A tritrophic effect of host plant on susceptibility of western flower thrips to the entomopathogenic fungus *Beauveria bassiana*

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Abstract

Adult female western flower thrips (*Frankliniella occidentalis*) were exposed 12–24 h to bean (*Phaseolus vulgaris*) and impatiens (*Impatiens wallerana*) leaf disks treated with *Beauveria bassiana* conidia and then transferred to clean bean or impatiens at various times post-treatment. Significantly greater levels of fungal infection were observed when thrips were treated on bean versus impatiens, but exposure to impatiens following treatment had no effect on fungal infection (percent mortality). This result, combined with observations of no inhibition of germination of conidia exposed to intact or macerated impatiens foliage, indicated that the negative effect of the impatiens host plant was not due to plant chemical compounds (antibiosis). Further observations revealed that insects acquired (picked-up) 75% more conidia from treated bean disks than from treated impatiens disks. This difference in dose acquisition was determined to account for the observed difference in percent mortality (15%) following treatment on the two host plants. Median lethal doses (LD$_{50}$) of *B. bassiana* were not significantly different on the two host plants, but median lethal concentrations were nearly 7-fold greater on impatiens. This difference was explained by disproportionate rates of conidial acquisition at measured rates of conidial deposition (an inverse relationship was observed between application rate expressed as conidia/mm$^2$ and the number of conidia acquired). The mechanism underlying the differential rates of conidial acquisition from bean versus impatiens was not determined.

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1. Introduction

Efficacy of hyphomycete pathogens is constrained by numerous biotic and abiotic factors. Despite the great potential of mycopathogens as insect management alternatives, application of these pathogens frequently results in poor or inconsistent pest control in the field and greenhouse and their use has not been widely adopted. Unfavorable environmental conditions, including low relative humidity, high temperature, rainfall, and solar radiation are most frequently cited as the principal constraints, and assessments of the biocontrol efficacy of fungal pathogens have focused on these factors (Inglis et al., 2002; Hajek, 1997; Glare and Milner, 1991; Goettel et al., 2000). Recent studies, for example, have identified insect host body temperature as a key efficacy determinant in rangeland grasshoppers (Inglis et al., 1996; Thomas and Jenkins, 1997), and detailed study of temperature relations is generating increasingly more precise criteria for selection of fungal isolates with greater tolerance for the high temperature conditions commonly encountered in the field and greenhouse.

In any selected agricultural system, however, a great many factors directly and indirectly determine efficacy of biocontrol agents, and few of these have been as thoroughly investigated as temperature and moisture. Biotic factors in particular, have received relatively little study. Among the most important yet poorly studied biotic factors are the multi-trophic interactions among the host plant, insect pests, and insect pathogenic fungi. Plants are known to produce a diverse array of chemicals with
defensive properties (Cowan, 1999), and sequesteration of these chemicals by insects is a well-known defense strategy. These materials typically make the insect toxic or otherwise unpalatable to vertebrate and invertebrate natural enemies, and use of this strategy is often advertised by bold warning colors. Entomologists and insect mycopathologists have postulated a similar phenomenon in which antifungal phytochemicals sequestered by insects confer resistance to fungal infection (Gould and Massey, 1984; Ramoska and Todd, 1985; Gallardo et al., 1990; Tallamy et al., 1998; Poprawski et al., 2000; Poprawski and Jones, 2000). It is also commonly hypothesized that antifungal compounds in leaf exudates or leaf volatiles may come into direct contact with conidia of entomopathogenic fungi and inhibit the germination or infection processes (Brown et al., 1995; Vega et al., 1997; Lacey and Mercadier, 1998; Inyang et al., 1999a,b). Yet, few of the studies reporting these phenomena have unequivocally demonstrated acquisition/sequestration of a phytochemical compound at a concentration in or on the body of an insect that confers significant resistance to an entomopathogenic fungus. Additionally, few alternative mechanisms of host plant effects on efficacy of entomopathogenic fungi have been examined, for example, differential levels of phyllosphere humidity or rates of conidial acquisition (pick-up) from treated plant surfaces. Inyang et al. (1998) reported that a slightly greater percentage of flea beetle larvae acquired conidia when exposed to treated leaves of oilseed rape compared to Chinese cabbage or turnip, but the insects treated on cabbage and turnip showed greater rates of mortality. It was consequently hypothesized that the oilseed rape possessed fungistatic compounds that interfered with fungal infection.

Ugine et al. (2005a) showed that Beauveria bassiana (Balsamo) Vuillemin (strain GHA) was virulent against adult female western flower thrips (WFT), Frankliniella occidentalis (Pergande), exposed to bean leaf foliage treated with low dosages of conidia. However, direct application of B. bassiana against WFT infesting greenhouse-grown crops of garden impatiens, Impatiens wallerana (Hook) F., repeatedly showed poor efficacy (Ugine et al., 2006). Thus, studies were conducted to determine if host plant, beans versus impatiens, affected thrips susceptibility to B. bassiana and to elucidate the mechanism underlying the putative host plant effect.

2. Materials and methods

2.1. Insects

Western flower thrips adults collected from a Cornell University research greenhouse were used to establish a laboratory colony. Thrips were continuously reared on excised leaves of red kidney beans (Phaseolus vulgaris L.) in small plastic containers (16 cm x 16 cm x 6 cm) with snap-on lids. A hole (10.5 x 10.5 cm) was cut in the lid and covered with thrips-proof organdy (mesh openings of 95 μm) (Sefar America Inc., Kansas City, MO), to allow for ventilation. Containers were maintained at 28 ± 1°C under a 16:8 h light/dark regime.

Adult female western flower thrips (25–30) were added to a rearing container and allowed to oviposit for 24 h. Eleven days after initial infestation of a rearing container, the thrips population comprised primarily newly eclosed adult thrips (<24 h old). From this population, adult female thrips were collected via aspiration into modified 1 ml centrifuge tubes (bottoms removed and covered with screening). The vials were then randomly assigned to the treatments associated with an assay. For additional details of the rearing/selection processes, see Ugine et al. (2005a).

2.2. Plant material

Impatiens plants, var. ‘SuperElfin; white’, used in all experiments were purchased as plugs though a commercial plug producer (Cal Seedling Company, Arroyo Grande, CA). Plugs were sown with Pro-Mix BX (Premier Horticulture Inc., Quakertown, PA) into standard 36-cell flats (one plug/cell, each cell measuring ca. 6 x 5 x 5.5 cm), grown at 25°C (range of 17–30°C) in a greenhouse without supplemental lighting.

Red kidney beans were sewn in 30-cm diameter pots, 20–30 plants per pot, using Pro-Mix BX. Pots were grown in a walk in environmental chamber with VHO fluorescent lighting, at 25°C, 16:8 h L/D.

2.3. General bioassay protocol

Leaf disks for use in assays were cut from the first true leaves of bean plants that were at least 12 days old, and from impatiens leaves located in the top 1/3 of the canopy. The abaxial surfaces of leaf disks (2 cm diameter) were spray treated with the appropriate conidial preparation using a Burgerjon spray tower (Burgerjon, 1956). The tower was fitted with an air-atomizing nozzle (Fluid Cap 2850 + Air Cap 70) mounted in a 1/4 J nozzle body (Spray Systems Co., Wheaton, IL) connected to a regulator valve providing a constant airflow of 10 l/min. Spray targets were positioned on a rotating turntable (33 rpm) during application, and spray deposition at the level of the target surface was approximately 0.01 μl/mm² (resulting from spraying of a 5 ml aliquot). Two leaf disks, which were allowed to dry before being added to one-ounce portion cups, were placed with their abaxial surfaces together, between two filter paper disks. Each piece of filter paper was moistened with 35 μl deionized water. For a more detailed description of the leaf-disk application bioassay and procedures, see Ugine et al. (2005a). A randomly selected group of six newly emerged adult female thrips was added to each assay cup, which was then sealed with Parafilm “M”® (Pechiney Plastic Packaging, Chicago, IL) and capped with the plastic snap-on lids provided by the manufacturer. In all tests described herein, bioassay cups and petri dishes with conidial deposits were incubated at
25 ± 1 °C in a growth chamber under a natural light regime (incubator with glass-door in a laboratory with unshaded windows).

2.4. Fungal preparations

Conidia of *B. bassiana* strain GHA were produced and formulated by Emerald BioAgriculture Corp. (Butte, MT) using proprietary methods and ingredients. A clay-based wettable powder (WP) formulation (BotaniGard 22WP) containing 4.4 × 10^10 conidia/g was used in all assays and was stored at 4 °C. Stock suspensions were prepared by mixing ca. 15–133 mg wettable powder in 20 ml aliquots of deionized water in 50-ml plastic centrifuge tubes. One gram of glass beads (2.0 mm diameter) was added to each tube, and the tubes were agitated on a wrist action shaker (Model BT, Burrell Scientific, Inc., Pittsburgh, PA) set at maximum speed (6.7 oscillations/s) for 15 min. The resulting suspensions were either used as prepared or diluted to produce lower concentrations.

2.5. Quantification of spray application rates and acquired doses

Numbers of conidia deposited on the leaf disks were estimated from sample deposits collected on polystyrene petri dish lids (90 mm diameter) placed on the target platform of the spray tower during each spray application. The lids were situated at the same radial distance from the platform center as the leaf disks. To quantify the conidia, a drop of lactic acid (85%) containing acid fuchsin (1 mg/ml), was placed onto the petri dish lid, covered with a coverslip, and viewed at 400x magnification. Numbers of conidia/mm^2 were determined using the procedure described by Wraight et al. (1998), except that 5 replicate counts were made per dish lid.

Conidia attached to both the dorsal and ventral surfaces of adult female thrips were enumerated following the protocol described by Ugine et al. (2005a). Thrips were mounted in lactic acid/acid fuchsin and lightly compressed between two glass cover slips taking care not to rupture the body wall. They were then examined under a phase contrast microscope at 400x magnification. Both the dorsal and ventral surfaces of each thrips were examined. Conidia-like objects on spray-carrier treated thrips (carrier controls) were also quantified, and the mean numbers of conidia on fungus-treated insects were corrected for these “background” numbers by subtracting the mean number of conidia-like objects from the count for each insect.

Additionally, a petri dish with yeast extract agar (1%) was sprayed along with the leaf disks in each test. The inoculated plates were sealed with Parafilm and incubated for 24 h at 25 °C. The spray deposits were then stained with acid fuchsin, and the first one hundred conidia encountered under phase contrast microscopy (400x) were scored for germination. This procedure was repeated at five randomly chosen locations per petri plate, and numbers of conidia in all assays were corrected for viability. In all cases, germination was ≥ 89 percent.

2.6. Effects of rearing host plant and treatment host plant on susceptibility of thrips to *B. bassiana*

To characterize/quantify host plant effects, adult female thrips were collected from the greenhouse-grown impatiens described above as well as from the laboratory colony maintained on bean. The greenhouse population of thrips had been maintained on impatiens for 3–5 months prior to use in assays. Thrips from these two sources were exposed to *B. bassiana*-treated bean or impatiens leaf disks for 24 h. Following treatment, treated leaf disks in each assay cup were removed and replaced with clean bean disks, and the cup was immediately resealed with parafilm and capped. Hereafter, the plant on which the thrips were reared prior to bioassay will be referred to as the rearing host, and the fungus-treated plant to which the thrips were exposed will be referred to as the treatment host. The resulting experimental design was a two-way factorial design (rearing host plant × treatment host plant). The comparative virulence of the BotaniGard 22WP was quantified against adult female thrips in a series of 5-dose bioassays. The WP was applied to leaf disks as aqueous suspensions containing 6.63, 2.21, 0.74, 0.22, and 0.02 mg powder/ml in the first two replicate bioassays, and due to high levels of mortality at several of the high doses, the range of concentrations was reduced to 0.74, 0.22, 0.02, 0.007, and 0.002 mg powder/ml for the subsequent four replicate assays. These reduced concentrations were predicted to produce depositions ranging from ca. 1 to 100 conidia/mm^2. Two to three replicate cups (12–18 adults) were assembled for each application concentration and a spray carrier blank (carrier ingredients without conidia). The carrier blank was applied at only one concentration, that corresponding to the highest concentration of the WP. The six bioassays were conducted on three occasions (dates) (2 replicate assays/date) over a period of several weeks. Assay cups were incubated for 5 days (24 h treatment + 96 h post-treatment) at 25 °C.

2.7. Effects of treatment host plant and exposure to impatiens on susceptibility of thrips to *B. bassiana*

A series of single-dose bioassays was conducted to determine if thrips' susceptibility to *B. bassiana* was affected by: (1) the duration of post-treatment exposure to impatiens foliage, and (2) the host plant on which thrips were exposed to *B. bassiana* conidia: beans versus impatiens.

Four treatment groups of assay cups were assembled. Each group comprised 8–10 cups, each with six adult female thrips taken from the laboratory colony on beans. As described for the virulence assays, all cups were incubated a total of 5 days at 25 °C. Treatment of the different groups is described below and outlined in the top half of Fig. 1.
**Group 1.** Thrips were introduced into assay cups with *B. bassiana*-treated bean leaf disks treated at a rate of 0.04 mg/ml. After 12 h the treated disks in half of the chambers were replaced with untreated bean disks, and the treated disks in the other half were replaced with untreated impatiens leaf disks. The insects remained on these disks for the remainder of the test. Half of the insects were thus exposed to untreated impatiens foliage for 108 h.

**Group 2.** Treatments were identical to group 1, except that the initial exposure to *B. bassiana* was for 24 h. Ultimately, half of the insects were exposed to impatiens foliage for 96 h. Because thrips were held on the treated bean disks for twice as long as in group 1, the application concentrations were halved (0.02 mg/ml) in an attempt to deliver equal dosages of conidia in each treatment group (assuming a constant rate of acquisition). The 0.02 and 0.04 mg/ml rates are identified as 1× and 2× rates in Fig. 1.

**Group 3.** Thrips were exposed to the 1× rate of *B. bassiana* for 24 h as in group 2, but after 24 h, the treated bean disks in all cups were replaced with clean bean disks. After an additional 24 h, the bean disks in half the chambers were replaced with clean bean disks and those in the other half were replaced with clean impatiens disks, resulting in exposure to impatiens foliage for 72 h.

**Group 4.** Thrips were exposed 24 h to bean foliage treated with carrier blank at the 2× rate. Then, the leaf disks were replaced with clean bean or impatiens disks.

To investigate effects of the treatment host plant on *B. bassiana* efficacy, the entire protocol described above was repeated exactly, except that the thrips were treated by exposure on impatiens leaf disks and each of the host plant (leaf disk) changes was to the alternative host plant (see lower half of Fig. 1). This protocol resulted in exposure to impatiens leaf disks for 12, 24, 48, and 120 h. Thrips used in these assays were also taken from the laboratory colony on bean.

The entire series of assays was repeated on three occasions over a period of ca. 1 month. On one occasion, the 48 h post-treatment transfers were mistakenly made after 24 h, resulting in an incomplete block design. In the presentation of results, the host plant to which the thrips were transferred after treatment is referred to as the transfer host.

### 2.8. Antibiosis

To determine if *B. bassiana* conidia were inhibited or inactivated by antifungal compounds associated with the leaf cuticle, conidia were sprayed onto five bean and five impatiens leaf disks. The leaf disks were then pressed into 1% yeast extract agar (2 ml) contained in 35 mm diameter petri dishes immediately after the spray droplets dried (0 h) and 24 h after the initial spray. An additional treatment was included to investigate whether any compounds found within the leaf tissue might be inhibiting or inactivating conidial germination. One macerated bean or impatiens leaf disk was added per 2 ml yeast extract agar immediately prior to the agar solidifying. Groups of five bean and five impatiens leaf disks were then pressed into these crude agar mixtures at the 0 h (1 leaf press per agar dish). Leaf pressed agar dishes were incubated at 25°C for 16–18 h. After incubation, a drop of lactic acid/acid fuchsin was applied to each plate and covered with a coverslip. The first one hundred conidia encountered under phase contrast microscopy (400×) were scored for germination.

### 2.9. Host-plant effects on dose acquisition and susceptibility of thrips to *B. bassiana*

To determine if thrips acquired different numbers of conidia as a function of host plant and to assess potential
effects of differential acquisition on fungal efficacy, a series of twelve four-dose bioassays were conducted on each of three dates over a period of ca. one month. Adult female thrips from the laboratory colony were exposed 24 h to *B. bassiana*-treated bean leaf disks and then transferred to clean bean disks. The same procedure was repeated using impatiens leaf disks. Mortality was recorded after a total of 5 days. Six assays were conducted using each host plant (two replicate assays per date); however, in two of the six impatiens assays, mortality of thrips was too low for estimation of LC$_{50}$s and LD$_{50}$s.

BotaniGard 22WP was applied at concentrations ranging from 0.002 to 6.63 mg powder/ml. These concentrations were predicted to produce depositions ranging from ca. 1 to 3000 conidia/mm$^2$. When spraying each concentration in each assay, the bean and impatiens leaf disks were placed together on the spray tower turntable to ensure that equal dosages were applied to each host plant. A total of two or three replicate assay cups were assembled for each application rate (12–18 females/rate). One insect from each cup within an application concentration group was removed 24 h post-application and was examined microscopically to determine the acquired dose as described above. Dose was ultimately reported as the mean number of viable conidia per adult female thrips. Adult females were incubated for only 24 h before dose counts were made despite the fact that conidial acquisition after 24 h could potentially contribute to mortality, given that adults do not molt. However, it was determined in preliminary assays that acquisition of conidia after 24 h did not significantly increase mortality within the timeframe of the bioassay (5 days). Each assay included 2–3 replicate cups of thrips exposed to the spray carrier containing no fungal conidia (carrier controls) at application rates equivalent to the highest rate of WP applied in the assay. Enumeration of conidia attached to thrips cuticle was a time-consuming process, and it was not possible to process large numbers of thrips during each assay. Therefore, to more accurately estimate acquisition rates, an additional series of assays was conducted in which acquired conidia were quantified as described above, but the assays were terminated at 24 h (not monitored for mortality assessments).

### 2.10. Statistical analysis

Computations for all experiments were made using the statistical software package JMP version 4 (SAS Institute, 2002). Analysis of variance (ANOVA) was conducted on the percent mortality of each treatment for the host plant switching experiments. Treatment mortalities were corrected for control mortality using Abbott’s correction (Abbott, 1925) and were transformed using the arcsine square root function prior to analysis of variance (ANOVA). Tests were conducted over time, and the test dates were considered as experimental blocks. Means separations, where applicable, were carried out using the Tukey HSD test. Estimates of the LD$_{50}$ and LC$_{50}$ of *B. bassiana* were obtained using probit analysis, carried out by the statistical program POLO-PC (LeOra Software, 1987). Treatment mortalities were corrected for control mortality by the POLO program. LC$_{50}$ and LD$_{50}$ values associated with the different host plant treatments were further analyzed by ANOVA, an approach that must take into account a number of complications. Median lethal concentration/dose is derived essentially as a ratio of two normally distributed variables (probit regression slope and intercept). Ratios typically have non-normal distributions, and those derived from normally distributed variables fit a distribution known as the Cauchy distribution. The Cauchy distribution is characterized by heavy tails and is often described as being outlier prone (Zimmerman, 1995). This attribute tends to inflate treatment variances, and simulation studies have demonstrated that ANOVA of Cauchy-distributed data results in a conservative test (alpha is lower than nominal and power to detect truly significant differences among means is reduced) (Zimmerman, 1995). Under these conditions, nonparametric ranking tests, which tend to minimize effects of outliers, exhibit greater power than conventional ANOVA and are strongly recommended over ANOVA (Zimmerman, 1995). However, there is no universally proven nonparametric alternative to multi-way ANOVA with interaction (Conover, 1999; Zar, 1999). In analyzing the data presented here, we have adopted the approach recommended by Conover (1999). Replicated LC$_{50}$ and LD$_{50}$ values were first analyzed by conventional ANOVA (in this case following log transformation) and then the analysis was repeated following rank transformation. ANOVA of rank-transformed data produces a test equivalent to the nonparametric Kruskal–Wallis test (Conover, 1999). If the results from the two analyses agree with respect to significance of main effects and interactions, the results of the conventional ANOVA are accepted as reliable.

### 3. Results

#### 3.1. Effects of rearing host plant and treatment host plant on susceptibility of thrips to *B. bassiana*

The LC$_{50}$ values generated from multi-dose assays of thrips reared on beans versus impatiens and exposed to *B. bassiana* on beans versus impatiens in all combinations are presented in Table 1. ANOVA revealed significant effects of test date, treatment host, and significant date × rearing host and treatment host × rearing host interactions. There were, however, no effects of rearing host, and the date × treatment host and three-way interactions were not significant (Table 2). These results were confirmed by the nonparametric test conducted on the ranks of the LC$_{50}$ values (Table 2). The LC$_{50}$ value for thrips exposed to *B. bassiana* on impatiens was 7.1 times greater compared to thrips exposed to *B. bassiana* on beans (grand means of 73.5 ± 14.7 and 10.4 ± 2.2, respectively). Further investigation of the treatment host × rearing host interactions
revealed a significant effect of treatment host and no effect of rearing host or treatment host × rearing host interaction on each test date. These results were also confirmed by a nonparametric test conducted on the ranks of the LC50 values for each date (Table 3). Slopes of the LC regression lines were not significantly affected by any main effect or interaction (Table 2).

3.2. Effects of treatment host plant and exposure to impatiens on susceptibility of thrips to B. bassiana

Thrips were exposed to B. bassiana for 12 or 24 h on treated bean or impatiens leaf foliage and then transferred to untreated bean or impatiens foliage at various time intervals. Exposure duration refers to the length of time thrips were exposed to impatiens foliage during an assay.

A preliminary analysis revealed that the number of times leaf disks were changed in the assay cups had no significant effect on thrips mortality \((F_{1,32} = 0.14, P = 0.71)\). Treatments that were equivalent with the exception of number of leaf disk changes (see Fig. 1) were therefore combined for subsequent analyses of thrips exposure to impatiens. Because of the differences on the times of exposure to impatiens in the bean treatment host versus impatiens treatment host data sets (Fig. 1), separate analyses were conducted to investigate this effect. ANOVA revealed that exposure to impatiens for 0–108 h following treatment on beans had no significant effect on fungal virulence \((F_{2,5} = 0.05, P = 0.95)\) (Table 4). In this test, date and the date × exposure duration interaction were also not significant \((F_{2,10} = 2.3, P = 0.15\) and \(F_{5,10} = 2.3, P = 0.15\), respectively). Similarly, ANOVA indicated no effect of impatiens exposure for 12–120 h in the impatiens treatment host assays \((F_{2,5} = 0.9, P = 0.46)\), and again, both date and the date × exposure duration interaction were not significant \((F_{1,5} = 5.8, P = 0.06\) and \(F_{5,5} = 1.7, P = 0.3\), respectively) (Table 4). It warrants note that the degrees of freedom in the above tests were reduced as a consequence of the incomplete block design.

A single analysis including all treatments from both data sets was conducted to assess the effect of the treatment host plant (bean versus impatiens). ANOVA revealed a significant effect of treatment host \((F_{1,26} = 10.2, P = 0.004)\) and no date × treatment host interaction \((F_{2,26} = 0.17, P = 0.85)\). Treatment of thrips on bean resulted in mortality 15 percentage points higher than treatment on impatiens (65.8% versus 51.1%) (Table 4).

3.3. Antibiosis

Percent germination of conidia that were pressed into 1% yeast extract agar after 0 and 24 h of exposure to bean and impatiens foliage did not differ significantly from one another (0 h: \(F_{1,8} = 1.4, P = 0.27\); 24 h: \(F_{1,8} = 0.14, P = 0.71\)). Mean (±SE) percent germination after 0 and 24 h was 94.0 ± 1.1% versus 89.6 ± 3.3%, and 94.6 ± 1.5% versus 95.5 ± 1.0%, from bean and impatiens foliage, respectively. Additionally, there was no significant difference in percent germination of conidia exposed to agar containing macerated bean versus impatiens foliage pressed at the 0 h \((F_{1,8} = 3.3, P = 0.11)\). Mean percent germination was 98.0 ± 1.3% and 95.0 ± 1.5% on agar containing beans and impatiens foliage, respectively.
3.4. Host-plant effects on dose acquisition and susceptibility of thrips to B. bassiana

Across the range of dosages applied, thrips exposed to treated bean leaf disks acquired approximately 75% more conidia than thrips treated on impatiens (Table 5). *Beauveria bassiana* was not equally efficacious on the two host plants (Table 6). There was a significant effect of both date and host plant on the LC50 estimates and there was no interaction between host plant and date (Table 7). The LC50 estimate for adult female thrips exposed to *B. bassiana* on impatiens foliage was 6.7 times greater compared to thrips exposed to the fungus on bean foliage (Table 6). Additionally, there was no effect of date or host plant on the slopes of the LC probit regression lines, although there was a significant interaction between host plant and date (Table 7). Estimates of LD50 values were not significantly affected by host plant or by the host plant × date interaction, while the main effect of date was statistically significant (Table 7). The mean LD50 on beans and impatiens was 34.5 and 26.8 conidia per insect, respectively (Table 6). There was no effect of host plant or the host plant × date interaction on the slopes of the LD regression lines. The results of all analyses (ANOVA’s) conducted on LC50 and LD50 estimates were confirmed with a nonparametric test conducted on the ranks of the estimates (Table 7). The slopes of LC regression lines were significantly lower on both host plants compared to the slopes of LD regression lines (Bean: $F_{[1,6]} = 9.7, P = 0.02$; Impatiens: $F_{[1,6]} = 8.7, P = 0.03$) (Table 5).

4. Discussion

Differential susceptibility of insects to entomopathogens as a function of host plant has most frequently been ascribed to host plant chemistry. In this study we investigated the role of host plant on susceptibility of western flower thrips exposed to *B. bassiana* on garden impatiens compared to kidney bean foliage to determine if host plant chemistry affects thrips susceptibility, and after finding no evidence of an effect of host plant chemistry, it became our objective to investigate the mechanism underlying the host plant effect.

In the first experiment, a set of tests was conducted to determine if rearing thrips on beans versus impatiens affected their susceptibility to fungal infection when exposed to *B. bassiana* treated bean or impatiens foliage. We determined that LC50 values were significantly affected by the host plant on which thrips were exposed to *B. bassiana*, the LC50 values were lower when thrips were treated on beans; however, this effect was complicated by a significant treatment host × rearing host interaction (Table 2). This interaction may be of some interest. When insects were reared on beans and tested on beans, they appeared less susceptible to infection compared to when they were reared on impatiens and tested on beans (LC50 13 versus 8 conidia/mm², respectively) (Table 1). Similarly, when
thrips were reared on impatiens and tested on impatiens, they appeared less susceptible to infection compared to when they were reared on beans and tested on impatiens (LC₅₀ 91 versus 56 conidia/mm², respectively) or tested

### Table 4
Mortality of thrips treated with a single dosage of B. bassiana conidia on bean versus impatiens leaf disks and exposed for varying periods of time to impatiens leaf disks during a five-day bioassay

<table>
<thead>
<tr>
<th>Treatment Host</th>
<th>Percent control mortality ± SEa</th>
<th>Corrected percent treatment mortality ± SEb</th>
<th>Weighted meanc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bean</td>
<td>0 h (3) 16.8 ± 4.7 A</td>
<td>0 h (8) 65.2 ± 6.4 a</td>
<td>65.8 ± 3.7 A</td>
</tr>
<tr>
<td></td>
<td>12 h (3) 120 h (3) 12 h (3)</td>
<td>72 h (2) 62.6 ± 12.4 a</td>
<td></td>
</tr>
<tr>
<td>Impatiens</td>
<td>7.0 ± 1.4 A</td>
<td>96 h (3) 69.4 ± 4.5 a</td>
<td>65.8 ± 3.7 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>108 h (3) 65.8 ± 8.6 a</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5
Numbers of conidia acquired by western flower thrips treated on bean versus impatiens leaf disks in laboratory bioassays

<table>
<thead>
<tr>
<th>Application rate (mg WP per ml)</th>
<th>Dosage (conidia/mm²)</th>
<th>Conidia / insect ± standard error (number of thrips examined)</th>
<th>Ratio: (beans/impatiens)</th>
<th>Dose acquisition rate on beana</th>
<th>Dose Acquisition rate on impatiensa</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1.9 ± 0.6 (11)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>0.002</td>
<td>4 ± 2.5</td>
<td>4.2 ± 1.5 (15)</td>
<td>1.08</td>
<td>1.050</td>
<td>0.097</td>
</tr>
<tr>
<td>0.02</td>
<td>9 ± 1.4</td>
<td>8.5 ± 1.9 (32)</td>
<td>5.2 ± 1.4 (32)</td>
<td>0.944</td>
<td>0.0578</td>
</tr>
<tr>
<td>0.22</td>
<td>70 ± 8.4</td>
<td>18.8 ± 4.2 (32)</td>
<td>10.5 ± 2.4 (33)</td>
<td>0.269</td>
<td>0.015</td>
</tr>
<tr>
<td>2.21</td>
<td>779 ± 61.2</td>
<td>40.6 ± 5.6 (34)</td>
<td>19.1 ± 2.6 (32)</td>
<td>0.052</td>
<td>0.025</td>
</tr>
<tr>
<td>6.63</td>
<td>3184 ± 188.9</td>
<td>82.9 ± 18.3 (20)</td>
<td>48.0 ± 7.6 (21)</td>
<td>0.026</td>
<td>0.015</td>
</tr>
<tr>
<td>Weighted mean</td>
<td></td>
<td>28.5 ± 3.7</td>
<td>16.4 ± 2.0</td>
<td>1.74</td>
<td></td>
</tr>
</tbody>
</table>

### Table 6
LC₅₀ and LD₅₀ estimates (±standard error) from bioassays of B. bassiana against adult female western flower thrips on bean or impatiens foliage

<table>
<thead>
<tr>
<th>Treatment host plant</th>
<th>No. of assays</th>
<th>LC₅₀ ± SE</th>
<th>Slope ± SE</th>
<th>Mean χ² (range)</th>
<th>LD₅₀ ± SE</th>
<th>Slope ± SE</th>
<th>Mean χ² (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bean</td>
<td>6</td>
<td>179.0 ± 53.0</td>
<td>0.71 ± 0.17</td>
<td>1.3 (0.26–2.79)</td>
<td>34.5 ± 8.6</td>
<td>1.62 ± 0.40</td>
<td>2.8 (0.22–9.1)</td>
</tr>
<tr>
<td>Impatiens</td>
<td>4</td>
<td>1195.5 ± 350.0</td>
<td>0.98 ± 0.22</td>
<td>4.3 (2.14–7.09)</td>
<td>26.8 ± 8.5</td>
<td>1.66 ± 0.26</td>
<td>4.3 (2.1–6.0)</td>
</tr>
</tbody>
</table>

a Mean of the heterogeneity χ² (2 degrees of freedom) values from the replicated bioassays.

### Table 7
ANOVA of LC₅₀ and LD₅₀ estimates, ranks of the LC₅₀ and LD₅₀ estimates, and slopes of probit regression lines from bioassays of Beauveria bassiana against adult female thrips exposed to treated bean or impatiens foliage

<table>
<thead>
<tr>
<th>Factor</th>
<th>LC₅₀s</th>
<th>LC₅₀s: rank transformed</th>
<th>LC Slopes</th>
<th>LD₅₀s</th>
<th>LD₅₀s: rank transformed</th>
<th>LD slopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date (D)</td>
<td>F₁[2,6] = 20.8, P = 0.002</td>
<td>F₁[2,6] = 9.8, P = 0.01</td>
<td>F₁[2,6] = 1.2, P = 0.35</td>
<td>F₁[2,6] = 48.4, P = 0.0002</td>
<td>F₁[2,6] = 12.1, P = 0.008</td>
<td>F₁[2,6] = 1.9, P = 0.24</td>
</tr>
<tr>
<td>Treatment host plant (TH)</td>
<td>F₁[1,6] = 79.2, P = 0.0001</td>
<td>F₁[1,6] = 32.4, P = 0.001</td>
<td>F₁[1,6] = 0.29, P = 0.61</td>
<td>F₁[1,6] = 0.51, P = 0.50</td>
<td>F₁[1,6] = 0.05, P = 0.83</td>
<td>F₁[1,6] = 0.23, P = 0.65</td>
</tr>
<tr>
<td>D x TH</td>
<td>F₁[1,5] = 2.3, P = 0.19</td>
<td>F₁[1,5] = 1.8, P = 0.24</td>
<td>F₁[1,5] = 5.6, P = 0.04</td>
<td>F₁[1,5] = 0.50, P = 0.51</td>
<td>F₁[1,5] = 0.39, P = 0.56</td>
<td>F₁[1,5] = 0.9, P = 0.23</td>
</tr>
</tbody>
</table>
on beans (LC50 91 versus 8 conidia/mm^2). One possible explanation for this response might be that despite the polyphagous nature of western flower thrips, when they are reared for many generations, or mature from egg to adult on a given host plant, they prefer that host over others. Therefore, when thrips are removed from their original (rearing) host plant (e.g., impatiens) and placed on a different host (bean), they may be somewhat agitated for some time before settling to feed, and this could result in greater acquisition of conidia and consequently greater fungal infection. It is important to note, however, that the existence of a significant host-plant switching effect is not clearly supported by the data. First, the result that originally pointed to such an effect, the treatment host × rearing host interaction, was only marginally significant (P = 0.03–0.05) (Table 2). Second, the apparent differences in the abovementioned LC50 values were not statistically significant (there was no main effect of rearing host (P = 0.56)). Finally, conducting the analyses by treatment date (to remove the problematic date × rearing host interaction) indicated no significant rearing-host effects or treatment host × rearing host interactions (Table 3). Additional replication would be required to unequivocally demonstrate a significant host-plant switching effect.

In the experiment investigating effects of treatment host and exposure to impatiens, there was never a significant reduction in thrips infection levels, as measured by percent mortality, when thrips were exposed to impatiens foliage after becoming inoculated with B. bassiana conidia on bean disks. However, thrips treated on impatiens leaf disks were significantly less susceptible to infection than thrips treated on bean disks, a result that concurred with the findings in our initial experiment investigating effects of rearing and treatment hosts. The lack of a plant species effect on viability of B. bassiana conidia combined with the observation of no significant host-plant-related differences in LD50 provides evidence that host plant chemistry was not the cause of the observed difference in susceptibility of thrips treated on the different host plants. Hence, we hypothesized that the differential susceptibility on bean versus impatiens foliar age was the result of thrips acquiring conidia at different rates depending on the host plant on which they are exposed to B. bassiana.

This alternative hypothesis was supported by our findings in the test that investigated acquisition of conidia by adult female thrips from bean versus impatiens foliage. Thrips exposed to B. bassiana-treated bean foliage acquired approximately 75% more conidia than thrips exposed to B. bassiana-treated impatiens foliage. This difference seems the most likely explanation for the significantly lower susceptibility of thrips exposed to B. bassiana-treated impatiens leaf disks compared to bean leaf disks (Table 4). Thrips exposed to treated bean disks exhibited mortality nearly 15 percentage points higher than thrips exposed to treated impatiens. This difference is very close to the predicted level obtained by doubling the LD50 dose in the linear probit regression model \( Y = a + bx \), where \( Y \), probit mortality; \( a \), the intercept; \( b \), slope; and \( x \), log dose. For impatiens, given the equation \( 5 = 2.629 + 1.66(1.4281) \) (Table 6), a 75% increase in dose would be expected to increase mortality by approximately 16 percentage points.

The above calculations show that the difference in percent mortality resulting from treatment on bean versus impatiens can be accounted for by the observed difference in dose acquisition on the two host plants, but determining the expected effect of the different acquisition rates on the bean versus impatiens LC50 values, which differ by a factor of nearly seven, is more difficult. Ugine et al. (2005b) reported that probit slopes for LC regressions were lower than the slopes for LD regressions in tests of B. bassiana against western flower thrips (i.e. exposure of thrips to B. bassiana-treated bean leaf disks) and suggested that this occurred because the rate of conidial acquisition, defined as the number of conidia per insect with respect to the number of conidia per mm^2 of leaf surface, decreased as the number of conidia per mm^2 increased. The same relationships were found in the current study. Mean slopes of the LC50 and LD50 lines were 0.85 and 1.64, respectively (Table 6), and as concentration of conidia on the leaf disks increased nearly 800-fold, the numbers of conidia acquired per thrips increased only 12–20-fold (Table 5). This phenomenon may lead researchers to erroneous conclusions if an attempt is made to characterize and compare inherent virulence of pathogens using assays in which the entire dose or a large proportion of the dose is acquired secondarily from a treated substrate. Because of the inverse relationship between dosage and acquisition rate, acquisition of conidia is not directly proportional to concentration. As concentration increases, the effective (acquired) dose also increases, but at a slower rate, and the result is a depression of the regression coefficient (slope) to a level potentially much lower than would be observed in the same assay if the doses were applied directly to the insects. Such reductions in slope can have profound effects on estimates of lethal concentration estimates (especially at the LC90 or LC50 levels).

To further investigate this phenomenon, we attempted to correct for the disproportionate rates of acquisition by applying an adjustment factor based on low-dose acquisition rates and their associated infection levels. We know that the acquisition rate is highest at those values that have the lowest number of conidia per mm^2 (see Ugine et al., 2005b and Table 6). Thus, we identified the lowest dosage (conidia/mm^2) within each LC assay that yielded significant mortality; and inserted into the equation of a line \( Y = a + bx \) the slope from the LD probit regression line, the log of the selected low dose, the probit value for the percent mortality associated with the selected dose, and solved for the intercept. We then used the common slope from the LD regression lines, the newly calculated intercept, and the percent mortalities achieved at each dose to recalculate the doses expected from the observed percent mortalities. When these adjusted doses, along with their respective observed mortalities, were subjected to probit
analysis, the mean LC50 values for thrips exposed to *B. bassiana*-treated bean and impatiens leaf disks that would be expected if acquisition were directly proportional to the conidia/mm² dosage, were 68.9 ± 16.5 and 105.2 ± 38.2 respectively. Using these values, it is then possible, by solving for Y (probit mortality), to determine the percent mortality expected if bean leaf disks were treated at the dosage required to infect 50% of thrips treated on impatients (105 conidia/mm²). Solving for Y yields a value of 5.3, which corresponds to 62% mortality, an increase of 12% over the 50% level; this is again a value close to the observed 15% difference in mortality of thrips exposed to *B. bassiana* conidia on the two host plants.

The mechanism underlying the greater rate of conidial acquisition on bean versus impatients is not known; however, it may relate to leaf-surface topography. The leaf veins of beans are external, and create sharply defined ridges across the abaxial surface (underside) of the leaf. Being thigmotactic, thrips may frequent the bases of these ridges, and if these areas were to collect greater numbers of spores during the spray application process, this could translate into enhanced dose acquisition (spore pick-up). Veins of impatients leaves are internal and both the upper and lower surfaces of the leaf are markedly smoother and waxier than those of bean. Inyang et al. (1998) also reported reduced pick-up of conidia by mustard beetle larva feeding on the smooth, waxy leaves of Chinese cabbage and turnip versus the roughly textured leaves of oilseed rape. Leaf surface hydrophobicity (waxiness) could also affect spray droplet deposition patterns, which might in turn affect levels of conidial aggregation. Ugine et al. (2005a,b) hypothesized that spore acquisition might be affected by levels of conidial aggregation. An alternative explanation is that thrips are for some reason more active when feeding on beans than impatients. Any increase in activity on a conidia-treated leaf surface would obviously have potential to increase pick-up of conidia.

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**References**


