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# Targeted precision biocontrol and pollination enhancement in organic cropping systems

**Zielgenaue biologische Krankheitsbekämpfung und Verbesserung der Bestäubung in ökologischen Anbausystemen**

**FKZ: 11OE016**

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# Final Report

## „BICOPOLL - Targeted precision biocontrol and pollination enhancement in organic cropping systems“

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## □ Kurzfassung (deutsch):

„BICOPOLL - Zielgenaue biologische Krankheitsbekämpfung und Verbesserung der Bestäubung in ökolgischen Anbausystemen“; Dr. Otto Boecking & Victoria Kreipe (authors); LAVES – Bee Research Institute Celle, Herzogin-Eleonore-Allee 5, 29221 Celle, Germany; otto.boecking@laves.niedersachsen.de (Text: 2.000 Zeichen)

Die ökologische Beerenproduktion leidet unter dem Mangel an effektiven Krankheitsbekämpfungsmitteln und gleichzeitig unter nicht ausreichender Bestäubung zur Ertragssteigerung. Im Erdbeeranbau ist Grauschimmel *Botrytis cinerea* als Pilzerkrankung die entscheidende Problemkrankheit, die zu erheblichen Einbußen führen kann. Fungizide dürfen im ökologischen Anbau nicht eingesetzt werden. Hier bieten sich biologische Verfahren an, wie der Einsatz von Antagonisten. Zur Bekämpfung des Grauschimmels werden antagonistisch wirkende Pilze erfolgreich eingesetzt. Damit diese Pilz-Sporen (in Pulverform) zum richtigen Zeitpunkt vor der Infektion der Erdbeerblüte auf der Blüte appliziert werden, bieten sich Bienen geradezu an. Sie übernehmen die Aufgabe eines *flying doctor*, während sie die Blüten bestäuben. Dazu passieren sie einen Dispenser am Flugloch, in dem das Pilzspulver eingefüllt ist. Wir haben Honigbienen zur (i) gezielten und präzisen Ausbringung von Antagonisten auf die Erdbeerblüten eingesetzt, um damit dem Grauschimmel zu begegnen und gleichzeitig (ii) den Bestäubungserfolg zu steigern. Zentrale Frage war, wie bekommt man Bienen dazu in Erdbeerplantagen zu fliegen und bei der Bestäubung der Blüten gleichzeitig das Pilzsporen-Pulver zu verteilen? Eine Aufstellung von Bienenvölkern direkt an ein Feld der Zielpflanzen bedeutet noch lange nicht, dass diese auch dort hinfliegen. Dazu bedurfte es zunächst der Beantwortung wichtiger grundlegender Fragen, bevor ein solches System in der Praxis einsatzfähig ist. Wir haben postuliert, dass kleine Bienenvölker (Individuen-arm) eher in der näheren Umgebung um ihren Stock sammeln, wenn man sie mit großen Völkern vergleicht. Dazu wurden kleine und große Völker aus Kunstschwärmen erstellt und mit Geschwisterköniginnen ausgestattet. Während der Erdbeerblüte wuchsen diese Völker an. An aufeinander folgenden Erfassungstagen im Zeitraum 2012 - 2014 zeigte sich mittels Pollenfallen bzw. am Flugloch abgefangenen Flugbienen, dass die kleinen Völker tendenziell mehr Sammlerinnen in den nahen Erdbeeren entsendeten als es die großen taten. Damit wurde erstmals unsere Hypothese mit Daten unterstützt. Bevor man aber daraus

Schlüsse und Empfehlungen für die Praxis ableiten kann, bedarf es einer Wiederholung und Verifizierung dieser Ergebnisse.

## □ Abstract (English):

„BICOPOLL - Targeted precision biocontrol and pollination enhancement in organic cropping systems“; Dr. Otto Boecking & Victoria Kreipe (authors); LAVES – Bee Research Institute Celle, Herzogin-Eleonore-Allee 5, 29221 Celle, Germany; otto.boecking@laves.niedersachsen.de (Text: 1.670 characters)

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. The BICOPOLL project aimed to change this and to improve the yield and quality of organic strawberry production significantly and thus farm economics. We used honeybees to (i) target deliver a biological control agent (fungus antagonist) to the flowers of the target crops (strawberries) to provide control of the problem diseases grey mold (*Botrytis cinerea*) and to (ii) improve the pollination of this organic horticultural crops. The use of bees has many environmental and economic benefits compared to spraying fungicide like in conventional farming systems. As bees, that actually forage in the target crop, is a key essential requirement for the entomovectoring technology, the main focus of this project was to determine, which factors can affect foraging ranges of honeybees and to examine how to steer the bees to the target crop (strawberry), even if the nectar or pollen rewards are less attractive compared to competitive other plants in the near surrounding. We hypothesized that small hives (number of individual bees per colony) forage closer to their hive and are thus more suitable for the bio-control of less nectar and pollen producing crops (like strawberries) than big hives, with higher numbers of individual bees per colony. Our investigations during 2012 – 2014 showed that on average smaller bee hives showed slightly higher proportions of Fragaria-pollen collecting bees than bigger bee hives based on both population estimates at the beginning and at the end of the investigation period. Thus our scientific hypothesis seems to be supported by our findings. However, before jumping to general conclusions or even recommendations for practical use it is indeed necessary to prove and verify these findings.

**Content**

# 1. Introduction

## 1.1 Subject of the project

Grey mold is the most common fruit rotting pathogen of strawberry and one the most important biotic threats for the organic berry and fruit production in general. It is a major problem during bloom and on ripening, mature and harvested fruit, particularly during wet weather. However, effective disease and pest management tools for organic farm systems are still lacking. The pathogen *Botrytis cinerea* is a necrotrophic fungus that infests more than 200 different crops worldwide, from which vegetables and small fruits like grapes, strawberries and rasp- and blackberries are the most endangered (Williamson et al., 2007). This fungus is probably the most ubiquitous pathogen worldwide. *B. cinerea* survives the winter in dead or dying leaf tissue and plant debris. In spring, the fungus produces spores that are disseminated to susceptible plant parts by wind and splashing rain or irrigation water. Under cool and wet conditions, fungal spores germinate and infect the blossoms and leaves. For conventional farming systems there are a lot of chemical fungicides available, but resistances increased since most of the products have to be applied several times per season and the genetic plasticity of *Botrytis cinerea* is high. For organic farmers the only control measurements are to limit the growth and distribution of the fungus using cultivation practices, like mulching with straw to reduce humidity or to increase plant spacing to ensure air movement and good lighting conditions (Williamson et al., 2007).

Additionally, both wild and domesticated pollinator densities rapidly decline i.a. due to the global intensification of agriculture (e.g. Potts et al., 2010). However, 35 % of the world's crop production relies on animal pollination and thus bee pollination by honey-, bumble-, and wild bees is a key ecosystem service (e.g. Klein et al., 2007, Potts et al., 2010). In strawberries, adequate pollination increases the market values by 40 % to 50 % per fruit compared with wind and self-pollination, respectively.
Intensively pollinated flowers produce fruits of higher weights, less malformations, longer shelf-life and improved post-harvest quality due to lower sugar-acid-ratios and a more intense coloration (Vaissiere et al., 2011; Klatt et al., 2014). However, due to high disturbances of suitable landscapes, habitats and nesting sides and thus reduced pollinator abundance and activities in the agricultural fields, natural pollinator densities are often too low and pollination quantitatively and qualitatively inadequate to guarantee

maximum food quality (Biesmeijer et al., 2006).

## 1.2 Aims and objectives

This project was based on the pilot call (first call) of CORE Organic II
http://www.coreorganic.org/corenews/sep06/COREcall_%20final_draft_060904.pdf
The BICOPOLL project aimed to overcome disease management and pollination deficits with targeted precision bio control and pollination enhancement involving honey bees, bumble bees, and solitary bees. Bees are able to carry a plenty of microscopic particles like pollen and fungal spores, but also bacteria and viruses (Shafir et al., 2006; Kevan et al., 2008). Hence, foraging activities of bees will be used to deliver biological control agents (BCA) to the flowers of target crops in order to antagonize pests and pathogens like *Botrytis cinerea* and to increase the pollination success and finally fruit and yield quality, simultaneously. Therefore the fungus spores (antagonists) must be added into specially designed dispensers that are fitted in front of the bee hives. The bees then pick up the spores between their body hairs and bring them to the flowers. This 'entomovectoring technology' is a promising approach in the sense of sustainable agriculture as it provides both ecological and economic profits. The use of bees has many environmental and economic benefits compared to spraying fungicide like in conventional farming systems. This highly innovative approach can solve some of the most difficult disease and pest problems in organic berry and fruit production. It offers solutions in an area where no solutions exist now. The aim of the project was to improve the efficiency of the entomovector technology via innovative research on bee management, components of the cropping system, and on the plant-pathogen-vector-antagonist–system. Moreover, the project aimed to investigate possibilities of expanding the use of this concept into other organic berry and fruit growing systems.
The BICOPOLL project was designed as a pan-European case study, including cooperation partners from Finland, Estonia, Belgium, Slovenia, Italy, Turkey and Germany. Usability and practicability of different bee species (*Apis mellifera*, *Bombus terrestris*, *Osmia cornuta* and *Osmia rufa*) to disseminate the biological control agent Prestop® Mix from Verdera, containing mycelium and spores of *Gliocladium catenulatum* (Strain J1446), were investigated. The project was divided into eight work-packages, one for the coordination and dissemination of results (WP1), and seven for specific scientific issues (WP2-8; Fig.1).
The main objective and scientific question for the LAVES bee institute was, how to steer

foraging bees to the target crop (WP2: Honey bees *Apis mellifera* as vectors and crop pollinators) - more in detail:

- to develop practical methods for steering foraging bees to the target crop,
- to optimize the required pollinator (hive) density for vectoring and pollination on the target crop,
- to determine the practical needs for managing beehives used to disseminate the biocontrol agents (BCA),
- if possible, to investigate the effect of vegetation management in and around strawberry fields on the success of targeted BCA vectoring and pollination,
- to develop a practical guide/handbook to the biocontrol/pollination service for berry growers and beekeepers at the end of the project period.

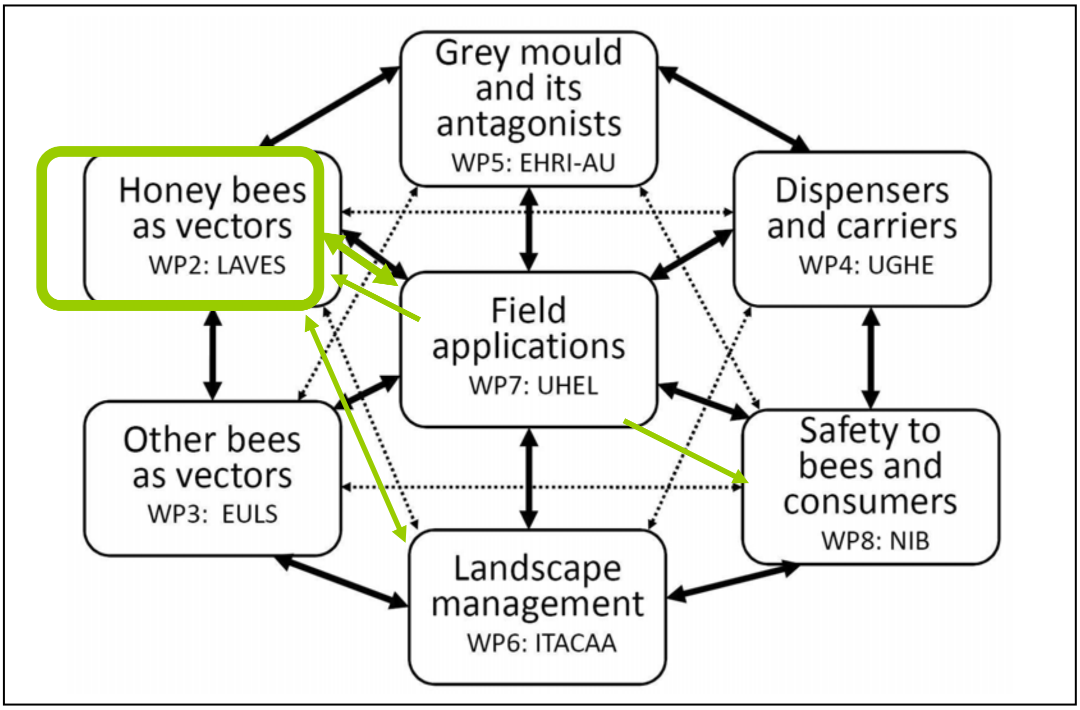


Figure 1: Cross-linkages within in the BICOPOLL consortium. The cross-linkages of the LAVES IB Celle are underlined with green color.

## 1.3. Planning and project procedure

The coordination of the consortium was organized by the Finnish partner from the University of Helsinki (UHEL). Annual meetings were organized in Helsinki (Kick-off meeting in 2012), Turkey, Antalya (2013), Italy, Bologna (2014) and a final meeting in Pålsböle, Åland (Finland in 2014).

**Project procedure:**
For the German participant the project was divided into twelve milestones, referring to honeybees as vectors (M1-4), vegetation management (M5-7), field applications (M8-9), technology transfer (M10-11) and safety of the bio-control agents (M12).

As bees, that actually forage in the target crop, is a key essential requirement for the entomovectoring technology, the main focus of this project was to determine, which factors can affect foraging ranges of honeybees and to examine how to steer the bees to the target crop (strawberry), even if the nectar or pollen rewards are less attractive compared to competitive other plants in the near surrounding. We hypothesized that small hives (number of individual bees per colony) forage closer to their hive and are thus more suitable for the bio-control of less nectar and pollen producing crops (like strawberries) than big hives, with higher numbers of individual bees per colony. For this, foraging behaviors of colonies of defined sizes were compared based on the bees' nectar and pollen resources, which they brought back into their hive. Nectar and pollen resources were determined using honey stomachs and pollen pockets (corbicula pollen). Further, to determine the influence of vegetation management in the surrounding area, the amounts of pollen from other competing crops were examined. In addition to that, we assessed the natural pollinator densities (wild bee, bumble bee and hoverfly) in comparison to honeybee densities in the target crop by the means of line-transects. These investigations were carried out on a study site in Metzingen/Lower Saxony (postcode 29351) throughout all three study years and additionally on a study site in Nienhagen/Lower Saxony (postcode 29336) in 2012.

Furthermore, we investigated the general attractiveness of the used strawberry cultivar by determining the amount of daily produced pollen and nectar as well as the nectar sugar-ratios (2013). In the last year (2014), we additionally tested the biological control agent Prestop® Mix for its compatibility for bees based on a larval toxicity test.

## 2. State of the art

Early trials on biological control of *Botrytis cinerea* have all relied on spraying the bio control agents (BCA's) on the target flowers. However, single sprayings cannot be adjusted to deliver the BCA's to inflorescences at different developmental stages. In contrast, bees colonize flowers with BCA's frequently at each stage of development through repeated flower and pollination visits over the entire course of the day and blooming period. For strawberries the entomovector technology has extensively been tested using honeybees and bumble bees to deliver the control agents *Gliocladium roseum* (e.g. Peng et al., 1992, Sutton and Peng 1993; Yu and Sutton 1997) and *Trichoderma harzianum* (e.g. Kovach et al., 2000; Bilu et al., 2003; Shafir et al., 2006; Albano et al., 2009). However, further investigations regarding efficiency and practicability are still needed as the success of the treatment is largely influenced by the bee species, the dispenser type, the carrier material and the attractiveness of the target crop, especially in open field experiments (Bilu et al, 2004; Shafir et al., 2006). A most comprehensive review about entomovectoring in plant protection can be found in Mommaerts & Smagghe (2011).

**Pollinator species**

The most efficient pollinator species depends on the target crop, the landscape context and cultivation system (greenhouse, open field) and is hence crucial for maximizing pollination and disease control. Albano et al. (2009) found that strawberry flowers in open fields pollinated by honeybees carry lower amounts of Colony Forming Units (CFU) of *Trichoderma harzianum* than flowers pollinated by bumble bees in greenhouses. They argue that honeybees probably loose high amounts of the BCA's through grooming behavior, flight activities and especially by visiting alternative crops in the surroundings. Consequently, in the open field only 33 % of the flowers pollinated by honeybees carried detectable vestiges of the control agent, whereas in the greenhouse (equipped with bumble bees) 100 % of the flowers were successfully colonized. Additionally, even solitary bees like the horn faced bee *Osmia cornuta* have already been shown to serve as pollinators and disseminators for delivering BCA's for the control of fire blight with the causal pathogen *Erwinia amylovora* (Maccagnani et al. 2004).

**Dispenser type**

Furthermore, an adequate distribution of the BCA's is not only affected by the formulation type, but also by the dispenser type. Up to now, there are two different dispenser types available, the one-way type (the chambers through which the bees enter or leave the dispenser are not separated) and the two-way type (the bees leave the hive through a chamber filled with BCA's and enter through a separated chamber without BCA's).

For the North American bumblebee *Bombus impatiens* the use of a one-way system as developed by Yu and Sutton (1997) was satisfactory, whereas for the main greenhouse pollinator in Europe, *Bombus terrestris*, the one-way systems showed very low efficacy (Maccagnani et al., 2004). More recently, Mommaerts et al. (2010) developed a new two-way dispenser for *B. terrestris*, realizing a 10 times higher loading of the passing bumble bees compared to earlier dispensers. This changed system even did not affect the foraging intensity of the bumblebees used. In general, an optimal dispenser should fulfil the following three parameters (a) loads the vector with a sufficient amount of the BCA product, (b) does not interfere with the foraging behavior, and (c) has long refilling intervals (>1 day) (Kevan et al., 2008; Mommaerts & Smagghe, 2011).

**Carrier material**

Carrier materials must be selected with respect to BCA stability (Hjeljord et al., 2000) and vector safety (Israel and Boland, 1993; Pettis et al., 2004). Early studies used pollen as BCA carrier substance (Thomson et al., 1992), polystyrene beads, which showed to be efficient but were expensive (Butt et al., 1998), corn meal (Peng et al., 1992), corn flour (Al-mazra'awi et al., 2006), bentonite (Kevan et al. 2008) and corn starch (Maizena-Plus) (Mommaerts & Smagghe 2011). As they are inexpensive, safe to bee brood (Pettis et al., 2004), do not induce grooming behavior (Kevan et al., 2008), and need no additional registration because they are of food grade qualified. However, to date there is still inadequate information on the potential of different carriers and their role in vector acquisition. Still data are lacking about potential side effects of the carrier material to the foraging bees that pass the dispenser.

**Attractiveness of the target crop**

Efficiency of the entomovector technology is furthermore affected by the target crop and it's highly depending onto the attractiveness to the pollinator species. Wild bees only have low foraging ranges up to a few of hundreds meters from their nest sides. Whereas foraging ranges of honeybees cover several kilometers, always searching for the most lucrative nectar and pollen resource, with mass dimensions. Accordingly, in early trials on testing the entomovector technology in open strawberry fields, honeybee densities in the strawberry fields were low (Albano et al., 2009), because strawberry flowers only produce low amounts of nectar and pollen in general.

However, foraging range also might depend on the hive size (number of individual bees per colony), following a theoretical strategy of optimal foraging. Although obtaining food provides the bee and the whole bee colony with energy, searching for and capturing the food require both energy and time. It might be useful, that the individual bee wants to gain the most benefit (energy) for the lowest cost during foraging, so that it can maximize its fitness. This theory helps to predict the best strategy that a bee and a bee colony can use to achieve this goal, whereby small bee hives might show lower foraging ranges than big hives. Hence, within the course of this project the LAVES Bee Research Institute Celle examined whether small hives forage more closer to their nest and are thus more suitable for the bio-control of strawberries than big hives.

## 3. Materials and methods

### 3.1 Study sites

**Study site 1: Metzingen/Eldingen (2012 – 2014)** – see Figure A-1 in the annex
Main study trials were conducted on fields of a local organic farmer in Metzingen/Eldingen (postcode 29351) in the district of Celle/Lower Saxony. The cultivated variety was Fragaria x ananassa 'Korona', planted in monoculture as double rows using refrigerated plants. Plant spacing was 25 cm within the row, 56 cm between the rows and 150 cm between double rows. Cultivation was divided into a 1.5 ha one-year-old and a 0.8 ha two-year-old field in 2012 and a 1.6 ha one-year-old and a 0.5 ha two-year-old part in 2013. In 2014 there was only a one-year-old field covering 1.7 ha. Hedge structures around the fields included *Cornus sanguinea*, *Rosa canina*, *Salix alba* and *Trifolium repens*. Competing crops within a 3 km radius were *Brassica napus* in 1.8 km distance from the hives during all three study years and additionally *Trifolium incarnatum-Viccia cracca* right next to the strawberry field in the last year. To limit growth and distribution of *Botrytis cinerea* the farmer mulched the fields with straw.

**Study site 2: Nienhagen/Wathlingen (2012)** – see Figure A-2 in the annex
Additionally, to investigate the influence of other competing crops to the bees foraging behaviour, an extra trial was conducted using a strawberry field of a conventional farmer in Nienhagen/Wathlingen in the district of Celle in 2012. The field was divided into a one-year-old and a two-year-old part. Cultivated varieties were Fragaria x ananassa

'Donna', 'Kimberly' and 'Clery'. Competing crops within a 3 km flight radius were two blooming *Asparagus officinalis* fields in 450 m and 690 m distance and a flower stripe in 70 m distance to the hives.

**Table 1:** Overview of the study sites in the district of Celle in Lower Saxony from 2012-2014.

| study year | study site | size | cultivar | competing crops within 3 km radius (distance to the hives) |
|---|---|---|---|---|
| **2012** | Metzingen | 1.5 ha (one-year-old field)<br>0.8 ha (two-year-old field) | Korona | *Brassica napus* (1.8 km) |
| **2012** | Nienhagen | (one-year-old field)<br>(two-year-old field) | Donna, Kimberly, Clery | Flower stripe (70 m), *Asparagus officinalis* (450 m, 690 m) |
| **2013** | Metzingen | 1.6 ha (one-year-old field)<br>0.5 ha two-year-old field | Korona | *Brassica napus* (1.8 km) |
| **2014** | Metzingen | 1.7 ha (one-year-old field) | Korona | *Trifolium incarnatum-Vicia cracca* (250-300 m), *Brassica napus* (1.8 km) |

### 3.2 Experimantel bee hives

Food preferences and flower allocation of hives differing in size were compared to determine the influences of hive size on foraging behavior and flying range. Colonies of defined sizes were prepared using artificial swarms as starting material. Three small colonies using 1200 g and three large colonies using 2500 g bees were prepared by weighting the bees with a scale in the first year; four small using 600 g and four large using 1000 g in the second year and five small using 300 g and five large using 500 g bees in the last year. All hives were equipped with sister queens in order to have the same genetic basis. The bees were filled into small (Mini-Plus-Beuten) and into large polystyrene hives (Segeberger-Beuten) onto frames equipped with wax-foundations. At the starting point the bees were fat with liquid sugar in order to boost their development. Hives were positioned next to the strawberry fields at the beginning of the flowering phase of the strawberries. Resulting population sizes were verified using the Liebefeld method (Imdorf et al. 1987; Imdorf and Gerig, 1999) of estimation the bee population (number of open and capped brood cells and the number of adult bees) once at the beginning and once at the end of the trial respectively (Tab. 2).

**Table 2:** Preparation of the experimental colonies (population estimates).

| year | site | artificial swarm | population estimate | hive no. | adult bees | capped brood | open brood | ∑ brood | pollen | bees/brood |
|---|---|---|---|---|---|---|---|---|---|---|
| 2012 | Metzingen | - | 15.05.2012 - | 1 | 4494 | 8410,5 | 6207,75 | 14618,25 | 133,5 | 3,25 |
| | | - | - | 2 | 5376 | 9478,5 | 6141 | 15619,5 | 133,5 | 2,91 |
| | | - | - | 3 | 4704 | 9345 | 6741,75 | 16086,75 | 400,5 | 3,42 |
| | | - | - | 4 | 19525 | 14087,5 | 16296 | 30383,5 | 1050 | 1,56 |
| | | - | - | 5 | 9405 | 7525 | 12775 | 20300 | 875 | 2,16 |
| | | - | - | 6 | 34705 | 20650 | 24150 | 44800 | 5687,5 | 1,29 |
| | | - | 06.06.2012 - | 1 | 12978 | 8677,5 | 9879 | 18556,5 | 0 | 1,43 |
| | | - | - | 2 | 11424 | 6808,5 | 7209 | 14017,5 | 0 | 1,23 |
| | | - | - | 3 | 8442 | 4939,5 | 7609,5 | 12549 | 0 | 1,49 |
| | | - | - | 4 | 25795 | 20737,5 | 14525 | 35262,5 | 437,5 | 1,37 |
| | | - | - | 5 | 15510 | 9800 | 8925 | 18725 | 0 | 1,21 |
| | | - | - | 6 | 25795 | 19862,5 | 22050 | 41912,5 | 1050 | 1,62 |
| | Nienhagen | - | 23.+24.07.2012 - | 117 | 17517 | - | - | 18025 | - | - |
| | | - | - | 116 | 25872 | - | - | 27475 | - | - |
| | | - | - | 4 | 27447 | - | - | 22663 | - | - |
| | | - | - | 1 | 16968 | - | - | 6942 | - | - |
| | | - | - | 2 | 16212 | - | - | 12015 | - | - |
| | | - | - | 9 | 21714 | - | - | 16220 | - | - |
| 2013 | Metzingen | 08.05.2013 600 g | 17.05.2013 (9 days later) | 116 | 2273 | 0 | 4872,75 | 4872,75 | 200,25 | 0,47 |
| | | 680 g | | 108 | 2489 | 0 | 3270,75 | 3270,75 | 200,25 | 0,76 |
| | | 620 g | | 142* | 3424 | 0 | 2603,25 | 2603,25 | 200,25 | 1,32 |
| | | 600 g | | 112 | 2377 | 0 | 1602 | 1602 | 0 | 1,48 |
| | | 1020 g | | 115 | 7155 | 525 | 14350 | 14875 | 612,5 | 0,48 |
| | | 1160 g | | 5 | 6200 | 1400 | 11900 | 13300 | 700 | 0,47 |
| | | 1120 g | | 141 | 7600 | 525 | 14525 | 15050 | 1225 | 0,50 |
| | | 1080 g | | 102 | 7660 | 0 | 14875 | 14875 | 437,5 | 0,51 |
| | | 08.05.2013 600 g | 18.06.1013 (41 days layter) | 116 | 6226 | 15619,5 | 6942 | 22561,5 | 66,75 | 0,28 |
| | | 680 g | | 108 | 5660 | 8811 | 5673,75 | 14484,75 | 534 | 0,39 |
| | | 620 g | | 142 | 6576 | 14284,5 | 9078 | 23362,5 | 467,25 | 0,28 |
| | | 600 g | | 112* | 2592 | 0 | 0 | 0 | 667,5 | |
| | | 1020 g | | 115 | 13955 | 20825 | 19075 | 39900 | 2362,5 | 0,35 |
| | | 1160 g | | 5 | 17115 | 17412,5 | 19862,5 | 37275 | 2537,5 | 0,46 |
| | | 1120 g | | 141 | 15090 | 24150 | 14175 | 38325 | 2450 | 0,39 |
| | | 1080 g | | 102 | 19805 | 21525 | 16012,5 | 37573,5 | 4900 | 0,53 |
| 2014 | Metzingen | 14.04.2014 300 g | 05.05.2014 (21 days later) | 200 | 1863 | 3604,5 | 2670 | 6274,5 | - | 3,37 |
| | | 300 g | | 201 | 1494 | 4272 | 2403 | 6675 | - | 4,47 |
| | | 300 g | | 202 | 1502 | 4806 | 1335 | 6141 | - | 4,09 |
| | | 300 g | | 203 | 1251 | 2136 | 1068 | 3204 | - | 2,56 |
| | | 300 g | | 204 | 1637 | 4672,5 | 2670 | 7342,5 | - | 4,49 |
| | | 300 g | | 205 | 2584 | 5540,25 | 1001,25 | 6541,5 | - | 2,53 |
| | | 500 g | | 206 | 2720 | 12600 | 4550 | 17150 | - | 6,31 |
| | | 500 g | | 207 | 1955 | 7000 | 2275 | 9275 | - | 4,74 |
| | | 500 g | | 208 | 2555 | 11550 | 4025 | 15575 | - | 6,10 |
| | | 500 g | | 209 | 4150 | 9975 | 2975 | 12950 | - | 3,12 |
| | | 500 g | | 210 | 2645 | 9100 | 1575 | 10675 | - | 4,04 |
| | | 500 g | | 211 | 2805 | 15225 | 8575 | 23800 | - | 8,48 |
| | | 14.04.2014 300 g | 26.05.2014 (42 days later) | 200 | 5335 | 8143,5 | 7209 | 15352,5 | - | 2,88 |
| | | 300 g | | 201 | 4884 | 6408 | 6274,5 | 12682,5 | - | 2,60 |
| | | 300 g | | 202 | 4096 | 6808,5 | 2202,75 | 9011,25 | - | 2,20 |
| | | 300 g | | 203 | 2010 | 3070,5 | 2736,75 | 5807,25 | - | 2,89 |
| | | 300 g | | 204 | 5037 | 7876,5 | 7075,5 | 14952 | - | 2,97 |
| | | 300 g | | 205 | 7870 | 10813,5 | 10546,5 | 21360 | - | 2,71 |
| | | 500 g | | 206 | 8015 | 0 | 0 | 0 | - | 0,00 |
| | | 500 g | | 207 | 9710 | 17850 | 12600 | 30450 | - | 3,14 |
| | | 500 g | | 208 | 11195 | 19600 | 16975 | 36575 | - | 3,27 |
| | | 500 g | | 209 | 7765 | 12425 | 6825 | 19250 | - | 2,48 |
| | | 500 g | | 210 | 11385 | 15575 | 14000 | 29575 | - | 2,60 |
| | | 500 g | | 211 | 16160 | 21525 | 16100 | 37625 | - | 2,33 |

### 3.3 Pollination level in the field: pollinators along line-transects (2012-14)

To assess the pollinator diversity and occurrences in the strawberry fields, honeybee, wild bee, bumble bee and hoverfly densities were measured by counting all visible pollinators between two double rows along 100 m line-transects. Various transects were set evenly distributed across the field in the first year. In the second and third study year line-transects were arranged as continuous lines from the beginning to the end of the field to determine, whether honeybee densities decrease with distance to the hive. Pollinator densities along transects were assessed several times a day respectively and recorded as bees/insects per minute and 100 m transect.

### 3.4 Foraging range of the bee hives: bee and pollen samples (2012-14)

To compare foraging ranges of the small and big experimental bee hives, samples of homecoming forager bees were taken using a hand net, shock-frozen with $CO_2$ snow and stored at -20°C until further preparation durin g the experimental period in Metzingen, 2012. Proportions of Fragaria-pollen collecting bees were determined through pollen analyses from the honey stomachs and pollen loads under the microscope (according to the standard protocol of the LAVES IB Celle). However, as there was only little strawberry pollen in the honey stomachs, sampling of homecoming forager bees was replaced by sampling pollen with pollen traps in Metzingen 2013 and 2014. Samples were taken several times a day in the first and second study year and only once at the end of the day in the third year respectively. 2013 and 2014 all pollen sources were determined to identify the major competing crops. In order to investigate the possible influence of *Asparagus officinalis*, *Zea mays* and flower stripes as competing crops, bulk samples from all bee hives using $CO_2$ snow were taken in Nienhagen 2012.

### 3.5 Attractiveness of strawberries: Sugar concentrations of strawberry nectar (2013)

Attractiveness of strawberry flowers was examined by determining its nectar sugar ratios. Though only flowers excluded from pollinators were used (flowers from cages, tunnel or greenhouse), nectar volumes were too low to collect pure nectar with micro capillary tubes and to determine daily produced nectar volumes per flower. Hence, only sugar ratios of bulk samples could be determined. For this, three different methods for sampling nectar from flowers with low nectar amounts were applied.

a. Washing flowers in distilled water: 120 flowers out of a strawberry tunnel were washed in 200 ml of distilled water. After 60 min the sample was filtered and stored at -20℃ until further preparation.

b. Rinsing with a pipette using a known volume of distilled water: For the rinsing method frigoplants from the field were cherished in the greenhouse. During flowering 25 µm of distilled water were rinsed over the nectaries, let it on for a short moment and then removed. This procedure was repeated with a plenty of flowers until a sufficient volume to run the high-performance liquid chromatography analysis (HPLC) was obtained.

c. Centrifugation: 123 single flowers from the open field, which had been caged the day before in order to hinder flower visitation by pollinators, were trimmed, each placed in a calibrated centrifuge tube by pinning them onto the lid and then centrifuged at around 2,500 rpm for 5 min. Resulting nectar volumes were assembled (67 µl in total) and then filled up with 443 µl distilled water to run the HPLC analysis.

### 3.6 Side effects of the Prestop® powder to bee larvae: Toxicity test (2014)

To test the biological control agent Prestop® Mix, containing mycelium and spores of *Gliocladium catenulatum* (Strain J1446) for its compatibility for the bees, a larval toxicity test with a repeated exposure was performed according to the OECD draft guidance document (for test details see OECD, 2013, http://www.oecd.org/env/ehs/testing/Draft_GD_honeybees_rep_exp_for_2nd_CR_25_November_2013.pdf). We performed two test series. In the first run, 100 µg Prestop® Mix was fed per larvae and day and in the second run, as much of the Prestop® Mix powder as was barely soluble was fed together with the normal royal jelly food standard. Feeding of the test solution started at day 1 after grafting the young bee larvae into the laboratory test tubes. It must be emphasized here, that these first test on possible side effects of the Prestop® Mix powder to bee larvae are only first orientation tests and should be sorted as such only.

# 4. Results

## 4.1 Pollinators in the strawberry fields

Honeybees were the most abundant pollinators during all assessments, whereas hoverflies, wild and bumble bees, including inter alia Andrena sp., Osmia sp., Halictus sp., Lasioglossum sp., Nomada sp., *Bombus lapidarius*, *Bombus pascuorum* and *Bombus terrestris*, showed only low abundances in Nienhagen 2012 and Metzingen 2012 and 2013 respectively. However, in Metzingen 2014 honeybee densities did not differ from the other natural pollinator densities. All in all natural pollinator abundances were similar among study sites and years, but honeybee densities differed, being highest in Metzingen 2012 and lowest in Metzingen 2014 (Fig. 2a d). However, honeybee densities did not differ among line-transects in Metzingen 2013. In 2014 only two measurements could be obtained (Fig. 2e-f).

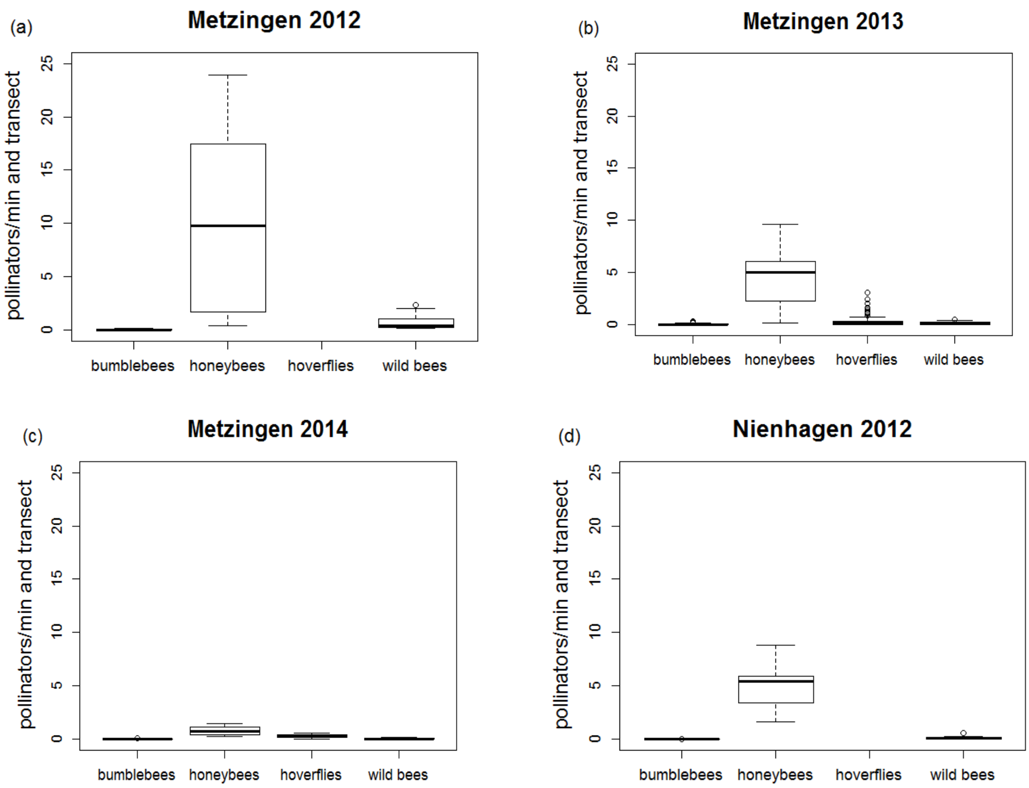


**Figure 2a-d:** Honeybee, wild bee, bumble bee and hoverfly densities per min, measured between two double rows along 100 m line-transects in Metzingen and Nienhagen from 2012 to 2014, visualized as box-and-whisker plots showing the median (horizontal line), interquartile ranges (box) and the 1.5 time ranges of the interquartile range from the box (dashed vertical lines). Notably, bees partly collected nectar and pollen at the same time.

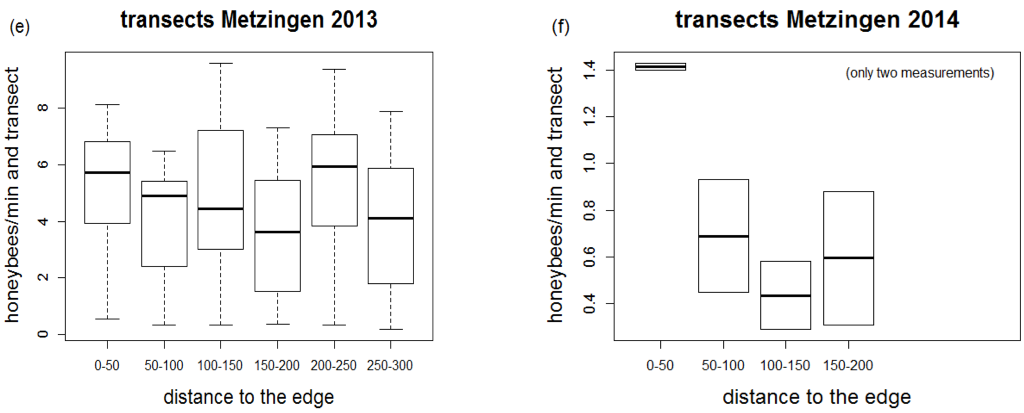


**Figure 2e-f:** Honeybee densities among six different transects in 0-50, 50-100, 100-150, 150-200, 200-250 and 250-300 meter distances to the edge of the field in Metzingen 2013 and among four different transects in 0-50, 50-100, 100-150, 150-200 meter distances in Metzingen 2014.

## 4.2 Bee and pollen samples

***Bee samples using $CO_2$ snow Metzingen 2012: Pollen vs. nectar collecting bees***

In total 86 bee samples including 4.384 homecoming forager bees were caught and prepared for the pollen analysis. Samples included high proportions of nectar collecting bees (median 56.55 %) and bees neither collecting nectar nor pollen (med 34.45 %, Fig. 3a). However, for most nectar collecting bees no inferences about foraging resources could be drawn, as most stomachs contained pollen from many different species. Only few could be determined as strawberry nectar due to pure strawberry pollen (med 0 %; Fig. 3b). Therefore, we changed the focus to the pollen collecting bees (med 43.45 %, Fig. 3a) for all following analyses during the course of the project.

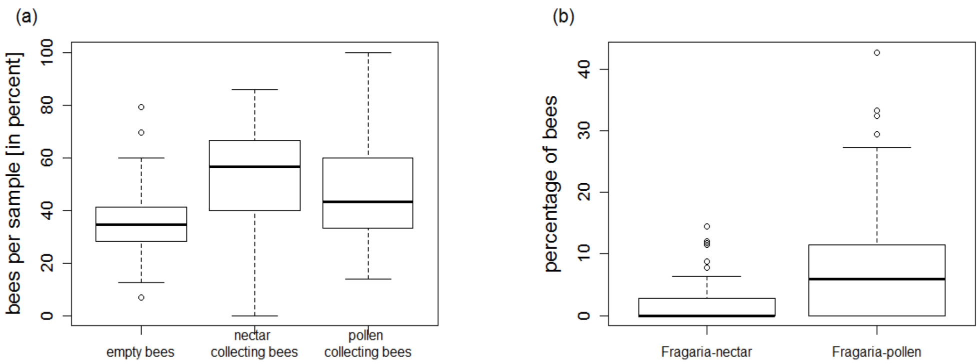


**Figure 3a-b:** Proportion of bees neither collecting nectar nor pollen (empty bees), nectar collecting and pollen collecting bees based on the $CO_2$ samples taken in Metzingen 2012.
**3b:** Proportions of Fragaria-nectar and Fragaria-pollen collecting bees based on the total number of bees which either collected pollen or nectar.

***Metzingen 2012: Pollen analysis based on pollen collecting bees***

In total 1253 individual pollen collecting bees were analyzed. Proportions of Fragaria-pollen collecting bees per sample varied depending on the time of the day, the sampling date and the bee hive (min 0%; max 42.6%). Median Fragaria-pollen proportions per bee hive were low to a maximum of 15 %, ranging from 0 % to 15.25 % (hive 1= 15.25 %; hive 2= 4.25 %; hive 3= 0 %; hive 4= 6.55 %; hive 5= 7.7 %; hive 6= 6.3 %; Fig. 4a). Further, Fragaria-pollen collecting bees decreased during the course of the flowering period, as it can be expected due to the ending of the blooming period (Fig. 4b). However, no differences in the proportions of Fragaria-pollen collecting bees could be obtained between the different experimental colonies (Fig. 4c-d).

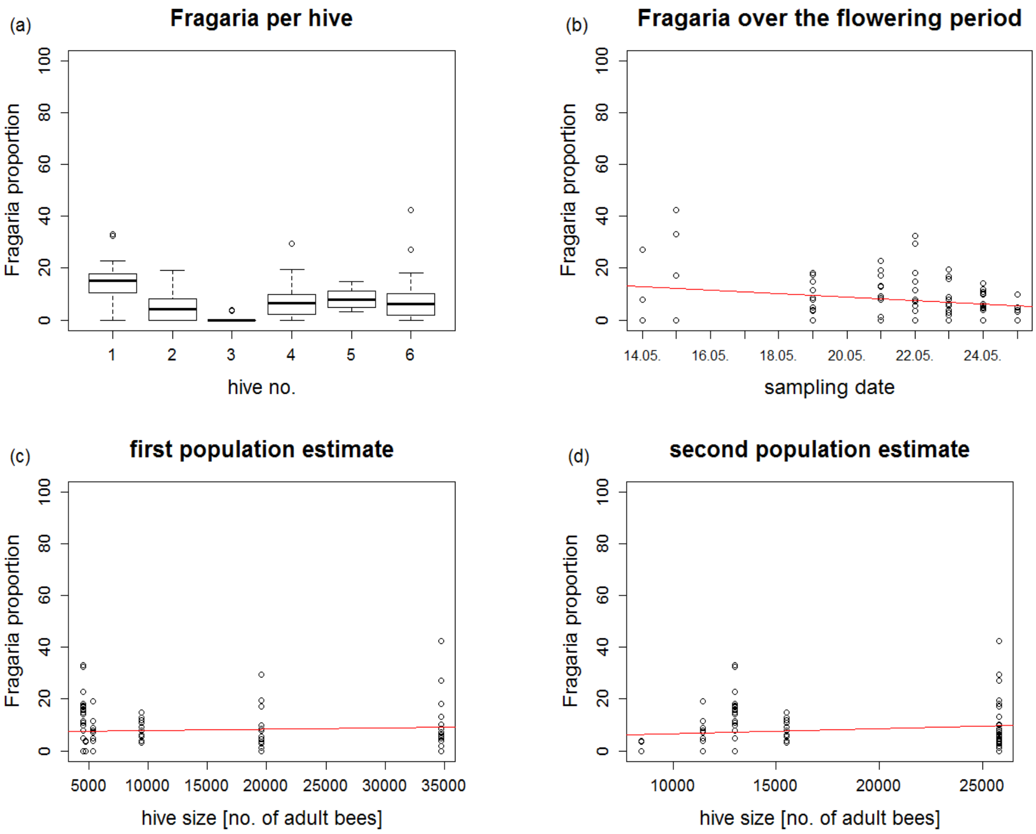


**Figure 4a:** Box and whisker plots of the proportion of Fragaria-pollen collecting bees per bee hive over the entire experimental period. **4b:** Proportion of Fragaria-pollen collecting bees depending on the sampling date. **4c-d:** Proportion of Fragaria-pollen collecting bees depending on the hive size once based on the first population estimate (at the beginning of the experimental period, 4c) and once based on the second population estimate (at the end of the experimental period, 5d:. Hive size is visualized as adult bees per hive.

***Pollen samples using pollen traps Metzingen 2013:***

Since we could find only low amounts of bees with pure strawberry pollen in their honey stomach in 2012, we restricted the analyses of determining the bees' pollen sources using pollen traps in 2013 and 2014 only. In 2013, 107 pollen samples including 54.363 individual pollen pockets were taken from two small hives (hive 108 and hive 116) and three large hives (hive 102, 115 and one sample from hive 141). Proportions of Fragaria-pollen collecting bees per sample were highly variable, depending on the time of the day, the sampling date and the bee hive (min 0 %; max 100 %). Median Fragaria-pollen proportions per bee hive were higher than in 2012, ranging from 10.67 % to 52.42 % (hive 102=19.29 %; hive 108=52.42 %; hive 115=10.67 %; hive 116=49.94 %; hive 141=50.77 %; see Fig. 5a).

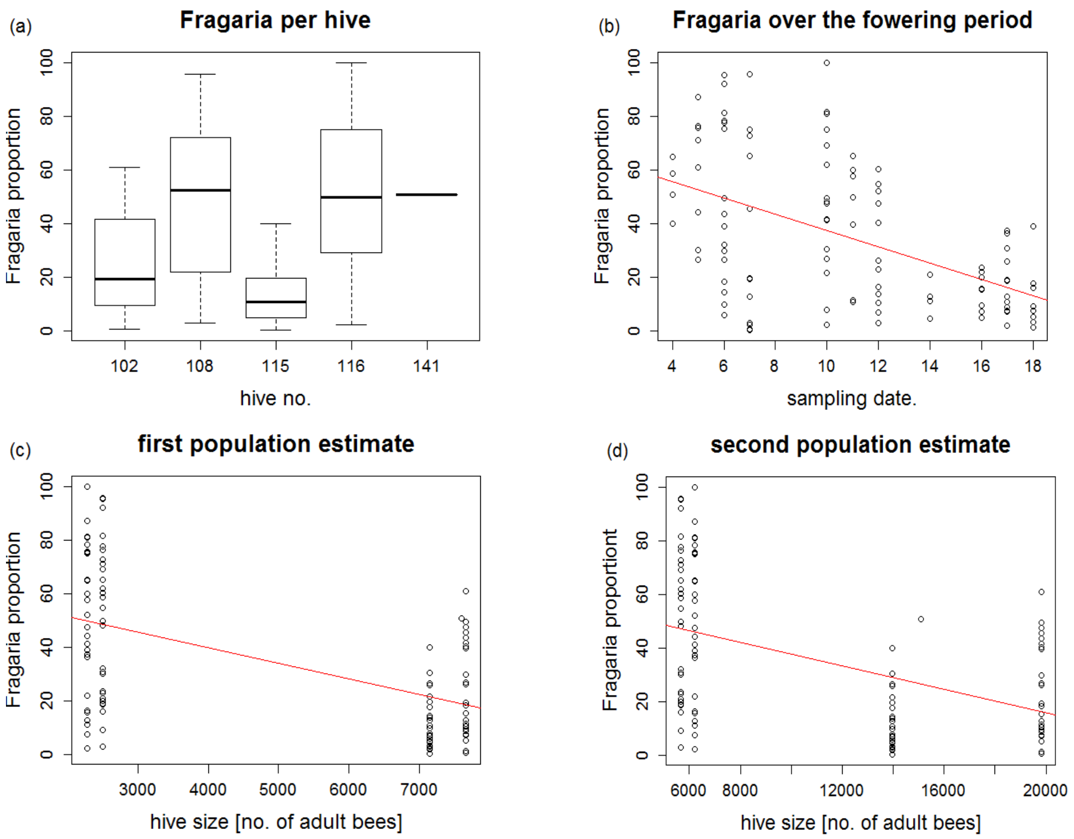


**Figure 5a:** Box and whisker plots of the proportion of *Fragaria*-pollen collecting bees per bee hive over the entire experimental period. **5b:** Proportion of *Fragaria*-pollen collecting bees depending on the sampling date. **5c-d:** Proportion of *Fragaria*-pollen collecting bees depending on the hive size once based on the first population estimate (at the beginning of the experimental period, 5c) and once based on the second population estimate (at the end of the experimental period, 5d). Hive size is visualized as adult bees per hive.

On average Fragaria-pollen collecting bees decreased during the course of the flowering period (Fig. 5b) and big hives showed lower proportions of Fragaria-pollen collecting bees than small hives, based on both population estimates (Fig. 5c-d).

Furthermore, full pollen analyses indicated that besides Fragaria there were no major competing crops. Instead, the bee's pollen sources were highly variable during the experimental period including a wide range of different pollen species. Next to Fragaria, most pollen resources originate from different Rosaceae (including *Rosaceae* sp., *Rosa* sp., *Rubus* sp. *Prunus* sp. *Pyrus* sp. *Pyracantha* sp. and *Potentilla* sp.), *Trifolium repens, Cornus sanguinea* and *Papaver* sp. (Fig. 6). Despite the fact that the *Brassica napus* field was in the closer vicinity, Brassica pollen proportions were low for all bee hives.

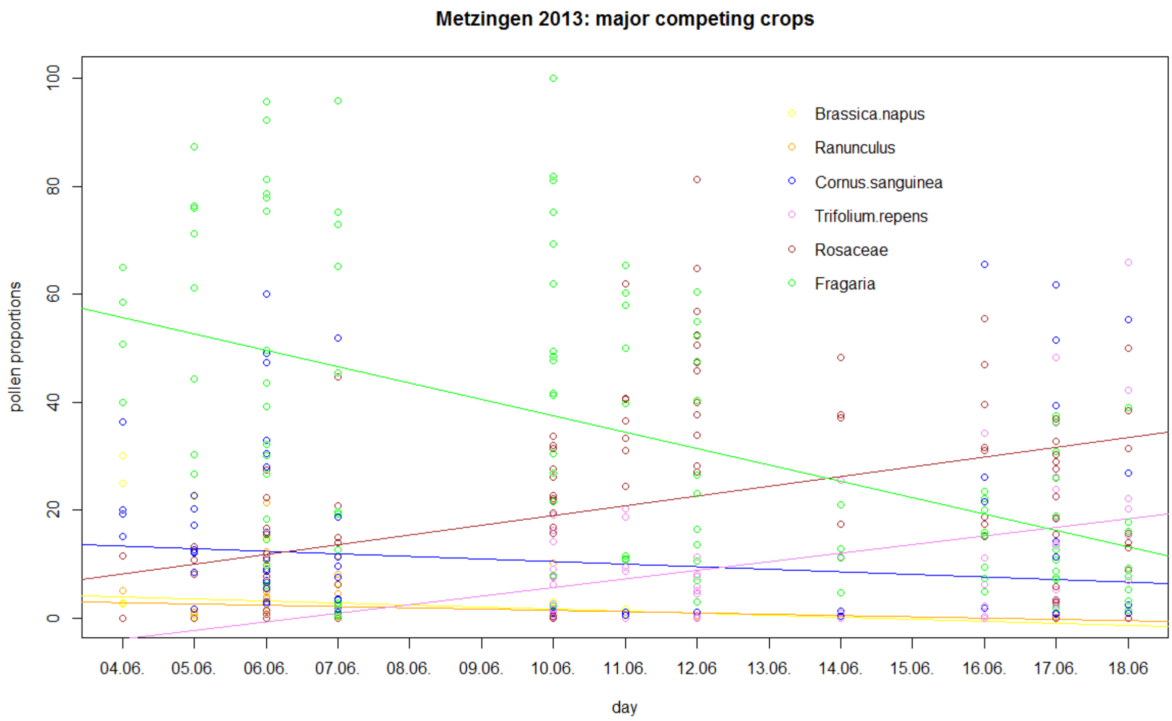


**Figure 6:** Proportion of the most collected pollen species.

### *Bee samples using pollen traps Metzingen 2014:*

In total 90 pollen samples including about 153.798 individual pollen pockets were taken at seven different study days during the flowering period of the strawberries. Proportions of Fragaria-pollen collecting bees per sample were highly variable depending on the sampling date and the bee hive (min 0 % - max 87 %). Median Fragaria-pollen proportions per bee hive were low, ranging from 1.2 % to 13.5 % (hive 200=9.35 %; hive 201=3.3 %; hive 202=10.35 %; hive 203=13.5 %; hive 204=8.7 %; hive 205=10.6 %; hive 206=1.2 %; hive 207=2.5 %; hive 208=2.4 %; hive 209=1.6 %; hive 210=6.4 %; hive 211=3.9 %; see Fig. 7a). Comparing foraging behavior of hives differing in size, on average smaller bee hives showed slightly higher proportions of Fragaria-pollen collecting bees than bigger bee hives based on both population estimates (Fig. 7c-d). However, Fragaria-pollen collecting bees decreased during the course of the flowering period for all bee hives (Fig. 7b).

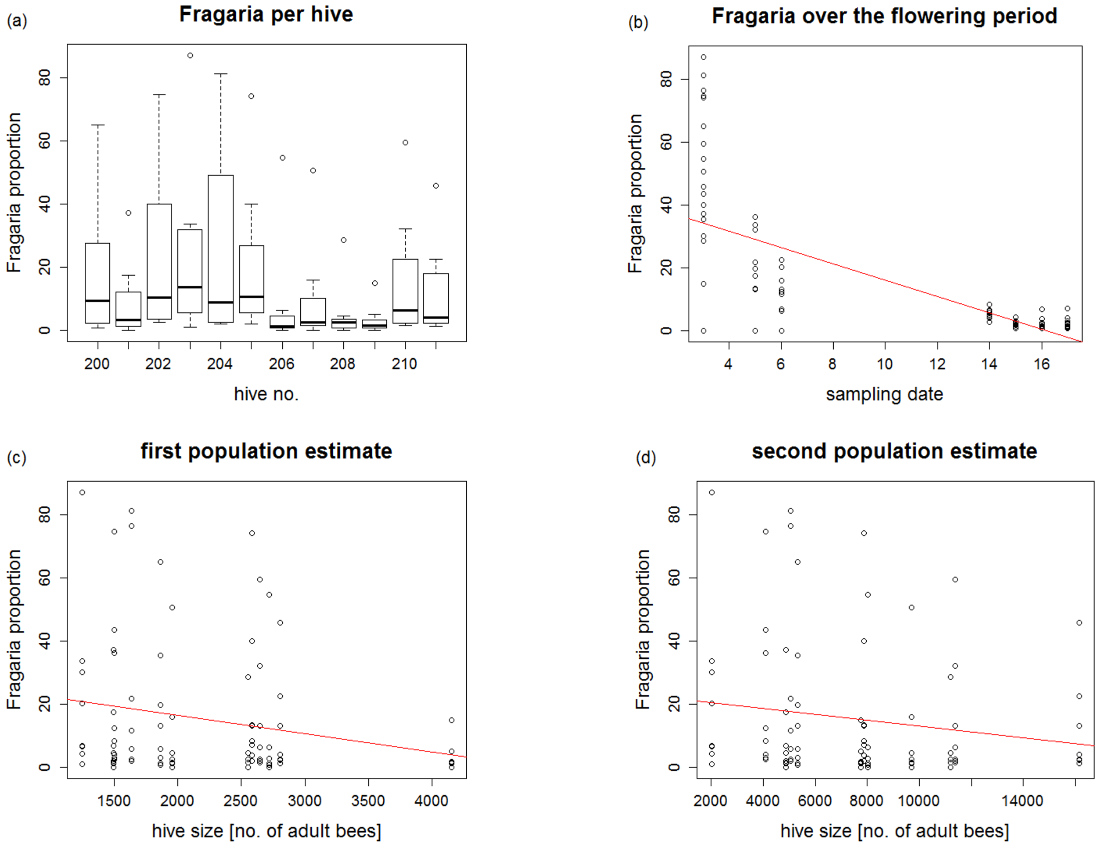


**Figure 7a:** Box and whisker plots of the proportion of *Fragaria*-pollen collecting bees per bee hive over the entire experimental period. **7b:** Proportion of *Fragaria*-pollen collecting bees depending on the sampling date. **7c-d:** Proportion of *Fragaria*-pollen collecting bees depending on the hive size once based on the first population estimate (at the beginning of the experimental period, 7c) and once based on the second population estimate (at the end of the experimental period, 7d). Hive size is visualized as adult bees per hive.

An overall pollen analyses indicated that main pollen sources changed in the course of the flowering period for all bee hives. In the first study days most abundant pollen sources were Fragaria and various Rosaceae. Other pollen sources, but less abundant were *Trifolium repens, Vicia cracca, Rhamnus sp., Acer* sp.,*Chelidonium major, Taraxacum* sp., *Cornus sanguinea, Campanula* sp., *Rumex* sp., *Ligustrum* sp., *Ilex aquifolium, Sambucus* sp., *Brassica napus, Aesculus* sp., *Papaver* sp., *Ustilaginomycotina* sp. and some Ericaceae and Pocaceae. However, *Trifolium incarnatum* proportions increased during experimental period reaching a maximum median of 92.35 % including all bee hives at the end of the experimental period (May 15, see Fig. 8).

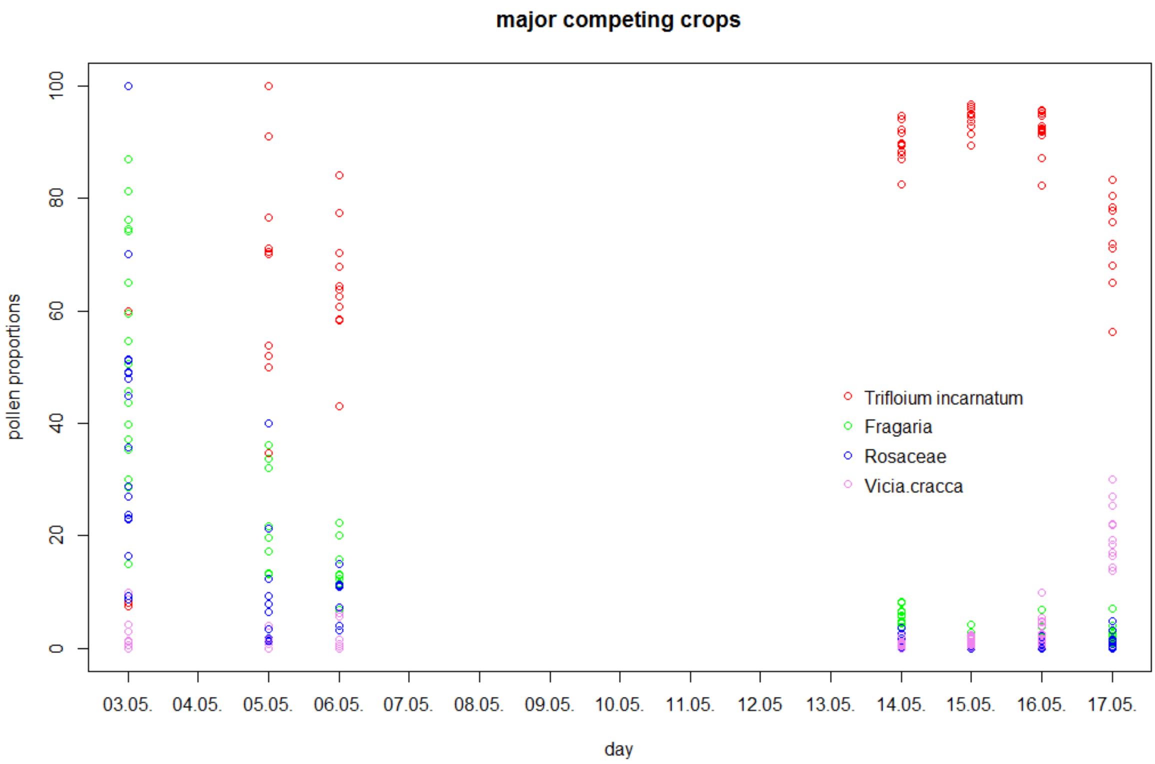


**Figure 8:** Pollen proportion of the most abundant pollen sources (*Trifolium incarnatum, Fragaria*, different Rosaceae and *Vicia cracca*) for all bee hives in Metzingen 2014.

***Bee samples using $CO_2$ snow Nienhagen 2012:***

In total eleven bee samples including about 509 individual pollen collecting bees were taken at five different study days during the flowering period of the strawberry field. Most abundant pollen were *Asparagus officinalis* (36.1 % to 67.8 %), followed by Fragaria (1.7 % to 34 %) and pollen from species included in the flower stripe (1.7 % to 29.2 %).

*Zea mays* proportions were low for all study days (0 % to 13.6 %; see Fig. 9). Other but less abundant pollen sources were *Trifolium repens*, *Matricaria chamomilla*, *Achillea* sp. and *Cirsium* sp..

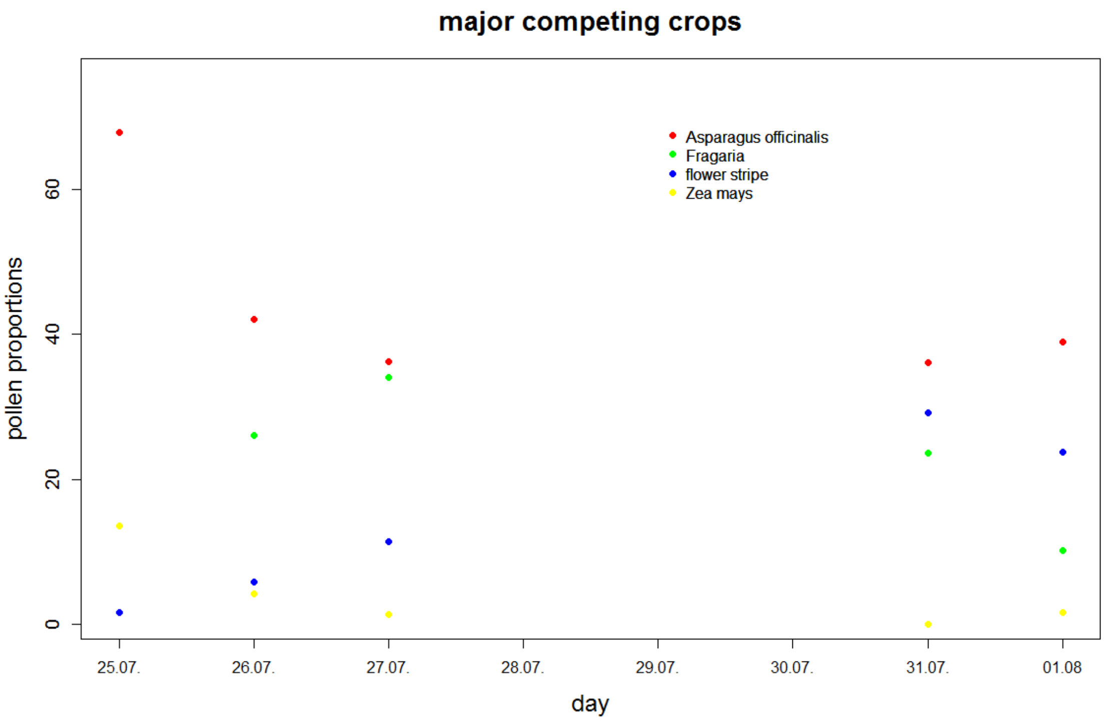


**Figure 9:** Proportional pollen sources including *Asparagus officinalis, Fragaria*, different flower stripe species as well as *Zea mays* for all bee hives in Nienhagen 2012. Flower stripe species include *Phacelia sp., Raphanus sativus* and *Sinapsis alba*.

## 4.3 Sugar concentration of strawberry nectar

The daily nectar volume was altogether too low in order to gain quantify results. Only a collective sample including many flowers was analyzed using HPLC.

## 4.4 Toxicity test (side effects of the Prestop® powder)

Visible effects of Prestop® Mix fed to developing bee larvae could be obtained from the standard larval laboratory tests. Even in the low concentration test series, while feeding only 100 µg/larva of the Prestop® Mix powder together with the standard larval food (royal jelly), some larvae showed symptoms of necrosis, but could develop up to adult bees (see the following pictures – Fig. 10a-c). However, their developmental time was prolonged compared to the untreated larvae. Bee larvae fed with the maximum solubility proportion of Prestop® Mix in the larval food (royal jelly) were all dead 10 days following their grafting into the laboratory test equipment (see Fig. 11a-b).

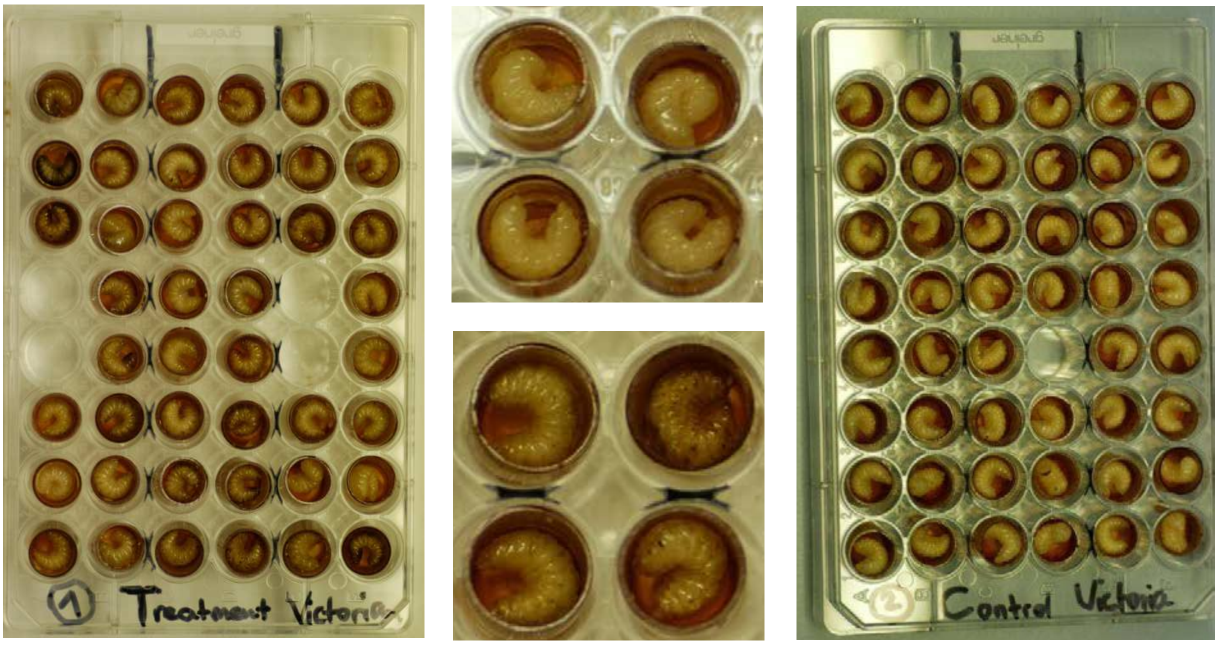


**Figure 10a-c:** Visible effects of Prestop® Mix fed to developing bee larvae could be obtained from the standard larval laboratory tests. Left: treatment (100 µg/larvae). Right: control. In the middle (above control), down: treatment, the developing larvae show symptoms of necrosis.

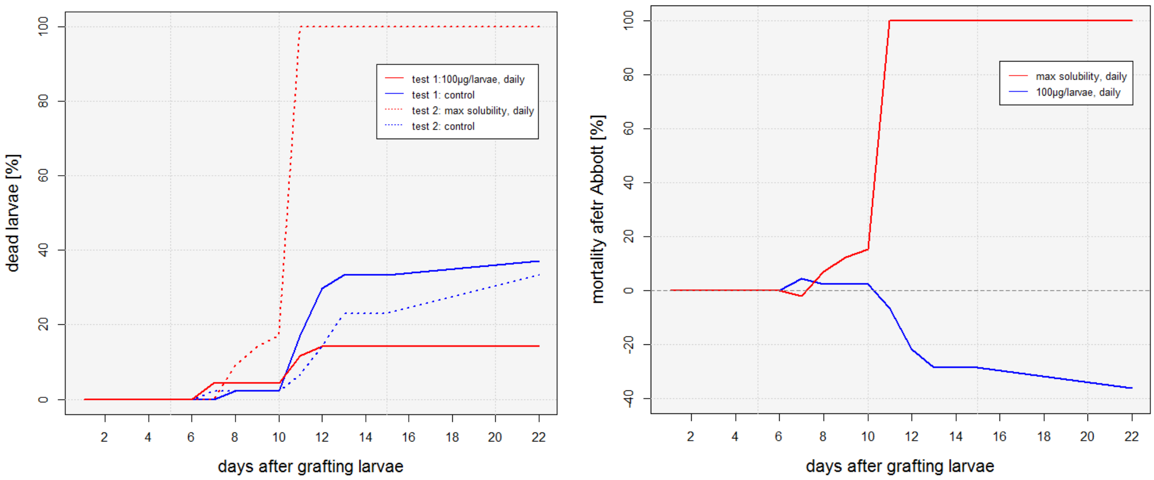


**Figure 11a-b:** Mortality rate of bee larvae fed with Prestop® Mix mixed to their standard larval food (royal jelly) under laboratory conditions with two concentrations (100 µg/larvae and maximum solubility proportion). 11b) Mortality rate of the two treatments corrected according to the Abbott's formula.

## 5. Discussion of the results

As bees forage in the target crops is a key requirement not only for the pollination purposes, but particularly for the transfer of the fungus antagonist to the flower, the main focus of this project part was to determine, which factors affect foraging ranges of honeybees and to examine how to steer the bees to the target crop, even if the nectar or pollen rewards are less attractive to nearby alternative crops, that might attract the bees more.

We hypothesized that small hives forage closer to the hive and are thus more suitable for the bio-control of less nectar and pollen producing crops like strawberries than big size beehives. For this, foraging behaviors of colonies of defined sizes were compared based on the bees' nectar and pollen resources obtained.

We started our experimental colonies with weighted adult bees obtained from artificial swarms in order to build up "small" and "large" colonies respectively. Sister-queens were used for all colonies in order to reduce genetic effects. As expected, from the "large" colonies more foraging bees could be counted regularly during the whole observation period if compared to the "small" colonies.

We could not find differences in the flight activity of the foraging bees within the strawberry field in relation to the hive position (near or 300 m from the hives).

Also 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. During all records along the line transects regularly honey bee foragers, wild bees, inclusive bumble bees and hoverflies could be obtained.

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.

Furthermore, we investigated the general attractiveness of the used strawberry cultivar by determining the amount of daily produced pollen and nectar as well as the nectar sugar-ratios.

The quantification of the steering of the bees to the target crop (strawberries) was confirmed based on pollen analysis from two sources, the pollen loads from bees (corbicula), which were collected in pollen traps installed in front of the experimental colonies and directly from individual homecoming foragers/pollinators entering the experimental hives.

In the first investigation period in 2012 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.
Based on the analysis of several thousand pollen samples and individually analyzed bees in the investigation-periods 2013-2014 we could show, that “small” bee colonies tend to forage more to the target plants (strawberries) near to their hive if compared to large colonies. On average smaller bee hives showed slightly higher proportions of Fragaria-pollen collecting bees than bigger bee hives based on both population estimates at the beginning and at the end of the investigation period in 2013 and 2014. However, before jumping to general conclusions or even recommendations for practical use, it is indeed necessary to prove and verify these findings.

Additionally, we tested the biological control agent Prestop® Mix for its compatibility for bees based on a larval toxicity test. The results showed, that even small amounts of this powder added to the bee larvae food result some necrotic symptoms in the developing bee and a slower developmental time compared to untreated control bee larvae. Feeding the maximum possible amount of powder together with the standard larval food killed al developing larvae. It must be emphasized here, that these first test on possible side effects of the Prestop® Mix powder to bee larvae are only first orientation tests and should be sorted as such only.

In contrast to other BICOPOLL partners, we did not use the fungus-antagonist as a practical field application since *Gliocladium catenulatum* was unauthorized till may 2014 in Germany.

With this project approach we were able to add new information concerning the principles, opportunities and constraints of a new approach to crop protection and yield improvement, which combines the benefits from the two major ecosystem services: insect pollination, and biological control of diseases and pests.

## 6. Possible benefits and applicability of the project results

**Foreseeable Economic benefits:**

Also this German project part within the transnational COREorganic II project and all the other partners of this BICOPOLL consortium built up and boosted the knowledge in the field of entomovectoring and raised new open till now not fully answered aspects and questions, however the project prospects a highly promising approach to plant protection in principle. The concept combines two key ecosystem services, biological control and pollination, via ‘entomovectoring’ where pollinating, flower visiting insects are utilized to disseminate beneficial micro-organisms to target crops.

Many current plant production systems rely heavily on repeated applications of chemical pesticides, with well-known negative side effects. This reliance is due, in particular in many berry and fruit production systems, to the lack of effective alternative disease and pest management tools, such as biological control methods. Furthermore, the reliance of agricultural production on adequate insect pollination is increasing, while the populations of pollinating insects have been declining in many parts of the world - notably in Europe and in North-America. Thus, crop production frequently is suffering from inadequate insect pollination, resulting in lower and highly variable yields.

Strawberry alone is of great interest, as EU is the biggest producer of strawberries in the world, and of the single member countries, Spain is number two producer after the USA. Turkey is the third most important strawberry producer in the world Italy is on place 9, Germany on place 10, and Belgium on place 19 in global strawberry production. In total, strawberry area in the EU was 111’801 ha in 2008 (FAO 2011). In terms of economic importance, strawberry is in Germany the 15th most valuable agricultural commodity (after a long list of top-ranking animal-based products such as meat, milk, eggs, etc), and ranks similarly among top 20 agricultural commodities in Finland (12th), Estonia (15th) and Belgium (16th).

Grey mould (*Botrytis cinerea*) is the most important biotic threat to the crop, and conventional growing uses more fungicides on strawberry than on any other crop, usually 3-8 treatments per season. The industry is concerned about the slow progress in the development of biological control methods (biofungicides) against Botrytis, as the chemical fungicides rapidly lose their ability to control the disease. Currently organic strawberry growers have no means of preventing grey mould on their crop, and consequently, they occasionally lose the harvest almost entirely. Conventional growers suffer 10-20% pre-harvest crop losses to grey mould on the average, even up to 25-35% despite the numerous fungicide treatments [Strømeng G.M. (2008) Aspects of the biology of *Botrytis cinerea* in strawberry (Fragaria x ananassa) and alternative methods for disease control. Doctoral thesis at NMBU Norway].

Given that our approach to use small honeybee colonies for this special pollination and biological control service via entomovectoring will be widely be confirmed by other researchers, this will eventually change and boost the principle service provided by specialized pollination beekeepers later.

From these above mentioned facts it can be concluded, that the projects results can benefit organic farming systems on a real economic level, if this approach is proved itself in practice. Moreover, this approach is widely applicable in various horticultural and agricultural contexts, and is particularly suited to organic farming systems.

**Scientific value:**

Our scientific hypothesis that small hives forage more closely to their nest and are thus more suitable for the pollination service and bio-control of strawberries than big hives is based on a theoretical strategy of optimal foraging. Although obtaining food provides the individual bee and the whole bee colony with energy, searching for and capturing the food require both energy and time. It might be useful, that the individual bee wants to gain the most benefit (energy) for the lowest cost during foraging, so that it can maximize its fitness and together with nest mates the fitness of the whole bee colony. This theory helps to predict the best strategy that a bee and a bee colony can use to achieve this goal, whereby small bee hives might show lower foraging ranges than big hives. As we know today this is the first trial to prove or to disprove this scientific hypothesis. Hence, our findings are new based on the investigations within this project. We will publish the results soon in order to inspire additional research work in this segment.

## 7. Planned and realized objectives

| work package | | planned methods | realized |
|---|---|---|---|
| **WP2: Honey bees as vectors and crop pollinators** | | | |
| **Task 2.1.** Steering of foraging bees to the target crop | 2.1.1. Colony size:<br>Bee colonies differing in size (number of individual bees) are compared for foraging range and location<br><br>2.1.2. Amount of brood and pollen stores | a.) Bees will be quantitatively recorded along line-transects<br>b.) Pollenanalyses from the honey bee stomach and the bee cuticle, to confirm steering bees to the target crop<br>c.) a.) Larvae and pollen stores will be manipulated | a.) 1. and 2. year<br><br>b.) 2012: stomach not feasible due to high contamination → 2013-14 only pollen traps<br><br>c.) not realized due to time limitations during the short test period determined by the relative short blooming period of strawberries |
| **Task 2.2.** Vegetation management in and around strawberry fields | The impact of white clover between strawberry rows on the foraging activity is assessed (collaboration with Task 6.2) | a.) Frequent direct counts in ‘treated’ and ‘untreated’ sections of the fields similar to 2.1.1. | a.) not realized due to the fact that the field management of the owner, where we performed our tests, did not allowed a change of his system |
| **Task 2.3.** Dynamics of nectar and pollen production in the target crop | Nectar and pollen quantity, quality and availability during the course of a day | b.) Nectar will be extracted from strawberry flowers with microcapillary tubes throughout the day, and over the lifetime of a flower. Sugar content will be analysed with HPLC.<br>c.) Pollen availability is monitored via similar sampling | b.) 2013: daily nectar volume too low to quantify → collective sample including many flowers were analysed using HPLC<br><br>c.) 2013: no feasible quantification method available, measuring errors too high to get reliable results |

| | | | |
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| **WP6: Landscape Management supporting BCA dissemination and pollination** | | | |
| **Task 6.2.** Vegetation management in and around strawberry fields | the impact of white clover between strawberry rows on the BCA dissemination and pollination activity by managed (Apis, Bombus, Osmia) and wild bees is assessed | a.) frequent direct counts in 'treated' and 'untreated' sections of the fields<br><br>b.) Additional variables are introduced by managing (removing or leaving) competing vegetation (e.g., Taraxacum) in and around the fields | a.) This task was the main focus and realized by the project partners ITACAA in Italy and our part was an advisory task only<br><br>b.) Determining competing crops in and around the strawberry field including a three km radius (2012: Asparagus and flower strips; 2013: *Brassica napus*; 2014: *Trifolium incaranatum* and *Brassica napus*) |
| **WP7: Field applications of the entomovector technology** | | | |
| **Task 7.1.** Practical execution of a joint field experiment on strawberry | 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) | a.) 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<br>b.) BCA treated, and untreated control, with four replicate assessment plots on each farm. At the time of actual berry- | a.) This task was realized as a practical field triial since it is not clarified whether BCA harms bee larvae and adult bees. Furthermore *Gliocladium catenulatum* was unauthorized till may 2014 in Germany<br><br>b.) See above |

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| | | 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 | 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<br>a.) **management system** [required management practices, inputs, machinery, labour, management flexibility];<br>b.) **economic factors** [yields, operating costs, administrative costs, aspects of farm/product competitiveness]; and<br>c.) **social factors** [non-pecuniary social effects such as business opportunities, requirements for education and training, social cohesion via improved collaboration] | a.) see above<br>b.) see above<br>c.) see above |
| **Task 7.3.** Enhancing project PR | promoting BICOPOLL | a.) organizing at least once per year an event at the field trial | a.) The idea to present the project and first results during a field day to strawberry farmers could not be |

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| | | site, where the public and professional media are invited, along with grower and beekeeping organizations<br><br>b.) Publishing at least once per year a popular article on the topic/trial for a national professional magazine (berry-grower's and/or beekeepers' magazines). | achieved, because its realization was in time conflict with the necessary field work of these farmers at the time of the strawberry blossom.<br><br>b.) Since our investigations is only part of the whole cooperation work with the other project participants only a joint publication would make sense. However, this project opened new questions also in the field of practical application of this method a publication would have been to early<br>Interview about the BICOPOLL projet at the Radio: Interview in Deutschlandradio 21.3.2013: „Bienen sollen Erdbeeren vor Krankheiten schützen“ im Program ”Forschung Aktuell”.<br>http://www.dradio.de/dlf/sendungen/forschak/2116324/ |
| **WP8: Safety of the entomovector approach to bees and consumers** | | | |
| | | | a.) Also this task was the main focus and realized by the project partners NIB in Slovenia, we tested the biological control agent Prestop® Mix for its compatibility for bees based on a larval toxicity test.<br>It must be emphasized here, that this first test on possible side effects of the Prestop® Mix powder to bee larvae was only a first orientation test and should be sorted as such only. |

## 8. Summary

Many current plant production systems rely heavily on repeated applications of chemical pesticides in order to reduce the plant disease problems, with well-known negative side effects. This reliance is due, in particular in many berry and fruit production systems, to the lack of effective alternative disease and pest management tools, such as biological control methods and this is mainly a large problem for organic farming systems. One of the worst economically relevant problem is grey mold (*Botrytis cinerea*) worldwide. It is the most common fruit rotting pathogen of strawberry and one the most important biotic threats for the organic berry and fruit production in general. It is a major problem during bloom and on ripening, mature and harvested fruit, particularly during wet weather. This fungus is probably the most ubiquitous pathogen worldwide.

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.

The BICOPOLL project aimed to change this and to improve the yield and quality of organic strawberry production significantly and thus farm economics.

The concept developed and used combines two key ecosystem services, biological control and pollination, via entomovectoring where pollinating and flower visiting insects are utilized to disseminate beneficial micro-organisms to the target crops.

We used honeybees to (i) target deliver a biological control agent (fungus antagonist) to the flowers of the target crops (strawberries) to provide control of the problem diseases grey mold (*Botrytis cinerea*) and to (ii) improve the pollination of this organic horticultural crops. The use of bees has many environmental and economic benefits compared to spraying fungicide like in conventional farming systems.

As bees, that actually forage in the target crop, is a key essential requirement for the entomovectoring technology, the main focus of this project was to determine, which factors can affect foraging ranges of honeybees and to examine how to steer the bees to the target crop (strawberry), even if the nectar or pollen rewards are less attractive compared to competitive other plants in the near surrounding. We hypothesized that small hives (number of individual bees per colony) forage closer to their hive and are thus more suitable for the bio-control of less nectar and pollen producing crops (like strawberries) than big hives, with higher numbers of individual bees per colony. Our investigations during 2012 – 2014 showed that on average smaller bee hives showed

slightly higher proportions of Fragaria-pollen collecting bees than bigger bee hives based on both population estimates at the beginning and at the end of the investigation period. Thus our scientific hypothesis seems to be supported by our findings. However, before jumping to general conclusions or even recommendations for practical use, it is indeed necessary to prove and verify these findings.

In contrast to other BICOPOLL partners, we did not use the fungus-antagonist as a practical field application since *Gliocladium catenulatum* was unauthorized till may 2014 in Germany.

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## 10. **Publications** – based on the project findings

**■ Scientific publications:**

**Boecking O. and Kreipe V.** (2013) *Flying doctors* zur Grauschimmel Bekämpfung und als Bestäuber im ökologischen Erdbeeranbau – erste Untersuchungsergebnisse im BICOPOLL Projekt. 60.AG Bienentagung in Würzburg (Germany). Apidologie Abstract

**Boecking O. and Kreipe V.** (2014) *Flying doctors* zur Grauschimmel Bekämpfung und als Bestäuber im ökologischen Erdbeeranbau – neue Untersuchungsergebnisse im BICOPOLL Projekt. 61.AG Bienentagung in Marburg (Germany). Apidologie Abstract

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).

**■ Talks at national and international conferences:**

**Boecking O. and Kreipe V.** (2013) *Flying doctors* zur Grauschimmel Bekämpfung und als Bestäuber im ökologischen Erdbeeranbau – erste Untersuchungsergebnisse im BICOPOLL Projekt. 60.AG Bienentagung 19. bis 21. März in Würzburg (Germany).

**Boecking O. and Kreipe V.** (2014) *Flying doctors* zur Grauschimmel Bekämpfung und als Bestäuber im ökologischen Erdbeeranbau – neue Untersuchungsergebnisse im BICOPOLL Projekt. 61.AG Bienentagun 25. bis 27. März in Marburg (Germany).

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■ **Interview at the Radio**:

Interview im Deutschlandradio 21.3.2013: „Bienen sollen Erdbeeren vor Krankheiten schützen“. Dr. Otto Boecking und Prof. Dr. Heikki Hokkanen. Program ”Forschung Aktuell”. http://www.dradio.de/dlf/sendungen/forschak/2116324/

■ **Book publication:** (in preparation)

Hokkanen HMT, **Boecking O,** Menzler-Hokkanen I and G Smagghe (2016) Precision biocontrol and enhanced pollination of crops: exploiting synergy of ecosystem services. Springer, series: Progress in Biological Control (in preparation)

The book describes in detail the concept, state-of-the-art, gaps in knowledge, and prospects of a highly promising new approach to plant protection. This approach is widely applicable in various horticultural and agricultural contexts, and is particularly suited to organic farming systems. The concept combines two key ecosystem services, biological control and pollination, via ‘entomovectoring’ where pollinating (flower visiting) insects are utilized to disseminate beneficial micro-organisms to target crops. The aim of the book is to collate all current knowledge and to stimulate further work on the topic. The book is based on ongoing research work in the EU-ERA-NET project BICOPOLL (“Targeted precision biocontrol and enhanced pollination”); the editors and chapter authors are all partners in that project.
Many current plant production systems rely heavily on repeated applications of chemical pesticides, with well-known negative side effects. This reliance is due, in particular in many berry and fruit production systems, to the lack of effective alternative disease and pest management tools, such as biological control methods. Furthermore, the reliance of agricultural production on adequate insect pollination is increasing, while the populations of pollinating insects have been declining rapidly in many parts of the world; notably in Europe and in North-America. Thus, crop production frequently is suffering from inadequate insect pollination, resulting in lower and highly variable yields.
In this book we will present and analyze all available information concerning the principles, opportunities and constraints of a new approach to crop protection and yield improvement, which combines the benefits from the two major ecosystem services: insect pollination, and biological control of diseases and pests. 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 crops. We will analyze how to improve the efficiency of the entomovector technology via innovative bee management, manipulation of bee behavior, components of the cropping system, and on the plant-pathogen-vector-antagonist–system, and investigate possibilities of expanding the use of the concept into different growing systems. 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. This is a highly innovative approach to solving some of the most difficult disease and pest problems in crop production, offering solutions often in areas where no solutions as yet exist. Furthermore, the entomovector approach represents the only significant breakthrough in sight for improving plant protection in organic cropping systems, particularly in high-value crops.

We will present as a detailed example a case study on protecting strawberries from its main biotic production stressor, the grey mould fungus *Botrytis cinerea*. Strawberry alone is of great interest, as EU is the biggest producer of strawberries in the world, and of the single member countries, Spain is number two producer after the USA. Turkey is the third most important strawberry producer in the world and of the other countries involved in this book proposal, Italy is on place 9, Germany on place 10, and Belgium on place 19 in global strawberry production. In total, strawberry area in the EU was 111'801 ha in 2008 (FAO 2011). In terms of economic importance, strawberry is in Finland the 12th most valuable agricultural commodity (after a long list of top-ranking animal-based products such as meat, milk, eggs, etc), and ranks similarly among top 20 agricultural commodities in Germany (15th), Estonia (15th), and Belgium (16th). Grey mould (*Botrytis cinerea*) is the most important biotic threat to the crop, and conventional growing uses more fungicides on strawberry than on any other crop, usually 3-8 treatments per season. The industry is concerned about the slow progress in the development of biological control methods (biofungicides) against Botrytis (AAFC, 2009), as the chemical fungicides rapidly lose their ability to control the disease. Currently organic strawberry growers have no means of preventing grey mould on their crop, and consequently, they occasionally lose the harvest almost entirely. Conventional growers suffer 10-20% pre-harvest crop losses to grey mould on the average (Stromeng, 2008), even up to 25-35% (IPMCenters, 2011) despite the numerous fungicide treatments.

Early trials on biological control of Botrytis have all relied on spraying the biocontrol agents (BCA) on strawberry flowers, with poor results. The same is true for many other similar systems. Spraying cannot be adjusted to deliver the BCA to the inflorescence at the different developmental stages of flowers and at the right time in order to prevent grey mould growth. In contrast bees, as an essential component of the pollination system, will colonize the flowers with the BCA and achieve disease suppression naturally, via frequent pollination visits at each inflorescence at the right time. Despite the promising results of the first studies on the use of pollinating insects – the honeybee as the first one – to spread the BCA to the flowers instead of spraying (Peng et al., 1992), the development of this approach has not progressed far. The dispensers may not have been adequate, or the BCA has not functioned (e.g., Stromeng, 2008). Recently a more systematic development of the 'entomovector technology' (Hokkanen & Menzler-Hokkanen, 2007) has taken place, with focus on developing the component technologies such as the dispensers and carrier substances (see Mommaerts et al. 2011a). With functioning dispensers and improved, new BCA available, excellent results have been obtained (Hokkanen et al., 2012).

Several researches have shown that the choice of the most efficient pollinator is crucial for maximizing pollination and disease control at the same time, e.g. Maccagnani et al (2005, 2006b) with solitary bees (*Osmia cornuta*) and honey bees in delivering BCA for the control of fire blight Erwinia amylovora. This is the most serious bacterial disease in apple and pear, and has during the last four decades spread throughout Europe. Osmia spp. were studied also as carriers for BCA against the fire blight (Maccagnani et al. 2005, 2006b), and a prototype of a dispenser has been developed (Maccagnani et al 2006b). A promising approach, insufficiently investigated up to now, is combining the primary BCA dissemination and secondary BCA dissemination from flower to flower by pollinating insects (Maccagnani et al. 2005). Osmia cornuta proved also to be an excellent fruit pollinator, and much more efficient than the honey bee (Maccagnani et al. 2006a). The main difficulty is to synchronize female emergence and nesting activity with pear blossoming, which could be achieved by rearing and management of Osmia, and by ensuring the availability of adequate ecological infrastructures in the orchard landscape (Maccagnani et al. 2006a).

**ANNEX I** Study sides

**Study site 1**: Metzingen/Eldingen (2012 – 2014)


Figure A-1: Main study site (Metzingen/Eldingen) in the district of Celle in Lower Saxony including the strawberry fields used in this project, the positions of the experimental bee hives and the location of the *Trifolium incarnatum* field in 2014.

**Study site 2**: Nienhagen/Wathlingen (2012)

Figure A-2: Study site 2 (Nienhagen/Wathlingen) in the district of Celle in Lower Saxony including the strawberry field used in this project, the positions of the experimental bee hives and the locations of the two Asparagus fields as well as the flower stripe.