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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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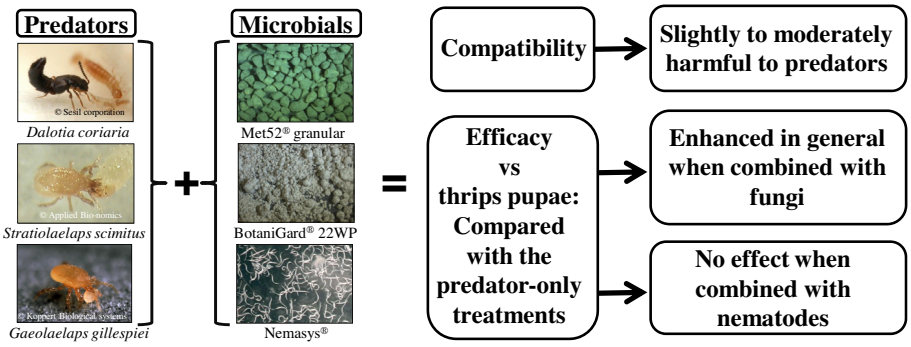
HIGHLIGHTS

- 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)