Biological control of *Lygus lineolaris* (Hemiptera: Miridae) and *Frankliniella occidentalis* (Thysanoptera: Thripidae) by *Bombus impatiens* (Hymenoptera: Apidae) vectored *Beauveria bassiana* in greenhouse sweet pepper

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Received 5 July 2005, and in revised form 17 October 2005; accepted 1 November 2005

Available online 18 January 2006

Abstract

The ability of bumble bees to disseminate conidia of *Beauveria bassiana* (Balsamo) Vuillemin from hive-mounted dispensers to greenhouse sweet peppers for the control of tarnished plant bug (TPB) and western flower thrips (WFT) was investigated in greenhouse trials using large screened enclosures. Samples collected from the enclosures (four treatments) on two sampling dates showed that 97, 90, 91, and 42% of the collected bees, flowers, leaves, and TPB, respectively, showed detectable densities of *B. bassiana* on the first sampling date. On the second sampling date, 99, 96, 87, and 30% of collected bees, flowers, leaves, and TPB, respectively, showed detectable densities of the fungus. Mean mortalities of TPB collected from cages treated with *B. bassiana* were 34 and 45% compared to 9 and 15% in the controls on the first and second sampling dates, respectively. Mean infection rates of WFT were 40 and 34% compared to 3% in the controls on the first and second sampling dates, respectively. These results indicate that bumble bees are an effective means of vectoring the fungal conidia to the crop. The pollinator vector technology is a novel example of integrating agro-ecosystem processes of pollination and pest management, that reduces the reliance on insecticides and increases crop yields and quality.

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Keywords: *Beauveria bassiana*; Bee vectoring; *Bombus impatiens*; Dispensers; *Frankliniella occidentalis*; *Lygus lineolaris*; Pollination

1. Introduction

Lygus lineolaris (Palisot de Beauvois) (Hemiptera: Miridae), the tarnished plant bug (TPB), is a serious pest for many agricultural crops and is becoming an increasingly important pest in greenhouse vegetable crops in Canada (Howard et al., 1994). The TPB is associated with weedy habitats near and inside greenhouses and the number of TPB collected from such habitats were correlated with their number inside nearby greenhouses (Gillespie et al., 2003).

The western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is a major pest of greenhouse sweet pepper, *Capsicum annuum* L. (Solanaceae) (Shipp et al., 1991, 1998). WFT causes direct yield loss by feeding on, or ovipositing in, developing fruits which results in bronzing and silvering of the fruit (Shipp et al., 1998). Although biological control measures have been adopted for management of WFT on greenhouse sweet peppers, chemical insecticides are required sometimes for effective control (Shipp et al., 1998). Insecticidal treatments, however, are the main control measures for other pests, such as the TPB, which are difficult to control with biological control agents (Shipp et al., 1998). The use of insecti-

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cides for TPB or WFT control can disrupt biological control programs for other greenhouse pests, such as aphids. In addition, the development of pesticide resistance has been reported for both WFT (Immaraju et al., 1992) and TPB (Snodgrass, 1996a,b).

The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Hypomycetes) has been shown to be an excellent potential biological control agent for many agricultural pests (Colorado potato beetle, European corn borer, codling moth and greenhouse pests) (Goettel et al., 1990; Lewis et al., 1996; Poprawski et al., 1997). Once its infective propagules come in contact with the host, they penetrate directly through the cuticle. This route of exposure is essential for the control of pests with sucking mouthparts, which are unlikely to ingest the microbe during feeding (Dunn and Mechals, 1963). Moreover, *B. bassiana* is safe for pollinators (Kevan et al., 2003) and other beneficial organisms under field conditions (Goettel et al., 1990).

Previous reports have demonstrated the susceptibility of TPB to *B. bassiana* (Bidochka et al., 1993; Liu et al., 2002; Steinkraus and Tugwell, 1997). The fungus causes mortality to TPB through disintegration of the insect cuticle and muscle tissues (Bidochka et al., 1993).

Beauveria bassiana has also been found to be virulent to WFT. Gindin et al. (1996) screened 16 entomopathogenic fungal isolates belonging to four species including *B. bassiana* for their virulence against WFT and onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). All tested isolates caused mortalities between 58.2 and 89.5% for the WFT and 96.0 and 100% for the onion thrips (Gindin et al., 1996). Jacobson et al. (2001) tested two commercial formulations of *B. bassiana* Naturalis-L (Troy BioSciences Inc., USA) and BotaniGard WP (Mycotech, Butte, MT, USA), against WFT and the non-target biological control agent *Neoseiulus* (*Amblyseius*) *cucumeris* Oudemans (Acari: Phytoseiidae) on greenhouse cucumbers. Both products reduced populations of immature WFT by 87 and 75%, respectively, compared to untreated control but showed no adverse effect on *N. cucumeris* (Jacobson et al., 2001). *B. bassiana* combined with insect attractants such as Lure Insect attractant (Setre Chemical Co., Memphis, TN, USA) and sugar was also evaluated against WFT (Ludwig and Oetting, 2002). Although the attractants did not enhance the efficacy of *B. bassiana*, high populations of WFT were reduced by the application of the fungus (Ludwig and Oetting, 2002). More recently, Shipp et al. (2003) investigated the influence of greenhouse microclimate on the efficacy of *B. bassiana* against greenhouse pests and their natural enemies including the WFT. In greenhouse trials, the commercial product BotaniGard ES (Emerald Bio-Agriculture Corp., Salt Lake City, UT, USA) which is based on *B. bassiana* strain GHA suppressed the population density of WFT compared to the untreated control (Shipp et al., 2003).

A novel method for the application of microbial control agents against agricultural pests using bee pollinators has

been developed after honey bees were evaluated as vectors of fungi that were antagonistic to gray mold, *Botrytis cinerea* Pers. (Moniliaceae) on strawberries in Ontario (Peng et al., 1992) and the bacterium, *Pseudomonas fluorescens* (Trevisan) (Pseudomonadaceae), which is antagonistic to fire blight *Erwinia amylovora* (Burrill) (Enterobacteriaceae) in apples and pears in western USA (Thomson et al., 1992). Later, the same principle was used successfully against gray mold on raspberries using both honey bees and bumble bees as vectors (Yu and Sutton, 1997). This technology was tried in other countries when the fungus *Trichoderma harzianum* Rifai (Hypocreaceae), which is antagonistic to gray mold, was applied to strawberries by honey bees in Italy (Maccagnani et al., 1999) and by bumble bees and honey bees in the USA (Kovach et al., 2000).

The first report on using bee pollinators to deliver microbial control agents against insect pests was by Gross et al. (1994), who showed that honey bees effectively vectored *Heliothis* nuclear polyhedrosis virus to crimson clover against *Helicoverpa zea* (Boddie) (Lepidoptera: Tortricidae) (Gross et al., 1994). Later, the entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.) Sorokin (Hypomycetes), was vectored successfully by honey bees to winter and spring canola for the control of the pollen beetle, *Meligethes aeneus* F. (Coleoptera: Nitidulidae), in the UK (Butt et al., 1998). The bee-delivered spores resulted in mortalities of 47 and 64% compared to 5 and 12% in the controls on winter and spring canola, respectively (Butt et al., 1998).

Given the virulence of *B. bassiana* to TPB and WFT, and the widespread use of bumble bees as pollinators for many greenhouse crops including sweet peppers (Shipp et al., 1994), bumble bees, *Bombus impatiens* (Hymenoptera: Apidae), were investigated as a vector for *B. bassiana* for the control of WFT and TPB on greenhouse sweet peppers.

2. Materials and methods

2.1. Insect and plant cultures

Newly emerged adults of TPB were obtained from a laboratory culture maintained in the insectary at the AAFC, Southern Crop Protection and Food Research Centre (SCPFRC), London, ON, Canada. This culture was established from field-collected TPB adults on alfalfa at the SCPFRC research farm and kept in cages containing green beans, potato sprouts and romaine lettuce at 24 °C, 60% relative humidity (RH) and 16 h photoperiod.

Colonies of the bumble bee, *B. impatiens* (BioBest Canada, Leamington, ON, Canada), were used during the experiment. Each colony consisted of a queen and 40–50 workers. The bees in each colony were transferred from their cardboard colony box to a wooden colony box (32 cm × 23 cm × 24 cm) that allowed the use of an inoculum dispenser. Bees were kept in the wooden boxes for 48 h before use in the trials to allow for acclimation to the new chamber.

The inoculum dispenser was similar to that used by Yu and Sutton (1997) with a slight modification. The dispenser consisted of a wooden box (21 cm × 120 cm × 8.5 cm height) that was divided horizontally into two compartments by a 7-mm thick sheet of Perspex. The lower compartment had a sliding tray with a zigzag passageway in which the inoculum was spread 5 mm deep. Each of the lower and upper compartments were connected to the main chamber of the hive by a hole 13 mm in diameter. The hole connecting the upper compartment with the main chamber was supplied with a tapered tube that projected inside the main chamber allowing the bees to enter the main chamber but not to go out. This insured that all exiting bees left through the lower compartment where the formulated inoculum was placed. After crawling through the inoculum dispenser, the bees exited via a hole made in the corner of the Perspex sheet to the upper compartment where they left the hive through the hole in the front of the dispenser. Returning bees entered via the hole in the front of the upper compartment and proceeded to the hole in the main chamber of the hive without contacting the inoculum in the lower compartment. The modification to the dispenser involved replacing the front wooden side of the dispenser with a sheet of Perspex. This allowed more light to enter through the dispenser facilitating bees exiting the main chamber of the hive to the dispenser and then to the outside. Each hive was used for two successive sampling dates. Before the vectoring trials and between sampling date trials, the bees were supplied with pollen mixed with 50% wt./wt. sugar syrup.

Sweet pepper (cv Edison) plants were grown in a 1:1 mixture of sand and peat moss in 20-cm pots. Initially, the plants were maintained in a separate greenhouse compartment until the first and second flower cluster stages and then transferred to the screened cages for the vectoring trials. Day/night temperature set points for the greenhouse were 22/19 °C and 70% RH. Greenhouse climate was maintained by Argus Control Systems (Argus Control Systems, White Rock, BC, Canada). Plants were irrigated and fertilized with the Harrow Fertigation Manager (Labbate Climate Control Systems, Leamington, ON, Canada) according to standard commercial practices (Ontario Ministry of Agriculture and Food, 2001).

2.2. Inoculum preparation

Beauveria bassiana strain GHA (BotaniGard WP, Emerald BioAgriculture, Salt Lake City, UT, USA) was used in the vectoring trials. For each replicate, to assess the number of colony forming units (CFU) per unit weight, six 0.1 g samples were taken at random, suspended in 100 mL sterile distilled water and 0.1% Tween 80, and then agitated on a rotary shaker at 125 rpm for 2 h. Three 0.1 aliquots of 10-fold serial dilutions of each suspension were spread on oatmeal agar Petri plates amended with 550 µg/mL Dodine, 400 µg/mL penicillin G, 1000 µg/mL streptomycin sulfate and 5 µg/mL crystal violet (Beilharz et al., 1982), incubated at 25 ± 1 °C for 4–5 days, after which colonies of *B. bassiana*

were counted and recorded. To determine the viability of the conidia, six 0.01 g samples of conidia were suspended in 100 mL distilled water and 0.1% Tween 80. Then, 200 µL of the conidial suspension was added to 1 mL of Sabouraud's dextrose broth amended with 1% yeast extract in a sterile test tube. The suspension was incubated at 24 ± 1 °C for 24 h. After this, four groups each consisting of 200 conidia were examined for percentage germination using a hemacytometer and a compound microscope. Inoculum from the commercial product was prepared by mixing the dry conidia with corn flour (particle size 45–90 µm) to a concentration of 1 × 10⁹ CFU/g.

2.3. Vectoring trials

Vectoring trials were done at the Greenhouse and Processing Crop Research Centre, Harrow, ON, Canada, in the spring of 2003. Two adjacent climate-controlled greenhouse compartments (8.5 m × 13 m) were used for the trials. Two translucent screened cages (1.8 m × 4 m × 2 m height) were placed inside each greenhouse compartment. Fine mesh screening was used to contain the bees as well as the target pests on the plants. Sixty-four potted pepper plants were placed in two double rows inside each of the screened cages. The experimental design consisted of a complete randomized block design with each trial being replicated over time (block). Within each trial, all treatments were randomized among the cages. Treatments were: (1) viable *B. bassiana* inoculum + TPB + bumble bees, (2) viable *B. bassiana* inoculum + WFT + bumble bees, (3) heat-inactivated *B. bassiana* inoculum + TPB + WFT + bumble bees and (4) TPB + WFT only. Plants in three of the cages were infested with newly emerged *L. lineolaris* adults at a rate of four adults per plant (treatments 1, 3, and 4). Adults of WFT were introduced at a rate of 30 thrips per plant into three cages (treatments 2, 3, and 4). TPB and WFT adults were allowed to feed on the plants for 24 h and then were checked to ensure survival. Next, three bumble bee colonies in the modified wooden boxes each equipped with an inoculum dispenser were placed inside three of the cages between the two double rows of pepper plants (treatments 1, 2 and 3). Two dispensers were filled each with 25 g of the prepared inocula (treatments 1 and 2), while the dispenser in the remaining colony was filled with 25 g corn flour inoculated with the heat-inactivated conidia (treatment 3). During the experiment in both the viable and heat-inactivated treatments, the dispensers were refilled after a 72-h interval.

After filling the dispensers, the bumble bees were allowed to forage on the flowering pepper plants. To assess dissemination of *B. bassiana*, samples were collected 3 days after the dispensers were placed on the hives. On each sampling date, 10 bumble bees, 10 fully opened pepper flowers, 10 pepper leaves, 10 TPB adults and 50 WFT adults were collected from each treated cage. Bumble bees were collected directly after they left the hive through the dispenser. Flowers and leaves immediately adjacent to the bee colony were not selected in the sampling process. To estimate via-

ble conidia (CFU) on the bumble bees, flowers, leaves, TPB and WFT, samples were agitated individually in flasks containing 100 mL sterilized distilled water and 0.1% Tween 80 on a rotary shaker for 2 h except for the WFT samples that were vortexed in 1-mL centrifuge tubes. Three 0.1 aliquots of 10-fold serial dilutions of each suspension were spread on oatmeal agar Petri plates that were prepared as described in Section 2.2. To assess mortality of TPB, adults were collected from cages dedicated to treatments 1, 3, and 4. Five subsamples, each containing 10 TPB, were collected per treatment by means of an aspirator and plastic vials. Each subsample was placed in a ventilated PVC cylinder (10 cm diameter $\times$ 20 cm height) lined with moist filter paper and incubated at 24 °C and a 16-h photoperiod. Fresh organically produced lettuce leaves were provided as food for the caged insects. Mortality was recorded on days 1, 4, and 7 after collection. Dead insects were removed and placed in Petri plates lined with moist filter paper to encourage external growth of conidia. To assess the infection rate in WFT, adults were collected from cages dedicated to treatments 2, 3, and 4. Five subsamples, each containing 10 WFT adults, were collected per treatment using an aspirator and plastic vials. The subsamples were surface sterilized by immersing in 70% ethanol for 15–20 s, followed by 5% bleach containing 0.3% NaOCl amended with 0.05% Tween 20 for 3 min and two rinses of sterile distilled water amended with Tween 20 for 1–2 min. The surface-sterilized WFT adults were then placed in water agar plates and incubated at 25 °C for 5–6 days. After the incubation period, all test individuals were examined using a dissecting microscope for the presence of *B. bassiana*.

The experiment was replicated five times. Each replicate consisted of a new set of plants, freshly prepared inocula and new batches of insects. Between replications, the greenhouse compartments were heated to 35–37 °C for 72 h and fumigated with the insecticide Nicotine (MGS Horticultural, Leamington, ON, Canada) to eliminate any carryover of TPB and WFT between replicates. Mean ($\pm$ SE) daily temperature and RH in the cages were maintained at 21.0 ± 0.1 °C and $67.2 \pm 5.1\%$ RH using the Argus Greenhouse Management System (Argus Control System) to ensure similar conditions between the two greenhouse compartments. Temperature and RH were monitored inside the cages using shaded temperature/humidity probes (Hycal,

Elmonte, CA, USA). Probes were installed ca. 60 cm above the ground at the middle of the plant canopy.

2.4. Statistical analysis

Percentage mortalities of TPB adults and infection rates of WFT adults were arcsin square root transformed and analyzed using a mixed linear model in a split plot design with sampling dates (the subplots) and treatments (the whole plot) (PROC MIXED, SAS Institute, 1999). Means were then separated using polynomial contrasts where the mortality and infection rates in the *Beauveria*-treated cages were compared to the average mortality from both the heat-inactivated *Beauveria* and non-*Beauveria* treatments. Another contrast was made to compare the effects of the heat-inactivated *Beauveria* and the non-*Beauveria* treatments. Data on the estimated values of *B. bassiana* CFU were $\log(x+1)$ transformed and subjected to ANOVA to test for differences in CFU counts between the two sampling dates (PROC ANOVA, SAS Institute, 1999). For both CFU data and mortality and infection rate data, means were compared only if significant differences were found by the *F*-test. The type I error rate (α) was set at 0.05 level for all the tests.

3. Results

Bumble bees leaving the hive on the two sampling dates efficiently acquired *B. bassiana* conidia supplied via the hive dispensers. Essentially, all the bee samples had detectable levels of the fungus (Table 1). Inocula carried by the emerging bees ranged from 0 to 2.1×10^6 and 0 to 2.3×10^6 CFU/bee on the first and second sampling dates, respectively. The bumble bees were also effective in delivering the inoculum to the flowers of greenhouse sweet peppers. Ninety percent (± 5.2) of the flowers on the first sampling date and 95.6% (± 2.4) on the second sampling date contained detectable densities of the fungus (Table 1). Densities of CFU per flower were from 0 to 1.4×10^5 and 0 to 2.1×10^5 on the first and second sampling dates, respectively. Inoculum of *B. bassiana* was also detected on the leaves of the crop. Percentages of the leaf samples with detected densities of the fungus were 91.3% (± 3.0) and 86.7% (± 4.4) on the first and second sampling dates, respectively (Table 1). Densities of

Table 1
Mean ($\pm$ SE) number of colony forming units (CFU)/insect or plant and mean ($\pm$ SE) percentage of bumble bees, sweet pepper flowers and leaves, TPB adults and WFT adults with detectable densities of *Beauveria bassiana* as collected from treatments containing bumble bees and *B. bassiana*

Sample	First sampling date		Second sampling date	
	Mean	Percentage	Mean	Percentage
Bees	4.5×10^5 (5.3×10^4)	97.0 (1.5)	3.8×10^5 (7.9×10^4)	98.9 (1.1)
Flowers	1.8×10^4 (4.7×10^3)	90.0 (5.2)	3.2×10^4 (9.0×10^3)	95.6 (2.4)
Leaves	3.2×10^4 (7.9×10^3)	91.3 (3.0)	3.6×10^4 (1.4×10^4)	86.7 (4.4)
TPB	586.7 (252.7)	42.0 (12.4)	708.3 (461.6)	30.0 (14.7)
WFT	1013.5 (502.1)	NA	1031.6 (285.5)	NA

CFU for WFT were based on groups of 10 WFT per group.

the fungus were 0 to 1.7×10^5 and 0 to 2.0×10^5 CFU/leaf on the first and second sampling dates, respectively. With respect to TPB adults, ca. 30–40% of the bugs showed detectable amounts of the fungus on the two sampling dates (Table 1). Densities of CFU per adult TPB were from 0 to 5.5×10^3 on the first sampling date and 0 to 8.2×10^3 on the second sampling date. Adult WFT also acquired the bee-delivered inocula with densities of CFU per subsample of 10 WFT adults ranging between 133 and 2267 and 180 and 2246 on the first and second sampling dates, respectively. No significant differences were found in the density of *B. bassiana* conidia on bumble bees ($F_{1,17}=0.1$, $P=0.75$), leaves ($F_{1,15}=0.2$, $P=0.66$), flowers ($F_{1,17}=1.53$, $P=0.23$), TPB ($F_{1,7}=0.23$, $P=0.65$) and WFT ($F_{1,6}=0.44$, $P=0.53$) between the first and the second sampling dates (Table 1).

Percentage mortalities of TPB collected on the two sampling dates from the cages with or without *B. bassiana* delivered by bumble bees are presented in Table 2. Statistical analysis showed that the effects of sampling dates ($F_{1,9}=2.92$, $P=0.12$) and the interaction between sampling dates and treatments ($F_{2,9}=3.42$, $P=0.08$) on TPB mortality were not significant. However, significant differences in TPB mortalities were found among the treatments ($F_{2,8}=37.85$, $P<0.001$). Further means testing of TPB mortality in the *Beauveria*-treated cages with those from the heat-inactivated *Beauveria* and non-*Beauveria* treatments, and TPB mortality in the heat-inactivated *Beauveria* to non-*Beauveria* treatments revealed that adult TPB collected from the treatments receiving the living conidia of *B. bassiana* had significantly higher mortalities than did TPB collected from cages with the heat-inactivated or non-*B. bassiana* treatments ($F_{1,8}=75.59$, $P<0.001$). No significant difference in mortality of TPB was found between the heat-inactivated *Beauveria* and the non-*Beauveria* treatments ($F_{1,8}=0.11$, $P=0.75$). Most of the mortality occurred 3–7 days after collection of the TPB and the final cumulative mortalities 7 days after collection of the TPB ranged from 34 to 45% over the two sampling dates (Table 2).

The higher mortality of TPB collected from the living *Beauveria* treatment compared to the heat-inactivated *Beauveria* and non-*Beauveria* treatments was confirmed by the high percentage of dead TPB that developed external mycosis. The mean percentage of mycosed insects was 90% (± 3.1) and 91% (± 3.0) for the first and second sampling dates, respectively. Few mycosed insects were observed in the heat-inactivated *Beauveria* and non-*Beauveria* treat-

ments from the first and the second sampling dates (Table 2).

Similar results were obtained from analysis of the WFT infection rates (Table 3). The effect of sampling dates and the interaction effect between treatments and sampling dates were not significant at the 0.05 level ($F_{1,12}=1.11$, $P=0.31$ for sampling date and $F_{2,12}=0.15$, $P=0.86$ for the interaction effect). The infection rate for the viable *Beauveria* treatment was significantly higher than the average values from the heat-inactivated *Beauveria* and the non-*Beauveria* treatments ($F_{1,12}=33.89$, $P<0.001$) (Table 3). No significant differences were detected by comparing WFT mortalities for the heat-inactivated *Beauveria* treatment with non-*Beauveria* treatment ($F_{1,12}=0.2$, $P=0.67$).

The frequency distribution of bumble bees with detectable densities of *Beauveria* collected from the cages supplied with the living *B. bassiana* is presented in Fig. 1. Only 2% of the bumble bees left the hive without detectable densities of the fungus and almost one-third of the bees carried between 1 and 1×10^5 CFU/bee. The percentage of bees with *Beauveria* decreased with an increase in the density of CFU per bee above 10^5 and 9% of the bumble bees carried more than 10^6 CFU/bee. Mean CFU per bee for all sampled bees was 4.2×10^5 ($\pm 6.6 \times 10^4$) and the median was 2.5×10^5 (Fig. 1).

The frequency distribution of greenhouse sweet pepper flowers sampled on the first sampling date showed that 73% of the flowers had CFU densities within the ranges 1 to 10×10^3 (34%), 10.1×10^3 to 20×10^3 (26%) and 30.1×10^3 to 40×10^3 (13%) CFU/flower (Fig. 2). Ten percentage of the sampled flowers had no detected densities of the fungus. Few flowers had inoculum densities within the ranges of

Table 3

Mean infection rate ($\pm$ SE) of WFT adults on greenhouse sweet pepper plants caged without bumble bees, with bumble bees and with bumble bees and *B. bassiana* for two sampling dates

Treatment	Infection rate	
	First sampling date	Second sampling date
WFT + bees + <i>B. bassiana</i>	39.5 $\pm$ 11.8 a	34.1 $\pm$ 6.9 a
WFT + bees + heat-inactivated <i>B. bassiana</i>	3.4 $\pm$ 2.6 b	3.1 $\pm$ 2.6 b
WFT only	2.2 $\pm$ 2.2 b	0.5 $\pm$ 0.5 b

Means within columns and rows with different letters are significantly different at 0.05 level using polynomial contrast.

Table 2

Mean percentage ($\pm$ SE) mortality and mycosis of TPB adults on greenhouse sweet pepper plants caged without bumble bees, with bumble bees and *Beauveria bassiana* for two sampling dates

Treatments	First sampling date		Second sampling date	
	% Mortality	% Mycosed	% Mortality	% Mycosed
TPB + bees + <i>B. bassiana</i>	33.6 $\pm$ 6.6 a	90.0 $\pm$ 3.1	45.0 $\pm$ 3.9 a	91.0 $\pm$ 3.0
TPB + bees + heat-inactivated <i>B. bassiana</i>	9.2 $\pm$ 2.7 b	6.0 $\pm$ 4.4	15.3 $\pm$ 3.2 b	14.5 $\pm$ 7.5
TPB only	14.8 $\pm$ 4.1 b	0	9.0 $\pm$ 1.9 b	1.7 $\pm$ 1.7

Means within columns and rows with different letters are significantly different at 0.05 level using polynomial contrast.

Fig. 1. Frequency distribution of collected bumble bees with detected densities of conidia of *Beauveria bassiana* after emerging from hive-mounted dispensers filled with 1×10^9 CFU of *B. bassiana* per gram of corn flour.

Fig. 3. Frequency distribution of sampled sweet pepper flowers with detected densities of conidia of *Beauveria bassiana* from the cages containing bumble bees and *B. bassiana* on the second sampling date.

Fig. 2. Frequency distribution of sampled sweet pepper flowers with detected densities of conidia of *Beauveria bassiana* from the cages containing bumble bees and *B. bassiana* on the first sampling date.

4×10^4 to (1×10^5) . Mean CFU per flower for all sampled flowers on the first sampling date was $1.8 \times 10^4 (\pm 4.7 \times 10^3)$ and the median was 1.2×10^4 (Fig. 2). On the second sampling date (Fig. 3), a small percentage (4%) of the sampled flowers did not have any *B. bassiana*. The frequency of flowers with CFU per flower between 1 and 1.0×10^4 , and between 1.0×10^4 and 2.0×10^4 was 31 and 29%, respectively. Few flowers with inoculum densities within the range of 4.0×10^4 to (1.0×10^5) were sampled from the cages. Mean CFU per flower for all sampled flowers on the second sampling date was $3.2 \times 10^4 (\pm 9.0 \times 10^3)$ and the median was 1.9×10^4 (Fig. 3).

The frequency distribution of greenhouse sweet pepper leaves (Fig. 4) with detected inocula of *B. bassiana* pooled

over the sampling dates showed that 11% of the sampled leaves had no detectable *B. bassiana*. The percentage of leaves with *Beauveria* decreased with increasing density of CFU per leaf. The mean CFU per leaf for all sampled leaves was $3.4 \times 10^4 (\pm 1.1 \times 10^4)$ and the median was 1.3×10^4 (Fig. 4).

A large percentage of TPB (64%) showed no detected amounts of the fungus. The percentage of TPB with CFU densities from 1 to 1×10^3 and 1×10^3 to 2×10^3 were 20 and 8%, respectively. Mean CFU per TPB for all sampled TPB adults was $6.5 \times 10^2 (\pm 3.6 \times 10^2)$ and the median was 0 (Fig. 5).

Fig. 4. Frequency distribution of sampled sweet pepper leaves with detected densities of conidia of *Beauveria bassiana* from cages containing bumble bees and *B. bassiana* pooled over the two sampling dates.

Fig. 5. Frequency distribution of collected TPB adults with detected densities of conidia of *Beauveria bassiana* from the cages containing bumble bees and *B. bassiana* pooled over the two sampling dates.

4. Discussion

This study provides the first report of using bumble bees to vector a microbial fungal biological control agent for insect control on a greenhouse crop. *B. impatiens* effectively vectored *B. bassiana* from the inoculum dispensers to greenhouse sweet pepper plants and subsequently to the target pests. Bumble bees in the collected samples were coated with hundreds and thousands of *B. bassiana* conidia as they left their hives. The pooled mean of 4.2×10^5 CFU/bee for the two sampling dates was equivalent to 0.42 mg inoculum/bee. Yu and Sutton (1997) estimated that bumble bees carried 0.4 mg of *Clonostachys (Gliocladium) roseum* Bainier (Hypomycetes) inoculum when they emerged from similar dispensers.

The high percentage of flowers ((90%) on which *B. bassiana* was detected (Table 1) on both sampling dates indicated that the bumble bees efficiently vectored the control agent. Similar results were found by Yu and Sutton (1997), who found that *C. rosea* was recovered from 84% of the raspberry flowers treated with inoculum vectored by bumble bees. Bumble bees carried much larger amounts than they deposited on the flowers. Mean CFU per flower in the first and second sampling dates of 1.8×10^4 and 3.2×10^4 , respectively, indicated that the amount detected on flowers constituted only 4.0 and 8.4% of the amount carried by the bees. Similarly, Peng et al. (1992) reported that one-tenth of the inoculum was detected on the flowers compared to the density detected on the bees when honey bees were used as vectors of *C. rosea* to strawberry for suppression of gray mold.

The density of inoculum deposited on flowers was variable and ranged from 0 to 2.1×10^5 CFU/flower (Figs. 2 and 3). This variability might be attributed to several fac-

tors including: the variable amounts of inocula carried by the bees, bee vectoring dynamics, orientation of flowers and redistribution of inocula by the bees, other organisms and air currents. Successive visitation to flowers during a foraging trip might result in a decline of the dose carried by the foraging bees and thus, would affect inoculum transfer for subsequently visited flowers. Moreover, repeated visitation by more than one foraging bee to the same flower might result in more inocula deposited on that particular flower compared to the less visited ones. This variability is common in bee delivery systems and was reported by previous investigators (Peng et al., 1992; Yu and Sutton, 1997). Mathematical models of pollen transfer (e.g., exponential decay), which might resemble conidial transfer, are frequently used to study patterns of spatial dispersal of pollen among the flowers of crops (Iwasa et al., 1995). Those models are not applicable to the current results because flower sampling in this study was done randomly, whereas collection of data for the pollen models requires sequential sampling of flowers from the pollen source (Iwasa et al., 1995; Cresswell et al., 1995). Moreover, some factors included in the model such as the total number of flowers on the plants, the proportion of visited flowers and the number of visits for individual flowers were not considered in this study.

Beauveria bassiana was also disseminated from the inoculum dispensers to the leaves of the crop. The pooled average of the two sampling dates of 3.4×10^4 constituted 8.0% of the amount carried by the bees (Fig. 4). The presence of the inoculum on the leaves might be attributed to sedimentation of conidia from flying bumble bees as well as to redistribution of conidia by the bees, air currents and other living organisms. Observations of bumble bee behavior during the experiment showed that some bees flew directly from the dispensers to the leaves where they groomed.

The bee-delivered *B. bassiana* was acquired by the TPB feeding on the crop. This was indicated by the percentages of sampled TPB with detected densities of the fungus (Fig. 5). The amount of conidia detected on the TPB adults, as expressed by the pooled mean of the two sampling dates (Fig. 5), constituted 3.6 and 2.0% of the amount detected on the flowers in the first and second sampling dates, respectively. Although these percentages are low, substantial mortality of TPB was observed in the cages treated with *B. bassiana* compared to the untreated ones (Table 2). The fungus was considered as the cause of the death of TPB adults because an high percentage of insect cadavers developed external mycosis (Table 2). Low mortalities were observed in samples collected from the heat-inactivated *Beauveria* or the non-*Beauveria* treatments which indicated that neither the carrier, fine corn flour, nor the bumble bees affected the survival of the bugs and their susceptibility to the entomopathogen.

Similar to the results of TPB mortality, the infection rates of WFT adults collected from the living *Beauveria* treatment were higher compared to those of WFT adults collected from the heat-inactivated *Beauveria* or the non-*Beauveria* treatments (Table 3). This was further empha-

sized by the detection of bee-delivered conidia on WFT adults collected from cages supplied with living *Beauveria*. The pooled mean of the two sampling dates of 1023 CFU/10 WFT constituted 5.7 and 3.2% of the amount detected on the flowers on the first and second sampling dates, respectively. Very low infection rates (0.5–3.4%) were recorded from WFT adults collected from the heat-inactivated *Beauveria* or the non-*Beauveria* treatments. The infection is likely attributed to contamination by personnel while conducting the experiment.

Results indicated that the density of *B. bassiana* propagules on bumble bees, pepper flowers and leaves, TPB and WFT did not differ between the two sampling dates. Differences on the target pests might be detected after longer periods of bee vectoring with continued filling of the dispensers to cope with depletion of the inocula.

Very little is known about the impact of *B. bassiana* on bumble bees. The bumble bees, *B. pratorum* (L.) and *B. terrestris* (L.), have been reported as hosts for *Beauveria* (Goettel et al., 1990). Although *B. bassiana* infected some bumble bee workers during the course of the study, no epizootic of *Beauveria* was observed in the colonies. Careful monitoring during and after the trials showed no adverse impact on colony health, brood production and activity of bumble bees. However, studies are warranted on the effects of *B. bassiana* on bumble bees when applied as a dry formulation.

This study demonstrated the potential of using bumble bees as a novel method for the application of *B. bassiana* to greenhouse sweet pepper for the control of TPB and WFT. More research is needed to determine the optimum concentration of *B. bassiana* for maximum infection levels in TPB and WFT and how often the dispensers need to be refilled. In addition, using bumble bees for the pollination of greenhouse sweet pepper has resulted in a positive impact on fruit quality and yield (Shipp et al., 1994). Other researchers have found *B. bassiana* to be effective against several insect pests including *Lygus* spp. (Bidochka et al., 1993; Liu et al., 2002; Noma and Strickler, 1999; Steinkraus and Tugwell, 1997) and WFT (Gindin et al., 1996; Jacobson et al., 2001; Shipp et al., 2003). By using bumble bees as a means of delivering *B. bassiana*, one not only reduces pest populations at a single point in time, but also has a continuous impact on the pest populations over time because bees are disseminating the control agent on a daily basis. This continuous impact is critical in greenhouse systems where conditions are favorable for pest reproduction. Using bee pollinators as the means for the delivery and dissemination of microbial control agents on greenhouse crops is a novel way of integrating agricultural practices (i.e., pollination and pest control) that will result in better quality and quantity of crops.

Acknowledgments

We thank Emerald BioAgriculture (Salt Lake City, UT, USA) for providing *B. bassiana*. The study was funded by

Al-Balqa Applied University, Jordan and Natural Sciences and Engineering Research Council/Biocontrol Network, Canada. For laboratory and field assistance, we thank P. Kelly, G. Wilson, S. Nathan, M. Adjaloo, K. Wang and K. Wharram.

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