The Potential of Bee Vectoring on Coffee in Brazil

Juliana Macedo, Blandina Viana, Breno Freitas, Adriana Medeiros, Peter G. Kevan, and Carlos H. Vergara

1 Introduction

Coffee is already part of people's routine life. Around 2.58 billion cups of coffee are daily consumed (Bacon [2005](#page-13-0)). Most of the coffee produced in the world comes from smallholding, and it is considered the main source of economic resources for many poor families that lives in rural communities (FAO [2015\)](#page-14-0). Nearly five hundred million people are involved on coffee trade, right from the plantation of coffee to final consumption (DaMatta et al. [2007](#page-13-1)).

Despite high production and demand from consumers, coffee production around the world is strongly affected by disease and pest attacks. Actually, this is considered as one of the primary factors that lead to coffee yield reduction in the main coffee-producing countries (Oliveira et al. [2014\)](#page-15-0). For instance in Brazil, the world's largest producer of Arabica coffee, annual losses due to pests and diseases are around 0.4 million tons (Oliveira et al. [2014\)](#page-15-0). To compensate losses and to raise agricultural production and productivity, many farmers increase the use of chemical inputs (Wilson and Tisdell [2001](#page-16-0)). However, this can result in direct and indirect economic losses related to obtaining and using pesticides which can harm the human health and natural enviroment.

J. Macedo $(\boxtimes) \cdot B$. Viana $\cdot A$. Medeiros

Instituto de Biologia, Universidade Federal da Bahia-UFBA, Salvador, Brazil

B. Freitas

Departamento de Zootecnia-Centro de Ciências Agrárias, Universidade Federal do Ceará-UFC, Fortaleza, Brazil

P. G. Kevan School of Environmental Sciences, University of Guelph, Guelph, ON, Canada

C. H. Vergara

Department of Chemical and Biological Sciences, Fundacion Universidad de las Americas Puebla, Puebla, Mexico

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The deleterious effects of pesticides on human and environmental health, including wild pollinators, have been discussed in the scientific literature (Fischer and Moriarty [2011;](#page-14-1) Janssen [2011\)](#page-14-2), in relation to the development of resistance to major coffee diseases and pests like leaf rust (caused by *Hemileia vastatrix* Berk. and Br) (Silva et al. [2006](#page-16-1)) and the Coffee Berry Borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) (Brun et al. [1989\)](#page-13-2). Henry and Feola ([2013\)](#page-14-3), studying pesticide use among smallholder coffee producers in Jamaica, found that the majority of farmers suffer from at least one health symptom associated with pesticide handling, because safety practices were scarcely adopted. According to them, there was also the risk that other household members and the wider local community be exposed to pesticides. Despite that, the cost related to chemical control associated with this type of management makes clear the need of a new concept in agriculture involving a severe reduction in the use of chemical inputs (Nicolopoulou-Stamati et al. [2016](#page-15-1)), especially to control pests and diseases in coffee agrosystem. The implementation of environmentally friendly practices which are safer for the environment and human health and biodiversity, and capable of increasing crop yields quantity and quality is necessary to ensure long-term food security and profitability for coffee production.

In the early 1990s, a novel sustainable method for pest control using bees as a vector of microbial control agents against agricultural pests and diseases was proposed (Peng et al. [1992\)](#page-15-2). The Bee vectoring Technology (BVT) used managed bees to deliver microbial control agents to plants against plant pathogens and insect pests of crops (Peng et al. [1992;](#page-15-2) Kevan et al. [2008](#page-14-4)). Bee vectoring technology has several advantages over spraying, it requires low amounts of inoculum, decrease the need of external inputs, it reduces the cost and labor-intensive and minimizes nontarget organisms exposure (Kevan et al. [2003\)](#page-14-5). This technology combines two complementary ecosystem services, pollination, and pest control, and it might increase the potential for 'win–win' scenarios contribute to increasing crop yields, and ensure environmental safety. The most studies about bee vectoring focus in pest and disease that affects the flowers and leaves, few studies reveal the potential of these approaches in pest and disease that affects the fruits directly. Therefore, based in some studies results and a couple of information we believe that BVT can be a technique that contributes to pollination and at the same time with pest control in coffee crops as demonstrated in other crops. In this chapter, we discuss the potential use of managed bees as vectors of microbial agents to coffee berry borer control and challenges.

2 Bee Vectoring Technology (BVT)

Bee vectoring is a technology that uses managed pollinating bees to disperse beneficial microbial agents to flowering plants for the control of insect pests and suppression of plant diseases (Peng et al. [1992](#page-15-2); Kevan et al. [2008](#page-14-4)). This approach is possible due to the interaction between the following components: the crop, the pest (weed, disease, or herbivore), the pollinator (vector), the biocontrol agents, the powdery product, the dispensers, and the security for the environment and the human health (Kevan et al. [2008\)](#page-14-4). The vector is the bee species that has a high rate of flower visitation and deposition capacity of the microbial control agent (MCA) on the target crop. The selection of MCA depends on target crop pest or disease, and it must be safe for bees and the environment. In general, the powdery MCA formulations of a commercial product is often used in BVT approach (Mommaerts and Smagghe [2011\)](#page-15-3). The powdery MCA formulations are often mixed with a carrier or diluted to reduce concentration and maximize the contact with MCA and bee bodies (Kevan et al. [2008;](#page-14-4) Al-Mazra'awi et al. [2007](#page-12-0)). Designed dispensers fitted in front of the beehives make possible the contact between bees and MCA. Thus, when bees pass through the control agent provided in dispensers fitted in the beehive entrance, they pick up the inoculum of microbial agents control (fungi, bacteria, and viruses) on their bodies and hairs. Then, when bees visit flowers to collect nectar and pollen and during self-grooming on the leaves of plants, they deposit the inoculum powder on the flowers and leaves of the target crops (Kevan et al. [2008](#page-14-4)).

Some studies report the success of bee vector technology (Carreck et al. [2007;](#page-13-3) Mommaerts et al. [2010](#page-15-4)). Hokkanen et al. ([2015\)](#page-14-6) conducted a study in five countries on the management of strawberry grey mold caused by *B. cinerea* with the biocontrol fungus, *Gliocladium catenulatum* vectored by honey bees or bumble bees targeting strawberry cultivation in open fields. By the results, under heavy disease pressure bee vectoring provided on average a 47% disease reduction, which was a similar result to multiple fungicide sprays. However, under light disease pressure, biocontrol decreased grey mold by an average of 66%, which was more efficient than fungicide sprays. Other studies found similar results, where the use of bees as vectors of MCA was effective against pest or disease in many crops (Kovach et al. [2000;](#page-14-7) Maccagnani et al. [2005](#page-15-5); Shafir et al. [2006\)](#page-16-2).

3 Coffee Market

Coffee is considered the second most important commodity in the world after oil (Daviron and Ponte [2005\)](#page-13-4). Brazil is the most significant world producer and international trade of coffee, followed by Vietnam and Colombia (FAO [2015\)](#page-14-0). According to the ICO 2016 report, the total consumption of all importing countries was estimated at 104.9 million bags (60 kilograms or 132.276 pounds of coffee). The world consumption in 2015 suggests a steady increase to 152.1 million bags (ICO [2016](#page-14-8)). The average annual growth rate remains at a healthy 2% over since 2014, highlighted by an increase in consumption in exporting countries. The world's largest consumers are the European Union and the United States, both demanding around 42 and 24.4 million bags, respectively. The European Union shows an average consumption growth of 0.8% per year since 2012, but the USA continues to show an even more significant increase in coffee consumption by an estimated average rate of 3.2 % (ICO [2016\)](#page-14-8).

4 Coffee Botany

All natural *Coffea* species are native to tropical and subtropical Africa. The genus *Coffea* is a member of the family Rubiaceae (Davis et al. [2006\)](#page-14-9). Three species of *Coffea* are most commercially traded, *Coffea arabica*, *Coffea canephora* (commonly known as "*robusta*" coffee), *Coffea liberica* (liberica) and var. *dewevrei* (excelsa) (Davis et al. [2006](#page-14-9), Ngo et al. [2011](#page-15-6); FAO [2015](#page-14-0)). *Coffea arabica* is responsible for approximately 60% of the global coffee production, while the other 40% *Coffea canephora* (FAO [2015\)](#page-14-0). *Coffea liberica* and other forms represent an irrelevant proportion of the entire global production (Donald [2004\)](#page-14-10).

The *C. arabica* species is native to southwestern Ethiopia. Production is successful at elevation ranging of 900–1500 m (Davis et al. [2006\)](#page-14-9). *C. canephora* originated in the lowlands of equatorial Africa where it grows naturally between (50–)250– 1500 m (Davis et al. [2006;](#page-14-9) DaMatta et al. [2007](#page-13-1)).

Arabica coffee typically presents one main trunk, and Robusta coffee is typically multi-trunked (Vieira [2008\)](#page-16-3). In both species, the trunks develop above the soil and the plant produces horizontal plagiotropic branches, on which blooming and production occur (Fig. [1\)](#page-3-0) (Vieira [2008\)](#page-16-3). The flowers are produced in inflorescences on the axes of plagiotropic branches (Vieira [2008\)](#page-16-3). The flowers of both species are hermaphrodite, and they have five white petals, an elongated corolla tube (Klein et al. [2003b](#page-14-11)). There are five stamens, two-branched stigma, and an inferior ovary of

Fig. 1 Coffee crop in Chapada Diamantina-Brazil (Photo: Helione Barreira)

two chambers and one ovule per chamber (Klein et al. [2003b\)](#page-14-11). *Coffea arabica* is allotetraploid, self-fertile and this species does not need cross-pollination. On the other hand, Robusta coffee is diploid and self-sterile (requires cross-pollination). The flower opens in the morning and the stigma is already receptive when anthesis occurs (Free [1993;](#page-14-12) Klein et al. [2003b\)](#page-14-11). After that, the pollen starts shedding (Ngo et al. [2011\)](#page-15-6).

The flowering phenology and the number of plants blooming per year are influenced by precipitation and region's latitude (Vieira [2008](#page-16-3)). The flowering period is stimulated by first rainfall events in the seasons followed by a dry period, and it may result in more than one bloom (Alvim [1985](#page-13-5); Vieira [2008\)](#page-16-3). In Brazil blooming occurs during the spring (e.g., from September to December in the main Chapada Diamantina coffee production areas) (Fig. [2](#page-4-0)).

The fruit of coffee is an ellipsoid drupe, their size vary with the cultivar or variety planted and cultivation conditions (Vieira [2008](#page-16-3)). In arabica coffee, ripe fruits are red or yellow (Fig. [3\)](#page-5-0), in robusta plants, more hues occur (Vieira [2008\)](#page-16-3). Robusta less susceptible to attacks by pests and disease, produces more berries, and the quality of the beverage is lower when compared to Arabica. (Willson [1999;](#page-16-4) DaMatta et al. [2007;](#page-13-1) Reiger [2006](#page-15-7); Ngo et al. [2011](#page-15-6)).

Fig. 2 The flowering of *C. arabica* in Chapada Diamantina-Brazil (Photo: Acário Cordeiro)

Fig. 3 *Coffea arabica* ripe and green coffee berries in Chapada Diamantina-Brazil (Photo: Catalina Angel)

5 Coffee Pollination

Robusta coffee is self-incompatible, and *C. arabica* is self-fertile and many studies have recorded that wild and managed bees play an important role in pollination of both species (Fig. [4](#page-6-0)) (Willmer and Stone [1989;](#page-16-5) Badilla and Ramírez [1991;](#page-13-6) Raw and Free [1977;](#page-15-8) Klein et al. [2003a](#page-14-13), [b](#page-14-11); Ricketts [2004](#page-15-9); Ngo et al. [2011](#page-15-6); Saturni et al. [2016;](#page-15-10) Nunes [2017;](#page-15-11) Hipólito et al. [2018](#page-14-14)). Honey bees and stingless bees are the most abundant flower visitors during mass-flowering (Willmer and Stone [1989](#page-16-5); Ngo et al. [2011\)](#page-15-6). Krishnan et al. ([2012\)](#page-14-15) have conducted an experiment to compare the contribution of self, wind and insect pollination to fruit set in *C. canephora*. This author's reported that the number of flowers that development in fruits was highest when hand cross-pollinated (44%), followed by open- (insect and wind combined; 33%) and wind- (22.1%) pollination treatments. The flowers from open-pollinated treatments received almost the double of pollen grains than wind-pollinated flowers. The pollination provided by bees increased fruit production by 50% in *C. canephora*. In India, Boreux and collaborators [\(2013](#page-13-7)) found that bees contributed significantly to coffee production by increasing the number of berries produced in *C. canephora*. However, this is related to the initial flower number. The visitation by bees can increase berry production by more than 25%. According to Classen et al. ([2014\)](#page-13-8), bees contribute significantly increased fruit weight of coffee by an average of 7.4% in *C. arabica*. Bagging experiments conducted by Nunes ([2017\)](#page-15-11) with *C. arabica* in Brazil show that the rates of pollen deposition on stigmas and growth of the pollen tube were higher when the flower was visited by *Apis mellifera scutellata* Lepeletier, 1836 than those by spontaneous self-pollination. Thus, a single visit from *A.*

Fig. 4 Honey bee, *Apis mellifera scutellata Lepeletier, 1836*, visiting *Coffea arabica* flower during the blooming (Photo: Helione Barreira)

mellifera contributes to fruit development with weight, height, and width more regular. A recent study on coffee farms of *C. arabica* in Chapada Diamantina shows similar results, coffee flower visitors improved the yield on average 30% (Hipólito et al. [2018\)](#page-14-14). All results reveal the importance of pollination services providing by management and wild bees to increase the yields.

6 Coffee Pests

The natural characteristic perennial coffee plant (*Coffea* spp.) facilitates attacks by some insects and diseases (Barrera [2008\)](#page-13-9). The coffee root, trunk, foliage, and berry are susceptible to attack both in plantation and post-harvesting. In most cases, the pests weaken the plant, reducing yield or affecting the quality of grains (Barrera [2008\)](#page-13-9).

Among the pest that attacks coffee plants, the coffee berry borer (*Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae: Scolytinae) is the worst pest threatening coffee crop throughout the worldwide (Barrera [2008;](#page-13-9) Vega et al. [2015](#page-16-6)). *Hypothenemus hampei* originating from Africa now is considered cosmopolitan (Barrera [2008](#page-13-9)). This beetle causes direct damage to the coffee because attacking berries in all development phases, especially those with more than 20% dry matter (Damon [2000](#page-13-10)). Fruits attacked show a little hole in its apical portion, located at the center or ring of the berry's ostiole (Barrera [2008;](#page-13-9) Vega et al. [2015\)](#page-16-6). Usually, injured fruits fall and rot prematurely. All these damages lead to a reduction in yield and affecting bean quality (Barrera [2008\)](#page-13-9). In Brazil, this pest causes annual losses around at US\$215–358 million (Oliveira et al. [2013;](#page-15-12) Infante et al. [2013;](#page-14-16) Vega et al. [2015\)](#page-16-6).

For some years, the synthetic insecticide Endosulfan $(C_0H_6Cl_6O_3S)$ was used in many countries against CBB. The application of the CBB population's levels up to 80% (Aristizábal et al. [2016](#page-13-11)). Despite the ease of application in the field and insecticidal efficacy, the misuse of Endosulfan resulted in indirect economic losses, leading to social and environmental consequences (Lubick [2010](#page-14-17); Infante [2018](#page-14-18)). Due to

the effects of pesticides on human and environmental health, some countries have banned the use of endosulfan (Janssen [2011](#page-14-2)). Since Brazil forbade the use of Endosulfan, the infestation levels of CBB have reached alarming levels (Brazil [2015\)](#page-13-12). Alternatively, another insecticides such as pirimiphos-methyl, fenitrothion, chlorpyrifos, and fenthion, have been used with success against the CBB (Bustillo-Pardey [2002](#page-13-13)). A variety of strategies have been proposed to reduce the infestation levels of CBB (Vega et al. [2015;](#page-16-6) Infante [2018](#page-14-18)). Many studies revealed the efficacy of adopting the Integrated Pest Management (IPM) methods to control CBB (Aristizábal [2005;](#page-13-14) Benavides et al. [2012\)](#page-13-15). Infante [\(2018](#page-14-18)) summarized several additional methods.

Among the techniques used in IPM, the biological control with the entomopathogenic fungus *Beauveria bassiana* plays a major role in controlling CBB. This fungus is considered a natural controller of CBB because it is found infecting the *H. hampei* in all coffee plantations where CBB has arrived (Benavides et al. [2012\)](#page-13-15). *Beauveria bassiana* is considered as an environmentally safe bioinsecticide, no deleterious effects on humans and the environment and has a low impact on non-target organisms including CBB natural enemies (Zimmermann [2007;](#page-16-7) Aristizábal et al. [2016\)](#page-13-11). This fungus attack their host insects usually percutaneously (Zimmermann [2007\)](#page-16-7).

The use of *B. bassiana* for CBB control is carried out through one or more flood applications of large numbers of aerial conidia in dry or liquid formulation (Mascarin and Jaronski [2016](#page-15-13)). The inundative application is performed using traditional spray methods and recently by autoinoculation traps (Mota et al. [2017\)](#page-15-14). Despite that the autoinoculation trap provided high levels of *H. hampei* mortality in the field, the traps only attract a small amount portion of the insects in the field (Mota et al. [2017;](#page-15-14) Infante [2018](#page-14-18)). The efficacy of autoinoculation traps at long-term control of *H. hampei* and the cost-benefit of this strategy need to be investigated.

In inundative applications of *B. bassiana* by spray application, a high concentration of conidia ranging from 1×10^{11} to 1×10^{12} conidia/ha in aqueous suspension has been used (Benavides et al. [2012](#page-13-15); Mascarin and Jaronski [2016](#page-15-13); Nakai and Lacey [2017\)](#page-15-15). As summarized for Nakai and Lacey ([2017\)](#page-15-15) the mortalities rates of CBB by spray application of *B. bassiana* in fields trials ranged from 10% to 90%. A variety of factors influence the sucess of *B. bassiana* against CBB, such as the temperature, altitude, humidity, formulation, application equipment, strain, concentration, virulence, infestation level and location of CBB (inside or outside of fruit) (Mascarin and Jaronski [2016;](#page-15-13) Nakai and Lacey [2017](#page-15-15)).

According to Vega et al. [\(2015](#page-16-6)) most of the studies about mortality rates of CBB by spray application of *B. bassiana* in fields trials do not include the cost-benefit analysis. In the field, high concentrations of *B. bassiana* are spread by spray application, and this increases the cost of CBB control (Benavides et al. [2012\)](#page-13-15). Besides that, spray applications cause negative impact on conidia viability of the microbial control agent (Nilsson and Gripwall [1999](#page-15-16)). This method requires ready access and much water throughout the plantation, labor work, and machinery (Vega et al.

[2015\)](#page-16-6). Thus, it is essential to develop cost-effective and low impact practices for *B. bassiana* field application on coffee. Below we discuss how the adoption of the Bee Vectoring Technology can improve the efficacy to delivery *B. bassiana* spores against CBB, and improve the initial, maturation and harvest fruit set.

7 Can Pollinators Help to Control Diseases/Pests on Coffee?

The close relationship between coffee and bees has been described above. Among coffee flower visitors, the honeybee is the most frequently reported one in the literature as an important pollinator for *C*. *arabica* and *C*. *canephora*. Overall, the terms "increased production", "the most dominant visitor", "the most frequent flower visitor", "the primary pollinators" and "important pollinator" are frequently cited in studies that investigated the role of honey bees to improve coffee yields in many sites around the world (Roubik [2002a,](#page-15-17) [2002b](#page-15-18); Ricketts [2004](#page-15-9); Veddeler et al. [2006](#page-16-8); Bos et al. [2007](#page-13-16); Vergara et al. [2008;](#page-16-9) Ngo et al. [2011\)](#page-15-6). Raw and Free ([1977](#page-15-8)) reported that coffee brushes caged with honeybees showed higher yields of berries in *C. arabica*. In *C. canephora*, Klein et al. ([2003a](#page-14-13)) and Krishnan et al. [2012](#page-14-15) concluded that *Apis* spp., not only *A. mellifera*, are the most common visitors to coffee flowers.

As mentioned above, honeybees visit the coffee crop efficiently. Their interactions with the coffee plant covers one crucial assumption for BVT success: the close vector-plant interactions. Moreover, honeybees have a large foraging range (up to 3 km in radius) facilitating the spreading of biocontrol agents in large areas (Mommaerts and Smagghe [2011;](#page-15-3) Abou-Shaara [2014](#page-12-1)). Thus, honeybees have the needed requirements to be employed as a vector for disseminating microbial agents control on coffee crops.

Honey bees have been used in many studies to investigate their ability to disseminate some microbial control agents in both greenhouse conditions and open field cultivation (Peng et al. [1992](#page-15-2); Butt et al. [1998;](#page-13-17) Carreck et al. [2007;](#page-13-3) Johnson et al. [1993](#page-14-19)). In the study of Dedej et al. ([2004\)](#page-14-20) using honey bees as a vector of the bacterium *Bacillus subtilis* against mummy berry disease incidence in flower infection by *Monilinia vaccinii-corymbosa*, they found that bee-vectored agent Serenade reduced the incidence of mummy berry disease. Combining the results available in the literature on the success of honey bees for coffee pollination and vectoring of other crops we believe that the honey bee has a high potential as a vector of microbial agents control against the pest and disease in coffee.

BVT also requires that microbial control agents need to be safe to pollinator/ vector. Regarding coffee pests, the entomopathogenic fungus *Beauveria bassiana* can be used for dissemination by honeybees for coffee berry borer control. Several studies reported the effects of *B. bassiana* on *A. mellifera* (Alves et al. [1996;](#page-12-2) Al-Mazra'awi et al. [2007](#page-12-0); Meikle et al. [2008\)](#page-15-19). These effects are conditioned to conidia concentration of *B. bassiana*, the strain and the types of exposition (Al-Mazra'awi et al. [2007;](#page-12-0) Potrich et al. [2018\)](#page-15-20). It is necessary to quantify the effects of this fungus on the honeybees before the trial to better understand the optimal concentration of *B. bassiana* that poses the least for this vector and causes high mortality of CBB.

Some studies demonstrate the efficacy of honeybees to vectoring *B. bassiana* (at rates 1×10^9 conidia/g) against some pests. The potential of dissemination of *B*. *bassiana* by honeybees for control of Tarnished Plant Bug *Lygus lineolaris* (Al-Mazra'awi et al. [2007](#page-12-0); Palisot de Beauvois) on canola was investigated by Al-Mazra'awi et al. ([2006\)](#page-12-3). The bees effectively vectored the inoculum from the hives to the crop and these results indicated that bees might provide a novel means for applying *B. bassiana* to manage *L. lineolaris* in canola. According to these authors, the benefits are better pollination, reduction in pest pressure of *L. lineolaris*, and reduced reliance on insecticides.

The results mentioned above show the capacity of honey bees to spread the fungus *B. bassiana* to many crops around the world against some pests. These results refer to pests and diseases that attack the flowers. It is surprising that one fundamental question remains unanswered: how bees can help control a pest that has a cryptic life? The process that leads to bee-vectoring *B. bassiana* to infecting the CBB remains unclear. Almost all life cycle of the *H. hampei* occurs inside of the coffee berry which difficult their control (Barrera [2008\)](#page-13-9). In the field, post-harvest fallen berries in the ground are a source of new infestations because they are reservoirs for adult insects and larvae (Castaño et al. [2005;](#page-13-18) Benavides et al. [2012](#page-13-15)). Few months after plants are blooming, when conditions are appropriate occurs the massive adult emergence of the old coffee berries (Barrera [2008](#page-13-9)). Those adults mate with their siblings and fly, repeating the entire cycle (Benavides et al. [2012](#page-13-15)). According to Cure et al. ([1998\)](#page-13-19), control measures need to be carried out between the end of harvesting and the appearance of the first fruits of the early maturation of the crop. Generally, *B. bassiana* is applied when female *H. hampei* are just starting to penetrate the berries at the beginning of the year or in fallen berries on the ground (Damon [2000;](#page-13-10) Aristizábal et al. [2016\)](#page-13-11). According to Alves [\(1998](#page-12-4)), insect vectors are essential to inoculation and infection of others insects that live in sheltered places as CBB, because the former insect is capable of dispersing the fungus across the farm.

Bees spread the inoculum on the flowers and leaves of the crop, maybe in the soil too. A coffee stand has one or more blooming periods, and sometimes the vectorization of the fungus by bees can happen more than once. These repeated applications might increase the natural population of *B. bassiana* in an agrosystem. The adults of CBB are infected by bee-vectored *B. bassiana* through: (1) fallen berries contaminated with bee-vectored biocontrol agent; (2) Other insects visiting the coffee plants and then disseminating *B. bassiana* between host insects; (3) Alternative hosts may be infected and produce spores that also infect the CBB; (4) by wind currents.

Ureña and Chuncho ([2008\)](#page-16-10) investigated the ability of honey bees to deliver *B. bassiana* to coffee crops targeting *H. hampei*. Their results show that honey bees vectoring of *B. bassiana* spores can provide a coffee berry protection against berry borer infestation in coffee fields. The inoculum of *B. bassiana* used in the experiment had a concentration at 6.5×10^{10} colony forming units (CFU) per gram. The average percentages of infested berries with *B. bassiana* infection in field trial increased after the fungal dispersion by bees, but the inoculum distribution was not

Fig. 5 The inoculum dispenser (see Peng et al. [1992](#page-15-2)). The dispenser is loaded with inoculum (*Beauveria bassiana* + Vectorite) and attached to the beehive in field trial in coffee crop (Chapada Diamantina-Brazil) (Photo: Juliana Macedo)

homogeneous. Ureña and Chuncho ([2008\)](#page-16-10) also found that when bees vectored the fung, the average number of infested berries with *B. bassiana* infection was 43%, exceeding that provided by spray fungal suspension (23–30%). In some sample plots, when bees vectored the fung, the population of *B. bassiana* increased and reduced the population of coffee berry borer. Moreover, the bees vectored *B. bassiana* spores at a distance up to 200 m from the hives. According to Ureña and Chuncho [\(2008](#page-16-10)), an apiary of 4 bee hives can cover 12.5 ha of coffee homogeneously.

In Brazil (Macedo, personal communication, December 25, 2017), some experiments are developing (Fig. [4\)](#page-6-0). Honey bee hives, *A. mellifera scutellata,* were used during the experiment. Dispensers similar to those used by Peng et al. [\(1992](#page-15-2)) were used in field trials (Fig. [5](#page-10-0)). Preliminary results show that honey bees can deliver *B. bassiana* spores to coffee at a distance up to 350 meters from hives (Fig. [6](#page-11-0)). The low percentage of fungal colony forming units (CFU) of *B. bassiana* were observed on the leaves and flowers sampled in a field trial. The low amount of conidia on coffee flowers and leaves (Fig. [7](#page-11-1)) might be attributed to rain during the field trial. Overall, the results show that that microbial biocontrol can be vectored at long distances by bees into coffee fields and the dissemination of *B. basssiana* spores by bees during blooming can contribute to the regulation of CBB populations. As a result, rise the CBB control, and increase the fruit set by pollination service, and protect the coffee berries during maturation/ripening yield.

Fig. 6 Graph of the concentration of conidia of *Beauveria bassiana* as log_{10} CFU/ml of field collected samples of coffee flowers and leaves versus the distance of beehives in fields trials in Brazil

Fig. 7 Frequency of distribution of sampled flowers and leaves with the concentration of conidia of *Beauveria bassiana* in fields trials in Brazil

8 Challenges

Although the evidence compiled in this chapter points out that using BVT on coffee can be considered a suitable management method in pest control, there are many gaps to explore in this field of science in order to better understand the multitrophic relationships (between agent and vectors), and dynamics of this practice.

As mentioned above, the use of bees as a vector of biocontrol agents for crop protection is possible. The bees can disseminate the inoculum to flowers and leaves, but we need to assess the distribution, deposition of bee-vectored *B. bassiana* in coffee plants a long time. This helps to estimate the persistence and recovery of this fungus in the coffee plants and environment. The development of research is essential to study the efficacy of mixing strains of *B. bassiana* with other agents (e.g. *Metarhizium anisopliae*) for bee-vectoring and their effects on the health of bees. This can help improve CBB control.

Coffee plantations around the world are grown under a wide range of conditions (e.g., shade levels and sun light). These conditions can affect the viability of the control agents used (positively or negatively), as well as the foraging of bees, and this topic need further investigation. In unshaded coffee production, sunlight and warmth affect the post-application persistence of *Beauveria bassiana*. Spore shelflife and longevity need to be improved to enhance their persistence in the field. The implementation of BVT in different spatial and temporal scales, as well as landscapes effects on the effectiveness of BVT use, and different management techniques, also need to be investigated. The evidence compiled in this chapter points out that BVT can be considered a suitable management method to coffee IPM.

9 Final Considerations

The impacts of intensive agriculture are clear. Thus, a new approach like BVT is necessary. BVT increases pest control efficiency and crop productivity. This approach should be employed as a part of the Integrated Pest Management (IPM) combined with other non-chemical pest control methods for coffee berry borer control. Some years ago, BVT could be considered only as a management perspective, but nowadays BVT is a reliable method for pest control in some crops (apple, strawberry, canola), and has a great potential for coffee plantations.

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