Compatibility of an entomopathogenic fungus with a predator and a parasitoid in the biological control of greenhouse whitefly

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RESEARCH ARTICLE

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An ongoing debate in biological control consists of whether interference between biological agents can disrupt pest control. This study investigated the outcome of interactions between the entomopathogen *Beauveria bassiana* with the whitefly predator *Dicyphus hesperus* and the parasitoid *Encarsia formosa,* as well as their effect on the control of the greenhouse whitefly *Trialeurodes vaporariorum* on greenhouse tomato crops. Our objective was to determine whether the generalist *B. bassiana* would disrupt biological control by interfering with *D. hesperus* or *E. formosa*. In experimental greenhouses, whitefly, parasitoid and predator populations were established, and over 27 days, tomato plants were sprayed with three applications of the *B. bassiana* based product BotaniGard® (5.13×10^3 conidia/ mm^2) or water (control). Populations of greenhouse whitefly and biological control organisms were regularly monitored in control and *B. bassiana*-treated compartments. Overall, 10.6% of all whiteflies in treated compartments were infected, and 0.98% were both infected and parasitized. There were 31.7 and 22.3% fewer immature and adult whiteflies, respectively, on *B. bassiana*-treated plants relative to controls. Parasitism by *E. formosa* and predation by *D. hesperus* occurred at rates of 7.5 and 2.5%, respectively, in *B. bassiana*-treated compartments, and 5 and 6%, respectively in control compartments. Our study suggests that applications of *B. bassiana* for short-term biological control of greenhouse whiteflies are compatible with the concurrent use of *E. formosa* and *D. hesperus* on greenhouse tomato crops.

Keywords: *Trialeurodes vaporariorum*; *Dicyphus hesperus*; *Encarsia formosa*; *Beauveria bassiana*; BotaniGard®; biological control; intraguild interaction

Introduction

Biological control is an effective strategy for control of the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), a polyphagous pest of many field and greenhouse crops worldwide (Byrne and Bellows 1991). Biological control of the greenhouse whitefly has for many decades depended on inundative releases of the specialist parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) (van Lenteren, van Roermund, and Sütterlin 1996; van Lenteren 2000; Avilla, Albajes, Alomar, Castañé, and Babarra 2004). However, winter

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conditions, such as low temperatures, short days and low light intensity, may limit the efficacy of *E. formosa* (see Zilahi-Balogh, Shipp, Cloutier, and Brodeur 2006, 2009 and references therein). Two non-exclusive strategies are being considered to circumvent these problems: manipulate greenhouse climatic conditions to favour the actions of natural enemies and combine two or more species of biological control agents with complementary attributes to reduce whitefly populations.

Predatory mirids from the Dicyphinii tribe are frequently used in Europe as biological control agents on vegetables (Alomar and Albajes 1996). They are particularly appealing due to their zoophytophagous feeding habits which may sustain predator populations on crops when prey density is low (Gillespie and McGregor 2000). Plant-feeding predators are good candidates in crops tolerant to small levels of herbivory. In Canada, a large proportion of greenhouse tomato producers use *E. formosa* and *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) for inundative releases to suppress whiteflies in greenhouses (Murphy et al. 2002), and some may also combine these parasitoids with releases of the native mirid predator, *Dicyphus hesperus* Knight (McGregor, Gillespie, Quiring, and Foisy 1999; Sanchez, Gillespie and McGregor 2003; Shipp and Wang 2006). *Dicyphus hesperus* preys upon many species of small insect pests (Kelton 1980; Henry and Wheeler 1988; Gillespie, McGregor, Quiring, and Foisy 2000; Shipp and Wang 2006). It represents a general and long-term biological control strategy where the introduced predator becomes established and reproduces on a crop as long as environmental conditions are appropriate, and adequate resources are available (Sanchez et al. 2003).

In situations where whitefly populations approach injurious levels, entomopathogenic fungi may be an effective means of pest reduction. Microbial biological control agents, which share several characteristics of conventional chemical pesticides (i.e., speed of kill, application method, storage capacity) may provide a rapid and substantial reduction of pest populations (Lacey, Frutos, Kaya, and Vail 2001). In particular, it has been shown that the ubiquitous and generalist *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) may effectively reduce whitefly populations (Wraight et al. 2000; Kirk, Lacey, and Goolsby 2001). *Beauveria bassiana* has been isolated from over 700 invertebrate host species around the world (Feng, Poprawski, and Khachatourians 1994; Butt, Jackson, and Magan 2001). However, the fungus may infect non-target natural enemies (Goettel, Poprawski, Vandenberg, Li, and Roberts 1990), and could therefore disrupt existing whitefly biological control programs.

Complex relationships might occur between entomopathogens and arthropod natural enemies that exploit an herbivore. Such intraguild interactions are widespread within communities of biological control agents and are likely to have an impact on the efficacy of biological control (Rosenheim, Kaya, Ehler, Marois, and Jaffee 1995). Interactions between fungi, parasitoids and predators are mostly asymmetric, in favour of the entomopathogens (Brodeur and Rosenheim 2000). Fungal infection may be lethal or sublethal to all developmental stages of predators and parasitoids. For example, survival of developing *E. formosa* was found to be jeopardized by *Aschersonia aleyrodis* Webber (Deuteromycotina: Coelomycetes) infection when the pathogen was applied in the first 3 days following parasitism (Fransen and van Lenteren 1994). On the other hand, arthropod natural enemies are also known to feed on entomopathogens. Studies with Pell, Pluke, Clark, Kenward,

and Alderson (1997) showed that aphids heavily infected by *Pandora (Erynia) neoaphidis* (Remaudière & Hennebert) Humber, were consumed by coccinellid and carabid beetles. Similarly, Askary and Brodeur (1999) observed that aphid parasitoid larvae feeding on host tissues also ingest fungal spores. Finally, an entomopathogen may be more effectively transmitted to the target pest in the presence of a parasitoid or predator (Roy and Pell 2000). As the outcome of such multispecies interactions has only begun to be examined, the limited number of quantitative population studies to date remains insufficient to understand and predict the compatibility of fungal infection, parasitism and predation.

In this study, we examined the compatibility of BotaniGard® 22 WP, a formulation based on conidia of *B. bassiana*, strain GHA, with the parasitoid *E. formosa* and the predator *D. hesperus*. We assessed densities of whitefly natural enemies and measured both whitefly parasitism and predation following applications of BotaniGard. The effect of *B. bassiana* on biological control of greenhouse whitefly was also determined through a comparison of control versus pathogen-treated compartments in which populations of *T. vaporariorum*, *E. formosa* and *D. hesperus* had been established. This study was conducted in large greenhouse compartments over a 2-month period. These experimental conditions provide sufficient scale and complexity for expression of a wide range of potential interspecific interactions (Messing, Roitberg, and Brodeur 2006) and a realistic measure of resulting pest population performance.

Materials and methods

Experimental design

Tomatoes *Lycopersicon esculentum* Mill. (Solanaceae, cv. Rhapsodie, Syngenta Seeds, Boise, ID), were grown in two, 12×6.4-m glasshouses at the Pacific Agriculture and Agri-Food Research Centre (PARC) in Agassiz, British Columbia (Lat $49°14'$N, long $121°44'$W). Tomatoes were seeded in mid-December and seedlings were planted on rockwool slabs on January 15 of the following year. Plants were arranged in two central rows and two lateral single rows in each glasshouse. Plants within rows were 40 cm apart, central rows being separated by 50 cm, and central and lateral rows by 100 cm. During the experiment, plants were regularly maintained by removing lateral growing stems, by winding and lowering the growing primary stem around a hanging plastic cord, and by removing the oldest shrivelled leaves from the bottom of each plant. Each house was subdivided longitudinally in two and latitudinally in four sections, to give eight 3×1.6-m compartments. Compartments were separated from each other using a Visqueen microfibre cloth (Oxfordshire, UK) that allowed for air flow, while restricting movement of introduced organisms and delineated BotaniGard treatments. This cloth was suspended from a ceiling infrastructure in order to separate compartments as well as to cover their ceilings. The experiment was planned as a randomized complete block design. Each glasshouse represented a block with eight compartments of 10 plants each, giving a total 80 plants per house. Within each glasshouse were four replicate compartments for each of two randomly assigned treatments: (1) parasitoids+predators+water spray and (2) parasitoids+predators+BotaniGard spray. Over the course of this study, only natural lighting was used. Temperature was

set at 22°C during the day and 18°C at night, and relative humidity (RH) at 70%. As of August 16th, or 8 months after seedlings were planted, the hourly temperature and RH were recorded using a two-channel temperature and relative humidity data logger (HOBO Onset Computer Corp. Bourne, MA). Over the course of the experiment, the temperature ranged from 16.8 to 37.4°C in house 1 and from 15.3 to 36.8°C in house 2. The relative humidity for this period ranged from 24.3 to 78.2% in house 1 and from 25.75 to 84.8% in house 2. The mean temperature and relative humidity were of 23.0°C and 57.92%, respectively, for house 1 and of 22.4°C and 59.3% for house 2. No difference in temperature ($F_{1,864} = 1.01$, $P = 0.42$) nor relative humidity ($F_{1,864} = 1.1$, $P = 0.06$) was observed between houses. An average relative humidity of $67.4 \pm 3.2\%$ was achieved in the 12 h following the pathogen treatment by closing greenhouse vents in order to promote germination of conidia.

Three releases to all compartments (Table 1) allowed for a rapid and large build-up of whitefly (*Trialeurodes vaporariorum*) populations. Whiteflies, obtained from Applied Bionomics Ltd, B.C., Canada, were introduced either by placing fourth instar pupae onto the lowest leaf of tomato plants as in the first introduction (August 16) or by introducing adults in the case of the latter two (August 29, September 1). By the third introduction, whiteflies had colonized the entire height of the tomato plants. On September 24 and 25th, when whitefly populations were well established on tomato plants of all compartments, initial releases of *E. formosa* and *D. hesperus* were made. These releases were then followed by one larger parasitoid and two smaller predator releases in October (Table 1). *Encarsia formosa* was first released as adults received from Biobest® Biological systems (Westerlo, Belgium) and subse-

Table 1. Release schedule for greenhouse whitefly and natural enemies over the course of the study.

Organism	Release date	Corresponding week number	Number of organisms released/compartment
Trialeurodes Vaporariorum	August 16	0	300
	August 29	0	300
(pupae and adults)	September 1	0	100
Encarsia formosa	September 24 (adults)	3	200
	October 8 (pupae)	5	400
Dicyphus hesperus (adults)	September 24	3	100
	October 9	5	50
	October 15	6	50
Beavueria bassiana (BotaniGard®)	September 25	3	7.31×10^7 conidia/mL
	October 1	4	8.60×10^7 conidia/mL
	October 22	7	8.85×10^7 conidia/mL

Indicated are the numbers of organisms released per compartment on each date and the concentration of conidia within *Beauveria bassiana* suspensions.

quently by hanging parasitized whitefly cards from Applied Bionomics (Victoria, BC, Canada). On each of three haphazardly selected tomato plants per compartment, *E. formosa* adults and pupae were released in equal numbers onto the lowest leaf situated 50–80 cm above ground level. *Dicyphus hesperus* predators were subsequently released in equal numbers onto the mid canopy of three haphazardly selected tomato plants per compartment.

Dicyphus hesperus predators were obtained from a laboratory colony at PARC, which was initially established in 1999 with individuals collected from white stem hedge nettle, *Stachys albens* A. Gray (Lamiaceae) in the foothills of the Sierra Nevada Mountains at an elevation of ca. 300 m near Woody, CA USA (Lat 35°42.9′N, long 116°49.1′W). Colonies were maintained in screened wooden cages containing tobacco plants at $24.8 \pm 1.5°C$ ($\pm$SD), under a 16-h photoperiod, and were fed eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) from Biobest®. In our experiment, adult predators, approximately 4-days-old from emergence, were introduced onto tomato plants at a 50/50 ratio of males/females.

The BotaniGard® or water (control) treatments were first applied following the first release of parasitoids and predators (Table 1). Second and third applications of these were made 5 and 26 days after the initial application, reasoned by high whitefly densities. During applications, all surfaces of tomato plants were sprayed to runoff.

To determine fungal deposition rate (conidia per mm^2), 5% water agar blocks were pinned on the underside of three haphazardly selected leaves from the third bottom canopy levels of two plants (see sampling for description of canopies) and in each of the eight *B. bassiana* compartments before application of the entomopathogen. These agar blocks were collected after application and the average number of conidia per mm^2 was calculated from microscopic observation with a $40 \times$ objective. Pathogen applications were made using a hand held pressurized sprayer (11.4 L Model # 65010, Hudson & Industrial, Chicago, IL, USA). A small quantity of the fungal suspension was taken from the sprayer early, mid and late during the course of each pathogen application in order to determine the actual conidial concentration through enumeration using a haemocytometer (Table 1). The viability of conidia was also determined by spraying the spore suspension onto three 0.005% Benlate® (benomyl, wettable powder 50%, E.I. DuPont de Nemours and Co., Wilmington, DE) amended potato dextrose agar (PDA) plates. Benlate® facilitated counting germinated conidia as it halts cell division during mitosis that otherwise leads to contaminant fungal overgrowth to occur (Goettel and Inglis 1997). Plates were sealed with Parafilm and incubated at 25°C for 2 days, after which the proportion of germinated conidia from a total of 500 conidia from each plate was determined. Viability of conidia was high, i.e., 97.5, 96.3 and 93.2% for *B. bassiana* treatments 1, 2 and 3, respectively.

Sampling

Non-destructive sampling of insects on tomato plants was performed using a hand held magnifier of about $3\times$. Sampling was conducted twice a week starting on September 6th, 22 days before the first *B. bassiana* treatment, in order to determine pre-treatment conditions. Two plants from each compartment were haphazardly selected for sampling, plants in culture only having a single primary stem. Leaves of a similar size from each of four canopy levels ($=4$ leaves $\times 2$ plants/compartment) were

sampled for insects by gently turning them over to reveal organisms on the underside. Shrivelled bottom canopy leaves were excluded from counts. Care was taken to avoid disturbing adult whiteflies and natural enemies by limiting unnecessary movement of plants. Vertically stratified sampling allowed measurement of the abundance of all organisms at all life stages. Although population estimates are based on numbers of fungus-, predator-, and parasitoid-killed whiteflies that accumulated over periods of time, especially for bottom leaves, this sampling method provides reliable estimates of mycosis, predation and parasitism on selected sample date post-treatment, especially when comparing treatment effects. Plant canopies were stratified by enumerating leaves starting from the top, where canopy 1 = leaves 1 to 5; canopy 2 = leaves 6 to 9; canopy 3 = leaves 10 to 12; canopy 4 = leaves 13 to 15. Entire leaves, composed of several leaflets, were sampled by counting all organisms, even at high densities. The following life stages and species of insects were identified: whitefly eggs, whitefly nymphal stages N1 and N2 (N1–2) and N3 and N4 (N3–4), adult whiteflies, adult parasitoids and the black pupae or mummies of parasitized whiteflies, adult *D. hesperus*, whitefly pupae that had been preyed upon by *D. hesperus*, infected N1–2 whiteflies, infected N3–4 whiteflies, and infected and parasitized whiteflies. Whitefly consumed by *D. hesperus* appeared as an empty but complete exoskeleton which adhered to the leaf. These pupal capsules were distinguishable from those left behind by emerged adult whitefly by the fact that the top surface of latter was often dislocated or torn apart.

The proportion of infected whitefly was determined under laboratory conditions from destructive samples collected in the greenhouse. Two haphazardly selected third canopy leaflets from each compartment with at least 30 immature whiteflies upon them were collected from each compartment, placed in a 15-cm diameter Petri dish on a moistened filter paper, and incubated at 25°C. Leaflets were kept moist by adding water to the filter paper as needed. Following a 5-day incubation period, whitefly that showed signs of infection such as sporulation or redness due to *B. bassiana*'s production of oosporein, were enumerated and the proportion of infected whitefly over the total number of whitefly per leaflet was thus estimated.

Statistical analysis

The effects of treatment and time (sample date) for data collected following initial pathogen treatment were evaluated using a repeated measures ANOVA (linear mixed model using the PROC MIXED option of SAS 1999) of: whitefly eggs, N1–2 nymphs, N3–4 nymphs, whitefly adults, adult parasitoids, adult predators, parasitized whiteflies, predator-consumed whiteflies and infected whiteflies. The sample unit was defined as a greenhouse compartment and the spatial dependence between the compartments within each glasshouse was accounted for by including the house effect as a random block factor in the main plot part of the model. Moreover, the most appropriate temporal dependency structure was selected by choosing the structure with the smallest Akaike Information Criterion (AIC) (Akaike 1974) among all tested (Littell, Milliken, Stroup, and Wolfinger 1996). Following a significant treatment by time interaction, multiple comparisons based on a Least Significant Difference (LSD) were performed to identify differences between treatments within a time interval. To achieve normality assumptions, the dependent

variables were square root transformed. The homogeneity of variance was verified by graphical visualization of residuals against predicted values.

Results

In both glasshouses, we were successful in establishing and maintaining populations of *T. vaporariorum*, *E. formosa* and *D. hesperus*. Dead parasitoids and predators were occasionally found on leaves following the application of either water (control) or *B. bassiana*. A block or house effect was observed for the following dependent variables: N3–4 whitefly, adult parasitoid, parasitized whitefly and *B. bassiana* infected + parasitized whitefly (Tables 2 and 3). This effect may be attributed to the possible microclimatic differences within the glasshouses or to initial differences in the establishment of whitefly populations. An average spore deposition of $5.13 \pm 0.36 \times 10^3$ conidia/mm^2 (mean $\pm$ SEM) was observed in the eight *B. bassiana* treated compartments following its application.

Impact of **B. bassiana** *on whitefly population dynamics*

A treatment by time interaction was observed for N3–4 and adult whitefly classes (Table 2). This interaction probably originated from high mortality of early whitefly instars (Figure 1), which depleted subsequent cohorts. Compartments treated with *B. bassiana* had $31.7 \pm 19.7\%$ and $22.3 \pm 0.34\%$ ($\pm$SEM) fewer pupal and adult whiteflies, respectively, than control compartments (Figure 1). Infected whiteflies were more abundant in treated than control compartments (Figure 2c). Furthermore, a significant treatment by time effect was observed, whereby infected whiteflies increased as time increased following initial BotaniGard® treatment (Table 3).

Impact of BotaniGard® *on parasitism*

A first analysis of data generated for *E. formosa* abundance revealed exceptionally high adult parasitoid densities in two of the BotaniGard® treated compartments on sample date 6.1 (0.58, LSD, $F_{1,141}=37.44$, $P<0.01$) (Figure 2a). These outlying values did not reflect the general trend over time following pathogen treatment and thus were possibly caused by sampling of exceptional aggregations of *E. formosa* parasitoids on low leaves of certain tomato plants. After removing these two outlying values from the analysis, *E. formosa* parasitoid density was comparable in both BotaniGard® treated and control compartments throughout the experiment (Table 3, Figure 2a). The mean number of parasitized whitefly per leaf was significantly greater in treated than in control compartments (Table 3, Figure 2b). Similarly, the proportion of immature whitefly that were both parasitized and infected was greater in treated compartments compared to controls (Table 3, Figure 2d).

Impact of BotaniGard® *on predation*

The density of *D. hesperus* did not differ between *B. bassiana* treated and control compartments (Table 4, Figure 3a), but the proportion of immature whitefly consumed by *D. hesperus* in treated compartments was significantly lower than in control compartments (Table 4, Figure 3b). Furthermore, a significant effect of

Table 2. Repeated measures analysis of variance for effects of BotaniGard® treatment on the abundance of different developmental stages of the greenhouse whitefly, *Trialeurodes vaporariorum* per leaf.

Dependent variable	Sources of variation	df	*F*-value	*P*
Egg				
	House	1	1.65	0.2215
	Treatment	1	13.04	0.0032
	Main plot error	13		
	Time	10	15.42	<0.0001
	Treatment*time	10	1.37	0.2001
	Sub-plot error	140		
	Total	175		
N1–2				
	House	1	1.58	0.2315
	Treatment	1	20.76	0.0005
	Main plot error	13		
	Time	10	12.20	<0.0001
	Treatment*time	10	0.82	0.6109
	Sub-plot error	140		
	Total	175		
N3–4				
	House	1	0.80	0.0001
	Treatment	1	32.33	<0.0001
	Main plot error	13		
	Time	10	2.21	0.0204
	Treatment*time	10	4.05	<0.0001
	Sub-plot error	140		
	Total	175		
Adult whitefly				
	House	1	2.33	0.1509
	Treatment	1	5.25	0.0393
	Main plot error	13		
	Time	10	10.43	<0.0001
	Treatment*time	10	7.06	<0.0001
	Sub-plot error	140		
	Total	175		

treatment over time was observed in the proportion of whiteflies consumed by *D. hesperus* (Table 4, Figure 3b). Following the first BotaniGard® treatment, there was a significant decrease in the whiteflies consumed beginning sample date 5.1 ($F_{1,141} = 14.57$, $P = 0.0002$) (Figure 3b).

Partitioning whitefly mortality

The mortality of all immature whiteflies sampled during this experiment was categorized for each mortality factor. In the BotaniGard® treated compartments, *B. bassiana* caused an average mortality of 10.6% of all immature whiteflies (Figure 4).

Table 3. Results of a repeated measures analysis of variance for effects of BotaniGard® treatment on the proportion infected whitefly, *Encarsia formosa* density, proportion parasitized, or parasitized and *Beauveria bassiana* infected whitefly per leaf.

Dependent variable	Sources of variation	df	*F*-value	*P*
Infected whitefly				
	House	1	4.12	0.0633
	Treatment	1		<0.0001
	Main plot error	13		
	Time	10	2.12	0.0266
	Treatment*time	10	2.25	0.0179
	Sub-plot error	140		
	Total	175		
E. formosa (n/per leaf)				
	House	1	4.68	0.0497
	Treatment	1	0.75	0.4020
	Main plot error	13		
	Time	10	6.56	<0.0001
	Treatment*time	10	4.11	0.0979
	Sub-plot error	140		
	Total	175		
Proportion parasitized whitefly				
	House	1	18.15	0.0009
	Treatment	1	11.17	0.0053
	Main plot error	13		
	Time	10	6.09	<0.0001
	Treatment*time	10	2.95	0.0022
	Sub-plot error	140		
	Total	175		
Proportion infected and parasitised whitefly				
	House	1	7.34	0.0179
	Treatment	1	51.81	<0.0001
	Main plot error	13		
	Time	10	4.22	<0.0001
	Treatment*time	10	4.64	<0.0001
	Sub-plot error	140		
	Total	175		

While parasitism was a greater source of whitefly mortality in treated compartments than in control compartments, predation was observed less frequently in BotaniGard® compartments (Figure 4). A very low proportion of whiteflies (0.98%) found in treated compartments, were both infected and parasitized.

Discussion

Our results indicate that *B. bassiana* may be compatible with existing biological control programs for greenhouse whiteflies. When applied along with the combined
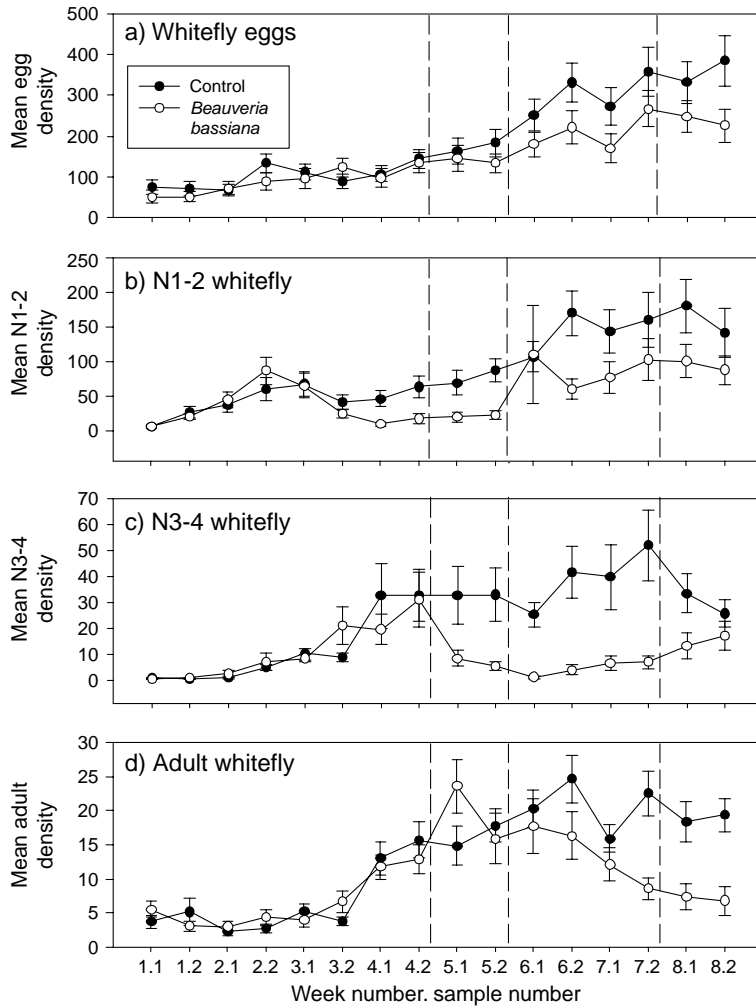
Figure 1.   Effect of BotaniGard® treatment on the density per leaf of (a) eggs; (b) N1–2; (c) N3–4, and (d) adults of the whitefly *Trialeurodes vaporariorum*. Y-axis values are the mean density $\pm$ SEM of samples ($n = 8$) taken at each of four plant canopy levels. Vertical lines indicate BotaniGard® treatments. The x-axis represents the sample date, whereby the first number stands for weeks and the second for the bi-weekly repetition of sampling.

release of *E. formosa* and *D. hesperus*, BotaniGard® contributes to a more effective reduction of *T. vaporariorum* populations, without significantly reducing natural enemy populations. *Beauveria bassiana* did not appear to affect numbers of *E. formosa* and parasitism rates were higher in treated than in control compartments. It is apparent however, that *B. bassiana* interfered with *D. hesperus*. Though the abundance of *D. hesperus* was similar for control and BotaniGard® treated compartments, there was a significant decrease in predation on immature whiteflies in treated compartments. This reduction in predation may have resulted from partial competitive exclusion of the predator by the fungus, as a result of the capacity of
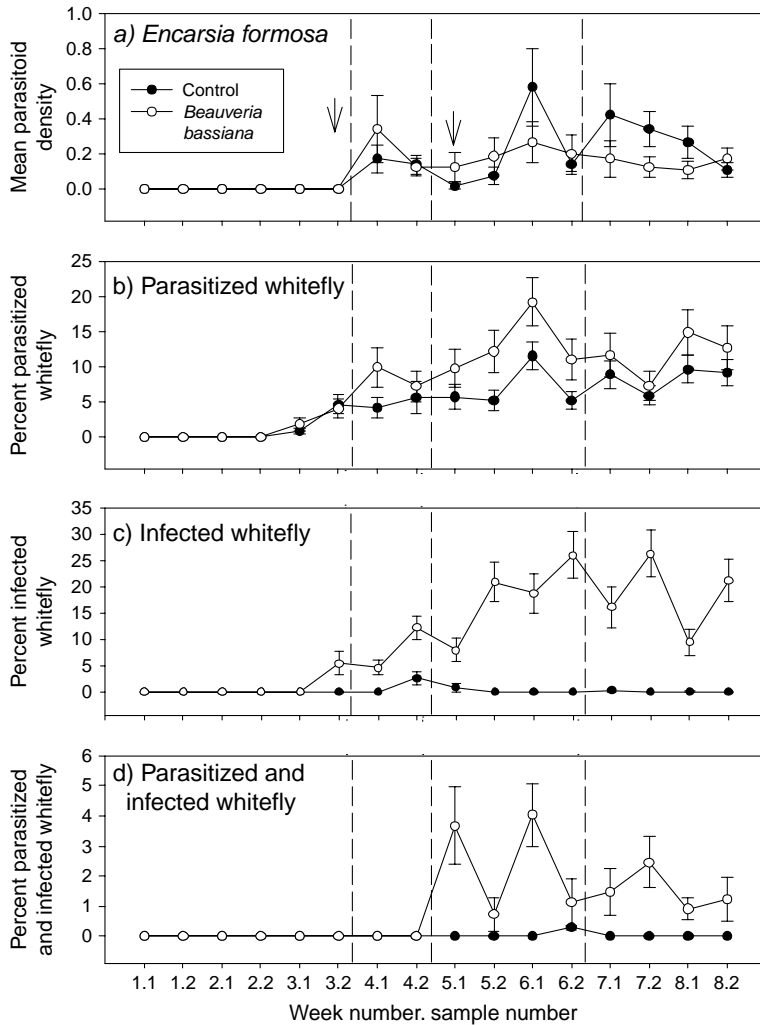
Figure 2. Impact of BotaniGard® treatment on (a) the density of adult *Encarsia formosa*; (b) the percent of whiteflies parasitized by *E. formosa*; (c) the percent of whiteflies infected by *Beauveria bassiana*; and (d) the percent of whiteflies per leaf that were both parasitized and infected. Y-axis values are the mean density $\pm$ SEM ($n = 16$) number of organisms per leaf sampled at four canopy levels, in four replicate compartments per house, and in two houses. Vertical lines indicate dates of BotaniGard® treatments. Arrows indicate dates of *E. formosa* releases.

D. hesperus to discriminate between healthy and fungus-infected prey (Labbé, Cloutier, and Brodeur 2006).

Whitefly–pathogen interaction

Over the course of the experiment and for all immature developmental stages, *B. bassiana* produced a relatively low mean whitefly mortality ($10.6 \pm 0.9\%$), which

Table 4. Probability values for a repeated measures analysis of variance for effect of BotaniGard® on the abundance of predator *Dicyphus hesperus* and on whitefly predation.

Dependent variable	Sources of variation	df	*F*-value	*P*
Dicyphus hesperus				
	House	1	–	–
	Treatment	1	3.89	0.0703
	Main plot error	13		
	Time	10	7.41	<0.0001
	Treatment*time	10	1.45	0.1635
	Sub-plot error	140		
	Total	175		
Predator consumed whitefly				
	House	1	–	–
	Treatment	1	233.33	<0.0001
	Main plot error	13		
	Time	10	14.41	<0.0001
	Treatment*time	10	2.85	0.0030
	Sub-plot error	140		
	Total	175		

may be accounted by low relative humidity directly following fungal treatment. An average RH of $67.4 \pm 3.2\%$ RH was achieved in both greenhouses 12 h following application of BotaniGard®. This relative humidity level was low compared to what is typically recommended for periods of BotaniGard® greenhouse applications (Shipp, Zhang, Hunt, and Ferguson 2003). Nevertheless, this treatment resulted in a marked decrease in density of the pest population relative to control compartments. The difference between BotaniGard® treated and control compartments was most evident after the second pathogen application. The effects of *B. bassiana* shortly after applications varied for whiteflies of different developmental stages and were not consistent between applications (Figure 1). For example, populations of N3–4 whiteflies sharply decreased following the first microbial application, remained fairly constant after the second application, but increased following the third application. Divergent patterns may result from differences in the susceptibility of each developmental stage of *T. vaporariorum* to *B. bassiana*, early instars being the most susceptible to infection (Siongers and Coosemans 2003). These patterns may also be influenced by the recruitment of individuals from the preceding developmental cohort in a population constituted of overlapping stages.

Parasitoid–pathogen interaction

Parasitoid abundance in BotaniGard® treated compartments was similar to that in control compartments excepting two outlier samples. Of interest, the number of parasitized whiteflies was consistently higher in treated compartments than in control compartments, leading to an increase of 2.5 percentage points in parasitism; from 5% in control compartment to 7.5% in treated ones. Although significant, such an increase in parasitism is not likely to contribute greatly to biological control of whitefly populations.
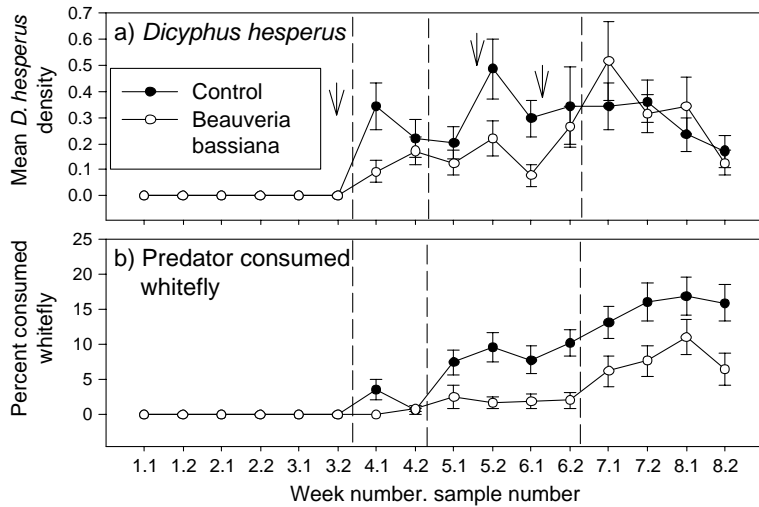
Figure 3. Impact of BotaniGard® treatment on (a) the density per leaf of predator *Dicyphus hesperus* (±SEM) and (b) on the density of whiteflies consumed by predators (±SEM). Values represent the mean (*n* =8) number of organisms on each of four canopy levels, in four replicate compartments per house and in two houses. Vertical lines indicate dates of BotaniGard® treatments. Arrows indicate dates of *D. hesperus* releases.

While this study did not examine either the lethal or sublethal effects of *E. formosa* infection, most documented pathogen–parasitoid interactions report that the cost of infection is typically dependent on dose or on the timing of infection (Brooks 1993; Brodeur and Rosenheim 2000). Another factor that may have contributed to the overall survival of *E. formosa* or *D. hesperus* was the relatively low humidity achieved during pathogen application. In their laboratory study, Shipp et al. (2003), found that application of BotaniGard ES on adult and immature *E. formosa* at 75% RH resulted in survival of 30.7 and 0.3%, respectively, whereas infections at 97% RH were 32.5 and 24.6%, respectively. In this study, a low RH may have reduced mortality of parasitoids and predators due to infection that may otherwise have been an important factor. Additional research is however required to reveal whether *B. bassiana* has the capacity to infect developing *E. formosa* under crop conditions, and to determine what optimal fungal concentration would maximize whitefly mortality and minimize parasitoid infection. In contrast, the timing of pathogen application may be less relevant to minimizing the detrimental effects on other biological control agents, particularly when microbials are used as short-term corrective measures. Although entomopathogens may interfere with arthropod biological control agents immediately after application, their long-term consequences on biological control are less clear. The capacity of the entomopathogen to survive in the absence of suitable or new hosts may consequently reduce their effectiveness over time. Furthermore, most greenhouse crops using biological control employ periodic, inoculative releases of specialized parasitoids and predators (Brodeur, Cloutier, and Gillespie 2002) which may renew their effectiveness.

Host discrimination by *E. formosa* females is likely to have been an important factor that led to a high proportion of parasitized whitefly observed in BotaniGard®
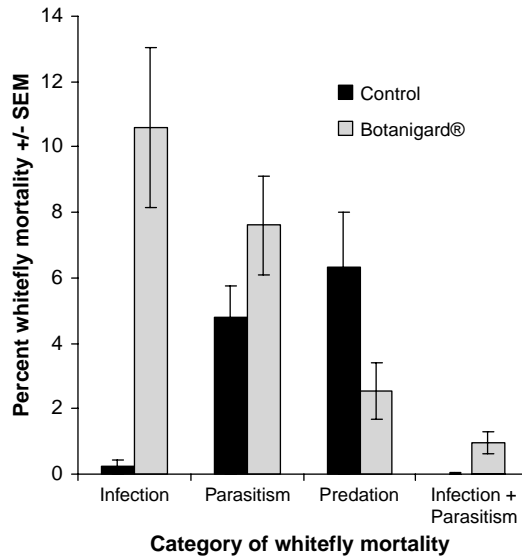
Figure 4.   Effect of BotaniGard® treatment over the course of the experiment on the mean percentage ($\pm$SEM; $n = 8$) of immature whitefly infected by *Beauveria bassiana*, parasitized by *Encarsia formosa*, fed on by *Dicyphus hesperus*, or both parasitized and infected.

treated compartments. Laboratory and field studies suggest that *E. formosa* has the capacity to discriminate between healthy and fungus-infected whiteflies and commonly avoid laying eggs in whitefly hosts infected by *A. aleyrodis* (Fransen and van Lenteren 1993) or *Lecanicillium muscarium* (formerly *Verticillium lecanii*) Zimm. (Lacey, Fransen, and Carruthers 1996; Jazzar and Hammad 2004). Infected host discrimination by parasitoids would reduce interference between *E. formosa* and *B. bassiana*, and lead to increased parasitism with BotaniGard® applications as we found here (50% increase).

Although not directly investigated in this study, an important consideration to make is the impact of parasitoid and predator interactions on the overall success of these organisms in biological control. Bennett, Gillespie, Shipp, and VanLaerhoven (2009) investigated the interactions among *E. formosa* and *D. hesperus* and showed that it was asymmetric. While *D. hesperus* was unaffected by *E. formosa* densities, *E. formosa* populations were reduced by the inclusion of *D. hesperus*. This type of interaction may help to explain why parasitism rates by *E. formosa* were low in our study despite the high release rates of parasitoids.

Predator–pathogen interaction

The abundance of *D. hesperus* was similar in control and *B. bassiana*-treated compartments, suggesting that the fungus did not readily infect the predator. Using a comparable experimental approach, Alma, Goettel, Roitberg, and Gillespie (2007) reached the same conclusion for the interaction between *D. hesperus* and the fungus *Paecilomyces fumosoroseus* strain Apopka-97 (Wize) Brown and Smith (Ascomycota: Hypocreales). However, we observed a significant decrease in the predation of immature whiteflies in *B. bassiana*-treated compartments relative to controls. Labbé

et al. (2006) examined prey selection of *B. bassiana*-infected whiteflies by immature and adult *D. hesperus* and found that the probability of feeding on infected prey depends on the timing of infection. Whiteflies were rejected late during infection when either hyphae or oosporein, a red pigmented compound produced by the fungus, were present in the whitefly. This delayed prey discrimination may have increased competition between *B. bassiana* and *D. hesperus* early following fungal application. We further observed that when foraging on tomato plants, *D. hesperus* was more abundant in regions where the proportion of infected whiteflies was high (data not shown). Such a distribution pattern would likely also contribute to increased competition between *D. hesperus* and *B. bassiana*. It is possible that when foraging in patches with unsuitable prey, *D. hesperus* could resort to phytophagy, which would reduce predation rates.

Biological control agents that differ in degrees of specificity towards hosts or prey, modes of action, functional and numerical responses, climatic ranges, and other biological attributes may complement each other and provide more consistent pest suppression in greenhouse crops. The development of effective pest control strategies in which microbial insecticides based on generalist entomopathogenic fungi such as *B. bassiana*, are integrated with arthropod natural enemies requires a comprehensive knowledge of the nature and outcome of trophic and guild interactions. Based on our knowledge of the discriminatory capacity of *E. formosa* (high discrimination) and *D. hesperus* (moderate discrimination) towards fungus-infected hosts (Labbé et al. 2006), we expect to observe an increase in whitefly mortality when the parasitoid or the predator are used in combination with the fungus.

Intraguild interactions are common within communities of biological control agents and will almost certainly influence the efficacy of biological control (Polis and Holt 1992; Rosenheim et al. 1995). Due to the diversity of natural enemies involved in guild interactions, from entomopathogens to zoophytophagous predators, and the numerous ecological factors that govern the relationships between these various species, a wide range of potential outcomes can be expected in systems with multiple biological control agents, from interference to synergism. Understanding and exploiting interactions among biological control agents is complicated by discrepancies between ecological theory, which emphasizes equilibria within arthropod communities and predicts increased pest densities, and empirical evidence, which often reveals that intraguild predation does not result in increased pest populations (see Janssen, Montserrat, HilleRisLambers, de Roos, and Sabelis 2006; Rosenheim and Harmon 2006). Manipulative experiments conducted at the appropriate scale and complexity level remain essential to test theoretical considerations.

In conclusion, although the application of BotaniGard® on tomato crops caused relatively low levels of whitefly infection, it resulted in a significant reduction of whitefly populations over time. *Beauveria bassiana* was compatible to a great extent with *E. formosa* and *D. hesperus*, the two most commonly used biological control agents of the greenhouse whitefly in Canada. Mortality caused by these natural enemies was similar in the presence or the absence of fungal treatments, although partitioned differently. In BotaniGard® treated compartments, increased parasitism by *E. formosa* balanced a reduction of predation by *D. hesperus*. Besides the intrinsic susceptibility of an arthropod natural enemy to fungal infection, its capacity to discriminate between healthy and infected hosts or prey appears to be a determinant

factor of compatibility. Overall, the significant reduction of whitefly density and lack of clear interference suggest that *B. bassiana* can be used jointly and inundatively along with a 'traditional' cocktail of whitefly biological control agents to effectively contribute to a practical integrated pest management strategy on tomato and possibly on other related crops. This is particularly the case when whitefly abundance is too high for predators or parasitoids to control alone.

Acknowledgements

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