outlined in section B 4e are effectively executed.

Task 1.2. Work-package coordination and management (all Participants)
Every partner is involved in project coordination and management as a WP leader, member of a WP Steering Committee (WPSC), and as a designated representative at the GA. They participate in the planning and timely execution of the work in the WPs and tasks, prepare documents and reports, and participate in project meetings and present their reports. All partners contributing to a WP participate in the planning via the WPSC.

Task 1.3. Organization of consortium meetings (UHEL, others)
Project kick-off meeting will be held in Helsinki (UHEL), and the annual project meetings in other partner countries. The final conference will present the project results to a wide audience. Smaller ad-hoc meetings around a WP or a specific question are organized as needed.

Task 1.4. Dissemination of results (all Participants): Description of activities is given in section B4e.

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

Project management in general has functioned well, all the intended activities have been carried out, including consortium meetings and the dissemination of results.

B- comments on deviations from the original plan:

During the peak field season it has turned out to arrange the envisaged bi-monthly (virtual) management meetings with all WP-leaders, as was planned. To our knowledge, this has not hampered the proper execution of the project.

WP 2	Honey bees as vectors and crop pollinators

Responsible partner: P2, LAVES, WP manager Otto Boecking

Original description of work:

The main objectives of **WP2** are:

- To develop practical methods for steering foraging bees to the target crop
- To optimise the required pollinator (hive) density for vectoring and pollination on the target crop
- To determine the practical needs for managing beehives used to disseminate BCA, and to develop a practical guide/handbook to the biocontrol/pollination service for berry growers and beekeepers
- To investigate the effect of vegetation management in and around strawberry fields on the success of targeted BCA vectoring and pollination (**see WP6, Task 6.2.**).

Task 2.1. Steering of foraging bees to the target crop

2.1.1. Colony size (LAVES): Pilot investigations indicate that small and specially prepared honey bee colonies pollinate closer to the site of their hive than large colonies. Bee colonies differing in size are compared for foraging range and location. Bees are quantitatively recorded along a line-transect (100 m within 10-15 minutes) within the strawberry field in spatial relation to the hive location. Steering to the target crop will be confirmed based on pollen analysis from the honey bee stomach and the bee cuticle, recovered from individual homecoming foragers/pollinators entering the hive.

2.1.2. Amount of brood and pollen stores (**UHEL**, LAVES): Honey bee foraging is steered by colony needs. Pollen is collected when there are abundant larvae, and pollen stores in the hive are low. These parameters are manipulated in hives at strawberry fields, and foraging patterns determined as in 2.1.1.

2.1.3. Pheromones and plant volatiles (**UHEL**, NIB): Several brands of “bee attractants”, based on bee’s own pheromones (e.g., citral and geraniol) and flower scents, are commercially available for attracting bees to target crops (orchards in particular). Their usefulness in the context of entomovectoring is studied, and tailor-made blends specifically for strawberry are developed and tested for improved performance. Impacts on honey bee foraging are determined as in 2.1.1.

Task 2.2. Vegetation management in and around strawberry fields (LAVES, UHEL**)**
In collaboration with Task 6.2, the impact of growing low-trimmed blossoming white clover between strawberry rows on the foraging activity by managed honey bees is assessed by frequent direct counts in ‘treated’ and ‘untreated’ sections of the fields, as in 2.1.1. (see also WP6, Task 6.2.).

Task 2.3. Dynamics of nectar and pollen production in the target crop (LAVES)
Nectar secretion dynamics (quantity, quality and availability during the course of a day) of the crop (strawberries) needs to be investigated for current varieties, to allow determination of the density of bees necessary to provide the pollination and disease control services. Nectar is extracted from strawberry flowers with micro capillary tubes throughout the day, and over the lifetime of a flower, and the sugar content analysed with HPLC. Pollen availability is monitored via similar sampling.

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

At LAVES, two complete blocks of experiments and data collections could be accomplished on spot of two different strawberry farms; one in the period May/June and another in July 2012. Many field data (inclusive weather data, potential competitive foraging crops in the bees’ foraging range, etc.) and bee samples could be collected. The quantification of the steering of the bees to the target crop (strawberries) was confirmed based on pollen analysis from the honey bee stomach and the bee cuticle, recovered from individual homecoming foragers/pollinators entering the experimental hives. The most important results:

- Although strawberries are obviously not as attractive to honey bees if compared with other crops, honey bees were frequently foraging in the strawberry field where we placed them.

- We could hardly detect any strawberry pollen in the bees' honey stomach, although for this N=4.384 bees were investigated individually with standard laboratory methods. From these data we can conclude, that obviously the honey bees search mainly for pollen and not for nectar, if they forage in strawberry blossoms depending on strawberry varieties. For the next investigation period we have to reconsider the sampling method and analysis of the foraging bees.

- Concerning our scientific hypothesis we gained only nonspecific results. From this first investigation period we could not find differences in the number of strawberry foragers, if we compare small with larger bee colonies, although all queens of these experimental colonies were genetically "sister" queens.

- The public awareness about this ongoing CORE organic II project, the interest of strawberry farmers and beekeepers in this approach could be raised with some PR activities.

At UHEL, efficacy of commercial bee attractants, and of sugar syrup feeding with fresh strawberry blossom scents, in steering honeybee (and other pollinator) foraging to the target crop (strawberry) was tested at two locations. No significant steering effects were observed, although honey bees visited the strawberry frequently regardless of the treatment. Some attractant effect in increasing bumble bee visits on apple flowers was observed.

B- comments on deviations from the original plan:

All planned working steps could be accomplished and the milestones for this reporting period could be achieved. As the result of this project part, we will supply data for the missing link of potential number of distributors (honey bees) for the antagonist powder and the time frame for the dispenser/antagonist application. No modifications or changes were necessary, with few on to the initial working-time and expenditure planning.

WP 3	**Other bees as vectors**
Responsible partner: P3, EULS, WP manager Marika Mänd	

Original description of work:

Objectives of WP3 are

- To determine the efficiency of bumble bees in vectoring BCA to strawberries, and in enhancing pollination, in the greenhouse and in open field cultivations
- To evaluate the specific requirements in the use of commercial bumble bees for targeted biocontrol and pollination on strawberries
- To assess the potential of using solitary bees (*Osmia* spp.) for targeted biocontrol and enhanced pollination in open field strawberry and pome fruit orchard situations

Task 3.1 Bumble bees as vectors in greenhouse conditions (UGHE)

3.1.1. Assessment of *Bombus terrestris* foraging

Study on the reliability of *B. terrestris* as vector will focus on their foraging behaviour under greenhouse conditions. Measured parameters include flight time, handling time, and number of flower visits per bee. All bumble bee workers will be labelled with opalith plates at the start of the experiment (before opening of the hive).

3.1.2. BCA dissemination capacity of *Bombus terrestris*

a) *Effect of distance to target flowers*: *B. terrestris* hives containing a queen, 20 workers and her brood, and the dispenser developed by Mommaerts et al. (2010) are used. Strawberry plants at various distances, with individually labelled flower buds are used for the test. Flowers and the bee are both individually collected after the first bumblebee visit. Plants placed at 5m-10m-15m-20m from the hive are assayed. 20 flowers/site are collected and analysed; the experiment is replicated twice.

b) *Effect of multiple bumblebee visits*: A similar experiment as above, but bumblebees are allowed to visit the flowers during the entire life-span of a flower. Thereafter all flowers are collected and the amount of BCA per flower is determined.

3.1.3. Efficacy in grey mould control

The efficacy of *B. terrestris* as vector to control *Botrytis cinerea* in the greenhouse is evaluated as under 3.1.2, using *Gliocladium catenulatum*. At each distance 20 plants with 100 labelled freshly opened flowers are inoculated with 2000 *B. cinerea* spores. Bee disseminated biocontrol is introduced at varying times, and the efficacy of control scored at two time points: at picking, and 2 days after picking (Mommaerts et al., 2011b).

Task 3.2 Bumble bees as vectors in open field conditions (EULS)

3.2.1. Management of bumble bee foraging

Commercially-produced bumble bee colonies (*B. terrestris*) are employed. Bee densities and colony locations in the strawberry field are manipulated to estimate the optimal number of colonies. Foraging distances are determined by recapturing marked individuals along transects, and by following individual bees. Foraging parameters (flight time, handling time, number of flowers visited) of individual bees are quantified in the open field. Foraging preferences are determined by sampling the pollen loads of returning foragers and the pollen stored in nests. Relative flower densities, reward availabilities and competition by other pollinators are analysed.

3.2.2. Dispersal distance and control effect of BCA

The number of BCA (*Trichoderma* and *Gliogladioum*) inocula on flowers is determined at varying distances from the hives. The proportion of healthy and infected fruits is calculated. Based on the experimental data and literature, a meta-analysis will be performed to evaluate the specific requirements for using commercial bumble bees for targeted biocontrol and pollination on strawberry.

Task 3.3 Solitary bees as vectors (ITACAA, UHEL)

3.3.1 Rearing and management of the solitary bees *Osmia cornuta* and *Osmia rufa*

Cocoons of *O. cornuta* and *O. rufa* will be overwintered under controlled conditions, and introduced slightly before initiation of flowering to a strawberry field and pear orchard, and manipulated to ensure the emergence of females and males at the onset of blooming. In May-June nesting tubes will be retrieved from the field. In September, cocoons of the new generation will be extracted and the reproductive success evaluated.

3.3.2. Efficacy of *Osmia* in delivering BCA from dispenser to target flowers (primary dissemination)

After the establishment of an *Osmia* population and the insertion of the dispenser, flowers at increasing distances from the nesting shelter (10, 50, 100, 200 m) and in the four directions (North, South, East, West) will be sampled and plated to evaluate the amount of BCA on the floral organs. Non-visited flowers from net-protected buds will act as negative controls. Sampling will be repeated three times during blooming.

3.3.3 Efficacy of *Osmia* in disseminating BCA from flower to flower (secondary dissemination)

The efficacy of pollinators in transferring the BCA from spray-inoculated flowers to the stigmas of newly opened ones will be determined at different time intervals after inoculation till the end of blooming. Flower samples will be collected and analyzed for the BCA; untreated and treated flowers will act as controls.

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

1. **Bumblebees as vectors in greenhouse conditions**

At UGHE the study was conducted in strawberry production greenhouses. Queen-right bumblebee hives equipped with special dispensers containing biofungicide Prestop-Mix were introduced to the study area. The *Botrytis* infection was measured in bumblebee vectored

Prestop-Mix treated and untreated strawberries. In addition, the bumblebee effectiveness in depositing CFUs on flowers was studied. The most important results:

- Vectoring of Prestop-Mix by bumblebees resulted in a higher crop production, as 71% of the flowers developed into healthy red strawberries at picking (preharvest yield) as compared with 54% in the controls. In addition, these strawberries were better protected, as 79% of the picked berries remained free of *B. cinerea* after a 2 day incubation (post-harvest yield), while this percentage was only 43% in the control.
- During first 60 s of the flight the bumblebees lost 81% of Prestop Mix they had achieved. The mean numbers of CFU per flower found in the first, second and third strawberry flowers visited were 23.4±0.4,14.7±6.3 and 16.5±2.0 respectively. The reduction of CFU between first three flowers was not significant. When comparing the CFU transporting efficiency at different distances (zones 0–8 m, 8–18 m and 18–21 m) also no significant changes were found.

2. Bumblebees as vectors in open field conditions

At EULS, the study was conducted in two strawberry farms. Bumblebee hives with Prestop-Mix containing dispensers were placed near strawberry fields. Two treatments were established: bee-delivered Prestop-Mix treatment and untreated control. Healthy and *Botrytis*-infected berries were counted from treated vs untreated plots as well as at different distances from the hives. The field data such as flower densities, nectar and pollen amount in strawberry flowers, flower visitors on strawberries etc. were collected. Pollen pellets (N= 270) from returning forager bumblebees were gathered and identified. The most important results:

- The corbicular pollen gathered by the bumble bees contained 25-40% of strawberry pollen and 1/3 of the foragers visited mostly or only strawberry during a foraging trip. The other dominantly-collected pollens belonged to the plant families *Rosaceae, Laminaceae* and *Ranunculaceae.*
- The rate of strawberry infection by grey mould decreased from 18% on the isolated control plots to 6% on the plots visited by bumble bees.
- There were no differences in proportion of infected berries within 100 m from the bumble bee hives.

3. Solitary bees as vectors

3.3.1 Rearing and management of the solitary bees *Osmia cornuta* and *Osmia rufa*

At ITCAA, cocoons of *O. cornuta* were overwintered under controlled conditions. Male and female cocoons were sexed on the base of their weight and dimensions, and two groups made of 50 males and 50 females were constituted. When the meteorological conditions turned favourable, males and females were brought gradually to 20°C under laboratory conditions for two days. When the first males emerged from cocoons, the cocoons, protected in small polystyrene boxes with respiratory holes were brought to the pear orchard slightly before blooming initiation. At that moment, the nearby peach (*Prunus cerasus*) orchard was also starting flowering. In May-June nesting tubes were retrieved from the field.

In September, cocoons of the new generation will be extracted and the reproductive success evaluated.

3.3.2. Efficacy of *Osmia* in delivering BCA from dispenser to target flowers (primary dissemination)

After the establishment of an *Osmia* population and the insertion of the dispenser, flowers at increasing distances from the nesting shelter (10, 50, 100, 200 m) and in the four directions (North, South, East, West) were labeled and protected with insect-proof net in order to prevent pollinator visits.

The unstable meteorological conditions, and the sudden increase of the air temperature made the blooming of peach and pear orchards overlap because pear blooming went very fast, and faster than the time needed to females to start their nesting activities. This prevented us to perform the trial on the distribution of the biocontrol agent *Bacillus amyloliquefaciens* on pear flowers (against the causal agent of the fire blight *Erwinia amylovora*), and no sampling of flowers was carried because when females were ready to an intense foraging activity the pear blooming was almost concluded.

3.3.3 Efficacy of *Osmia* in disseminating BCA from flower to flower (secondary dissemination)

For the same reasons explained above, it was not possible to perform the field experiments with *O. cornuta* in delivering the antagonist to the pear flowers, and to spread it from flower to flower as planned.

B- comments on deviations from the original plan:

Due to adverse meteorological conditions, it was not possible to perform the trials on the ability of *O. cornuta* to deliver the biocontrol agent from the dispenser to the pear flowers and from flower to flower.

We decided then to perform a study on the **capacity of each *O. cornuta* female to load the biocontrol agent,** and **to evaluate which part of the body is more efficient in loading the powdery formulation** of *Bacillus amyloliquefaciens* (Amylo-x ® Intrachem-Italia), used as a spray, to control *Erwinia amylovora* (the causal agent of the pear fire blight).

The amount of biopreparation that each individual *O. cornuta* female carries has been tested by capturing exiting *O. cornuta* females from the dispenser developed within the project. The dispenser was charged with 5 and 2.5 ml of powdery formulation. Eight bees per charge amount were sacrified and sectioned in head, thorax, abdomen and plated on proper medium. Petri dishes were incubated at 36°C and CFU were counted after 24 hours.

In both trials bees were able to collect powdery preparation at very high extent – 10^6 CFU for each body part. This means that potentially each bee can transport several millions of potential inoculum cells to the visited flowers, even when the charge is reduced to the half of the initial one.

WP 4	**Dispensers and carriers**
Responsible partner: P4, UGHE, WP manager Guy Smagghe	

Original description of work:

The aim of this WP is

- the development of carrier substances for optimal bee loading and transport capacity of the BCA to the crop
- the technical development of dispensers to ensure the easiness of their operation, technical reliability, survival of inoculum, improved protection from rain, humidity and heat, minimised need of filling, and effectiveness in separating ingoing from outgoing bees
- to determine the safety of inoculum carrier substances to honey bees, bumble bees and solitary bees

Task 4.1. Evaluation of carrier substances and their mixtures (UGHE)

The bumblebee body load using single carrier substances mixed with BCA at different ratios (5/95; 10/90; 25/75; 50/50; 75/25; 10/90 and 95/5) or, with mixtures of carriers with the BCA, is determined. The carriers considered include corn starch, wheat flour, potato starch, oat meal, cellulose and kaolin. The experiment is conducted in the laboratory with *B. terrestris* and the dispenser as developed by Mommaerts et al. (2010). As the reference BCA Prestop-Mix containing *G. catenulatum* J1446 will be used. The impact of carriers on bumblebee load is determined immediately after leaving the dispenser (=direct load) and after a flight in a flight cage. 20 bumblebee workers are collected at each point, and the experiment replicated twice.

Amount of BCA present on the body will be evaluated on each bee. In addition, the time a bumblebee spends in the dispenser (before leaving) is measured, and behavioural observations are taken (e.g., grooming) before bees leave the dispenser. The results will be used also for experiments in WP3.

Task 4.2. Biosafety of the optimal carriers and carrier mixtures (UGHE)

a) *Impact on bees*: The carrier substances may affect worker survival or the colony development. Assessment end points are evaluated with a new laboratory test (two-way miniature dispenser test, Mommaerts et al. (2011). Workers are exposed to the substances and the impact on worker survival followed daily for the first three days after exposure and then weekly for 5 weeks. As indicator of sublethal effects on reproduction, the number of drones produced per nest is weekly scored as described by Mommaerts et al. (2006) during 5 weeks.

b) *Impact on the BCA:* The carrier substance must not interfere with the viability of the BCA. This is assessed by incubating the various carrier substances and their mixtures together with the BCA at 24°C and 80% RH. Samples of the BCA are taken after 0 h, 12 h, 24h, 48 h, and 72 h of incubation, and the germination of the BCA determined from 100 conidia. Germination is compared with that of the reference product Prestop-Mix.

Task 4.3. Optimization of dispensers (**UGHE**, ITACAA, UHEL)

Various dispensers for honey bees, bumble bees and for solitary bees need to function optimally in order to be efficient, safe, and user-friendly. Key parameters are determined for the most promising dispenser types available, and improvements are suggested when appropriate. Parameters to be measured include (i) the influence of dispenser on the number of exiting/homing bees, and (ii) the ability of the dispenser in separating ingoing from outgoing bees. In addition (iii) the frequency of refilling is determined by collecting individual workers leaving the dispenser at 0 h, 1 h, 4 h, 8 h, 12 h, 24 h, 48 h and 72 h after filling the dispenser. The loads on different body parts of the bees (ventral part of the abdomen, thorax, and the legs) are analysed separately as in Mommaerts et al (2010).

Task 4.4. Development and functioning of a dispenser for *Osmia* nesting shelters (**ITACAA**, UHEL)

A new prototype is developed based on the model of Maccagnani et al. (2006). Steering the exiting females toward the dispenser positioned in the upper part of the shelter is the key point for development and various solutions are to be tested. The behaviour of the bees is monitored for 30 min 4 times per day over 7-10 days and the correct/incorrect use of the exit and entrance calculated. The experiment is repeated three times.

The efficacy of the dispenser and of the carrier substance in contaminating exiting females with the BCA is determined using a powdery preparation of *Bacillus amyloliquefaciens.* Ten exiting females are captured, killed and their body parts (head, abdomen and legs) analysed separately to determine the amount of inoculum at various times after dispenser loading (0-30 min, 180 min, 24 h) as in Maccagnani et al (2006). Performance in the field and/or polytunnel is studied on strawberry using Prestop-Mix and/or *Trichoderma* for *Botrytis* control.

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

1. Carriers
    a. Impact

The impact of different carriers on the acquisition of Prestop-Mix on the bumblebee body and the dissemination into the strawberry flower was assessed. This by blending Prestop-mix and Maizena-Plus, which is a corn starch based sauce thickener. The different mixtures had concentration ratios (w/w) of 100/0; 75/25; 50/50; 40/60; 25/75 and 10/90 Prestop-mix/Maizena-Plus. When the acquisition directly after the dispenser was tested, concentrations on the bumblebee body dropped together with decreasing amounts of Prestop-Mix added. However, after a flight of 60 seconds, the number of spores per bumblebee body showed no significant difference between the ratios 70/25, 50/50 and 40/60. Subsequently, the numbers of spores deposited by the bumblebee in the strawberry flower were examined for a 50/50 (w/w) ratio and for Prestop-Mix alone. Here a ten times higher deposit was measured when Maizena-Plus was added compared with Prestop-mix alone.

b. Interaction

Using laser diffraction it was seen that Prestop-mix has a wide size distribution, this due to the fact that this product contains spores, mycelium and other types of fungal cell structures which have a varying size range. Also other unknown formulation components cause different wide peaks in the laser diffraction pattern. Using microscopy, an attachment from the fungal spore to the Maizena-Plus granule was observed.

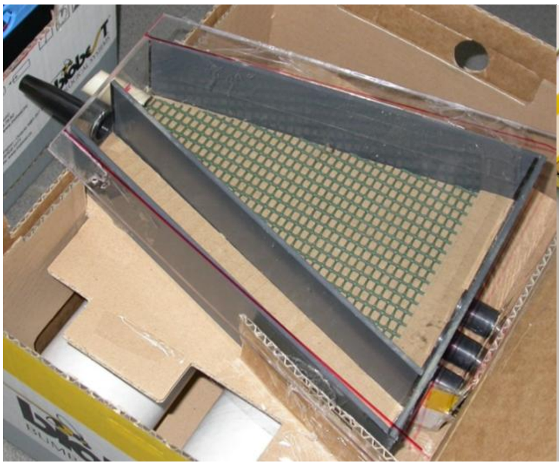
c. Safety

The safety of the bumblebee nest for a combined use with Prestop-mix was determined using a miniature dispenser test. Here, a miniature hive with five female bumblebees was used. The bumblebee workers were forced to forage for sugar water through a 20cm long miniature dispenser. Acute parameters as worker mortality and chronic parameters as drone production were determined to assess the powder formulation safety for the vector. This approach reflects the same exposure circumstances as a real dispenser. Using this test, no negative impact on the bumblebees was observed by Prestop-mix.

2. Dispensers

a. Bumblebee

The first experiments were conducted with the existing side-by-side passageway (SSP) dispenser. Dispensers like these have different compartments and this seemed too difficult for the bumblebees to pass through. In search of a better dispenser, the optimal length was established by allowing the bumblebees to walk through dispensers with a varying length from 5 to 40 cm. It was shown that a dispenser length of 20cm gave an optimal loading of microbial control agent on the bumblebee body. Second, a funnel (F) dispenser was developed with a different entrance and exit route (Fig 1). In order to prevent the bumblebees from using the exit holes as entrance, bumblebee-in-closers were mounted. The F-dispenser gave a ten times higher loading of the bumblebees than the SSP-dispenser. To easily use this in the field, a built-in dispenser was developed (Fig 1). Here, the dispenser is mounted on top of the lid of the bumblebee hive lid.



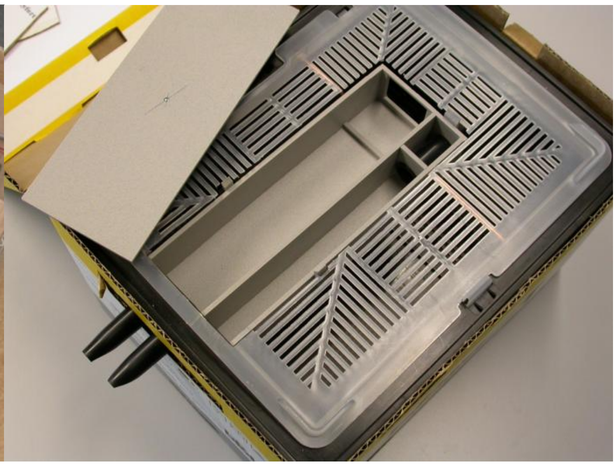


Fig 1. Picture of the funnel dispenser (left) and the built-in dispenser (right). The entrance and exit ways are separated. Bumblebees walk over a grid in order, filled with Prestop-Mix in order to get out. They enter the hive through a powder free path.

b. Honeybee

For the dispersion of Prestop-mix, the BeeTreat dispenser was used (Fig 2). To see what de influence of the mounting of the dispenser on the bee colony is, cameras were installed inside the dispenser. With an empty dispenser, it was clear that the dispenser space was used by workers for other functions such as ventilation of the hive and protection. When the dispenser was filled with powder, bees had problems passing the large amount of powder and fights between bees were observed. This behaviour was probably caused by the physical presence of the powder. Also, it was seen that honeybees had problems getting up the landing strip, a less steep angle and grooves on the landing strip can solve this problem.

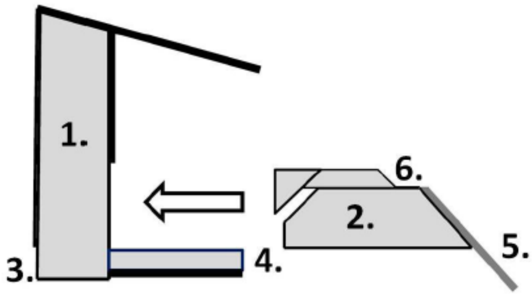


Fig 2. BeeTreat dispenser as developed by Heikki Hokkanen and Ingeborg Menzler-Hokkanen. 1 = body of the dispenser (back against the beehive); 2 = detachable steering part, to be inserted into the body; 3 = opening joining the dispenser with the hive opening; 4 = exit area for the bees; 5 = landing platform for the returning bees; 6 = entrance corridor for bees to return to the hive (crawl over the solid block 2 to access opening 3). The area between 3 and 4 forms the exit corridor, where the material to be dispensed is placed on the bottom. The solid block 2 forms the ceiling of the corridor. All parts are made of untreated wood or plywood, except 5, which is clear plexiglass to allow daylight to be seen from the hive opening at 3 (upper surface is slightly roughened for bees to get a grip).

c. Solitary Bee

For *Osmia cornuta*, a low cost dispenser was developed (Fig. 3). Therefore, per nest, 50 females and 100 males in their cocoons were released. After the mating and when the females started nesting, a metal grid was installed in front of the nest to oblige the females to get out passing through the dispenser. A free space above the dispenser gave female bees the possibility of returning to their nest without crossing the dispenser. Although some time was needed to learn how to enter the nest using the free space above the dispenser, it ended up being successful. Exiting and passing the dispenser did not result in any hindrance. This experiment shows that successful nesting activity and a good dispersion of microbial control agent can be obtained with the use of solitary bees.

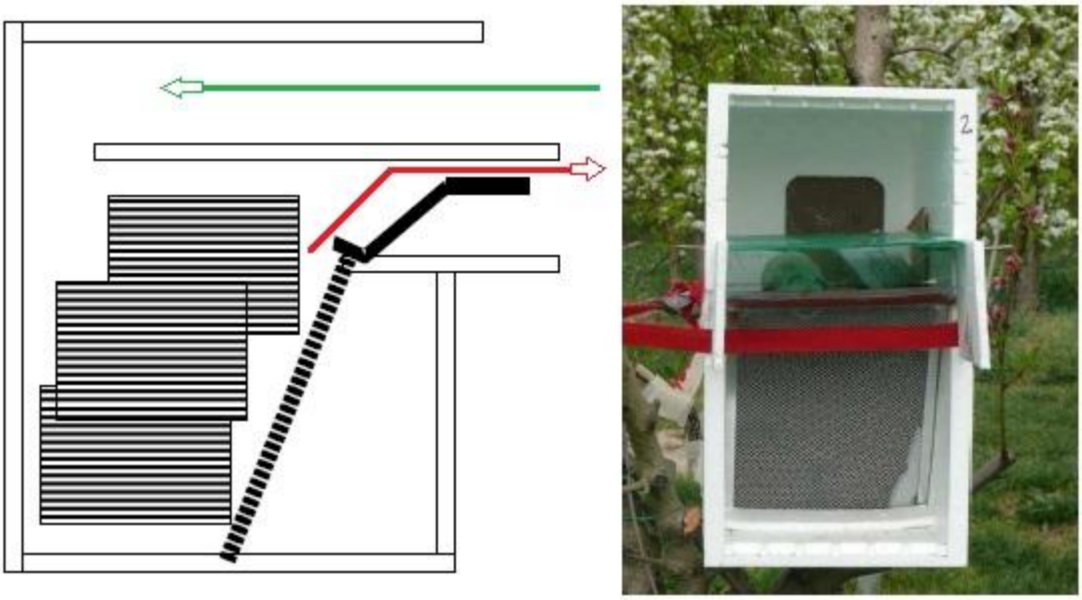


Fig 3. Side view of the nesting facility (left) and picture of the font view (right) developed by Bettina Maccagnani. The green arrow represents the entering route where the red arrow represents the exit route through the dispenser.

B- comments on deviations from the original plan:

No deviations necessary.

WP 5	**Suppression of grey mould by antagonists**

Responsible partner: P5, SDU, WP manager Cafer Eken

Original description of work:

WP5 focuses on the interaction between the plant (strawberry), the plant disease (*Botrytis*), and the BCA (*G. catenulatum* J1446*,* and as a comparison, *Trichoderma atroviride*). To understand the conditions for effective disease suppression, WP5 will evaluate:

- the importance of timing of flower colonization with *Botrytis* vs. BCA
- the effect of balance in numbers of spores between *Botrytis* vs. BCA in the colonization of the flower
- the impact of temperature on the disease suppression dynamics with the BCA
- the actual dynamics of the disease vs. BCA interaction under field conditions, while using bees to disseminate the BCA to the strawberry flowers

Task 5.1. Flower infection/disease suppression dynamics with *Botrytis* vs. *G. catenulatum* (**EHRS-SDU**)

Infection experiments are conducted in controlled conditions (T, RH) in the laboratory with *Botrytis* and the BCA, following the procedure of Stromeng et al. (2008). Strawberry flowers are inoculated with 10^3 spores of *Botrytis* mixed with BCA spores at 4 concentrations (0, 10^3, 10^4, and 10^5 BCA spores/flower) at three temperatures: 15, 22, 30°C. Another experiment examines the timing of the BCA and the pathogen arrival: *Botrytis* application at 10^3 spores/flower 2 days before the BCA (at 10^3 spores/flower), and then 1 day before, at the same time, 1 day after, and 2 days after the BCA application. Six replicates are used, each with 3 strawberry flowers.

Task 5.2. Flower infection/disease suppression dynamics with *Botrytis* vs. *T. atroviride* (**EHRI-AU**)

As Task 5.1, but with *T. atroviride,* and with a reduced testing schedule, based on the outcome of assays in 5.1.

Task 5.3. Disease vs. BCA dynamics under actual field conditions (**EHRS-SDU**, NIB, UHEL)

From selected field sites in WP7, the actual dynamics of BCA vs. *Botrytis* colonization will be assessed using the q-PCR methods developed by NIB in WP8. This will indicate whether the bee dissemination in the field has been adequate for effective disease management in terms of timing and quantity of BCA deposition onto the strawberry flowers. The results will give guidance for further refinement of the technique.

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

The biofungicide Prestop-Mix, based on *Gliocladium catenulatum* J1446 Gilman &Abbott (now *Clonostachys rosea* f. *catenulate* J1446 (Gilman &Abbott) Schroers), *Clonostachys rosea* f. *rosea* (Link: Fr.) Schroers, Samuels, Siefert and W. Gams (formerly *Gliocladium roseum* Bainier), *Trichoderma asperellum* Samuels, Lieckf. &Nirenberg and *Botrytis cinerea* Pers.: Fr. S-TR-20 (an infected strawberry at Erzincan, Turkey) were used for an agar plate assays.

One flower was placed on 15 ml of sterile 1.5 % water agar in 10-cm-diameter petri dishes. Each flower was inoculated at one point at the base of the petals with 10 µl drops of spore suspension containing 10^3, 10^4or10^5conidia/ml of *B. cinerea*, alone ore mixed with 10^3, 10^4or10^5conidia/ml of *Clonostachys rosea* f. *catenulate* J1446, *Clonostachys rosea* f. *rosea* or *Trichoderma asperellum.*

As a result; There were no significant differences between disease severity (%) of spore suspensions (10^3, 10^4,10^5conidia/ml) of *B. cinerea*. The *C.catenulata*10^5, *C.rosea*$10^5$and *T. asperellum*10^5exhibited a remarkably higher disease control activity on petioles, whereas disease control activity on strawberry petioles was not significantly different between 10^3 and 10^4treatments.

Pot Experiments; Strawberry (cv. Aromas) seedling were planted in 25-cm diameter plastic pots (1 seedling per pot) containing a soil mix.The plants were grown in a climate-controlled greenhouse where air temperature averaged 18° to 24°C during the day and 13° to 17°C at night. Pot experiments will be start soon.

FieldTrials; The experiment was located in Erzincan, Turkey. The frigo plants of ‘Aromas’ strawberry cultivars were used. Frigo plants were planted in raised and black polyethylene mulched beds of June in 2012 at distances of 25 ×30 cm. The strawberry plants were grown in the field using routine cultivation practices. No chemical fungicides were used.

The field contained 7564 plants and consisted of twenty one rows on raised beds. The beds were provided with trickle irrigation. One month, flowers and stolons were cut down for homogenous and strong growing was taken place. The experimental design was completely randomized with 4 repetitions. Each plot (no cage and cage) consisted of 70 strawberry plants planted in an area of 3.30 m × 4 m. The total yield of each plot was recorded and then divided by the total number of plants from each plot and expressed in g/plant. During the season strawberries were harvested 5 times.

Yield is very low for 2012.

Compared with cage experiments, the yield values of strawberry in no cage experiments were slightly low at 1.92 g/plant and 1.97 g/plant, respectively.

B- comments on deviations from the original plan:

No deviations necessary.

WP 6	Landscape Management supporting BCA dissemination and pollination

Responsible partner: P6, ITACAA, WP manager Bettina Maccagnani

Original description of work:

Objectives of WP6 are to

- Determine the impact of presence/absence of key ecological infrastructures (pollen and nectar food, nesting sites) on the colonisation efficacy of managed solitary bees in the target crop system, as well as their impact on the abundance of wild pollinators
- Establish the role of vegetation management using ‘push-pull’ strategy on enhancing managed and wild pollinator activity in strawberry fields
- Assess the benefits to fruit pollination of managing solitary bee populations

Task 6.1. Role of ecological infrastructures in supporting *Osmia* establishment (ITACAA)
To support the early establishment of *O. cornuta* and *O. rufa* in pear orchards the role of early flowering pollen/nectar plants will be studied: two orchards with *Prunus spinosa* edges and a variety of wild and/or implanted flowering plants in the margins and between rows; two orchards with a mix of *Brassica oleracea* cv. *botrytis* (with different ripening times from 30 to 120 days) and of *Brassica napus* var *oleifera.* The effect of the treatments on the establishment of solitary bees in the nesting shelters will be evaluated. Every two days, video-recordings will be performed at the onset of the nesting activity early in the day to count the number of nesting females. Larval cell production will be monitored by extracting and weighing the nested reeds. At the end of the reproductive cycle the sex-ratio and the female reproductive rate will be calculated.

Task 6.2. Vegetation management in and around strawberry fields (LAVES, UHEL)
In collaboration with Task 2.2, the impact of growing low-trimmed blossoming white clover between strawberry rows on the BCA dissemination and pollination activity by managed (*Apis, Bombus, Osmia*) and wild bees is assessed by frequent direct counts in ‘treated’ and ‘untreated’ sections of the fields. Additional variables are introduced by managing (removing or leaving) competing vegetation (e.g., *Taraxacum*) in and around the fields.

Task 6.3. Impact of ecological infrastructures on wild pollinators in orchards (ITACAA)
In orchards with different ecological infrastructures visual counts are made on the diversity and numbers of flying and flower visiting managed and wild pollinators (*O. cornuta*, honey bees, bumble bees, hover flies, other Diptera, etc.) during the blooming period, by walking for 10 min every 2 h (from 8.00 a.m. till 6.00 p.m.) along 4 chosen pear rows at increasing distance from the nesting sites.

Task 6.4. Pollination efficacy of managed solitary bee pollinators (ITACAA)
Every two days the percent of pear pollen present in 10 *Osmia* larval cell provisions is determined. Fruitlet-set, fruit-set, and seed-set are measured on five pairs of trees exposed to: (i) the maximum pollination treatment (natural pollination + *Osmia* sp; pairs of trees at increasing distances from the nesting sites within the flying range of *Osmia* females); (ii) natural pollination treatment (pairs of trees located outside the flying range of *Osmia* females, > 500 m from the nesting sites. At the popcorn stage, 30 inflorescence buds per tree are labeled and the flowers per bud counted, to evaluate fruitlet and mature fruit percent and the number of seeds per fruit, according to the pollination treatment.

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

In 2012 ITACAA started the research for **Task 6.1** - Role of ecological infrastructures in supporting *Osmia* establishment (ITACAA), and **Task 6.3** - Impact of ecological infrastructures on wild pollinators in orchards

One trial was planned in 2012 in order to maximize the foraging activity of *Osmia cornuta* on pear (*Pyrus communis*) by supporting its establishment in the orchard thanks to the presence of ecological infrastructures, whose period of flowering occurs before pear flowering.

Due to the adverse weather conditions it was not possible to carry on the research. After a very cold February (a lot of snow, with temperatures down to -13°C), suddenly the weather turned towards very warm temperatures.

Cocoons were immediately brought to the pear fields, but the flowering of the crops progressed much faster than emergence of the adults from cocoons, and the flowering of the two crops overlapped almost entirely.

This meant for us that it was not possible to use the peach flowering as the period necessary for *O. cornuta* females to emerge from cocoon, feed themselves and start with the nesting activity before pear flowering, and these behaviors were largely performed while pear had already started blooming.

This prevented the possibility to compare the behavior of *O. cornuta* females in the two ecological situations, and we decided not to go on with the collection of the data.

In September 2012 we started with the preparation of the fields in which the trials will be carried on in 2013.

Along one side of a pear field surrounded by extensive cultivations, a row of 50 m of *Brassica napus* and *Brassica oleracea* with different ripening times from 30 to 120 days were sowed.

We expect that *Brassica* will survive the winter at the stage of 6-7 leaves, and in this condition it will be flowering at the beginning of march, before pear bloom starts.

B- comments on deviations from the original plan:

The feasibility and practical utility of the researches on **Task 6.2** - Vegetation management in and around strawberry fields have been reconsidered by LAVES, because of the problems related to the difficulties caused to the workers at the moment of strawberry harvest by the presence of honeybees foraging on white clover and *Taraxacum* between the strawberry rows. A final decision on the opportunity to proceed in 2013 with these researches needs to be taken.
ITACAA started the study on the role or ecological infrastructures in sustaining the establishment and the foraging activity of bees on the target crops, and to evaluate their impact on the presence of wild pollinators in the orchard.

WP 7	**Field applications of the entomovector technology**
Responsible partner: P1, UHEL, WP manager Heikki Hokkanen	

Original description of work:

Objectives of WP7 are to:

- Test under realistic field growing conditions in all partner countries the technical applicability, practical and economic feasibility, and environmental soundness of the entomovector technology in controlling the grey mould on organic strawberry production, and in improving pollination of the crop
- Facilitate rapid field testing at a broad geographical scale of any improvements and innovations arising during the BICOPOLL project
- Facilitate an immediate and efficient technology transfer to all interested end-users (growers, grower organizations, beekeepers, researchers, advisory services, etc) in all partner countries
- Initiate a Europe-wide network of demonstration-farms using this completely new approach, highly attractive to the general public (TV, newspapers) and the professional media (electronic and printed)

Task 7.1. Practical execution of a joint field experiment on strawberry (**UHEL** + all others)
A standardized field trial on at least one (organic, where available) strawberry farm is established by all partners, using the concept already tested and applied in Finland (Hokkanen et al. 2011). The trial is established in the first study year, and continued throughout the project. Honey bees are used as vectors, and *Gliocladium catenulatum* as the standard BCA. Where feasible, an additional trial using another commercially available BCA (e.g., *Trichoderma* sp.) is

conducted for a comparison. A minimum of two treatments are included at each site: BCA treated, and untreated control, with four replicate assessment plots on each farm. At the time of actual berry-picking (usually every two days), data on grey mould incidence and marketable yield are obtained from each assessment plot (minimum: 4+4 plots on each farm). Additionally, honey samples from all disseminator-hives and nearby control hives will be collected annually at each location, and sent to NIB for analysis in WP8.

Task 7.2. Feasibility study of using the entomovector technology (UHEL + all others)
UHEL will carry out a feasibility analysis of the technology, based on data collected by all partners on the joint field experiment. Parameters to be assessed include (i) management system [required management practices, inputs, machinery, labour, management flexibility]; (ii) economic factors [yields, operating costs, administrative costs, aspects of farm/product competitiveness]; and (iii) social factors [non-pecuniary social effects such as business opportunities, requirements for education and training, social cohesion via improved collaboration].

Task 7.3. Enhancing project PR (**UHEL** + all others)
All partners devote specific attention to promoting BICOPOLL public relations by organizing at least once per year an event at the field trial site, where the public and professional media are invited, along with grower and beekeeping organizations. Every partner writes at least once per year a popular article on the topic/trial for a national professional magazine (berry-grower's and/or beekeepers' magazines).

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:
In the BICOPOLL-project we aim to demonstrate Europe-wide the applicability of the entomo-vectoring approach, using as a case study the control of strawberry grey mould *Botrytis cinerea*, with the biocontrol fungus *Gliocladium catenulatum*, vectored by honey bees or bumble bees. The joint trial targets strawberry cultivations in the open field, and ideally includes four treatments: untreated control, chemical fungicide, entomovectored biocontrol, and chemical + biocontrol combined. In organic fields, no pesticide treatments are included. The proportion of mouldy berries, and/or the marketable yield of healthy berries is recorded from each treatment, along with other parameters of local interest. In 2012 such joint field trial was carried out in Estonia, Italy and in the UK – besides Finland, where large areas commercially already use entomovectoring (see below).

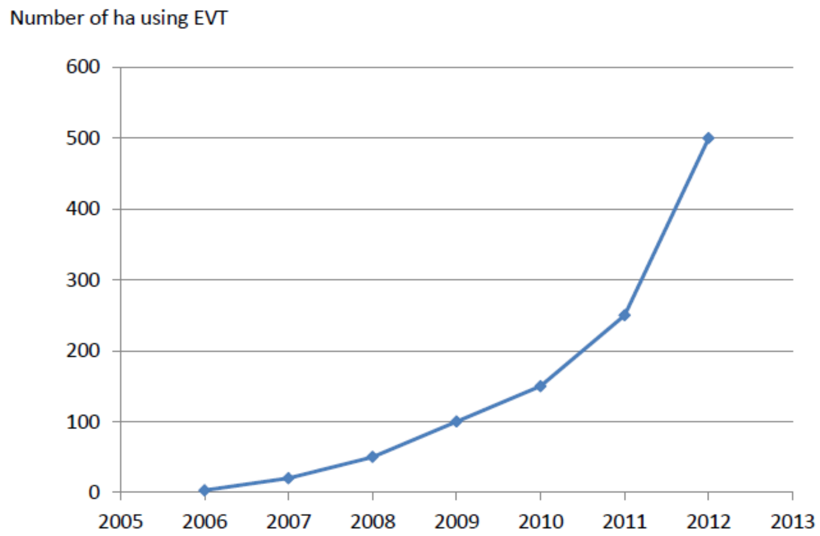


Fig. 1. Commercial uptake of honey bee vectored precision biocontrol on strawberry in Finland. The method has been approved for general use in 2008.

A conservative estimate for Finland is that close to 500 ha of strawberry cultivation was using the technique in 2012. The excellent control results have been presented earlier e.g. at the 63rd ISCP in Ghent in 2011. Field results in summer 2012 in Italy showed – despite extremely difficult weather conditions – significant reduction over untreated control (mean 39% mould) by biocontrol alone (13%), and by the combined treatment (11%), while chemical control (26%) did not differ significantly from the untreated control. In the UK trial entomovectoring by bumble bees resulted in control of the grey mould, which was as good as by the chemical control. In Estonia, field studies at very low pathogen pressure nevertheless showed significantly less grey mould, and higher marketable berry yields in plots entomovectored either by honey bees, or by bumble bees (separate field studies). So far, all field tests using entomovectoring and *Gliocladium catenulatum* (Prestop Mix) have shown excellent control results, and we intend to broaden these trials to include still other BICOPOLL partner countries in 2013 and 2014.

B- comments on deviations from the original plan:

Setting up demonstration field trials has not yet been possible in all partner countries due to lack of suitable strawberry growers, logistics, or other constraints. Further efforts will be directed to organizing such trials in all partner countries.

WP 8 Safety of the entomovector approach to bees and consumers

Responsible partner: P7, NIB, WP manager Andrej Cokl

Original description of work:

The main objectives of WP8 are

- To develop a quantitative PCR-method for detection of the control agent
- To test for possible direct and indirect effects of the BCA on honey bee foragers
- To determine the safety to consumers of products from beehives that have been used to disseminate BCAs

Task 8.1. Detection of biocontrol agent *Gliocladium catenulatum*

8.1.1. Development of q-PCR assay for the BCA (NIB)

The q-PCR assay for specific detection of biocontrol agent *Gliocladium catenulatum* strain J1446 is designed. Analysis of already available related sequences is performed in combination with de novo sequencing. The specificity of the new primers and probes is tested with pure cultures of *G. catenulatum* strain J1446 and with two other strains of *G. catenulatum* and related fungi. The assay is validated with determination of the limit of detection, limit of quantification, repeatability, and effect of sample matrix. Testing of different DNA extraction procedures for compatibility with q-PCR will also be done.

8.1.2. Quantitative assessment of the BCA in hive products and the environment (NIB, all other partners)

Strawberry blossoms, departing and returning foragers, and hive products including honey and pollen will be analyzed to quantify the presence of control agent from samples provided by all project partners.

Task 8.2. Do honey bees transfer the BCA successfully to the field? (NIB)

Departing and returning bees are sampled at the colony entrance to determine the quantity of spores transported to the field, and back to the colony by using q-PCR. The behaviour of foragers before departure will be observed with a video camera to determine whether workers carry the BCA to the field successfully. The time bees spend at the entrance before departing, and the occurrence and intensity of grooming are recorded.

Task 8.3. Returning time and loss of honey bees exposed to the BCA

The effect of control agent on homing ability of foragers is tested. Individually marked workers of known age exposed to the control agent in a similar way as in natural conditions, and control workers are released at the same distance from the colony. Returning time and the number of returned bees are recorded.

Task 8.4. Learning ability of honey bees exposed to the BCA
The effect of exposure to control agent on learning and memory formation in honey bee is tested in the laboratory on harnessed foragers of the same age. The olfactory conditioning of proboscis extension reflex (PER) is used to test differences between treated and untreated bees. Before learning experiments responsiveness to ascending sugar solution is tested to assure that bees do not differ in sugar threshold, which affects their learning abilities. Bees are conditioned to the olfactory stimulus once and tested for PER response by presenting odour alone 1 and 12 min after conditioning.

Task 8.5. Life span of honey bees exposed to BCA
Experiments are performed in cages. Bees are daily exposed to control agent in a similar way as in natural conditions to examine longevity of treated bees. The number of dead bees will be recorded on daily basis. Untreated bees serve as controls.

Task 8.6. Impact of BCA on *Osmia* (ITACAA)
The survival of 3 groups of freely foraging bees that for one week passed through a dispenser with *Bacillus amyloliquefaciens* is compared to that of bees passing through an empty dispenser, and the respective reproductive success is measured. The condition of their pollen collecting abdominal hairs is be evaluated under a stereomicroscope. The impact on larvae fed with pollen mixed with BCA is also assessed.

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

Task 8.1. Detection of biocontrol agent *Gliocladium catenulatum*
Efficient and specific qPCR assay for detection and quantification of *G. catenulatum* J1446 was developed (additional strains for specificity testing are needed). DNA extractions from difficult matrices (bees, BCA with carrier substance) were optimized.

Task 8.2. Do honey bees transfer the BCA successfully to the field?
The behaviour of forager bees in the dispenser without and with Prestop Mix (5 g) was observed by two video cameras. Observations show that Prestop Mix changes bee behaviour and results in waste of product and some loss of bees. Most of Prestop Mix was spent first hour after application. We suggest using less Prestop Mix at a time and improvement of the dispenser.

Task 8.4. Learning ability of honey bees exposed to BCA
The effect of exposure to Prestop Mix on learning and memory formation was tested in the laboratory on harnessed foragers. The olfactory conditioning of PER (proboscis extension reflex) was used to test differences between treated and untreated bees. Before learning responsiveness to sucrose solution was tested. Bees were conditioned to the olfactory stimulus once and tested for PER response by presenting odour alone one or 12 min after conditioning. No statistically significant differences were found between treated and untreated bees.

Task 8.5. Life span of honey bees exposed to BCA
Affect of Prestop Mix on mortality of honey bees in cages in incubator (34°C, 96 hours) was tested. Bees were exposed to high amounts of Prestop Mix for 15 s only first day (acute exposure) or every day (chronic). Control group was not exposed to Prestop Mix. Each day number of dead bees was counted. Exposure to high amounts of Prestop Mix (in laboratory conditions) increased mortality of honeybees.

B- comments on deviations from the original plan:

No deviations necessary.

4. Publications and dissemination activities

4.1 List extracted from Organic Eprints

(Publications affiliated to European Union > CORE Organic II > “project acronym”, grouped by EPrint type, with date of extraction)

The list can have these headers:

http://orgprints.org/view/type/

publications that are not allowed as open access should be deposited as “Visible to: Depositor and staff only.”. The funding bodies and project evaluators will be granted access to these during the evaluation. Guidance on the use of Organic Eprints can be found here: *http://orgprints.org/help/* .

4.2 Additional dissemination activities

(List dissemination activities that are not uploaded to Organic Eprints)

Hokkanen H, Aase A-L, Bevik D, Boecking O, Cokl A, De Meyer L, Dupont Y, Eken C, Karise R, Maccagnani B, Menzler-Hokkanen I, Mänd M, Smagghe G, Söderlund N, Tuncer S, Veromann E, Witzgall P (2013). BICOPOLL: Targeted Precision Biocontrol and Enhanced Pollination. *Apidologie* (in press).

Hokkanen, H. M.T. & Menzler-Hokkanen, I. (2013). Do not fight against *Varroa* - get rid of it! *Apidologie* (in press)

Hokkanen, H., Boecking, O., Cokl, A., Cotes, B., Eken, C., Karise, R., Krajl, J., Maccagnani, B., Menzler-Hokkanen, I., Mommaerts, V., Mänd, M., Smagghe, G., Söderlund, N., Tuncer, S., Veromann, E. and Witzgall, P., 2012. BICOPOLL: Targeted Precision Biocontrol and Enhanced Pollination. **64th International Symposium on Crop Protection** (May 22, 2012 Gent, Belgium): 230.

Hokkanen, H., Boecking, O., Eken, C., Cokl, A., Maccagnani, B., Mänd, M., Smagghe, G., 2012. Ecological Infrastructure Management for enhanced pollination and targeted precision biocontrol: the BICOPOLL Project. **1st ApiEcoFlora** (October 4th - 6th 2012, Republic of San Marino): 37-38.

Eken, C., Tuncer, S., Genç, T., Kadıoğlu, Z., 2013. Use of honeybees (Apis mellifera L.) to disseminate biocontrol agents to strawberries for grey mould (Botrytis cinerea Pers. ex Fr.). 1st Congress of Plant Protection Products and Equipment (April 2-5, 2013), Antalya, Turkey.

Eken, C., Tuncer, S., Genç, T., Kadıoğlu, Z., 2013. The use of honey bees (Apis mellifera L.) for biological control of plant diseases. V. Marmara Beekeping Congress (April 4-6, 2013), Bursa, Turkey.

De Meyer, L., Hokkanen, H., Menzler-Hokkanen, I., Maccagnani, B., Karise, R., Muljar, R., Mänd, M., Lahdenperä, M-L., Eken, C., Cokl, A., Boeckıng, O., Smagghe, G., 2013. Introduction to entomovectoring and FP7 project “BICOPOLL”. 65th International Symposium on Crop Protection (May 21, 2013 Gent, Belgium): 121.

Eken, C., Tuncer, S., Genç, T., Kadıoğlu, Z., 2013. Present situation of honey bees and bumble bees having potential entomovector technology in Turkey. 65th International Symposium on Crop Protection (May 21, 2013 Gent, Belgium): 123.

Bevk D. (2013). Čebele - včeraj, danes, jutri. *Gea*, 23 (5): 20-33 (“Bees – yesterday, today, tomorrov” A popular article published in Gea - the main Slovenian popular science magazine)

Pirc M., Bevk D., Čokl A., Ravnikar M., Dreo T. (2013) The development of new methods for monitoring biocontrol agent, *Gliocladium catenulatum* J1446, to control gray mold on

strawberries. Plant Protection Society of Slovenia, Bled, 5.-6. March 2013 p. 31-32. (Published scientific conference contribution abstract)

Maccagnani B., Ferrari R., Pozzati M., 2013. – Ecological Infrastructure Management Enhanced Pollination and Targeted Precision Biocontrol: The BICOPOLL Project. Bulletin of Insectology 66 (1): 26, 2013. http://www.bulletinofinsectology.org/pdfarticles/vol66-2013-026-026bicopoll.pdf

Agen.Ter – Ricerca: Presentation of the BICOPOLL project
http://www.agenter.it/ricerca.html
http://www.agenter.it/scheda-bicopoll.html

Tec.BiO – Il punto dei tecnici BIO - Impollinatori per trasportare microrganismi antagonisti di malattie – il Progetto BICOPOLL.
http://www.tecpuntobio.it/news.php
http://www.tecpuntobio.it/news.php?cat=6&sub=4&id=423
http://www.tecpuntobio.it/Documenti/progetto%20BICOPOLL%20-%20gennaio%202013.pdf

Il Divulgatore - Agricoltura, Alimentazione e Ambiente: Progetto BICOPOLL (Ecological Infrastructure Management for Enhanced Pollination and Targeted Precision Biocontrol)
http://www.ildivulgatore.it/
http://www.ildivulgatore.it/bicopoll.html

Maccagnani B., Ferrari R., Pozzati M., 2013. – *Gliocladium catenulatum* delivered by honeybees against *Botrytis cinerea* on strawberry. 1st Apimondia ApiEcoFlora Symposium, San Marino, 4 - 6 October 2012.

Technical staff information meeting, 18 October, 2012 Erzincan (Provincial Directorate of Ministry of Food, Agriculture and Livestock, Erzincan)

13 June, 2011; **Bereket TV** (http://www.bereket.tv/) Program of Tarım Bülteni (Agriculture Bulletin) on the Bereket TV

25 January, 2012: **Kanal B** (http://www.kanalb.com.tr/) Phone connection to the program of Sormak Gerek (need to ask)

There are a lot of newspapers samples about the BICOPOLL (Doktor Arılar).

Interview in Deutschlandradio 21.3.2013: Bienen sollen Erdbeeren vor Krankheiten schützen. Heikki Hokkanen and Otto Boecking. Program "Forschung Aktuell". http://www.dradio.de/dlf/sendungen/forschak/2116324/

4.3 Further possible actions for dissemination

- List publications/deliverables arising from your project that Funding Bodies should consider disseminating (e.g. to reach a broader audience)
- Indicate publications/deliverables that could usefully be translated (if this has not been done, and indicate target language)

4.4 Specific questions regarding dissemination and publications

- Is the project website up-to-date? Yes
- List the categories of end-users/main users of the research results and how they have been addressed/will be addressed by dissemination activities

Several workshops and training courses were organized, most of the time jointly with other funding sources, providing (i) cost-efficient use of funds and (ii) wider than expected target audience. During 2012 (until 31.5.2013) the following courses, workshops, and training sessions have been organized:
1. Kick-off meeting (jointly with the NordForsk project BICOPOLL-NET) and first project workshop: 9.-12.1.2012, Tvärminne Zoological Station, University of Helsinki, Finland.
2. Training sessions in entomovectoring and workshop on research collaboration at INRA-Guadeloupe and ASSOFWI research station, 29.2.-25.3.2012, in Guadeloupe, France.
3. BICOPOLL-meeting and symposium at Ghent, Belgium, 21.-24.5.2012
4. BICOPOLL training course in collaboration with BOVA MSc-course on "Causes and consequences of pollinator declines", Järvselg, Estonia, 5.-11.8.2012
5. BICOPOLL training course in collaboration with BeeNOVA MSc course on "Insect pollinators and pollination biology", 12-18.8.2012, Tvärminne Zoological Station, University of Helsinki, Finland.
6. BICOPOLL Annual Meeting and second project workshop, 3.-5.12.2012 in Tartu, Estonia.
7. BICOPOLL project meeting and local workshop in association with the Annual Meeting of the German Bee Research Institutes, 19.-23.3.2013 in Würtzburg, Germany.
8. Training session with the Aaland Islands Beekeepers' Association, 21.-22.4.2013, in Jomala, Åland.

In addition some high-profile demonstration and training sessions were held, for example in Finland on 20.8.2012 the project coordinator could explain and demonstrate pollination services and the importance of pollination (with a live, functional demonstration bumble-bee nest) in person for 20 min to the current Prime Minister of Finland (Jyrki Katainen), and the former PM of Finland (Mari Kiviniemi), in a private occasion.

BICOPOLL was also explained to staff of the Finnish Ministry of Agriculture and Forestry at their review of current research on 23.8.2012.

- Impact of the project in relation to main beneficiaries of the project results
  (Note: for the different categories of end-users/main users of the research results, explain how well the project has been able to reach these target groups, and any known impact)

  Main beneficiaries are the strawberry and other berry and fruit growers, as well as beekeepers. The rapid commercial uptake of the technology best illustrates the impact of the project. We expect rapid adoption of entomovectoring also in other countries, and in other crops, as soon as promising research results are disseminated widely.

5. Added value of the transnational cooperation in relation to the subject

(max 1 page, please describe the main advantages of the transnational research cooperation compared to a national research project approach in regard to the subject of the project. You may in particular expand on new research ideas raised by the project, research cooperation established during the project, research funding obtained etc.)

Complementary expertise by all partners greatly accelerates the accumulation of knowledge, necessary to make advances in an area like this. New research ideas, and new ideas for solving acute or encountered problems and for improving the concept are all added value from the transnational cooperation.

ANNEX 1: CHANGES IN WORK PLAN AND PROBLEMS ENCOUNTERED

Changes in consortium and work plan

(Type and reason for the changes, if any - Arial, size 11)
Note: the changes and the reasons should be indicated both in the WP sections and here.

Problems encountered, delays and corrective actions planned or taken, if any:
(Arial, size 11)

ANNEX 2: COST OVERVIEW AND DEVIATIONS FROM BUDGET

Project budget and costs in € (if in National currencies, please indicate):

Partner no.	1	2	3	4	5	6	7
TOTAL BUDGET	75000.00	122000	95865.00	99600	64200.00	66666.67	70000
Spent at Mid term	2491.65	61000	51128.00	50000	46740.08	31916.09	32000
Spent in 2nd period							
TOTAL SPENT	2491.65	61000	51128.00	50000	46740.08	31916.09	32000
DEVIATION							

Person months (PM) spent on the project:

Partner no.	1	2	3	4	5	6	7
TOTAL PM budgeted	12	18	24	24	12	16.2	18
Spent at Mid term	3	9	11.3	12	11.725	7.14	6.00
PM spent in 2nd period							
TOTAL PM SPENT	3	9	11.3	12	11.725	7.14	6.00
DEVIATION							

Reasons for major deviations in spending compared to original budget:
(Arial, size 11)

At Partner 1 (UHEL), other/own funds have been used during the first reporting period to carry out the tasks; therefore, funds have been saved to cover costs during the second part of the project.

ANNEX 3: RECOMMENDATIONS TO THE CORE ORGANIC CONSORTIUM IN RELATION TO LAUNCHING AND MONITORING OF FUTURE TRANSNATIONALLY FUNDED RESEARCH PROJECTS

(max ½ page)