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12 Efficacy of Spray Applications of Entomopathogenic Fungi Against
13 Western Flower Thrips Infesting Greenhouse Impatiens Under
14 Variable Moisture Conditions
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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 LC₅₀s of 193, 140, and 72 conidia/mm² 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 $\geq 80\%$ (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 $\geq 70\%$ in both foliage and flowers

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

Key Words: *Beauveria bassiana*, *Metarhizium* spp.; greenhouse efficacy; *Frankliniella occidentalis*; moisture conditions; relative humidity

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* (Pergande) (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 hours 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 generally 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. x 25 mm deep; Corning Life Sciences, Corning, NY). A smaller Petri dish (60 mm diam. x 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 (DH₂O). 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 yeast extract and 15 g agar/L (SDAY/2) in standard Petri plates. After incubation, cultures were dried by exposing open plates to air circulation for 1–2 days in a sterile containment hood at room temperature. Conidia were then

brushed from plates into standard no. 120 sieves (125x125 μm openings), dried overnight in the collecting pans over anhydrous CaSO_4 , transferred to glass vials, tightly sealed, and stored frozen at -15°C .

Larger quantities of conidial powders for greenhouse tests were produced essentially using the biphasic culture protocol described by Ugine (2011) with the following modifications. An agar plug from the growing edge of a fungal colony on SDAY/2 was inoculated into a 250-ml flask containing 100 ml DY broth (2% dextrose + 2% yeast extract). Flasks were incubated for five days on a rotary shaker at 150 rpm and 25°C .

Solid substrate (organic, thick-rolled barley flakes) was prepared in large mushroom spawn bags (flat dimensions: 20 x 56 cm; single-pleated sides expanding maximally to 12 cm) with a single 3.8 x 3.8 cm aeration patch (Fungi Perfecti, Olympia, WA). Each bag was filled with 200 g barley and 120 ml DH_2O . After mixing, the bags were placed in trays, and autoclaved for 15 min. After cooling in a sterile hood, the grain mass was crumbled by hand manipulation of the bags. Each bag was opened in the hood, and the entire culture from one DY culture flask was added. The bag was closed in such a way as to trap a large volume of air, and the top was folded over several times and secured with two spring clips. The barley was agitated to disperse the inoculum, and the bags were incubated 8–10 days at 25°C with 16-h daily photoperiod.

After incubation, bags were prepared for drying by cutting off one corner and draining as much condensed water as possible. The bags were weighed and then laid flat on racks in a HEPA-filtered containment hood. A slit was cut down the center length of each bag, and the cultures were left to dry with constant air circulation through the containment hood. Cultures were dried until they had lost 60–65% of their total weight (requiring ca. 7–10 days, depending on ambient RH). Dry conidia were harvested from crumbled substrate using a large sieve

(standard no. 100; 150 µm openings). Without further drying, conidial powders were sealed in 50 ml tubes and stored as described above. A different production batch of conidia was used for each greenhouse test; two or more batches were used in some tests. Prior to use, conidial powders were quantified. Five stock suspensions, each comprising a precisely weighed sample of powder (3.5–4.5 mg) in 100 ml water with Silwet surfactant at 0.02%, were prepared and quantified via hemacytometer. Mean concentration (range) of total conidia per mg of unformulated conidial powder was 1.25×10^8 ($1.21\text{--}1.38 \times 10^8$) for *B. bassiana* strain GHA, 4.85×10^7 ($3.42\text{--}6.69 \times 10^7$) for *M. brunneum* stain F52, and 4.75×10^7 ($4.44\text{--}4.95 \times 10^7$) for *M. anisopliae* strain ESC-1). For viability determinations, several small aliquots (8 µl) of suspension were dropped onto duplicate plates of freshly prepared 0.5% yeast extract agar and placed in a sterile containment hood until all water was absorbed. Plates were sealed with Parafilm and incubated for ca. 18 h at 25°C. Two or three counts of 100 randomly selected conidia (via transect scan) on each plate were made and averaged. During bioassays, samples of conidia were collected on agar plates placed on the spray target. Numbers of conidia per mg dry conidial powder determined from the hemacytometer counts were adjusted for viability.

2.3. Bioassays

Laboratory bioassays of fungal virulence were conducted using the treated-leaf-disk protocol of Ugine et al. (2005). Stock suspensions of each fungus were prepared by addition of 20 mg conidial powder to 20 ml DH₂O with 0.01% Silwet[®] surfactant. A series of 3-fold dilutions was then prepared using 0.01% Silwet solution. Each replicate assay comprised three doses. In assays of *B. bassiana* strain GHA, the first dilution in the series was employed as the high dose, with the next two dilutions serving as the medium and low doses. Doses of the

Metarhizium spp. were similarly prepared, except that the stock suspension served as the high dose. The ventral surfaces of bean leaf disks were sprayed with fungal suspension using a Burgerjon spray tower as described by Ugine et al. (2005) and allowed to dry. Samples of all spray deposits were collected on clean polystyrene Petri dish lids and stored until quantification using the method of Wraight et al. (1998). Control disks were sprayed with 0.01% Silwet. A pair of disks was then placed in each assay cup, treated surfaces together and sandwiched between two moistened filter paper disks (see Ugine et al., 2005).

Within 8–9-days after infestation, rearing dishes from the thrips colony contained large numbers of mixed-age nymphs. Dishes were opened under a low-power microscope, and early-second-instar nymphs were picked from the leaf surface using a wet paintbrush and transferred to the filter paper on top of the leaf disks in the assay cups. The cups were capped and sealed with Parafilm®, creating a constant high-humidity environment. Mortality was scored after incubation for 5 days at 25°C under 16-h daily photoperiod. Twelve nymphs were placed in each cup; each assay comprised two pseudoreplicate cups each of the high, medium, and low doses and 4–6 controls (total of 120–144 thrips). All assays were conducted independently, on different days, with different generations of thrips.

2.4. Greenhouse tests

Tests 1–8 (Table 1) were conducted in a partitioned greenhouse with small compartments measuring 2.75 x 7.30 m located at the USDA-ARS R.W. Holley Center in Ithaca, NY.

Impatiens walleriana var ‘Super Elfin Series White XP’ (Germania Seed, Chicago, IL), a variety with single-form flowers, were potted in a peat and composted pine bark-based medium (Metro-Mix 360, Sun Gro Horticulture, Anderson, SC) in plastic pots measuring 7-cm deep x 10 cm top

diam. (Dillen 4TW AZA, Myers Industries, Middlefield, OH). Research plots (ca. 0.9 x 1.7 m) comprised 12 rows of 7 plants. Spacing was minimal, and plants quickly developed a closed, dense canopy. Plants were contained in large trays with slow drainage for flood irrigation. Two plots (presumed to be non-independent pseudoreplicates because of the small size of the greenhouse compartments) were established on separate benches in each compartment. For relative humidity manipulations, water was applied to the greenhouse concrete floors from periodic operation of fine-mist spray nozzles mounted under the benches and directed downward to minimize wetting of plant foliage. Spray intervals were adjusted to keep the floor constantly wet during the desired period of time following fungus treatments. Plants were sub-irrigated daily or as needed; water contained a 20-10-20 fertilizer (Peters[®] Professional, Everris NA, Dublin, OH) at 200 ppm. Supplemental lighting from 400-Watt high-pressure sodium lamps (Philips C400S51/ALTO Ceramalux lamp, Philips Lighting, Mountain Top, PA) was provided as needed in late afternoon and evening to maintain a 15-h daily photoperiod. Greenhouse environmental controls were set to maintain temperatures between 15 and 25°C; however, the cooling systems were inefficient, and during peak hours of insolation during warm months, temperatures rose above 30°C. Environmental conditions (temperature and relative humidity) in each GH were monitored at 30 min intervals using electronic data recorders (HOBO[®] data loggers, Onset Computer, Bourne, MA) placed under shade beside each test plot.

Applications were applied using a hand-held, mist-blower spray gun (MaxCharge[™] electrostatic spraygun, with disk insert 0.4, Electrostatic Spraying Systems, Watkinsville, GA) operated at 103 kPa (15 psi) and delivering ca. 2.5 ml/sec. The gun was operated without the electrostatic generator (batteries removed) to represent basic mist-blower technologies. Unformulated conidia of all fungi, suspended in 0.01% Silwet, were applied at a rate equivalent

to a high rate of the commercial BotaniGard WP product of 0.45 kg/378.5 L/0.093 ha (label rate of 1 lb/100 gal/10,000 ft²). As the BotaniGard product is formulated at 4.409×10^{13} conidia/kg, this translates to a rate of 2.14×10^{14} conidia in a volume of 4074 L/ha. The treatment dose was delivered via a 15-sec spray pass per row of impatiens, with the spray directed at an approximate 45° angle into the canopy from a height of ca. 20 cm above the canopy. The high spray volume was intended to maximize penetration of the crop, with runoff to the substrate having potential to infect the quiescent prepupal and pupal stages in the soil. On small plants, the application could be considered a light sprench. The two plots in each greenhouse compartment were treated with independently prepared (replicated) conidial suspensions or carrier-control solutions. All tests included a pathogen-free, spray-carrier control of 0.01% Silwet. Tests were generally conducted on different days in randomly-assigned greenhouse compartments. In cases where two fungi were tested simultaneously, each treatment and control was assigned to a separate compartment. Two of the tests included an untreated control. Other details of the spray program in each test are presented in Table 1.

Thrips populations were sampled on the morning before each spray application and then twice during the last week of the test. One fresh flower with pollen (Ugine et al., 2006) and one short (1.5–2 cm) section of vegetative stem, comprising the terminal cluster of new foliage and one expanded leaf, were cut from one randomly selected plant from each row (n = 12). Plants for flower vs. foliage samples were independently randomized. The 12 flowers were collected in a single container of 80% ethanol, and the process was then repeated for the foliage samples. Samples were thoroughly dissected under a low-power microscope and total numbers of thrips recorded (nymphs + adults). Numbers were ultimately expressed as total thrips per flower or stem.

2.5. Statistical analyses

Bioassay data were analyzed by probit analysis (Finney, 1971) using the SAS[®] 9.3 software (SAS Institute, 2011). Efficacy of the spray program in each test was expressed in terms of the percent reduction in the thrips population in treatment plots relative to the control plots; this percent reduction is referred to as percent control. Percent control was based on the final sample of each test, with the exception of Test 2, in which the final samples of foliage indicated an abrupt loss of control (see Fig. 2A). In this case, efficacy estimated from the foliage samples was based on mean thrips collected in the last three samples. In the two tests that included untreated controls, thrips numbers were greater in the spray-carrier vs. untreated control plots (compartments), and control was calculated using the means of the two controls. Analysis of variance of the log LC₅₀s from bioassays and regression analyses estimating the proportion of total variation in percent control across tests attributable to specific environmental parameters (R-squared) and standard error of the regression (standard error of the estimate) were conducted using JMP[®] Pro 12.0.1 software (SAS Institute, 2015). In the latter analyses there was no evidence of nonlinearity, and no transformations were applied. Hypothesis tests of differences in efficacy between *Beauveria* and *Metarhizium* spp. were conducted with proportion control transformed to empirical logit: $\log(p/[1-p])$, with the smallest non-zero proportion (0.2 from test 10B) added to the numerator and denominator of the transform as recommended by Warton and Hui (2011).

Data from a number of our previous tests (Ugine et al. 2007a) were re-analyzed for direct comparisons to those of the present study. Tests considered most comparable were those with short spray intervals and those that included foliage samples; although, few foliage samples were

collected during our earlier study, and the foliage and flower sampling was not conducted concomitantly as in the present study. Efficacy data presented for Test 9 in Table 1 are derived from the day-21 post-application results shown in Fig. 2 of Ugine et al. (2007a). Application rate was ca. half that applied in the present study (4.9×10^{13} vs. 2.15×10^{13} conidia per ha) and spray volume was much lower (935 vs. 4074 L/ha), but sprays were more frequent (3-day vs. alternating 3–4-day intervals). No foliage samples were collected. Test 10 and Test 11 data are from Figs. 8 and 9, respectively, of Ugine et al. (2007a). Results from foliage samples (whole, small pre-flowering plants) are from early treatments (A tests), and results from flower samples are from late treatments (B tests). Applications were applied at five-day intervals at a rate of 2×10^{13} conidia in 3740 L/ha. In the test shown in Fig. 8 of Ugine et al. (2007), efficacy estimated from foliage samples (whole vegetative plants) was calculated from the samples collected before initiation of the late sprays (day 20). Efficacy from flower samples was calculated from the day 44 data (from flowers sampled 23 days after initiation of the late-spray treatments). Efficacy estimated from foliage samples reported for Test 11B in Table 1 was calculated from the day-16 results (last sample before flowering) shown in Fig. 9 of Ugine et al. (2007a). Efficacy from flower samples was determined from the day-37 results (from flowers sampled 17 days after initiation of the late-spray treatments).

3. Results

3.1. Laboratory bioassays

Viabilities of all conidial powders used in bioassays of *B. bassiana* strain GHA were high, ranging from 87 to 90%. *Metarhizium* conidia were unstable when repeatedly removed from

frozen storage and exposed to air. Viability of *M. brunneum* strain F52 averaged 66% (range 49–95%), and viability of *M. anisopliae* s.l. strain ESC-1 averaged 73% (range 44–89%). There was, however, no significant correlation between *Metarhizium* viability and LC_{50} ($r = -0.647$, $P = 0.12$, $n = 7$). Nevertheless, the fungi were all virulent against western flower thrips under ideal laboratory conditions of constant high humidity and moderate temperature, with LC_{50} s ranging from 72 to 193 viable conidia/mm² treated leaf surface (Table 2). *Metarhizium* strain ESC-1 exhibited the lowest LC_{50} , but differences were not statistically significant.

3.2. Greenhouse tests

All conidial powders of *B. bassiana* used in the greenhouse tests were highly viable (mean 88%, range 81–95%) except for the powder used in the first application of test four (76%). After the above-describes experience with poor viability of *Metarhizium* powders, conidia were used within a short time after production, and storage tubes were not opened repeatedly. Viability averaged 88% (82–96%). Spray application programs and resulting levels of thrips control (efficacy) are summarized in Table 1. In the following results and discussion, the percent reduction in greenhouse thrips population relative to the control population estimated from foliage/flower samples will be referred to as control (or efficacy) on foliage/flowers. None of the fungi exhibited outstanding efficacy. Excluding Test 5, which was conducted under unusually harsh (dry) conditions, mean efficacy across all tests of the present study (through Test 8) for *Beauveria* vs. *Metarhizium* was 71 vs. 74% against thrips populations on foliage and 52 vs. 56% against thrips on flowers. Augmentation of relative humidity during the 40-h period following each spray application (referred to henceforth as the post-spray period or PSP) had a large impact on efficacy. Across all tests of all fungi, efficacy in humidified greenhouses averaged 76% on

foliage and 63% on flowers vs. 41 and 32%, respectively, in non-humidified greenhouses. Augmentation of RH conditions to levels supporting $\geq 70\%$ control of thrips on both foliage and flowers prevented pest populations from increasing (Figs. 1 and 2).

Standard errors associated with some of the means in Figs. 1 and 2 were unexpectedly large, indicating substantial differences in pest populations between the two plots within each greenhouse compartment. Our initial disposition was to treat these plots as non-independent pseudoreplicates because the greenhouse compartments were small, and thrips movement was not restricted (plots were not caged). However, these observations reveal considerable independence and support our previous findings of limited movement between treated and control plots on separate benches in a larger greenhouse (Ugine et al., 2007a).

Environmental variables examined as potential predictors of efficacy are presented in Tables 3 and 4. Mean daily maxima and minima are included to provide information on range of conditions, but were not of interest as predictors of efficacy, as extreme conditions prevailed for only brief periods, and these variables provide no information with regard to duration of the conditions. Percentages of thrips control recorded in the tests of the present study (Tests 1–8) were regressed against each environmental variable. Percentages from our previous tests (Tests 9–11; Ugine et al., 2007a) were then added to the regression model. In most cases, these data were a good fit to the linear models; i.e., addition of the Ugine et al. (2007a) data had little effect on R-squared (R^2) and the statistical significance of the regressions (Tables 3 and 4; Figs. 3–9).

Prior to examining the relationships between fungal efficacy and environmental variables, preliminary regression analyses were conducted to determine if the activity of the *Beauveria* and *Metarhizium* strains differed significantly, which would call for independent analyses of the pathogen groups. The independent variable “fungus” (fungal genus) was included in these

regressions. In all cases, there were no significant differences in the slopes of the responses (P values for the fungus x environmental variable interactions ranged from 0.35–0.96 in regressions of efficacy on foliage and 0.32–0.99 on flowers), and this term was dropped from further analyses. In most cases, fungus also was not significant. The most notable exception was regression of percent control on flowers vs. mean RH. The clustering of the data points presented in Figs. 3B suggested that significantly different regression lines could be fit to the responses associated with *Metarhizium* vs. *Beauveria* (that *Metarhizium* was more effective than *Beauveria* on flowers with RH taken into account), and this was confirmed by the analysis ($F_{1,8} = 11.7$, $P = 0.009$). Also, percent control on flowers regressed on the alternative variable VPD was nearly significant ($F_{1,8} = 4.96$, $P = 0.068$). In these cases, separate fits of the *Beauveria* vs. *Metarhizium* data are presented in Figs. 3C and 4C. Fungus was also significant in regressions of efficacy on flowers vs. two other variables: mean hours $RH \geq 70\%$ ($F_{1,8} = 6.4$, $P = 0.036$) and mean hours $RH \geq 80\%$ during the last two post-spray periods ($F_{1,8} = 5.99$, $P = 0.040$). Mean hours $VPD \leq 0.8$ (the alternative variable to mean hours $RH \geq 70\%$) was nearly significant at $P = 0.07$. These variables were ultimately not selected as best predictors, and separate regressions are not shown. In all other analyses, fungus was not significant (P values from regressions with flowers ranging from 0.09 to 0.90 and from 0.09 to 0.96 with foliage), and in illustrated regressions, all fungi are fit to a single regression line. However, acknowledging potential differences between *Beauveria* and *Metarhizium*, the factor fungus was retained in all models.

Temperature in most tests was maintained within a range favorable for fungal infection (mean daily min/max range = 15.8–32.8) (Table 3), and under these conditions, the independent variable mean temperature was a weak predictor of efficacy; maximum R-squared (R^2) was 0.603 from foliage samples (regression not significant at $P = 0.10$) and ≤ 0.208 from flower

samples (Table 3). In all regressions with mean temperature, the standard error of regression (SER) was > 14 (average distance of observed values from the regression line was >14 percentage points).

Moisture conditions in the greenhouse were more accurate predictors of efficacy than temperature (Table 3). R^2 from regressions on mean RH ranged from 0.756 to 0.936 (Fig. 3), and regressions were consistently significant (P values ≤ 0.016). Mean vapor pressure deficit, as an alternative measure of moisture conditions, was a strong predictor of efficacy on foliage ($R^2 = 0.850\text{--}0.899$, $SER = 8.8\text{--}9.1$) (Fig. 4) but not on flowers ($R^2 = 0.581\text{--}0.62$, $SER = 16.3\text{--}17.0$). Regression of efficacy on flowers vs. mean VPD for tests 1–8 was not significant ($P = 0.089$).

Fungal efficacy was most strongly and consistently predicted by moisture conditions recorded during the 40-h post-spray periods (Table 4). R^2 from regressions of mean RH during PSP ranged from 0.741–0.908, which is similar to mean RH over all test days (cf. Fig 3 and Fig. 5), but mean VPD during PSP was a more accurate predictor of efficacy than mean VPD over all days (R^2 ranges: 0.711–0.912, $P \leq 0.042$ vs. 0.581–0.899, P values ≤ 0.089) (cf. Figs. 6 and 4). As observed with the overall means, mean RH during PSP was a stronger predictor of efficacy on flowers than VPD. However, in sharp contrast to relationships with overall mean moisture conditions, PSP moisture conditions were as strongly associated with efficacy on flowers as on foliage. R^2 across all variables and all tests in Table 4 (excluding the last two columns) averaged 0.807 for flowers vs. 0.784 for foliage.

The single best predictor of efficacy was mean hours during PSP with $RH \geq 80\%$. Regressions with this variable produced R^2 values ranging from 0.834 to a remarkable 0.959, with associated SER of 9.6 and 5.6 percentage points, respectively (Fig. 7, Table 4). Hours of $RH \geq 80\%$ was a markedly better predictor of efficacy than $RH \geq 70\%$, particularly as a predictor

of efficacy on flowers. Regressions of efficacy on hours of $RH \geq 70\%$ were very similar to those on overall mean RH, with the variable fungus being significant in both cases ($P = 0.036$ vs. 0.009 , respectively). Hours of extremely high moisture ($RH \geq 90\%$) were somewhat less predictive of efficacy than hours of $RH \geq 80\%$. During post-spray periods, hours of $VPD \leq 0.8, 0.5$, and 0.2 kPa predicted efficacy at nearly the same level of accuracy as hours of $RH \geq 70, 80$, and 90% , respectively (e.g., cf. Figs. 7 and 8).

One of the data points (61.5% control on foliage in test 10A) from the Ugine et al. (2007a) studies is a prominent outlier in Figs. 5, 7, and 8. An inspection of the environmental data from this test revealed that conditions were initially very dry, with moisture increasing markedly during the latter half of the test. This prompted investigation of an additional variable representing moisture conditions during just the last two post-spray periods (the two PSP before the final efficacy evaluation). Regressions of efficacy on hours of $RH \geq 80\%$ during the last two spray periods is presented in Fig 9. Efficacy was predicted with greater accuracy; R^2 increased from 0.821 to 0.902 and SER decreased from 10.0 to 7.4 (cf. Fig. 8). On the other hand, this variable was a weaker predictor of efficacy on flowers, with R^2 decreasing from 0.882 to 0.793 and SER increasing from 8.6 to 11.4. (Figs. 8 and 9, Table 4).

Inverse predictions of selected moisture conditions estimated to produce 70% and 80% control of thrips are presented in Table 5. Variables include mean RH, mean VPD, mean RH during PSP, mean VPD during PSP, mean hours $RH \geq 80\%$ during PSP, and mean hours $VPD \leq 0.5$ during PSP (Figs. 5–8, respectively). Estimates were generated from models including the independent variable fungus, but excluding the environmental variable x fungus interaction term. The regressions in Fig. 3C, in which fungus was clearly significant ($P = 0.009$) predict that achieving 70% control of thrips on foliage using *B. bassiana* strain GHA would require

maintenance of a mean RH of 78%, and that the same level of control on flowers would require a mean RH of 90% (Table 5). In contrast, 70% control with *Metarhizium* would require just 71 and 77%, respectively. VPD values predicted to support 70% control by *Beauveria* on foliage and flowers are 0.63 and 0.24 kPa vs. 0.89 and 0.69 for *Metarhizium*. Alternatively, achieving 70% control using either pathogen (means of similar estimates, effect of fungus not significant) is predicted to require elevating mean PSP humidity levels to ≥ 83 and 91% on foliage and flowers or by holding mean VPD at ≤ 0.5 and 0.26 kPa, respectively. The regressions predict that 70% control on foliage and flowers would require maintaining RH $\geq 80\%$ or VPD ≤ 0.5 kPa for ca. 26 and 34 hours, respectively. These inverse predictions assume that conditions in the greenhouses were generally inadequate to support significant efficacy without augmentation of moisture levels.

4. Discussion

In nearly all cases, addition of the data from Ugine et al. (2007a) to the regressions generated from the data of the present study had little effect on R^2 or significance (P), indicating that despite the differences in experimental protocols, the results from both studies fit the defined trends, confirming our hypothesis that moisture was the key factor limiting efficacy of *B. bassiana* in our previous study. Under manipulated RH conditions (RH augmented for 40 hours following each spray application), *B. bassiana* GHA held thrips to pre-treatment levels (Fig. 2B). As originally conjectured, *Metarhizium* spp. appeared to tolerate somewhat drier conditions than *B. bassiana* GHA. Predicted levels of moisture, in terms of mean RH and VPD, supporting 70%

control of WFT were 13 percentage points and 0.45 kPa lower for *Metarhizium* (Table 5). However, differences associated with other environmental variables were smaller and not statistically significant, and applications of *M. brunneum* F52 in a humidified greenhouse produced results similar to *B. bassiana* GHA, holding the thrips populations stable (Fig 1C). The apparently lower moisture threshold for activity of *Metarhizium* vs. *Beauveria* may not be low enough to translate to significantly greater pest control potential. Additional study is warranted.

We originally selected impatiens as the primary focus of our research beginning in the early 2000s because it was a leading bedding crop in the northeastern U.S. Markets for this plant have since declined due to a devastating mildew disease (Getter and Behe, 2013). Nevertheless, we have continued work with this plant because we consider it a challenging model host plant for use of fungal pathogens in protected crop environments. Leaves of impatiens are small, glabrous, and waxy, characteristics associated with lower and less stable moisture conditions in the phyllosphere (Jaronski, 2010). On vegetative plants, WFT rarely feeds on mature, fully expanded leaves, but rather prefer to feed in the dense clusters of new leaves at the meristems. In this microhabitat they are largely protected from direct spray applications, and as these new leaves expand, residual deposits of conidia representing secondary sources of inocula, become greatly diluted (Inyang et al., 1998). Impatiens are precocious flower producers, and in mature plants the overwhelming majority of adult WFT aggregate in the flowers, where they feed on abundant pollen (85% of all adults collected in the final samples from the tests of the present study were from flowers). Impatiens flowers, like those of most ornamental plants, are typically produced at the outer edge of or above the crop canopy, where ventilation and temperature are highest and moisture levels are lowest. The flowers of single-flower-form impatiens are flat, and the

androecium (pollen cap) is fully exposed to the ambient environment (Ugine et al., 2006). Protocols for greenhouse plant production generally call for maintenance of conditions as dry as plant growth requirements will allow while minimizing risk of plant disease outbreaks. In many greenhouse systems crops are well ventilated and sub-irrigated to prevent water from contacting leaf and flower surfaces. The above-described factors combine to produce a crop, in impatiens, with unusually dry canopy conditions. Our premise is that the definition of environmental conditions necessary to support activity of entomopathogenic fungi in single impatiens would be conservatively applicable to a broad range of ornamental and vegetable greenhouse crops.

Our observations of the critical importance of moisture conditions in fungal efficacy were not unexpected and are in general agreement with other studies. Shipp et al. (2003) reported $\geq 90\%$ control of WFT resulting from weekly applications of *B. bassiana* strain GHA (formulated as BotaniGard® ES) infesting cucumber in a greenhouse maintained at constant moderate temperature and high humidity (23–25°C and 86–92% RH). However, Shipp et al. (2003) also reported $>80\%$ infection of thrips exposed to direct sprays on cucumber-leaf undersides and then caged on the treated leaves in commercial greenhouses with moderate temperatures (means ranging from 17–21°C) and mean RH as low as 73%. In an unreplicated greenhouse test with mean temperature and RH of 20°C and 78%, Jacobson et al. (2001) also recorded good control of WFT (87%) on cucumber exposed to multiple high-volume (complete coverage) sprays of BotaniGard WP. In our tests of *B. bassiana*, mean humidities of 84–87% (tests 3 and 11B) were inadequate to support a high level of control, and our data estimate that 90% RH would be required to achieve just 70% control on flowers (Table 5). This inconsistency is almost certainly explained, in part, by

the differences in the crops (impatiens vs. cucumber). In a study of WFT infestations of cucumber, Higgins (1992) reported finding > 85% of the nymphal populations on the leaves. This contrasts sharply with our observations on impatiens; in the final samples of Test 1–8, 72% of all nymphs collected were found on the flowers. Cucumber produces large leaves that, when mature, tend to have more or less concave lower (abaxial) surfaces with abundant stomata and stout trichomes. These traits produce a protected, cool, humid microenvironment favored by thrips and highly favorable to activity of entomopathogenic fungi (Wraight et al., 2000). This microhabitat is also more exposed to efficient (hand-targeted) spray applications than other cryptic habitats commonly preferred by thrips. The significance of host plant species on efficacy of fungal biocontrol agents against greenhouse pests is well documented (Poprawski and Jones, 2001; Thungrabeab et al., 2006; Ugine et al., 2007b). Daily fluctuations in moisture conditions were likely an additional factor. Even during PSP periods of humidification we were unable to maintain constant levels of moisture as high as the 86–92% RH conditions described by Shipp et al (2003). In our small greenhouse compartments, heat from the lights and even brief periods of high insolation necessitated operation of the ventilation fans, and in the larger greenhouse bays used by Ugine et al. (2007a), the large evaporative coolers generated considerable air movement. Recognizing the difficulty of maintaining high-moisture conditions during daytime periods, as well as the need to minimize moisture as a deterrent to plant disease, we opted to attempt maintenance of high humidity for periods of ca. 40 hours. With sprays being applied in late afternoon, this necessitated humidification for just a single daytime period sandwiched between two nights (during which high RH is easily maintained in the absence of ventilation). Shipp et al. (2003) concluded from laboratory assays that “optimal

climatic conditions should be maintained for 2 d postspray for controlling *F. occidentalis*,” and this finding was confirmed by the studies of Mukawa et al. (2011).

With respect to moisture and ventilation effects on efficacy of fungal pathogens, it is noteworthy that these observations run counter to observations with another major group of greenhouse pest: the whiteflies. Numerous studies 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). We believe this is explained by the great differences in morphology/behavior of whiteflies vs. thrips. Whitefly larvae are scale-like and sedentary and intimately associated with the abaxial leaf surface and its humid boundary layer. Moreover, molting in these insects involves expanding and resettling on areas of leaf surface that may be contaminated with fungal conidia. Conidia in this situation are exposed to conditions highly favorable to germination and infection. Thrips larvae, in contrast, are highly mobile and far more cryptic than whitefly larvae, inhabiting dense clusters of new foliage or structures of flowers where they cannot be targeted directly with biopesticide sprays.

The observations that mean VPD and RH were equally strong predictors of efficacy on foliage, but that VPD was a weaker predictor on flowers (Tables 3 and 4) is difficult to explain. This anomaly may derive simply from the fact that percent control on flowers and VPD were more variable across tests than percent control on foliage and RH. Coefficients of variation of control on flowers vs. foliage = 51 vs. 32%, of mean VPD vs. mean RH = 37 vs. 15%, and of mean VPD during PSD vs. mean RH during PSP = 44 vs. 14%. It appears that high vapor pressure deficits recorded during dry conditions strongly bias the means. The average of the maximum VPD values recorded during each of the tests was 3.31 kPa; the average

of the minimum RH values was 30%. When the minimum VPD of zero (corresponding to 100% RH) is averaged with 3.31, the mean is 1.65; when the minimum RH of 30% is averaged with 100%, the mean is 65%. A VPD of 1.65 is representative of much drier conditions than a relative humidity of 65%. Percent control on flowers is likely affected more by ventilation than control on foliage, but we did not monitor ventilation and cannot account for this variable. Biased estimates of VPD and unaccounted-for effects could have generated spurious associations between some VPD and efficacy data.

Our findings of greater efficacy of fungi against thrips on impatiens foliage vs. flowers suggest not only that environmental conditions within the crop canopy are more favorable for fungal activity, but also that thrips eclosed on impatiens foliage in mature crops remain on the foliage at least long enough to become infected and succumb to mycosis. Studies by Ugine et al. (2006) have shown that immature thrips are not strongly attracted to pollen. Our findings that moisture conditions during the two PSP preceding a sample strongly predict efficacy on foliage, support these conclusions (Fig. 9A). The high-volume sprays applied in the present study likely penetrated the clusters of new foliage at the meristems and other cryptic sites within the crop canopy. Unfortunately, however, management of thrips is not possible without also achieving control of the populations in flowers. Flowers are extraordinarily attractive to adult female thrips, even before they have fully opened (Ugine et al., 2006), and the nectary and stems of fresh flowers are common oviposition sites (unpubl. observations). The population of thrips in flowers includes adult females of diverse age, which likely accounts for the poor prediction of efficacy on flowers by conditions following just two previous spray applications (Fig 9B). Considering thrips behavior and the dry environment they encounter feeding on the androecia of single-impatiens flowers, it is not surprising that thrips control in our tests required a very high level of

565 ambient humidity ($\geq 80\%$) for an extended period of 34 h following each spray. Presumably,
566 achieving complete control (population reductions) of thrips in impatiens would require near-
567 saturation conditions for a period approaching the 48 h suggested by Shipp et al. (2003).

568 In light of the inconsistencies in efficacy that have constrained mycoinsecticide use
569 against WFT, researchers have sought alternative approaches for their deployment.
570 Laboratory studies indicate that applications of spore suspensions targeting the prepupal
571 and pupal stages of this pest in the relatively stable, cool, moist environment of the
572 soil/potting medium, could be more effective (Helyer et al., 1995; Ansari et al., 2007, 2008).
573 Skinner et al. (2012) have demonstrated an alternative approach of inoculating the soil
574 surface with mycelial granules that undergo intensive sporulation post-application, but
575 implementation of this technology would have to address issues of increased risks of
576 inhalation exposure to greenhouse workers. Neither approach has been extensively tested
577 in open (uncaged) greenhouse crops. In preliminary greenhouse tests, we have recorded
578 50% reductions in WFT populations on potted impatiens following weekly soil-drench
579 applications of conidia under ambient RH conditions too low to support efficacy of the
580 intensive foliar spray programs described herein. (unpubl. data). It has been suggested that
581 an ideal biological control strategy would target both the soil- and foliage-dwelling stages
582 of WFT (Ansari et al., 2008), but efficacy of such dual applications, and the relative
583 contributions of each component toward thrips control have not been researched.

584 Finally, it warrants reiteration that we do not anticipate that efficacy of fungal pathogens
585 will, in all cases, be dependent upon ambient moisture conditions as high as those observed in
586 our tests. We selected impatiens as our study host specifically due to the challenge it poses to
587 activity of fungal pathogens. Much additional research is needed to assess the microbial

biocontrol potential of these agents against thrips infesting the exceedingly broad variety of crops produced in greenhouses under a great range of environmental conditions. In particular, ambient RH conditions can vary markedly across geographic regions and over different times of the year. Research is also needed to determine the potential of formulation (e.g., formulation in oil or oil-in-water emulsions) to enhance efficacy. This is particularly important in that we do not consider twice-weekly applications of entomopathogenic fungi to be a sustainable approach to thrips management. Such intensive spray programs put great pressure on pest populations to develop resistance and on pest managers to find alternative, less costly solutions. As concluded in our previous study (Ugine et al. 2007a), use of these pathogens for thrips management must be viewed in the context of well designed and rigorously implemented IPM systems.

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Figure legends

Fig. 1. Trends in western flower thrips populations treated with *Metarhizium brunneum* strain F52 during three greenhouse tests under variable moisture conditions. Relative humidity $\geq 80\%$ for an average of 6.8 (A), 28.6 (B), and 32.2 (C) hours during post-spray periods (see tests 5, 7, and 6, respectively, Table 4). Tests 6 and 7 were initiated prior to impatiens flowering. Vertical lines represent standard errors of means ($n = 2$); for clear presentation of standard errors, means are offset on the y-axis.

Fig. 2. Trends in western flower thrips populations treated with *Beauveria bassiana* strain GHA during two greenhouse tests under variable moisture conditions. Relative humidity $\geq 80\%$ for an average of 21.3 (A) and 36.2 (B) hours during post-spray periods (see tests 5, 7, and 6, respectively, Table 4). Vertical lines represent standard errors of means ($n = 2$); for clear presentation of standard errors, means are offset on the y-axis.

Fig. 3. Linear regressions of percent control of western flower thrips on mean relative humidity. Percent control determined from samples of impatiens foliage (A) and flowers (B) from plots treated with entomopathogenic fungi in 11 greenhouse tests described in Table 1; tests 4–8 (2011–2012), *Metarhizium spp.* (open squares); tests 1–3 (2011–2012), *Beauveria bassiana* strain GHA (open circles), tests 9–11 (2002–2004), *B. bassiana* GHA (filled circles); regressions fit to data from tests 1–8 (solid line) vs. all tests (dashed line). Graph C: regressions fit independently to *Beauveria* vs. *Metarhizium* data from Fig B.

Fig. 4. Linear regressions of percent control of western flower thrips on mean vapor pressure deficit. Percent control determined from samples of impatiens foliage (A) and flowers (B) from plots treated with entomopathogenic fungi in 11 greenhouse tests described in Table 1; tests 4–8 (2011–2012), *Metarhizium spp.* (open squares); tests 1–3 (2011–2012), *Beauveria bassiana* strain GHA (open circles), tests 9–11 (2002–2004), *B. bassiana* GHA (filled circles); regressions fit to data from tests 1–8 (solid line) vs. all tests (dashed line). Graph C: regressions fit independently to *Beauveria* vs. *Metarhizium* data from Fig B.

Fig. 5. Linear regressions of percent control of western flower thrips on mean relative humidity recorded for 40 hours following each spray application (post-spray periods). Percent control determined from samples of impatiens foliage and flowers from plots treated with entomopathogenic fungi in 11 greenhouse tests described in Table 1; tests 4–8 (2011–2012), *Metarhizium spp.* (open squares); tests 1–3 (2011–2012), *Beauveria bassiana* strain GHA (open circles), tests 9–11 (2002–2004), *B. bassiana* GHA (filled circles); regressions fit to data from tests 1–8 (solid line) vs. all tests (dashed line).

Fig. 6. Linear regressions of percent control of western flower thrips on mean vapor pressure deficit for 40 hours following each spray application (post-spray periods). Percent control determined from samples of impatiens foliage and flowers from plots treated with entomopathogenic fungi in 11 greenhouse tests described in Table 1; tests 4–8 (2011–2012), *Metarhizium spp.* (open squares); tests 1–3 (2011–2012), *Beauveria bassiana* strain GHA (open circles), tests 9–11 (2002–2004), *B. bassiana* GHA (filled circles); regressions fit to data from tests 1–8 (solid line) vs. all tests (dashed line).

Fig. 7. Linear regressions of percent control of western flower thrips on mean hours relative humidity $\geq 80\%$ during the 40-hour periods following each spray application (post-spray periods). Percent control determined from samples of impatiens foliage and flowers from plots treated with entomopathogenic fungi in 11 greenhouse tests described in Table 1; tests 4–8 (2011–2012), *Metarhizium spp.* (open squares); tests 1–3 (2011–2012), *Beauveria bassiana* strain GHA (open circles), tests 9–11 (2002–2004), *B. bassiana* GHA (filled circles); regressions fit to data from tests 1–8 (solid line) vs. all tests (dashed line).

770

771 Fig. 8. Linear regressions of percent control of western flower thrips on mean hours vapor
772 pressure deficit ≤ 0.5 kPa during the 40-hour periods following each spray application (post-
773 spray periods). Percent control determined from samples of impatiens foliage and flowers from
774 plots treated with entomopathogenic fungi in 11 greenhouse tests described in Table 1; tests 4–8
775 (2011–2012), *Metarhizium spp.* (open squares); tests 1–3 (2011–2012), *Beauveria bassiana*
776 strain GHA (open circles), tests 9–11 (2002–2004), *B. bassiana* GHA (filled circles); regressions
777 fit to data from tests 1–8 (solid line) vs. all tests (dashed line).

778

779 Fig. 9. Linear regressions of percent control of western flower thrips on mean hours $RH \geq 80\%$
780 during the 40-hour periods (post-spray periods) following the last two spray applications. Percent
781 control determined from samples of impatiens foliage and flowers from plots treated with
782 entomopathogenic fungi in 11 greenhouse tests described in Table 1; tests 4–8 (2011–2012),
783 *Metarhizium spp.* (open squares); tests 1–3 (2011–2012), *Beauveria bassiana* strain GHA (open
784 circles), tests 9–11 (2002–2004), *B. bassiana* GHA (filled circles); regressions fit to data from
785 tests 1–8 (solid line) vs. all tests (dashed line).

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Table 1. Greenhouse tests of three entomopathogenic fungi against western flower thrips on impatiens.

Greenhouse test (date)	Fungus - strain	Application intervals (no. of applications)	Thrips/stem or plant ^a (% control) ^b		Thrips/flower ^a (% control) ^b	
			Control	Treated	Control	Treated
1. 3–20 Jun 2011	<i>Beauveria bassiana</i> GHA	3–4 days (5) ^c	—	—	6.0	2.7 (55.0)
2. 10 Jan–2 Feb 2012	<i>B. bassiana</i> GHA	3–4 days (6) ^c	11.6	4.9 (57.8)	22.9	15.3 (33.2)
3. 3–22 Feb 2012	<i>B. bassiana</i> GHA	3–4 days (5) ^c	7.1	1.1 (84.5)	14.8	4.7 (68.2)
4. 3–20 June 2011	<i>Metarhizium brunneum</i> F52	3–4 days (5) ^c	—	—	5.4	3.2 (40.7)
5. 13 Dec 2011–6 Jan 2012	<i>Metarhizium brunneum</i> F52	3–4 days (6) ^c	4.9	3.7 (24.5)	6.0	8.3 (0)
6. 28 Feb–19 Mar 2012	<i>M. brunneum</i> F52	3–4 days (5) ^c	7.1	1.7 (76.1)	21.8	5.6 (74.3)
7. 10 Apr–3 May 2012	<i>M. brunneum</i> F52	3–4 days (6) ^c	7.8	2.0 (74.4)	14.3	6.9 (51.7)
8. 28 Feb–19 Mar 2012	<i>Metarhizium anisoliae</i> s.l. ESC-1	3–4 days (5) ^c	5.5	1.6 (70.9)	19.1	8.3 (56.5)
9. Ugine et al. (2007) 25 Jul–15 Aug 2002	<i>B. bassiana</i> GHA	3 days (7) ^d	—	—	19.3	14.6 (24.4)
10A. Ugine et al. (2007) 27 May–16 Jun 2003	<i>B. bassiana</i> GHA	5 days (4) ^d	2.6	1.0 (61.5)	—	—
10B. Ugine et al. (2007) 17 Jun–10 Jul 2003	<i>B. bassiana</i> GHA	5 days (4) ^d	—	—	13.0	10.3 (20.8)
11A. Ugine et al. (2007) 26 Jun–19 Jul 2004	<i>B. bassiana</i> GHA	5 days (4) ^d	0.43	0.25 (41.9)	—	—
11B. Ugine et al. (2007) 16 Jul–2 Aug 2004	<i>B. bassiana</i> GHA	5 days (3) ^d	—	—	2.13	0.87 (59.2)

^a Total thrips (nymphs + adults) per sample unit recorded from the final sample, with exception of test 2-foliage: total = mean number of thrips collected over the final 3 samples. Sample units: terminal section of stem, including meristem and 1 expanded leaf (2012); whole vegetative (pre-flowering) plant (2002–2004); fresh flower with pollen (all tests).

^b Percent reduction in thrips population relative to control(s).

^c Alternating 3- and 4-day intervals (applications made twice weekly). Applications applied at the rate of 2.15×10^{14} viable conidia in a volume of 4074 L aqueous carrier / hectare. Relative humidity was augmented following spray applications in tests 3, 6, 7, and 8 (data in bold type).

^d Applications applied at the rate of 4.9×10^{13} viable conidia in 935 L aqueous carrier/ ha (2002) or 2×10^{14} conidia in 3740 L/ ha (2003–2004) as described by Ugine et al. (2007).

Table 2

Table 2. Probit analysis of data from bioassays of three entomopathogenic fungi against early-second-instar western flower thrips.

Fungus	Number of assays	Range of doses (mortality) ^a	Mean LC ₅₀ ^{b c} (log LC ₅₀ ± SE)	Slope ± SE ^{bc}	Mean heterogeneity chi-square (range) ^d	Mean control mortality (%) (range)
<i>B. bassiana</i> strain GHA	6	9–474 (0–83)	193 (2.285 ± 0.223) a	1.163 ± 0.129 a	3.48 (0.05–12.6)	11.0 (2.8–15.8)
<i>M. brunneum</i> strain F52	4	17–297 (0–96)	161 (2.206 ± 0.385) a	1.29 ± 0.301 a	2.25 (0.02–6.2)	0.4 (0–1.7)
<i>M. anisopliae</i> s.l. strain ESC-1	3	24–326 (17–96)	72 (1.857 ± 0.049) a	1.825 ± 0.491 a	1.0 (0.3–1.7)	0.5 (0–1.4)

^a Range of doses (viable conidia/mm² leaf surface) tested and range of observed mortality (%) over all assays.

^b Median lethal concentration (LC₅₀) estimated from replicated three-dose bioassays (12 thrips/bioassay unit, 2 units/dose; 4–6 units of controls).

^c Means of log LC₅₀s and slopes followed by same letter are not significantly different (Welch ANOVAs: LC₅₀s F_{2,5.3} = 1.9, P = 0.24; slopes F_{2,3.8} = 0.75, P = 0.53).

^d Goodness-of-fit heterogeneity chi-square (Finney, 1971) with 1 degree of freedom.

Table 3. Environmental conditions during eleven greenhouse tests of entomopathogenic fungi against western flower thrips and accuracy of temperature and moisture variables as predictors of fungal efficacy.

	Percent control: foliage / flowers ^a	Mean temperature (°C)	Mean daily max. / min. temperature	Mean relative humidity (%)	Mean daily max. / min. RH	Mean vapor pressure deficit (kPa)	Mean daily max. / min. VPD
Test 1	– / 55.0	22.5	28.2 / 18.3	75	94 / 53	0.76	1.79 / 0.13
Test 2	57.8 / 33.2	21.1	27.5 / 16.4	77	94 / 60	0.64	1.50 / 0.14
Test 3	84.5 / 68.2	19.7	27.0 / 15.8	84	98 / 59	0.44	1.46 / 0.04
Test 4	– / 40.7	24.5	32.8 / 18.9	66	91 / 37	1.27	3.32 / 0.21
Test 5	24.5 / 0	22.8	25.1 / 20.5	45	62 / 29	1.53	2.21 / 0.98
Test 6	76.1 / 74.3	22.4	30.6 / 17.4	72	97 / 38	0.97	2.91 / 0.07
Test 7	74.4 / 51.7	20.6	25.3 / 16.6	71	98 / 36	0.78	2.07 / 0.04
Test 8	70.9 / 56.5	21.0	28.6 / 17.0	74	96 / 40	0.83	2.50 / 0.07
Tests 1–8 R ² ^b SER (P) Foliage Flowers		0.536 18.9 (0.317) <i>0.133</i> 25.7 (0.700)		0.936 7.1 (0.016) <i>0.815</i> 11.9 (0.015)		0.899 8.8 (0.032) <i>0.620</i> 17.0 (0.089)	
Test 9	– / 24.4	24.4	31.7 / 18.5	72	91 / 45	1.05	2.61 / 0.21
Test 10A	61.5 / –	20.9	24.0 / 19.3	63	76 / 49	0.93	1.49 / 0.55
Test 10B	– / 20.8	22.1	25.9 / 19.7	76	90 / 62	0.66	1.28 / 0.24
Test 11A	41.9 / –	22.6	29.4 / 17.0	65	85 / 43	1.07	2.33 / 0.32
Test 11B	– / 59.2	21.6	26.5 / 17.7	87	96 / 72	0.37	0.93 / 0.09
Tests 1–11 R ² ^b SER (P): Foliage Flowers		0.603 14.8 (0.100) <i>0.208</i> 22.4 (0.393)		0.820 10.0 (0.014) <i>0.756</i> 12.4 (0.004)		0.850 9.1 (0.009) <i>0.581</i> 16.3 (0.031)	

^a Percent control western flower thrips from tests of entomopathogenic fungi described in Table 1, data from samples of foliage vs. flowers (plain vs. italicized text).

^b Coefficient of determination (R-squared), standard error of the regression (SER), probability of type I error (P) testing significance of whole regression model (testing the hypothesis that all model parameters except the intercept are zero); tests 1–8, whole-model F-tests of foliage vs. flower data with 2,3 vs. 2,5 degrees of freedom; tests 1–11 with 2,5 vs. 2,8 df.

Table 4

Table 4. Environmental conditions following spray applications of entomopathogenic fungi against western flower thrips in eleven greenhouse tests and accuracy of temperature and moisture variables as predictors of fungal efficacy.^a

	Percent control: foliage / flowers ^b	Mean RH (%)	Mean VPD (kPa)	Mean hrs. RH ≥ 70%	Mean hrs. VPD ≤ 0.8 kPa	Mean hrs. RH ≥ 80%	Mean hrs. VPD ≤ 0.5 kPa	Mean hours RH ≥ 90%	Mean hrs. VPD ≤ 0.2 kPa	Mean hrs RH ≥ 80% following the last two sprays	Mean hrs VPD ≤ 0.5 kPa following the Last two sprays
Test 1	– / 55.0	81	0.57	30.8	29.6	23.7	22.8	13.7	11.2	23.8	23.5
Test 2	57.8 / 33.2	81	0.49	34.8	33.1	21.3	23.2	11.5	11.3	22.5	21.8
Test 3	84.5 / 68.2	95	0.16	38.9	38.3	36.2	35.6	31.4	29.7	36.0	34.8
Test 4	– / 40.7	77	0.91	25.2	24.9	19.4	19.2	13.1	10.2	14.3	15.7
Test 5	24.5 / 0	55	1.23	7.8	6.8	0.3	0.1	0	0	0	0
Test 6	76.1 / 74.3	84	0.59	32.6	32.2	29.5	28.9	24.3	23.6	27.3	27.3
Test 7	74.4 / 51.7	78	0.58	28.6	28.6	25.1	25.3	20.4	19.4	23.0	23.5
Test 8	70.9 / 56.5	88	0.40	33.0	33.0	31.0	30.8	28.3	27.5	29.3	29.0
Tests 1–8 R ² ^c		0.908 8.4 (0.028)	0.912 8.2 (0.026)	0.894 9.0 (0.034)	0.910 8.3 (0.027)	0.959 5.6 (0.008)	0.956 5.8 (0.009)	0.903 8.6 (0.030)	0.902 8.7 (0.031)	0.950 6.2 (0.011)	0.958 5.7 (0.009)
SER		0.835	0.718	0.801	0.812	0.900	0.866	0.805	0.763	0.872	0.888
(P):		11.2	14.7	12.3	12.0	8.7	10.1	12.2	13.4	9.9	9.2
Foliage		(0.011)	(0.042)	(0.018)	(0.006)	(0.003)	(0.007)	(0.017)	(0.011)	(0.006)	(0.004)
Flowers											
Test 9	– / 24.4	73	0.97	24.4	23.6	18.2	18.2	9.0	6.4	20.0	21.5
Test 10A	61.5 / –	62	0.91	15.7	19.4	8.2	8.5	2.0	1.3	16.3	17.0
10B	– / 20.8	76	0.65	31.0	32.0	16.3	15.9	3.8	1.9	25.2	23.5
11A	41.9 / –	65	1.00	16.2	22.4	9.6	10.2	3.6	3.2	14.3	15.0
Test 11B	– / 59.2	86	0.41	36.0	34.3	31.4	29.7	18.7	15.2	36.0	33.7
Tests 1–11 R ² ^c		0.741 12.0 (0.034)	0.789 10.8 (0.020)	0.726 12.3 (0.039)	0.783 11.0 (0.008)	0.834 9.6 (0.011)	0.821 10.0 (0.014)	0.795 10.7 (0.019)	0.786 10.9 (0.021)	0.902 7.4 (0.003)	0.915 6.8 (0.002)
SER		0.842	0.711	0.767	0.724	0.908	0.882	0.831	0.791	0.793	0.815
(P):		10.0	13.5	12.1	13.2	7.6	8.6	10.3	11.5	11.4	10.8
Foliage		(<0.001)	(0.007)	(0.003)	(0.002)	(<0.001)	(<0.001)	(<0.001)	(0.002)	(0.002)	(0.001)
Flowers											

^a Average relative humidity (RH), vapor pressure deficit (VPD), and number of hours of high humidity or low VPD recorded during the 40-hour post-spray periods (from the evening through the second night after each spray) from tests described in Table 1.

^b Percent control of western flower thrips from tests of entomopathogenic fungi described in Table 1.

^c Coefficient of determination (R-squared), standard error of the regression (SER), probability of type I error (P) testing significance of whole regression model (testing the hypothesis that all model parameters except the intercept are zero), data from samples of foliage vs. flowers (plain vs. italicized text).

Table 5. Estimated (inverse-predicted) environmental conditions supporting predicted levels of control of western flower thrips on impatiens in greenhouses.

Environmental Variables	ANOVA F-tests of main effect fungus ^a		Fungus	Predicted % control	Environmental moisture conditions required to achieve predicted control (95% confidence interval)	
	Foliage	Flowers			Foliage	Flowers
Mean relative humidity (%)	$F_{1,5} = 0.17$ $P = 0.23$	$F_{1,8} = 0.14$ $P = 0.009$	<i>Metarhizium</i>	70	71 (63 – 83)	77 (71 – 89)
			<i>Beauveria</i>	70	78 (69 – 90)	90 (84 – 100)
			<i>Metarhizium</i>	80	77 (69 – 94)	81 (74 – 96)
			<i>Beauveria</i>	80	84 (76 – 100)	94 (88 – 100)
Mean vapor pressure deficit (kPa)	$F_{1,5} = 4.4$ $P = 0.089$	$F_{1,8} = 4.5$ $P = 0.068$	<i>Metarhizium</i>	70	0.89 (1.08 – 0.62)	0.69 (0.96 – 0)
			<i>Beauveria</i>	70	0.63 (0.82 – 0.36)	0.24 (0.50 – 0)
			<i>Metarhizium</i>	80	0.73 (0.93 – 0.36)	0.53 (0.84 – 0)
			<i>Beauveria</i>	80	0.47 (0.67 – 0.10)	0.09 (0.39 – 0)
Mean RH during post-spray periods (PSP) ^b	$F_{1,5} = 0.03$ $P = 0.86$	$F_{1,8} = 3.7$ $P = 0.089$	<i>Metarhizium</i>	70	83 (70 – 100)	88 (83 – 97)
			<i>Beauveria</i>	70	83 (70 – 100)	95 (89 – 100)
			<i>Metarhizium</i>	80	91 (78 – 100)	93 (87 – 100)
			<i>Beauveria</i>	80	91 (79 – 100)	99 (93 – 100)
Mean VPD during PSP	$F_{1,5} = 0.08$ $P = 0.78$	$F_{1,8} = 2.1$ $P = 0.18$	<i>Metarhizium</i>	70	0.53 (0.81 – 0.06)	0.36 (0.59 – 0)
			<i>Beauveria</i>	70	0.47 (0.75 – 0)	0.15 (0.36 – 0)
			<i>Metarhizium</i>	80	0.33 (0.61 – 0)	0.22 (0.47 – 0)
			<i>Beauveria</i>	80	0.27 (0.55 – 0)	0 (0.24 – 0)
Mean hours RH $\geq 80\%$ during PSP	$F_{1,5} = 0.54$ $P = 0.50$	$F_{1,8} = 2.7$ $P = 0.14$	<i>Metarhizium</i>	70	27.4 (18.7 – 40.4)	32.4 (28.4 – 38.1)
			<i>Beauveria</i>	70	24.8 (16.1 – 37.8)	36.4 (32.6 – 42.0)
			<i>Metarhizium</i>	80	34.4 (25.6 – 52.5)	36.9 (32.3 – 43.8)
			<i>Beauveria</i>	80	31.8 (22.9 – 49.9)	40.9 (36.4 – 47.7)
Mean hours VPD ≤ 0.5 during PSP	$F_{1,5} = 0.29$ $P = 0.61$	$F_{1,8} = 2.1$ $P = 0.18$	<i>Metarhizium</i>	70	27.2 (18.2 – 41.0)	32.2 (27.7 – 38.8)
			<i>Beauveria</i>	70	25.3 (16.3 – 39.2)	36.1 (31.8 – 42.6)
			<i>Metarhizium</i>	80	34.1 (25.0 – 53.7)	36.6 (31.5 – 44.8)
			<i>Beauveria</i>	80	32.3 (23.2 – 51.9)	40.5 (35.6 – 48.6)

^a Main effect fungus = logit transformed percent control of western flower thrips on impatiens foliage vs. flowers achieved from spray applications of *Beauveria bassiana* strain GHA and two *Metarhizium* spp. in eleven greenhouse tests described in Table 1.

^b Mean conditions averaged across the 40-hour post-spray periods (from the evening through the second night after each spray) from the tests described in Table 1.

Figure 1 - Revised

Figure 2 - Revised

Figure 3 - Revised

Figure 4 - Revised

Figure 5 - Revised

Figure 6 - Revised

Figure 7 - Revised

Figure 8 - Revised

Figure 9 - Revised