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Compatibility of soil-dwelling predators and microbial agents and their efficacy in controlling soil-dwelling stages of western flower thrips *Frankliniella occidentalis*

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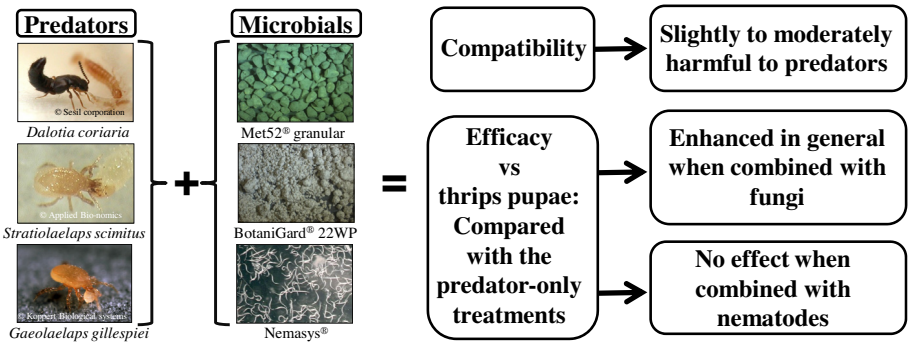
HIGHLIGHTS

- All of the biological control agents used in the study are commercially produced.
- Predator mortality from the microbial agents ranged from 2.93% to 60.95%.
- Efficacy against thrips was enhanced when predators and fungi were co-applied.
- Some combination treatments achieved >90% thrips pupal mortality.

GRAPHICAL ABSTRACT



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ABSTRACT

Western flower thrips (WFT) generally pupate in the soil. This laboratory study was designed to examine the compatibility of soil-dwelling predators with microbial biocontrol agents and assess their combined efficacy against pupating WFT, with a view to their integrated use. The following commercially available biocontrol agents were evaluated: a rove beetle, *Dalotia coriaria* (Kraatz); predatory mites, *Stratiolaelaps scimitus* (Womersley) and *Gaeolaelaps gillespiei* Beaulieu; entomopathogenic fungi, *Metarhizium anisopliae* (Metschnikoff) Sorokin (now classified as *Metarhizium brunneum*) strain F52 and *Beauveria bassiana* (Balsamo) GHA strain; and the nematode, *Steinernema feltiae* (Filipjev). Compatibility studies demonstrated mortality caused by the microbial agents ranging from 2.93% to 60.95% against the predators tested. In container studies, efficacy against WFT was significantly improved when the predators and fungi were combined, achieving >90% thrips mortality, compared to the treatments in which they were used separately. This was not observed with nematodes.

1. Introduction

The Canadian floriculture industry employs >43,000 full- and part-time workers, with farm-gate sales of >$1.4 billion (Statistics Canada, 2012). It is an industry whose product is valued solely on its esthetic quality, with minimal tolerance for pest damage. Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is a major impediment to the production of many economically-important greenhouse crops (feeding damage, transmission of plant viruses) (Kirk and Terry, 2003). Due to its high reproductive rate and cryptic habits, repeated applications of active compounds have traditionally been

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made to achieve control. WFT is now resistant to many classes of insecticide (Broadbent and Pree, 1997; Jensen, 2000; Thalavaisundaram et al., 2008) and few effective products are available to Canadian growers. As a result, biological control is increasingly practiced in Canadian floriculture, creating a paradigm shift in management philosophy and approach (Brownbridge et al., 2013; Murphy et al., 2011).

WFT eggs are laid in plant tissues and larvae (two instars) and adults feed on the foliage and flowers. Most larvae leave plants as late 2nd instars to pupate (pre-pupal and pupal stages), with up to 98% of thrips pupating in the soil depending on the host plant and prevailing environmental conditions (Berndt et al., 2004a; Broadbent et al., 2003; Buitenhuis and Shipp, 2008; Holmes et al., 2012; Steiner et al., 2011). These soil-dwelling phases are vulnerable to soil-dwelling predators and pathogens (Ansari et al., 2008; Berndt et al., 2004a; Buitenhuis and Shipp, 2005; Ebssa et al., 2001). Several studies have demonstrated good efficacy of soil-dwelling predators, i.e. *Dalotia* (=*Atheta*) *coriaria* (Kraatz), *Stratiolaelaps miles* (Berlese) and *Hypoaspis aculeifer* (Canestrini) (Berndt et al., 2004a,b; Carney et al., 2002; Echegaray and Cloyd, 2013); soil treatments of entomopathogenic fungi, i.e. *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) (Ansari et al., 2007, 2008; Brownbridge, 1995, 2006; Skinner et al., 2012); and entomopathogenic nematodes such as *Steinernema* spp. and *Heterorhabditis* spp. (Buitenhuis and Shipp, 2005; Ebssa et al., 2001, 2004, 2006; Premachandra et al., 2003a,b). However, when pest pressures are high, use of a single biocontrol agent rarely delivers the necessary level of control, requiring supplemental use of chemical sprays (which can disrupt a biocontrol program) or use of a suite of natural enemies to prevent damaging populations developing (Arthurs and Heinz, 2006; Brownbridge et al., 2013). Jacobson et al. (2001) showed that the concurrent use of foliar predators and fungal sprays can deliver benefits in terms of improved thrips control, and Ebssa et al. (2006) demonstrated similar improvements when foliar predators and *Steinernema feltiae* (Filipjev) were used together. While Premachandra et al. (2003a) found that combined treatments of nematodes and *H. aculeifer* significantly improved WFT control compared to individual applications of the same biocontrol agents, few studies have assessed the combined efficacy of soil-dwelling predators with entomopathogenic fungi or nematodes.

The current study was therefore done to (1) assess the compatibility of soil-dwelling predators with entomopathogenic fungi and nematodes, and (2) document the relative efficacy of soil-dwelling predators, entomopathogenic fungi and nematodes, including use of combined treatments of predators with entomopathogenic fungi or nematodes, against soil-dwelling stages of WFT. All of the biological control agents used in the study are commercially produced and readily available.

2. Materials and methods

2.1. Rearing of western flower thrips

A colony of WFT was maintained in a thrips-proof screened cage containing 8 potted (flowering) chrysanthemums (var. Brighton or Chesapeake). Plants were replaced every 6–8 weeks, placing infested foliage and flowers from the older plants alongside the new ones for 48–72 h (it was then removed) to allow thrips larvae and adults to migrate to the fresh plants. Cages were held in a glass house (22 ± 2 °C). Late 2nd instar thrips were collected from these plants using an aspirator prior to each experiment.

2.2. Predators

Dalotia coriaria were provided by Applied Bio-nomics Ltd. (BC, Canada) and Koppert Biological Systems BV (Netherlands) in packs of 500 insects in a one liter tube. The containers were kept in a 15 °C incubator until trials were set up. Adult beetles were used within three days of receipt. The remaining adults were used to establish a breeding colony to provide a supply of *D. coriaria* larvae. The breeding colony was maintained in a modified plastic bucket (Staphyline c Breeder Bucket System; Syngenta Bioline Ltd., UK). The bucket was half-filled with moistened peat moss, and the beetles were fed ground dry cat food (Purina® Friskies Party Mix) as needed. The colony was held in a diurnal incubator (16L: 8D h, 24 ± 1: 20 ± 1 °C).

The predatory mites *Stratiolaelaps scimitus* (Womersley) and *Gaeolaelaps gillespiei* Beaulieu were provided by Applied Bio-nomics Ltd., in one liter bottles containing a mixture of ca. 25,000 adults and nymphs. The bottle was stored at 15 °C and used within seven days of receipt. Only adults were used in this study.

2.3. Microbial control agents

2.3.1. Mycoinsecticides

The biopesticide Met52® granular (Novozymes Biologicals Inc., VA, USA) contains 9.0×10^8 colony forming units (CFU)/g of *M. anisopliae* Strain F52. Note that the fungus has been re-classified as *Metarhizium brunneum* (Petch) but the commercial product is still registered as *M. anisopliae*. The biopesticide is sold in one kg sealed bags (2% active on a rice carrier with 9×10^8 viable conidia/g guarantee). The viability of the conidia was determined prior to all fungal assays by preparing a stock suspension from 1.0 g of Met52 in 10.0 ml 0.01% aqueous Triton™ X-100 (EMD Chemicals Inc., NJ, USA) and plating 100 µl of 10^{-2} and 10^{-3} dilutions onto quarter-strength Sabouraud Dextrose agar (¼ SDA). Two replicate plates were inoculated per dilution and incubated at 25 ± 1 °C for 40 h. Three 22 × 22 mm glass cover-slips were then overlain, at random, onto the media. One hundred conidia per cover slip were counted under a phase-contrast microscope at 400× magnification and conidial viability determined.Viability data collected from each of the assays had a mean value of 73.4%.

BotaniGard® 22WP (BioWorks Inc., NY, USA) contains 4.4×10^{10} CFUs/g of *B. bassiana* GHA Strain. The viability of the conidia was determined as above, but readings were taken after 24 h. Viability was consistently >95%.

2.3.2. Entomopathogenic nematode

Nemasys® is based on *S. feltiae*, and was acquired from Becker Underwood (IA, USA). The nematodes are shipped in refrigerated packs containing 50 million infective juveniles (IJs). One quarter of the package was suspended in 1000 ml deionised water (ca. 12.5 million/1000 ml), and used to prepare a stock suspension containing five million nematodes/1000 ml. Nematode viability was assessed from the freshly made stock suspension, by conducting a live/dead count in a gridded Petri dish under a stereo microscope. Viability was always >95%.

2.4. Compatibility trial

To test the compatibility of *D. coriaria* and mycoinsecticides, sterile tight-fit Petri dishes (50 mm diam., PALL Corporation, MI, USA) were lined with filter papers (Whatman™, #1, 55 mm diam.). For each treatment (n = 15 dishes), the filter paper was inoculated with 0.3 ml of the following five treatments: (1) deionised water (control); (2) Met52 low (containing 1×10^5 conidia/ml); (3)

Met52 high (containing 1×10^7 conidia/ml); (4) BotaniGard low (containing 1×10^5 conidia/ml); (5) BotaniGard high (containing 1×10^7 conidia/ml). The Met52 treatments were prepared by washing conidia off the rice granules; once a suspension was obtained, the concentration was determined using an improved Neubauer hemocytometer and adjusted to achieve the desired test concentration. The WP formulation was used to prepare the BotaniGard treatments. One adult *D. coriaria* and ten late 2nd instar WFT were then introduced into the dish through an 8.0 mm diam. hole in the dish lid. The hole was sealed with a foam ear plug and the dishes were placed in a diurnal incubator (16L: 8D h, 24 ± 1: 20 ± 1 °C) for 48 h. *D. coriaria* were then transferred individually to clean dishes lined with filter paper moistened with 0.3 ml deionised water only. The beetles were provided with ten fresh late 2nd instar WFT every 48 h thereafter for 12 more days (14 days total observation period). Every 48 h, the status of *D. coriaria* and the number of thrips eaten were recorded. The experiment was replicated three times over time. The dead/alive counts for each replicate were pooled to obtain a total dead/alive ratio (where $n = 45$ or less, in cases where individuals were excluded from the analysis, i.e. individuals that died or escaped in the first 48 h of the trial), and analyzed using a Chi square test followed by a multi-comparison of proportions ($\alpha = 0.05$). Corrected mortality was also calculated (Abbott, 1925). Compatibility of *D. coriaria* and *S. feltiae* was not evaluated since a similar study has already been done (Jandricic et al., 2006).

To test the compatibility of *S. scimitus* and *G. gillespiei* with the mycoinsecticides, the same experimental set-up was used for the initial 48 h inoculation period but five adult mites and 40 late 2nd instar WFT were placed into each dish. For each replicate trial and each mite species, three dishes were set up (total 15 predatory mites) for each of the same five treatments used for *D. coriaria* above. Once set up, dishes were transferred to a diurnal incubator (16L: 8D h, 24 ± 1: 20 ± 1 °C) where they were held for 48 h. After that, mites were transferred to individual 2.0 ml cryogenic vials containing a sterilized strip of filter paper (7 × 35 mm, moistened with 30 μl of deionised water). For each mite species, $n = 15$ vials/treatment were set up, and ten late 2nd instar WFT were provided to each mite every three (*S. scimitus*) or five (*G. gillespiei*) days, for a further 12 days (total 14 days observation period). At each feeding time point, the status of the mites and the number of surviving thrips were recorded. The experiment was replicated three times over time. The dead/alive counts for each replicate were pooled to obtain a total dead/alive ratio ($n = 45$ or less, in cases where individuals were excluded from the analysis), and analyzed in the same manner as described for *D. coriaria* above.

Compatibility of the predatory mites with *S. feltiae* was assessed using the same experimental methodology as described above. The filter paper in the Petri dishes was inoculated by mixing 0.2 ml of the stock nematode suspension (containing approximately 1000 nematodes) with 0.1 ml of deionised water (total volume of 0.3 ml), which provided the recommended drench application rate of approximately 50 million IJs/100 m^2 or 50 IJs/cm^2. For each mite species, a total of 15 ($n = 15$) cryogenic vials were set up for each treatment. Dead individuals were placed on a glass slide in a Petri dish lined with a moist filter paper, and incubated at 25 °C and examined after three days for evidence of nematode emergence. The rest of the procedure was the same as outlined above, and data were similarly processed.

2.5. Efficacy trial

White opaque plastic cups with screw-on lids (120 ml, 5.5 cm diam., 7.0 cm height, QurPak®, VWR International) were used in the assays. The centers of the lids were cut out (4.8 cm diam.). Discs (ca. 4.8 cm diam.) were cut from standard yellow sticky cards and a ventilation hole (1.0 cm diam.) was made in the center of each. After removing the protective paper from one side of the card, the discs were placed onto the rim of thrips-inoculated cups, overlain with a piece of thrips-proof screen and held in place by the modified screw caps. This arrangement prevented the build-up of condensation inside the assay containers, but emergent thrips adults were still captured on the sticky cards.

Experiment 1: To determine the relative efficacy of *D. coriaria* and two mycoinsecticide treatments alone and in combination, ten treatment regimes were tested: (1) untreated control, (2) *D. coriaria* alone (one adult/cup), (3) *D. coriaria* and Met52 low rate (0.5 g/l of growing media), (4) *D. coriaria* and Met52 high rate (1.5 g/l of growing media), (5) Met52 low rate, (6) Met52 high rate, (7) *D. coriaria* and BotaniGard low rate (1.27 g/l of deionised water), (8) *D. coriaria* and BotaniGard high rate (2.54 g/l of deionised water), (9) BotaniGard low rate, (10) BotaniGard high rate. Note that the rates of the mycoinsecticides used were determined based on the manufacturers' recommended application rates. Each treatment consisted of eight replicate cups (each containing ca. 12 g of treated medium/cup, 3.0 cm deep), and the trial was repeated three times over time using three different batches of *D. coriaria* ($n = 24$ per treatment). The untreated control and *D. coriaria* alone treatments employed 160 g dry weight of sterilized dry growing medium (=one liter dry volume, Sunshine® Mix #1, Sun Gro Horticulture Canada Ltd., AB, Canada; sterilized by autoclaving after being dried in an oven at 85 °C for four days prior to use) that was moistened with 250 ml of deionised water and mixed thoroughly prior to being dispensed to the assay cups. Met52 was applied as granules which were weighed and mixed thoroughly with 160 g of sterilized dry growing medium; the treated substrate was then moistened with 250 ml of deionised water and mixed again before being transferred to the assay cups. BotaniGard 22WP was applied to the surface of the substrate as a spray; i.e., the weighed product was suspended in one liter of deionised water and then applied using a hand-held sprayer (SureSpray™, 8000 ml, Chapin International Inc., NY, USA). Because the registered application method for BotaniGard is as a foliar spray, this treatment was intended to simulate run-off/overspray onto the surface of the growing medium. The sprays ('high' and 'low' BotaniGard concentrations) were applied at a rate equivalent to 300 ml/m^2. The sprayer was calibrated prior to application, and 12 cups (already containing moistened medium prepared as described above for the untreated control; note: four extra cups were included for processing to determine the conidial concentration as described below) were sprayed. All treatments, except *D. coriaria*, were applied prior to the introduction of WFT larvae.

To determine the concentration of conidia in the growing medium, as well as to validate their virulence against insects, 3 × 5.0 g subsamples of the untreated control and mycoinsecticide-treated media were taken from each treatment batch. For each treatment, one subsample was dried at 85 °C for four days and then re-weighed to determine its dry weight. The second subsample was placed in a sterile 500 ml Erlenmeyer flask and 45 ml sterile 0.01% Triton X-100 was added. Flasks were capped with aluminum foil and placed on a rotary shaker at 175 rpm for ten mins. After shaking, the flask was left to stand for 1 min to allow the larger particulate matter to settle, and a sample of the supernatant was then filtered through sterile cheese cloth enclosed in a 5 ml pipette tip. A dilution series was prepared from the filtered supernatant and plated onto quarter-strength Sabouraud dextrose agar (SDA) supplemented with antibiotics (peptone 2.5 g, D(+)-glucose 10.0 g, agar 15.0 g, chloramphenicol 0.25 g, streptomycin sulfate 0.35 g, cycloheximide 0.125 g, deionised water 1,000 ml), two plates per dilution. Plates were incubated at 25 °C and *Metarhizium* and *Beauveria* colonies counted after ten days. The third subsample was placed in a sterile Petri dish (150 × 15 mm) along with 15 *Galleria mellonella* L. larvae (purchased from Recorp Inc., ON, Canada)

to validate the virulence of the mycoinsecticides in the treated medium. The Petri dish was sealed with Parafilm® and incubated at 15 °C for seven days. The number of surviving *G. mellonella* larvae was recorded at this time, together with the number of dead, infected larvae. Wax moth larvae are very sensitive to fungal infection and this simple test provided confirmation of the virulence of the fungal inoculum incorporated into the growing medium.

Twenty 2nd instar WFT were added to each assay cup on a piece of chrysanthemum leaf (ca. 20 × 20 mm) that was laid onto the surface of the growing medium; the leaf provided a source of food for the thrips larvae until pupation. At this time, the insects naturally moved from the leaf to pupate in the substrate. The cups were closed with screens only and placed in two large clear plastic storage containers lined with wet paper towel to prevent the growing medium from drying out. The cups were positioned in a randomized 8 × 5 array/container, and left undisturbed in a growth chamber (16L: 8D h, 24 ± 1: 20 ± 1 °C). Leaves were left in the cups for three days and then removed. Any larvae remaining on the leaf were removed as well and the number recorded in order to calculate the actual number of pupating thrips per container for the analyses. Immediately after the leaf was removed, *D. coriaria* was added to cups at a rate of one beetle per cup. Cups were then closed with the sticky disks in place; however, the sticky disks were omitted from cups with *D. coriaria* as the beetles are very mobile and in preliminary trials many became stuck to the disk. The cups were then returned to the growth chamber. In the event that 20 thrips did not sustain the *D. coriaria* adults for the duration of the trial, a small amount of artificial diet (1.0 mg of ground Purina Floating fish pellets/cup) was added to the cups four days after the beetles were introduced. Emerged WFT adults were counted seven days after the leaves were removed, and *D. coriaria* survival noted. Percent WFT pupal mortality was calculated for each cup

$$\%\ \text{pupal mortality} = [1 - (\text{number of emerged adults} \div \text{total number of larvae pupating})] \times 100 \quad (1)$$

and the mean percent pupal mortality calculated for each treatment. Since this trial was repeated three times, results were pooled ($n = 24$ per treatment), checked for normality and analyzed using the Kruskal–Wallis test followed by Dunn's test ($\alpha = 0.05$). Corrected mortality was also calculated (Abbott, 1925).

Experiments 2 and 3: The relative efficacy of *S. scimitus* and *G. gillespiei* with/without each of the mycoinsecticides was determined under the same ten treatment regimes used in the previous experiment. Separate trials were set up for each mite species and the same methodology described above was used for each, apart from the fact that two mites were introduced into each cup and no artificial diet supplement was added. Mite survival at the conclusion of the trials was not assessed owing to their minute size and difficulty in detecting them in the growing medium. The data were processed in the same manner as described above.

Experiment 4: The comparative efficacy of *D. coriaria* (adults and larvae), *S. scimitus*, *G. gillespiei*, and *S. feltiae* (Nemasys) in individual and predator-nematode combination treatments was determined using the same apparatus set-up and similar methodology to that described above. There were ten treatment regimes: (1) untreated control, (2) *S. feltiae* only (rate equivalent to 25 million IJs/100 m^2), (3) *D. coriaria* adult alone (one adult/cup), (4) *D. coriaria* larva alone (one late instar/cup, approximately 4.0 mm in size), (5) *S. scimitus* alone (two adults/cup), (6) *G. gillespiei* alone (two adults/cup), (7) *D. coriaria* adult and *S. feltiae*, (8) *D. coriaria* larva and *S. feltiae*, (9) *S. scimitus* and *S. feltiae*, (10) *G. gillespiei* and *S. feltiae*. Growing media were prepared in the same manner as previous trials. Note that the nematode application rate was different from the one in the compatibility trial where the recommended drench application rate was utilized. Here, we wanted to simulate the nematode sprench application method ("sprench" = spray drench, heavy foliage spray intended to run-off); therefore, the recommended foliage application rate for heavy WFT infestation levels was utilized. Virulence testing of the treated substrate using *Galleria* larvae was not done in this experiment. Twenty 2nd instar WFT were introduced into each cup and allowed to pupate in the growing medium as described above. *S. feltiae* was then applied by pipette (using a stock suspension of five million nematodes/1000 ml, 0.1 ml of the stock suspension in 0.9 ml of deionised water, resulting in ca. 25 IJs/cm^2). The treatments that did not require the nematode application also received 1.0 ml of deionised water to standardize the moisture level in the growing medium. The predators were then introduced into the respective treatment containers. The survival of the predators at the conclusion of the trials was not assessed. The resulting thrips pupal mortality data were pooled ($n = 24$ per treatment), checked for normality, and analyzed using a one-way ANOVA followed by the least significant difference test ($\alpha = 0.05$). Corrected mortality was also calculated (Abbott, 1925).

3. Results

3.1. Compatibility

Higher mortality levels were observed in *D. coriaria* that were exposed to mycoinsecticides than the control group (Table 1). There was a significant difference in mortality rates when all five treatments were compared; however, the post hoc test showed that mortality levels in the 'Met52 low', 'BotaniGard low', and 'BotaniGard high' treatments were not significantly higher than the control group. Mortality of *D. coriaria* was significantly higher in the 'Met52 high' treatment than the control group (Table 1).

S. scimitus and *G. gillespiei* reacted differently to the mycoinsecticides. *S. scimitus* was not significantly affected by either Met52 or BotaniGard (Table 1). In contrast, *G. gillespiei* was adversely

Table 1
Dalotia coriaria, *Stratiolaelaps scimitus*, and *Gaeolaelaps gillespiei* mortality 12 days after exposure to different Met52 and BotaniGard (shown as BG) concentrations for 48 h. Data were pooled from three replicate experiments and analyzed using a chi square test ($\alpha = 0.05$).

	Control	Met52 low	Met52 high	BG low	BG high
Dalotia coriaria					
Mortality	16.67%[a]	32.56%[ab]	51.11%[b]	33.33%[ab]	41.86%[ab]
C.M.*	0%	19.07%	41.33%	19.93%	30.23%
Infection confirmed	0%	2.33%	20.0%	2.38%	13.95%
Total *n*	42	43	45	42	43
χ^2 test	$\chi^2 = 12.33$	4 df	$p = 0.015$		
Stratiolaelaps scimitus					
Mortality	15.56%	33.33%	31.11%	35.56%	17.78%
C.M.*	0%	21.04%	18.42%	23.69%	2.63%
Infection confirmed	0%	4.44%	11.11%	6.67%	6.67%
Total *n*	45	45	45	45	45
χ^2 test	$\chi^2 = 7.95$	4 df	$p = 0.093$		
Gaeolaelaps gillespiei					
Mortality	23.17%[a]	51.11%[b]	46.67%[ab]	70.0%[b]	62.22%[b]
C.M.*	0%	36.37%	30.59%	60.95%	50.82%
Infection confirmed	0%	11.11%	15.56%	15.91%	37.78%
Total *n*	44	45	45	44	45
χ^2 test	$\chi^2 = 23.33$	4 df	$p < 0.001$		

Note: assigned lower case letters next to the mortality values show the results of the multi-comparison of proportions.

* Mortality corrected using Abbott's formula (Abbott, 1925).

affected by both of the mycoinsecticides. The post hoc analysis showed that mite mortality levels were significantly higher in all of the fungal treatments, except 'Met52 high', compared to the untreated control group (Table 1).

S. scimitus and *G. gillespiei* also reacted differently when exposed to the nematodes. *S. scimitus* was not significantly affected by the nematodes: the mortality value followed with the corrected mortality value in brackets in the control and *S. feltiae*-treated groups was 43.2 (0)% and 52.3 (16.8)%, respectively ($\chi^2 = 0.41$, 1 df, $p = 0.52$). In contrast, *G. gillespiei* was adversely affected by the nematodes: mortality in the control and *S. feltiae* treated group was 50.0 (0)% and 78.6 (57.5)%, respectively ($\chi^2 = 5.96$, 1 df, $p = 0.015$). No nematodes were observed emerging from isolated mite cadavers.

In general, for all the predatory species tested, the majority of deaths occurred around seven days post inoculation, often due to infection. Mean prey consumption rates were similar across all five treatments for surviving predators, i.e. 9–10 second instar WFT every two days for *D. coriaria*; 8–9 larvae every three days for *S. scimitus*; 7–8 larvae every five days for *G. gillespiei*.

3.2. Efficacy

The assay system developed performed effectively in the trials, and about 70% of emerged WFT adults were successfully captured on the modified sticky card; the remainder were easily collected from the surface of the growing medium or sides of the container using a moistened fine paint brush. Since this trial aimed to examine WFT pupal mortality, even dead WFT adults found on the surface of the growing medium were considered 'survivors' and included in the counts.

The CFU/g of dry medium data from Experiment 1, and Experiments 2 and 3 (combined) are summarized in Table 2 together with the virulence data of the mycoinsecticides, as validated in the *Galleria* trials.

Experiment 1: The mean WFT pupal mortality in the control groups was 38.2% (Fig. 1A and Table 3). There was significant variation in the efficacy of the different treatments ($H = 186.87$, 9 df, $p < 0.001$). The result of Dunn's test on the WFT pupal mortality data showed that all the treatments, except BotaniGard-only, were significantly different from the untreated control. Both Met52-only treatments significantly out-performed the BotaniGard-only treatments. The combination of *D. coriaria* and Met52 out-performed all other treatments.

Compared to the results obtained in the compatibility trials (Table 1), survival of *D. coriaria* was poor in the mycoinsecticide-treated cups. In the *D. coriaria*-only treatments, 75.0% of beetles survived, but survival decreased to 8.3% when used together with Met52 low, 12.5% with Met52 high, 12.5% with BotaniGard low, and 8.3% with BotaniGard high. However, the cause of beetle death was not determined. Beetles in the Met52 treatments could have died from fungal infection or starvation, as the fungus killed a significant proportion of the thrips, as indicated in the fungus-only treatments. However, beetle survival was equally poor in the BotaniGard treatments, and thrips survival was not significantly affected by the fungus. In compatibility trials, the beetles were in contact with mycoinsecticides for only 48 h; in contrast, the beetles were in contact with mycoinsecticides for up to seven days in the efficacy trial. This difference in the exposure period may have contributed to higher beetle infection levels, and the resulting poor survival of *D. coriaria* in this experiment.

Experiment 2: The mean WFT pupal mortality from control groups was 33.5% (Fig. 1B and Table 3). There was significant variation in the relative efficacy of the different treatments ($H = 196.71$, 9 df, $p < 0.001$). The result of Dunn's test on the WFT pupal mortality data showed that all the mite treatments resulted in significantly higher levels of WFT mortality than the untreated control. The efficacy of Met52- and BotaniGard-only treatments did not differ significantly; while WFT mortality in the BotaniGard-only treatments was similar to that obtained in Experiment 1, mortality in the Met52-only treatments was about 25% lower. This appears to be correlated with the observed difference in the Met52 CFU counts between the two trials (Table 2). *S. scimitus* alone consumed ca. 80% of the WFT in a cup. The combination of *S. scimitus* + mycoinsecticides out-performed all other treatments, but differences among mortality levels in the predator alone and predator + mycoinsecticide treatments were not significant.

Experiment 3: The mean WFT pupal mortality from control groups was 29.6% (Fig. 1C and Table 3). There was significant variation in the relative efficacy of the different treatments ($H = 191.8$, 9 df, $p < 0.001$). The result of Dunn's test on the WFT pupal mortality data were similar to those observed in Experiment 2. *G. gillespiei* alone consumed ca. 80% of the WFT in a cup.

Experiment 4: The mean WFT pupal mortality from control groups was 29.2% (Fig. 1D and Table 3). There was significant variation in the relative efficacy of the different treatments ($F_{[9,\ 230]} = 27.83$, $p < 0.001$). The result of the least significant difference test on the WFT pupal mortality data showed that mortality levels were significantly higher in all treatments than the untreated control. The efficacy of predator-only treatments can be summarized as (*D. coriaria* adult ≈ *G. gillespiei*) < (*D. coriaria* larva ≈ *S. scimitus*). The combination treatments (predator + nematodes) were no more effective than the predator-only treatments (Fig. 1D).

4. Discussion

4.1. Compatibility

The International Organisation for Biological Control (IOBC) has established a classification system for side-effects of pesticides on natural enemies on the basis of laboratory test results (Boller et al., 2005). The range of corrected mortalities for the predators tested against Met52 and BotaniGard ranged from 2.9% to 60.9% (Table 1) which, according to the IOBC system, may be considered as 'harmless or slightly harmful' (<30% mortality) to 'moderately harmful' (30–79% mortality), respectively. Only the high rates of the mycoinsecticides were moderately harmful to *D. coriaria*. Jandricic et al. (2006) found that *S. feltiae* was also not harmful to *D. coriaria* adults (4–6% corrected mortality), and were only 'slightly harmful' to third instar *D. coriaria* (26.9% corrected mortality) when exposed to 50 IJs/cm^2. All of the fungal and nematode treatments were harmless to *S. scimitus*. In contrast, all of the fungal and nematode treatments were moderately harmful to *G. gillespiei*. Although the compatibility of mycoinsecticides and soil-dwelling predatory agents has not been widely investigated, studies are available to validate the resilience of these predators. Cloyd et al. (2009) showed that *D. coriaria* was compatible with *Bacillus thuringiensis* subsp. *israelensis*, *Metarhizium*, flonicamid, and spinosad, based on mortality resulting from direct contact and prey consumption rates after the predator was exposed to the insecticides. Jandricic et al. (2006) reported that diflubenzuron was compatible with *D. coriaria*. No compatibility studies have been done on *G. gillespiei*, but Cabrera et al. (2004) further confirms the resilience of *S. scimitus* by concluding pyriproxyfen (insecticide), fosetyl-Al and mefenoxam (fungicides) are likely compatible under field conditions, since these chemicals caused little mortality of protonymphs and had no adverse effects on the development and reproduction of *S. scimitus* under extreme laboratory conditions. Jacobson et al. (2001) showed that thrips control was enhanced on pepper when the foliar-dwelling predatory mite, *Neoseiulus* (=*Amblyseius*) *cucumeris* Oudemans was used together

Table 2
Recovered colony forming units (CFU) per g of dry medium for mycoinsecticides applied in Experiments 1–3, and their virulence in terms of mortality (%) in *Galleria* larvae (grand mean from Experiments 1–3).

	Control	Met52 low	Met52 high	BG [a] low	BG high
CFU					
Experiment 1	0	$7.51 \pm 1.05 \times 10^6$	$18.49 \pm 3.87 \times 10^6$	$6.81 \pm 2.4 \times 10^6$	$16.64 \pm 0.91 \times 10^6$
Experiments 2 and 3 combined	0	$0.68 \pm 0.08 \times 10^6$	$5.87 \pm 2.71 \times 10^6$	$7.92 \pm 2.03 \times 10^6$	$17.7 \pm 1.47 \times 10^6$
Virulence trial	3.3 ± 2.3	84.4 ± 5.1	86.7 ± 3.4	78.9 ± 6.3	98.9 ± 1.1

[a] Abbreviation for BotaniGard.

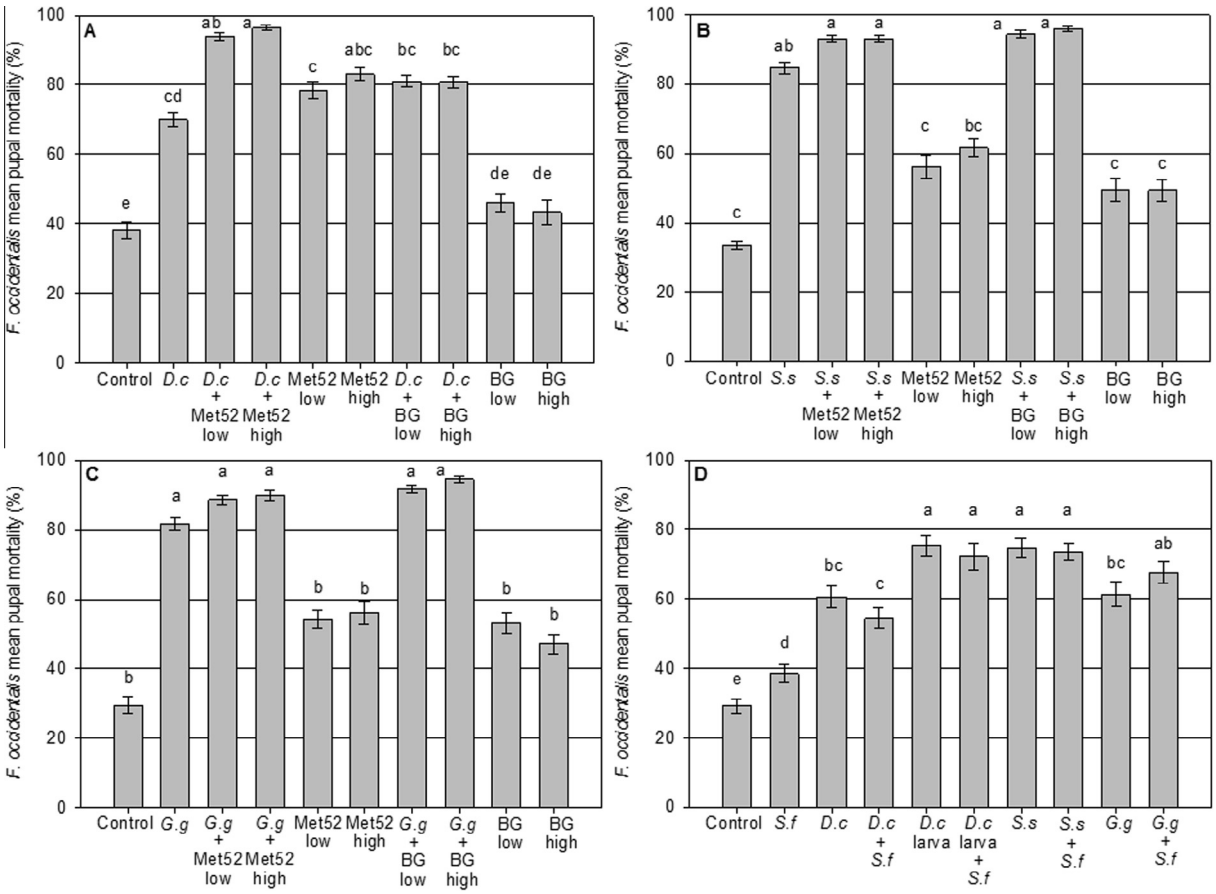

Fig. 1. *F. occidentalis* mean pupal mortality (%), with standard errors, from ten different treatment schemes (n = 24 cups/treatment). (A) *D. coriaria*, Met52, and BotaniGard; (B) *S. scimitus*, Met52, and BotaniGard; (C) *G. gillespiei*, Met52, and BotaniGard; (D) *D. coriaria* adults and larvae, *S. scimitus*, *G. gillespiei*, and *S. feltiae* (Nemasys). Assigned lowercase letters show the results of Dunn's test (A, B, and C; $\alpha = 0.05$), and the least significant difference test (D, $\alpha = 0.05$). Abbreviations: D.c (*D. coriaria*), BG (BotaniGard), S.s (*S. scimitus*), G.g (*G. gillespiei*), S.f (*S. feltiae*).

with *B. bassiana* (Naturalis®), and observed no significant effects of the pathogen on the predatory mites. However, susceptibility of mites to entomopathogenic fungi (both *Metarhizium* spp. and *Beauveria* spp.) has been demonstrated and control of pestiferous species with fungi has been achieved (Chandler et al., 2000, 2001; Kanga et al., 2003; Wekesa et al., 2005). Hence basic compatibility, and the biological significance of any negative interactions detected, are important considerations when integrating microbial and macrobial biocontrol agents.

4.2. Efficacy

The natural mortality levels observed in this study (29.2–38.2%) were not unexpected. Previous studies that employed similar experimental set-ups (where WFT larvae pupated in a plant growing medium) reported mortality levels in the untreated control ranging from 31.3% when a compost mix was used as the growing medium (Helyer et al., 1995), to 10.5–26.5% and 18–40.3% depending on the type of growing medium used (Ansari et al., 2007, 2008, respectively).

Despite the fact that efficacy testing was done under controlled, laboratory conditions, our trials used recommended release/application rates for each biological control agent, and provided some promising results (Fig. 1 and Table 3). When only predators were released, *D. coriaria* adults caused approximately 50% WFT pupal mortality (Table 3, Experiments 1 and 4), whereas late instar *D. coriaria* caused approximately 65% mortality (Experiment 4). *S. scimitus* showed variation in efficacy between Experiment 2 (Table 3, ca. 77% mortality) and Experiment 4 (ca. 64% mortality), and *G. gillespiei* showed similar variability in performance (ca. 74% mortality in Experiment 3, and ca. 45% mortality in Experiment 4). This variation may, in part, be due to differences in the sex ratios and fitness of the predators used in the different trials. Females are generally larger and eat more prey for reproduction.

Table 3
Corrected mortality[a] of *Frankliniella occidentalis* pupae in ten treatments from Experiments 1–4.

Predator	Control	Predator	Predator + Met52 low	Predator + Met52 high	Met52 low	Met52 high	Predator + BG[b] low	Predator + BG high	BG low	BG high
Experiment 1 *Dalotia coriaria*	0%	51.50%	90.23%	94.19%	65.16%	72.61%	69.28%	68.96%	12.73%	8.44%
Experiment 2 *Stratiolaelaps scimitus*	0%	77.14%	89.68%	89.78%	34.24%	42.45%	91.63%	94.04%	24.21%	23.83%
Experiment 3 *Gaeolaelaps gillespiei*	0%	74.09%	83.69%	85.41%	35.08%	37.73%	88.07%	92.25%	33.44%	24.86%
	Control	S. feltiae	D. coriaria	D. coriaria + S. feltiae	D. coriaria larva	D. coriaria larva + S. feltiae	S. scimitus	S. scimitus + S. feltiae	G. gillespiei	G. gillespiei + S. feltiae
Experiment 4	0%	13.23%	44.36%	35.80%	65.14%	60.72%	64.31%	62.81%	45.46%	54.30%

[a] Abbott's corrected mortality (Abbott, 1925).
[b] Abbreviation for BotaniGard.

For example, Berndt et al. (2004b) reported that female *Stratiolaelaps miles* ate about three to four times more WFT larvae than the males did. Thus although the total number of predators used in each trial was the same, the ratio of males:females was not, and could have affected relative WFT predation rates between the experiments. Alternatively, the age of the mites in different batches received from insectaries may have varied. Older mites are generally less fit and consume fewer prey than younger cohorts. Such variability in performance can in turn affect the success of a single-component biocontrol program and provides further justification for use of an integrated management approach.

When only mycoinsecticides were used, Met52 was more effective against WFT pupae in the growing medium than the BotaniGard spray treatment. This was predicted because the Met52 formulation and its application method are designed to work on insects in the soil. In contrast, the BotaniGard treatment was applied to simulate 'run-off' or overspray onto the surface of the growing medium, as may occur during a foliar spray. As such, the treatment was not directly targeted against pupating WFT and probably delivered insufficient conidia into the growing medium to control the pest. Met52 performed better in Experiment 1 (ca. 65–72% WFT mortality) than in Experiments 2 and 3 (ca. 35–40% mortality) (these were set up concurrently). This was correlated with differing CFU levels obtained in the growing media (Table 2). Essentially, Met52 was more efficacious in Experiment 1 where higher CFU levels were obtained, i.e. more infective conidia in the media, than in Experiments 2 and 3 where CFU levels were lower. Insect infection and mortality are dose-dependent, so these results are not surprising. The reasons behind the variation are unclear, since Met52 viability was, effectively, consistent between trials (75.6% in Experiment 1, 72.1% in Experiment 2 and 3). However, the three experiments were independent from each other and results were compared within each experiment, and were not compared across the experiments.

In all experiments, the 'high' rate (ca. three times more conidia) performed only slightly better (2–8%) than the 'low' rate. While efficacy, as measured by the rate and level of insect mortality, is related to concentration, differences in the 'high' and 'low' test concentrations used may not have been sufficient to affect insect mortality levels in the current experimental set-up. In spite of the inconsistent results, premixing Met52 does appear to provide good thrips pupal control. Results from Experiment 1 (65 and 72% WFT mortality at conidial concentrations of 4.5×10^8 and 1.35×10^9/l of dry growing medium, respectively) are comparable to those of Ansari et al. (2007, 2008). Their studies used the same *Metarhizium* strain (V275 = F52) as our study at a slightly higher rate (1×10^{10}/l of dry growing medium), and induced 72–91% mortality (2007) and 85–92% mortality (2008) in WFT pupae in growing media.

The number of BotaniGard 22WP CFUs recovered (Table 2), was consistent throughout Experiments 1, 2, and 3. Although observed pupal mortality levels were similar across all experiments, the corrected mortality levels (Table 3) varied (8–33% mortality). This inconsistency may be due to differences in natural mortality, as observed in the untreated controls. Skinner et al. (2012) also found that BotaniGard provided low levels of thrips control (ca. 15% mortality) even when the fungus was reformulated as mycotized millet grains, and mixed into the top layer of soil in potted plants. In contrast, Ansari et al. (2008) achieved 54–84% mortality when two experimental strains of *B. bassiana* were tested. In contrast to the simulated run-off application use in the current trial, their study premixed conidia into growing media at a rate that was more than 1×10^3 times higher than that used in our study.

Although *S. feltiae* alone significantly reduced the thrips population when compared to the untreated control (Fig. 1D), when corrected for natural mortality, the *S. feltiae* treatment provided only a 13.2% reduction in WFT survival (Table 3). Other studies have obtained variable results when testing the efficacy of commercial strains of *S. feltiae* against WFT soil-dwelling stages. In a Petri dish assay, Buitenhuis and Shipp (2005) achieved 44.6–53.6% control with 40 IJs/cm^2 and 59.7–62.0% with 80 IJs/cm^2. Ebssa et al. (2004) reduced WFT survival by only 2.6% using a similar experiment arena and test conditions to those used in our study, but an application rate of 200 IJs/cm^2. Interestingly Premachandra et al. (2003b) found the nematode provided 65% (nematodes applied 3 days after WFT late 2nd instars were released into the test arena) and 79% (nematodes applied 1 day after WFT late 2nd instars were released into the test arena) control when applied at 300 IJs/cm^2, using a similar experimental set up (but shallower soil) to the one in our study. These findings, together with our results, suggest that control is affected by the concentration of nematodes applied and that the nematode's host searching capacity may limit their ability to find and infect thrips pupae, especially when host numbers are (relatively) low. The most susceptible stages in the WFT life cycle are pre-pupae and pupae (Buitenhuis and Shipp, 2005); consequently, if nematodes are present in the soil at the time these stages are moving into and through the medium, the likelihood of their coming into contact with, and becoming infected by, infective juveniles is increased. In our study, nematodes were applied after WFT pupated, so infection would only have occurred if IJs could locate and enter a host. Timing of application and in-soil concentration of nematodes are important determinants of efficacy (Ebssa et al., 2004).

To our knowledge, this is the first study to compare the individual and combined use of soil-dwelling predators (*D. coriaria*, *S. scimitus*, and *G. gillespiei*) and mycoinsecticides (Met52 granular and BotaniGard 22WP), and soil-dwelling predators and entomopathogenic nematodes (*S. feltiae*). The combined use of predators and mycoinsecticides significantly improved the level and consistency of WFT control. Some combinations provided >90% mortality (Fig. 1 and Table 3). Mortality levels of 90–95% (among 20 thrips pupae in a cup) means only 1 or 2 pupa(e) survived to adulthood, which is probably approaching the maximum efficacy that can be achieved using biological control. Biologically, a predator or disease rarely eliminate an entire population of its prey or host. The enhanced efficacy levels observed in the combination treatments may be due to the combined effects of the individual treatments, i.e. predators and mycoinsecticides; however, it was not possible to calculate whether effects were antagonistic/additive/or synergistic from results in the current study as their calculation requires LC_{50} values for the individual treatments.

In contrast, the combined use of the predators and the nematodes did not work as well as predicted. In most cases, the combination treatments resulted in reduced efficacy compared to the predator alone treatments (Fig. 1D and Table 3). The combination of *G. gillespiei* and *S. feltiae* was the only one that exceeded the predator-only treatment. This may be due to the less-than-ideal timing of the nematode applications, as discussed earlier. If the efficacy of nematodes had been higher, the combination treatment might have had more pronounced additive effect. Alternatively, the reduced effect may have been due to predator feeding on the nematodes. Indeed, Brødsgaard et al. (1996) found that *Stratiolaelaps* (*Hypoaspis*) *miles* readily preyed on IJs of *S. feltiae*. Berndt et al. (2004b) reared and maintained a colony of *S. miles* with a saprophytic nematode, *Turbatrix silusiae* (de Man), and also found that *S. miles* reproduced better on a nematode diet than on a diet of thrips larvae. *G. gillespiei* is more of a soil-surface dweller than either *D. coriaria* or *S. scimitus* which are truly soil-dwelling predators. This difference in habitat may have influenced the level of contact between the predators and the nematodes.

Previous studies investigating the potential use of a combined release of predators and entomopathogenic nematodes found that thrips pupal mortality was significantly higher when *Hypoaspis aculeifer*, another commercially available soil-dwelling mite, was used with nematodes compared to individual releases of each natural enemy (combined treatment 71–82%; nematodes only 46–61%; mites only 46%), although effects were additive (Premachandra et al., 2003a). Use of nematodes with the foliar-dwelling mite, *N. cucumeris*, also provided superior control (83% mortality) to individual releases of each natural enemy (Ebssa et al., 2006). The authors noted that the foliar-dwelling mites increased the likelihood of thrips falling off the plant onto nematode-treated soil where they were liable to become infected. The current study further validates the benefit of combining predators and pathogens to control WFT, especially the combined use of soil-dwelling predators and a mycoinsecticide soil treatment.

There is still a need to examine the effect of the soil treatment with the mycoinsecticides on the fitness of predators and overall impact on a control strategy, e.g. through effects on longevity, reproductive performance, survival of progeny, etc. Greenhouse trials are currently ongoing to assess the strategic use of foliar-dwelling predators, soil-dwelling predators, mycoinsecticides, and entomopathogenic nematodes.

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