Different bees as vectors for entomovectoring with enhanced pollination and crop protection control: current practices, use-cases and critical view on transport

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Summary

Honeybees, bumblebees, and other insects have been used commercially for pollination for many years and microbial biocontrol agents have also been widely used in pest control. Pollinators and formulations of microbial pest control agents are routinely transported internationally on a large scale. A novel approach has been developed to use bees as vectors of microbial agents, by inoculating the surface of the pollinators using dispensers in modified hives. This innovation extends the market for these products and results in better yields. A successful entomovector system requires selection of the vector pollinator most appropriate for the crop and location, based on various criteria, in combination with a registered microbial agent. Currently, pollinators and microbial agents are packed separately and combined at the point of use. Local sourcing of the pollinator in the system reduces the need for long-distance shipping of these live insects and may improve efficiency due to local adaptation but will delay use and benefits of the system until research at each site/country catches up with the work already conducted in a few countries. In the meantime, clear guidance for innovative systems employing live insects could support the promising increase to food production.
Keywords

Crop protection – Microbial control – Pollination – Innovative biological systems

Introduction

Both pollinators and biocontrol agents (BCAs) are broadly commercialized internationally. In 2015, according to Van Lenteren et al. (1), the combined global area treated with invertebrate and microbial agents amounted to more than 30 million hectares. Commercial pollination by bumblebees alone was already estimated to be a € 55 million industry in 2004 with over a million colonies produced worldwide (2). In order to further optimize both international functions, pollination and application of microbial BCAs were combined through entomovectoring.

The entomovectoring system is a good example of man copying nature. It has been established for a long time that insects such as bees are able to transfer pollen and other microparticles such as bacterial cells and fungal spores from flower to flower, as demonstrated for *Erwinia amylovora* (3). Man has adopted this concept and used it for commercial pollination and, more recently, for the transport of microbial BCAs. Hokkanen and Menzler-Hokkanen (4) were the first to introduce the technical term ‘entomovector technology’ meaning the use of vectors such as insects for transporting microbial BCAs for plant protection. The entomovectoring system has the potential to increase the market further, but it also introduces new concepts that need to be understood when applying guidance on international risk assessment and shipping. For example, during transport, the vectors are packed separately from the BCA that must be applied via the dispenser when the beehive is installed in the greenhouse or on the field. The shipment itself does not differ or require extra management compared to standard pollinator transport. Misperception about the purpose and operation might occur during shipping, however, which is why detailed descriptions are necessary.
Already in 1992, the honeybee, *Apis mellifera*, was used to transfer the fungus *Gliocladium roseum*, a commercially available microbial BCA, for the protection of strawberry plants against the notorious plant pathogen *Botrytis cinerea* (5).

The entomovectoring system makes use of a unique combination to provide pollination and a protective service at the same time, by using pollinators such as the honeybee for the transport of microbial BCAs. As a result, the grower benefits from increased yields due to an increased seed set and protection against plant pathogens. There are other benefits from use of the entomovectoring system instead of spraying chemical control agents. Aside from avoiding chemicals that can have adverse effects on the environment or human health (6), the entomovectoring system can reduce water and electricity requirements for glasshouse production, resulting in a lower carbon footprint; this also fits within the framework of the European Green Deal (https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_en) (7).

Entomovectoring reduces costs and labour hours and decreases non-target organism exposure (8). In terms of hive management, the greatest requirement, apart from installation, is to refill the dispenser with BCA product, which takes approximately 15-30 seconds per hive, either twice a week for bumblebees or every 10 days for honeybees. Commercially available colonies require placement on a small stand, protection from rain (most of the hives have a paper-based coating) and access to pollen and nectar. Honeybees require year around maintenance to be applicable at the desired period with microbial BCAs. In addition, using pollinating insects for the delivery of the microbial BCAs assures that they will be disseminated directly onto the target location, that being the flowers from the moment they open (9). This is important as these are often the target organs for harmful plant pathogens (10, 11). Furthermore, less microbial BCA is wasted because during spraying a significant part of the product ends up on the ground.

The economic benefits of implementing entomovectoring with the honeybee and *T. harzianum* for the control of *B. cinerea* in field
strawberry plants were estimated in Colombia (12). Pollination resulted in an extra profit of 2987 $US/ha/year. The decrease of application of pesticides saved 1405 $US/ha/year, and a 1.88% reduction in losses due to *B. cinerea* compared to commercial treatment saved 872 $US/ha/year. Overall, the entomovectoring technology brought in utility close to 3710 $US per hectare, which is the sum of the three values mentioned above subtracting the costs for the implementation of the technology (1554 $US/ha/year) (12).

This review will provide a brief recap on how entomovectoring works, especially in relation to transport. Some historic cases are presented on the need to use local species in entomovectoring to protect pollinator biodiversity. Overall, this article aims to share information with stakeholders on the new concept of entomovectoring, its advantages and disadvantages, together with a critical view on transport of pollinators for the purpose of entomovector systems, demonstrating the need for clarity around any related regulations on export and import.

**Entomovectoring**

**Principles of entomovectoring**

Entomovectoring is an interplay of several parts (Fig. 1). A certain pollinator, referred to as the vector, must deliver a microbial BCA product to the crop under protection in a way that is safe for man, the vector, and the environment. To load the vector with a sufficient amount of colony forming units (CFU) of the microbial BCA, the vector must be loaded using a dispenser. For this, the vector walks through a device filled with the BCA formulation. The dispenser must provide a way to load the vector safely and sufficiently without altering the physiological behaviour of the vector too much and without compromising its health and safety (7). To ensure that the BCA has no adverse effects on humans, vectors, or the environment, risk assessments must be completed covering safety and product registration must be in place (13).

[Place Figure 1 here]
The vector

Proper application of the microbial BCA to the flowers has a significant contribution to the successful operation of the entomovectoring system (11). Matching the appropriate vector with the plant under protection is hence of high importance. The choice for the most suitable vector is dependent on several factors: the crop species, the crop visitation rate of the vector, the flying conditions for the vector, and the vector’s intrinsic capacity to disseminate microbial BCAs all impact the entomovectoring success (11).

The number of insect species that have already been used in the entomovectoring system is rather limited. The bottleneck is linking the commercial availability of the pollinator with the blooming period of the plant under protection as the pollinator life cycle is not always related to its blooming period (11). This is also why honeybees and bumblebees, two groups that are commercially available throughout the year, are very much in demand for use in the entomovectoring system, and have been the vector in many scientific reports investigating entomovectoring (13). For instance, Apis and Bombus workers differ in pollen deposition and removal as a result of different foraging behavior. Examples of crop-pollinator links were found for numerous species of the Asteraceae family that proved to be particularly attractive to honeybees and wild bees (15). Members of the Brassicaceae family were mostly disliked by solitary bees and bumblebees, but visited frequently by honeybees. Bumblebees were notably present on plants of the Leguminosae family (15). Apart from honeybees and bumblebees, solitary bees such as Megachile rotundata, Osmia bicornis, Osmia cornuta and Osmia lignaria have been used as vectors as well (16). Plans are being made for the use of stingless bees (Meliponini) for entomovectoring in Latin America. The stingless bee species Nannotrigona perilampoides has pollinated tomatoes successfully (17). However, more research and new multiplication methods are needed for many stingless bee species before they could be used commercially (2).
Two main pollinators in entomovectoring: Honeybees and bumblebees

Commercially, honeybees (*Apis mellifera*) have been used in North America, Europe, South America, and Asia (13, 18, 19). Bumblebees have been successfully deployed in North America (*Bombus impatiens*), Europe (*B. terrestris*) and Korea (*B. terrestris*) (9, 13, 18). The conditions under which the vectors must fly are very decisive for determining which vector would generate the best result for pollination. This is not different for the choice between bumblebees and honeybees. For example, it is known that honeybee workers do not fly, or only poorly, under rainy conditions and that rain affects their ability to disseminate BCAs (20, 11). Bumblebees that are described as bad-weather foragers (20) perform better under rainy conditions. In addition, honeybees have been described as less suitable for entomovectoring in greenhouse crops (21). However, bumblebees can tolerate higher temperature extremes better and are also more resistant to temperature fluctuations (20). This is why bumblebees are the most used pollinators inside greenhouses (22). Nevertheless, bumblebees are suitable as well for entomovectoring in open field (11).

Another important advantage that bumblebees have over the honeybee is the fact that they perform buzz pollination (20, 21). This is a very efficient pollination technique in which the flower stamens are shaken intensively by the vibrating wings of the insect, resulting in the formation of a cloud of pollen grains (23). Using this technique, bumblebees can pollinate flowers more efficiently than honeybees (22).

[Place Figure 2 here]

Case studies with honeybees *Apis mellifera*

The first study on entomovectoring was conducted by Peng *et al.* (5) for the protection of strawberry plants against *B. cinerea* with *Apis mellifera* and the microbial BCA *Gliocladium roseum*. This system was able to suppress *B. cinerea* on the flowers in the greenhouse and in the open field, except when the weather conditions were bad due to the resulting reduced foraging activity.
Honeybees and bumblebees were used for entomovectoring the fungus *Trichoderma harzianum* on strawberry plants in open fields for the control of *B. cinerea* (18). This entomovectoring system resulted in better control of *B. cinerea* than by conventional spraying. The more targeted and continuous application of the BCA at flower opening, in comparison to spraying which occurred before most flowers had opened, resulted in flowers being immediately protected at opening: the unprotected time is significantly less compared to spraying. Furthermore, entomovectoring contributed to on average 22% more seeds with a 26 to 40% (by weight) higher yield as a result (18). Entomovectoring with *T. harzianum* resulted in a level of control and a yield that were at least as good or better compared to the use of commercial fungicides plus pollination (18).

In another study (7), experiments were carried out on open field strawberry plants in Finland. During these experiments, honeybees loaded with Prestop-Mix with active component *Gliocladium catenulatum* increased the yield by 58% due to enhanced pollination and avoiding fungicides with potential negative effects on the seed set.

**Case studies with bumblebees *B. terrestris* and *B. impatiens***

Kapongo et al. (19) demonstrated how *Clonostachys rosea* transported by *B. impatiens* suppressed *B. cinerea* with 57% and 46% in the flowers and leaves of the tomato plant, respectively, and with 58.9% and 46.8%, respectively, in the flowers and leaves of the sweet pepper. Mommaerts et al. (24) investigated entomovectoring with Prestop-Mix, and the vector *B. terrestris* to combat *B. cinerea* on the strawberry plant under greenhouse conditions. They observed that the use of this BCA and vector could provide a significantly higher yield (2-2.5 times higher) compared to the control group without BCAs, even under conditions that were considered ideal for the fungus.

Nowadays there are companies which have developed business models based on entomovectoring technology. Examples are Biobest (Belgium), BVT (Bee Vectoring Technology, Canada) or Assatek (Finland). An example of a commercially available product is the
Flying Doctors® hive (Fig. 2a). This is a patented nest sold by Biobest and used especially for vectoring with B. terrestris. The dispenser is based on the Mommaerts dispenser (25).

**Other potential pollinators in entomovectoring: Solitary bees Osmia sp.**

Substantial research has already gone towards the pollination potential of native solitary bees (16). Wild bees can maintain significant populations providing ecosystems with a constant background pollination. So far, several manageable native solitary bees have been identified. *Peponapis pruinose* for example pollinates *Cucurbita pepo* very efficiently. Sampson and Cane (26) successfully bred a new adaptable pollinator, *Osmia ribifloris*, which was used in captivity to pollinate rabbiteye blueberry. The authors found that this insect pollinated the blueberries as efficiently as the honeybee.

Today, a few solitary bee species are commercially available. This is due to the limited possibility to develop mass reared populations of pollinators. Several difficulties occur such as the number of reproductive cycles, the duration of the diapause, and the adaptability to artificial nesting sites (16). In North America, *Megachile rotundata*, *Osmia lignaria*, and *Nomia melanderi* are available on a larger scale. *Osmia cornifrons* has been used for over 70 years in Japan for apple pollination. In Europe, *Osmia rufa* and *Osmia cornuta* are bred for orchard pollination (16). The genus *Osmia* consists of 300 species and for most of them their life cycle is related to the blooming period of the *Rosaceae* family. Members of the *Osmia* genus highly prefer *Rosaceae* pollen over other flowers (16). *Osmia* species have been used on several occasions for crop pollination. *O. cornuta* has been found to pollinate almond apple flowers more efficiently than *Apis mellifera* (27). Solitary bees can move more easily from tree to tree with respect to honeybees which makes them more suitable for pollination in orchards (16). Furthermore, solitary bees are better at maintaining their population level at a constant level in orchards compared to honeybees and bumblebees which makes them a reliable pollinator (28).
Case studies with solitary bees

Maccagnani et al. (29) studied the efficiency of two pollinators being *Apis mellifera* and *Osmia cornuta* as vectors for the BCA *Bacillus subtilis* BD 170 on apple cultivar ‘Golden Delicious’ for control of *Erwinia amylovora*, the causal agent of fire blight, under net screened tunnels. The authors studied secondary colonisation through transport from one flower to another. They found that in 50% of the cases, the BCA carry-over for *O. cornuta* was 10 to 100 times higher compared to *A. mellifera*. The solitary bee *O. cornuta* showed higher percentages of inoculated flowers in addition to a higher proportion of flowers with a high amount of BCA cells. These results are in line with the higher pollinating potential of solitary bees on crops of the *Rosaceae* family (29).

Maccagnani et al. (30) investigated the suitability of *O. cornuta* and *A. mellifera* to vector *Bacillus subtilis* BS-F4 for the control of fire blight on pear (*Pyrus communis*) cultivar Abbé Fétel. Overall flowers visited by *O. cornuta* showed a greater number of BCA than flowers visited by *A. mellifera*.

Transport of vectors: Impact of international import and export

Current status

As important pollinators, bumblebees (*Bombus* spp.) have been bred and used for pollination since the late eighties. In 1987, bumblebees were first used in Belgium for the commercial pollination of tomato plants (2). Already in 2006, Velthuis and Van Doorn (2) indicated commercial pollination by bumblebees as a € 55 million industry with over a million colonies produced worldwide in 2004. Companies such as Biobest produce bumblebees for several parts of the world (Table 1).
Potential risks in importing exotic vectors

The increasing commercialisation and the associated anthropogenic movement of commercial pollinators in and outside their natural ranges can have an impact on the native bee species (31). Moreover, commercial agricultural populations can impact the local bee fauna through several mechanisms (32). For example, they are said to spread harmful pathogens to native wild bee populations. In this sense, commercial populations act as pathogen reservoirs that can contaminate wild populations. Several commercial populations of bees have already been reported to contain elevated parasite loads as reviewed by Meeus et al. (31). Furthermore, in many cases the invasive bumblebee species have an overlapping foraging activity compared to the native bumblebee species. As such, they can replace the native species through competitive exclusion (33). Both competition for floral resources and nesting sites can have negative effects on the local bee fauna (32). Finally, the interspecific mating between invasive and native bumblebee species can have a detrimental impact on the native bee species (32).

The global trade of bees facilitates the introduction of potentially invasive species into new environments (see also Goka [34], this issue). Today, regulations are often still too regional to help in solving this global problem. For example, Norway has banned introduction of commercial bumble bees and solely uses species that are local or grown in their country to protect their native species. Estonia, on the other hand, has no national risk assessment concerning pollinator introduction. The reasoning is that while use of pollinator services or entomovectoring is not common in Estonia, there has not been any need for this specific risk assessment (private communication with the Estonian University of Life Sciences).

In Brazil, the introduction of alien species is forbidden and requires a license and specific authorization from the environmental agency (private communication with the Universidade Federal da Bahia in Brazil). Bumblebees for example have been shown to escape management, resulting in an invasive species in the wild as reviewed
by Aizen et al. (35). Chile participates in the trade of bumblebees for commercial pollination, which is believed to have spread two alien bumblebee species, *Bombus ruderatus* and *B. terrestris*, into Argentina, which bans the import of commercial alien bumblebees (35, 2). Arbetman et al. (36) and Maharramov et al. (37) found that *B. terrestris* introduced the pathogenic protozoan *Apicystis bombi* in the native species *B. dahlbomii*. Their conclusions were based on *A. bombi* samples from before and after the introduction of *B. terrestris* and they found that the pathogen had co-evolved with *B. terrestris* and had been one of the causes for the population collapse of *B. dahlbomii* (36). Another cause for the *B. dahlbomii* decline is the overlap in visited plant species compared to the two invasive *Bombus* species (38). Five years after arrival, *B. terrestris* has become the most abundant and widespread *Bombus* pollinator. Furthermore, 20 years after their introduction, *B. terrestris* and *B. ruderatus* have replaced the only native species *B. dahlbomii* which was formerly the most abundant pollinator in Patagonia (38).

The outperforming of *B. dahlbomii* can have detrimental effects on the local pollination networks as the plant-pollinator interactions have changed. The native bumblebee species *B. dahlbomii* is a more efficient pollinator for e.g. *Alstroemeria aurea* in terms of pollen quantity and quality deposited per flower visit. Due to shift towards *B. terrestris* and *B. ruderatus*, *Alstroemeria aurea* could suffer from a reduced fruit set and/or quality (32).

Next to the abovementioned cases in South America, similar cases also appeared in Asia. In 1992, *B. terrestris* was introduced in Japan for the pollination of greenhouse crops and nearly 20 years later, as a result of an adverse ecological impact, the species was banned. For example, Inoue and Yokoyama (33) observed the decrease in terms of population size of the two native bumblebee species *B. hypocrita sapporoensis* and *B. diversus tersatus* simultaneously with the increase of *B. terrestris*. The authors provided circumstantial evidence for the competitive exclusion of the two native bumblebees. Tsuchida et al. (39) reviewed the hybrid production between the invasive *B. terrestris* and the native *B. ignitus* and *B. hypocrita sapporoensis* in Japan. As a result of
interspecific mating, the reproduction of the native bumblebee species *B. ignitus* and *B. hypocrita sapporoensis* is reduced through the production of inviable eggs (39). As a result, the replacement of the native bumblebees by *B. terrestris* is accelerated.

In 1880, *B. terrestris* was introduced in New Zealand for the pollination of red clover and merely five years later, populations were found in the wild. Subsequently the pollinator expanded its territory at a rate of approximately 90 km/year becoming the main pollinating bumblebee (32). *B. ruderatus*, which was introduced around the same period, became ubiquitous as well although in some areas the *B. ruderatus* population has declined especially after *B. terrestris* introduction (32). International companies such as Biobest and BVT should hence pay attention to the local situation. BVT claims implementation to be fairly easy as European bees are relatively common around the world, or if not, there is typically some form of commercial pollinator available (private communication with BVT). The company only works with approved or available pollinators in every country. However, further work still must be done as international companies would need local partners in every country. However, in some countries (e.g. Estonia) no local bumblebee producers are present and rearing the local breeds for every country could be unpractical (private communication with the Estonian University of Life Sciences). Furthermore, IATA Live Animal Regulations have guidance on packaging honeybees and bumblebees, but no other pollinators. This could be a barrier to a developing market for some solitary or stingless bees, for example, which are not specifically mentioned in the guidance.

**Legislation, registration and the potential risk of BCAs**

Several micro-organisms including viruses, bacteria, and fungi have been suggested as possible microbial BCAs, and at least a dozen have already been tested. Registration requirements for microbial BCAs differ among countries. In the US, the Environmental Protection Agency (EPA) categorizes microbial BCAs as biopesticides that have to meet the standards of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) which prevents them from “causing
unreasonable adverse effects on human and environmental health” (40). This is not different for the BCAs used in entomovectoring. For example, Bee Vectoring Technology (BVT) only works with BCAs that are authorized by the respective country (private communication with BVT). In their legislation, the EPA encourages the development of biopesticides via shorter registration procedures and lower registration fees (40). In the EU, the approval of microbial BCAs is done at strain level by the European Commission. However, compared to chemical substances, guidance on risk assessment of microbial active substances is lacking. Also in the US, guidelines on chemical substances are more extensive than their microbial alternatives. Despite the lack of general guidance to the evaluation of microbial BCAs in the EU, the OECD Guidelines on the environmental safety evaluation of microbial BCAs are generally followed (40). Generally, the use of active microorganisms carries risk due to their potential to infect and multiply in host organisms and to produce toxic secondary metabolites upon contact with non-target organisms. These risks should be assessed, unless non-target organism exposure can be disproved (40).

Specifically for the entomovectoring system, harm to the vector is another point of attention (7). For example, \textit{Bacillus subtilis} QST713, the active component of Serenade, has an LC$_{50}$ of 5663 ppm for honeybees. However, honeybees directly sprayed would be exposed to approximately 8000 ppm, clearly above the LC$_{50}$. Nevertheless, EPA (41) found no adverse effects on pollinators, pollination or yields during field trials where Serenade was transported by honeybees. Mommaerts et al. (42) investigated the effect of AQ10 (\textit{Ampelomyces quisqualis}), Binab-T-vector (\textit{Hypocrea parapiilulifera} + \textit{T. atroviride}; 1/1), Prestop-Mix (\textit{Gliocladium catenulatum} J1446), Serenade (\textit{Bacillus subtilis} QST713), Trianum-P (\textit{Trichoderma harzianum} T22), Botanigard (\textit{Beauveria bassiana} GHA) and Granupom (\textit{Cydia pomonella} granulovirus), comprising five biofungicides and two bioinsecticides on \textit{Bombus terrestris}. Apart from Botanigard and Serenade, the BCAs had no adverse effects on the workers of \textit{B. terrestris} (42). Topical contact with Botanigard at its maximum field recommended concentration (MFRC) caused 92% mortality after 11 weeks, while the 1/10 MFRC killed 46% of exposed workers. Topical contact and oral delivery of
Serenade resulted in 88 and 100% worker mortality, respectively. Furthermore, upon treatment with their respective MFRC, nests were evaluated for sublethal effects over 11 weeks. Botanigard and Serenade gave rise to a significant decrease in drone production. Finally, Botanigard negatively affected the insect’s foraging behaviour when orally delivered at its MFRC.

It is clear that extra topical tests are very important to determine a system that does not harm the vector, specifically for the entomovectoring system where the vector comes in direct contact with the BCA. Furthermore, due to the increased globalisation for these emerging options, international guidelines should augment the national ones facilitating commercialization.

**Conclusions**

It is clear that entomovectoring combines the value of pollination with useful antipathogenic properties. Doing so, it further increases the international market for the several vector species used. Although, compared to regular pollinator transport, no extra management is needed during vector transport, misperception about the purpose, content, and risks might occur as BCAs and vectors are packed separately. It is important to provide a detailed description on the purpose for both the vector and the BCAs. Honeybees and bumblebees are the main pollinators for the entomovectoring system, and both are already treated as special cases in international transport. However, the abovementioned historic cases presenting pollinators that are also frequently used in the entomovectoring system confirm the value of developing future systems using local species, where feasible.

Despite the practical challenges in countries lacking local rearing facilities or relevant research, use of local species/populations for future entomovectoring avoids transport issues described in this thematic issue. Furthermore, these investments may help to protect pollinator biodiversity locally and also lead to higher efficiencies in the system. In the meantime, entomovectoring needs international guidelines for the registration of BCAs in order to deliver benefits sooner, including testing for (sub)lethal effects upon topical exposure.
With this review, we aim to share information with stakeholders on the use of pollinators as vectors in the new concept of entomovectoring, its advantages and disadvantages, together with a critical view on transport of pollinators, and to ask for more internationally recognized guidance on export and import of this category of live insects.

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41_1_09_Temmermans & Smagge - pre-print 19/24
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Table I

Bumblebee species produced by Biobest for several locations (from https://www.biobestgroup.com/en/biobest/pollination/things-to-know-about-bumblebees-7052/species-6674/).

| Species               | Production          | Availability                          |
|-----------------------|---------------------|---------------------------------------|
| Bombus terrestris     | Belgium, Turkey, China | Europe, Africa, Asia, Chile            |
| Bombus terrestris audax | Belgium             | UK                                    |
| Bombus canariensis    | Belgium             | Canary Islands                        |
| Bombus ignitus        | Belgium             | Japan                                 |
| Bombus impatiens      | Canada, Mexico      | North, Central and South America       |
| Bombus atratus        | Argentina           | South America                         |
| Bombus huntii         | Canada              | Western Canada                        |
Fig. 1
Schematic overview of the factors important for successful entomovectoring and their interplay (11, 14).
Fig. 2
(a) Flying Doctors® hive from Biobest (Westerlo, Belgium) (https://www.biobestgroup.com/nl/biobest/producten/hommelbest uiving-4456/); photo by authors. (b) Loading tray for Flying Doctors hive filled with BCA formulation; photo by authors. (c) Bumblebee hive for enhanced pollination in strawberry under protection; photo by authors.