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Efficacy of spray applications of entomopathogenic fungi against western flower thrips infesting greenhouse impatiens under variable moisture conditions

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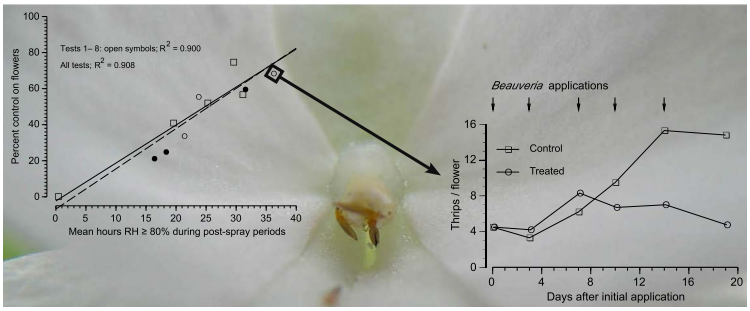
HIGHLIGHTS

- *Beauveria* and *Metarhizium* were applied vs. thrips on greenhouse impatiens.
- Control was highly dependent upon greenhouse moisture conditions.
- Hours of RH ⩾ 80% following sprays was the strongest predictor of control.
- Control ⩾ 70% on flowers and foliage prevented pest population increases.
- Maintenance of 80% RH for ca. 35 h after sprays was required to achieve 70% control.

GRAPHICAL ABSTRACT



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ABSTRACT

Laboratory bioassays of three entomopathogenic fungi (*Beauveria bassiana* strain GHA, *Metarhizium brunneum* strain F52, and *Metarhizium anisopliae* s.l. strain ESC-1) were conducted against 2nd-instar nymphs of *Frankliniella occidentalis.* All three fungi were highly virulent, with respective LC_{50}s of 193, 140, and 72 conidia/mm^2 of treated leaf surface. Efficacy tests were conducted against thrips infesting small plots of *Impatiens walleriana* on open greenhouse benches under variable moisture conditions. Multiple spray applications of fungal conidia suspended in 0.01% Silwet® were made at a high rate of 2×10^{14} conidia/ha at 3–5-day intervals, and efficacy was assessed from twice-weekly samples of both flowers and foliage. During tests, moisture conditions were modified via controlled sprays of water onto the concrete floor beneath the greenhouse benches for a period of ca. 40 h (from the evening through the second night) following each application (post-spray period). Efficacy of the pathogens expressed as percent reduction of the combined populations of thrips nymphs and adults relative to spray-carrier controls was ultimately correlated to greenhouse environmental conditions. Mean relative humidity (RH) over all test days was a strong predictor of thrips control on foliage ($R^2 = 0.820$) and flowers ($R^2 = 0.756$). Fungal efficacy was most strongly and consistently predicted by moisture conditions during the post-spray periods. The single best predictor was mean number of hours during post-spray periods when RH was ⩾80% (R^2 foliage = 0.834; R^2 flowers = 0.908). The three fungi produced similar levels of thrips control under comparable moisture conditions. Maintenance of 80% RH for ca. 25 h during post-spray periods resulted in >70% control of thrips in foliage. Substantially longer periods of high humidity (35 h) were required to achieve comparable control in flowers. Control ⩾ 70% in both foliage and flowers prevented pest

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populations from increasing. Aspects of the impatiens crop that create a challenge for thrips control using fungal entomopathogens are described and discussed.

1. Introduction

In previous greenhouse studies investigating the efficacy of the fungal pathogen *Beauveria bassiana* strain GHA (formulated as BotaniGard® 22WP) against western flower thrips *Frankliniella occidentalis* (Pergnade) (WFT) infesting greenhouse cultures of impatiens, we were unable to achieve effective levels of control. A primary hypothesis from the study was that greenhouse conditions were too dry to support fungal activity (Ugine et al., 2007a). Most of the tests were conducted in large greenhouse bays with effective sun-screens and efficient evaporative cooling systems. In one test conducted during the summer months, mean temperature was 21 °C and overall relative humidity averaged >80%. Such conditions would seem favorable for fungal activity against thrips, and yet, the pest populations were reduced just 32%. This suggests that daytime periods of reduced humidities, elevated temperatures, and high ventilation (to control temperature) had a strong negative impact on fungal efficacy. Little is known of the combined effects of these factors on fungal efficacy. Movement of air has a marked effect on its drying capacity and is difficult to control and quantify for experimentation, particularly above and within a crop canopy. Effects of ventilation on phylloplane microclimate add an additional level of complexity (see Jaronski, 2010). In studies of effects of moisture conditions on *B. bassiana* infection of *Rhodnius prolixus*, Luz and Fargues (1999) demonstrated that infection was dependent upon a near-saturation moisture threshold of 96%, and Fargues and Luz (2000) observed that daily fluctuations between optimal conditions of 97% humidity and 20–25 °C and even moderately lower humidity of 75% combined with a higher temperature of 28 °C were highly detrimental to efficacy. Harsher conditions than these are commonly encountered in greenhouses during daytime hours.

The environmental conditions supporting fungal infection of the blood-feeding vector *R. prolixus* obviously cannot be accepted as representative of conditions required for fungal activity against minute insect pests of crop plants. In contrast to the findings with *R. prolixus*, numerous studies of the whiteflies *Trialeurodes vaporariorum* and *Bemisia tabaci* have revealed that fungal infection of the larval stages of these insects is little affected by ambient humidity or ventilation (Wraight et al., 2000; Vidal et al., 2003; Fargues et al., 2003, 2005). Lack of correlation between ambient moisture conditions and infection has also been demonstrated with other insect pest-fungal pathogen associations (Marcandier and Khatchatourians, 1987; Fargues et al., 1997).

The primary objective of the present study was to determine if our previous failures to achieve effective control of WFT using *B. bassiana* can be attributed to unfavorable moisture conditions and to quantify the degree to which the microbial biocontrol potential of entomopathogenic fungi against WFT is dependent upon ambient moisture conditions. Original efficacy and environmental data from three greenhouse tests of the BotaniGard 22WP product against WFT reported by Ugine et al. (2007a) were reanalyzed for evaluations in the context of the current study. A second objective was to determine if maintaining high-moisture conditions over a period of ca. 40 h following spray applications would support sufficient fungal activity to achieve control. In addition to *B. bassiana*, various species/strains of *Metarhizium* have been found virulent against WFT (Gouli et al., 2009; Sengonca et al., 2006; Niassy et al., 2012). Fungi of the genus *Metarhizium* are enerally more tolerant of high-temperatures than *Beauveria* (Roberts and Campbell, 1977; Inglis et al., 1997), and because greenhouse humidity and temperature are closely interrelated (with lowest humidities associated with daytime high-temperature conditions), *Metarhizium* species were included as major study subjects. Prior to greenhouse testing, virulence of these fungi against WFT was confirmed in laboratory bioassays. Evaluations were conducted to reveal potential differences in efficacy of the *Beauveria* vs. *Metarhizium* fungi; however, it was not our objective to make rigorous comparisons. Such comparisons would require considerably greater numbers of directly comparable tests.

2. Materials and methods

2.1. Rearing of western flower thrips

A laboratory colony of WFT was established with adults collected from a Cornell University research greenhouse. Thrips were continuously reared on excised bean leaves (*Phaseolus vulgaris*) in large, polystyrene Petri dishes (150 mm diam. × 25 mm deep; Corning Life Sciences, Corning, NY). A smaller Petri dish (60 mm diam. × 15 mm deep) with a notch cut into the top of the sidewall for insertion of a bean leaf was affixed to the bottom of the large dish as a water reservoir. A large circle of dry filter paper was folded in half and placed in the bottom of the large dish, and the petiole of a single leaf (first true leaf) or the central leaflet of a trifoliate leaf was inserted into the reservoir, covered with absorbent cotton material from sanitary pads (Ugine et al., 2005) and saturated with distilled water (DH_20). The leaf was gently folded to fit within the dish space, and the reservoir was covered with a dish lid (also notched). Adult female thrips (15–20) were released onto the leaf and the dish was covered. The original dish cover, modified to include a 60-mm diam. opening fitted with fine screening (80 μm square openings) for ventilation, was inverted and placed on the dish (the smooth top surface of the dish lid outside the stacking ridge forming a tight seal against the rim of the dish bottom), and the lid was secured with four rubber bands. Dishes were held in a lighted incubator (25 °C, 16-h daily photoperiod). Water in the reservoir dishes was replenished daily (occasionally after 2 days). As the beans were produced in a non-quarantine greenhouse, the leaves used for rearing were not completely thrips-free, and there was a constant low-level infusion of thrips from the greenhouse population into the laboratory colony.

2.2. Fungal preparations

Three commercial strains of entomopathogenic fungi were selected for testing: *B. bassiana* strain GHA (basis of the current BotaniGard/Mycotrol® biopesticides) was cultured from a technical powder produced by Emerald BioAgriculture Corp., *Metarhizium brunneum* strain F52 (basis of the current Met52® products) was cultured from strain ARSEF 5198, and *Metarhizium anisopliae* s.l. strain ESC-1 (basis of the former Bio-Blast/Bio-Path products) was cultivated from Bio-Blast product produced by EcoScience Corp.

Unformulated conidia of *B. bassiana* strain GHA used in the laboratory bioassays were obtained from Emerald BioAgriculture. Conidia of the two *Metarhizium* strains were produced by culturing for 10–14 days at 25 °C on half-strength Sabouraud dextrose-yeast extract agar consisting of 20 g dextrose, 5 g Bacto™ neopeptone, 5 g