Evaluation of *Beauveria bassiana* for Management of Citrus Thrips (Thysanoptera: Thripidae) in California Blueberries

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ABSTRACT Citrus thrips, *Scirtothrips citri* (Moulton), is a plant-feeding pest most widely recognized for causing damage to citrus and mango fruits. This insect has broadened its host range to become a significant pest of commercial blueberries grown in the San Joaquin Valley of California. We evaluated *Beauveria bassiana* (Balsamo) for control of citrus thrips in blueberries grown under two watering regimes (drip irrigation with and without overhead sprinklers) and using two fungal formulations (commercially available spores in suspension vs. colonized seed) over two sampling periods, that is, for two 3-d periods after treatment. We found significant differences in thrips densities as a function of water regime treatment and fungal formulation. Thrips levels were reduced significantly with both fungal treatments at 3 d after treatment, but at 6 d, only results with colonized seed differed from the control treatment. These data suggest entomopathogenic fungi might be useful for control of citrus thrips on blueberries in particular situations (in organic production or as a resistance management tool) but that traditional pesticides will likely remain the preferred management option.

KEY WORDS biological control, *Beauveria bassiana*, pesticide alternative, resistance management

Citrus thrips, *Scirtothrips citri* (Moulton), has been recognized as a major pest of California citrus since the 1890s (Horton 1918) and is also known to scar mango fruits (Morse 1995). Historically, highbush varieties of blueberries (*Vaccinium corymbosum* L.) were grown in regions too cold for citrus production in California (Jimenez et al. 2005, Strik and Yarborough 2005). However, breeding efforts crossing northern highbush blueberries with several other *Vaccinium* species led to the development of several heat-tolerant highbush blueberry varieties (*V. corymbosum*). This enabled the establishment of a blueberry industry in the San Joaquin Valley, a region where both citrus and citrus thrips flourish (Jimenez et al. 2005, Strik and Yarborough 2005, Morse and Grafton–Cardwell 2012). The known host range of citrus thrips has broadened and in recent years, they have become a significant pest of blueberries planted in the San Joaquin Valley of California (Haviland et al. 2009). Citrus thrips feed on blueberry foliage during the middle and late portions of the season causing distorted, discolored, and stunted flush growth and poor development of the fruiting wood required to obtain the subsequent crop. Repeated pesticide applications of the few effective and registered pesticides to reduce thrips populations poses a concern regarding pesticide resistance management, and this issue is relevant not only to the blueberry industry but also for the 1,08,665 ha of California citrus, which has experienced repeated documented cases of pesticide resistance in citrus thrips populations (Morse and Grafton–Cardwell 2006, 2009, 2012). Currently, there are no integrated pest management (IPM) plans available for control of citrus thrips in blueberry, because of the recent nature of this crop–pest association.

With a limited number of pesticides available for citrus thrips control and the frequency of insecticide resistance shown by thrips, populations should be monitored carefully, treatments limited to levels of economic concern, and applications timed optimally (Morse and Grafton–Cardwell 2006, 2009, 2012; Morse and Hoddle 2006). Appropriate cultural practices and conservation of natural enemies should be practiced in concert with the use of pesticides only on an as-needed basis. Understanding citrus thrips' life history in the blueberry system to determine where and whether susceptible stages can be exploited, is one of the first steps in the development of alternative methods to the use of traditional insecticides.

On citrus, citrus thrips pupation occurs on the tree in cracks and in crevices; however, the majority of thrips drop as late second instars from trees to pupate in the upper layer of dry leaf litter (Grout et al. 1986, Schweizer and Morse 1996) and move upward onto the plant after adult eclosion. Propupae and pupae are rarely seen, move only if disturbed, and do not feed. Pupation in the upper layers of the soil surface may create the ideal interface for control using the entomopathogenic fungi *Beauveria bassiana* (Balsamo)

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Vuillemin because of this vertical movement of the citrus thrips. However, blueberry plants have much different plant architecture than citrus trees, and citrus thrips pupation behavior has yet to be studied on blueberries.

In the United States, pressure is increasing to move away from broad-spectrum insecticides and focus on alternative methods of control. Earlier work with *B. bassiana* determined that the commercially available strain, GHA (Laverlam International Butte, MT), was the most effective of six strains tested in laboratory trials against citrus thrips (Zahn and Morse 2013). The goal of this study was to determine whether this strain of *B. bassiana* could be used effectively against citrus thrips in California blueberry production. To achieve this objective, several factors of importance to fungal efficacy were measured before commencement of a field trial: 1) location of citrus thrips pupation on blueberry plants, 2) field pupal sampling methods, 3) fungal formulation and timing of application, and 4) density of product to use in the field. We then conducted a field trial measuring the potential utility of the GHA strain of *B. bassiana* in commercial blueberries for citrus thrips management as a possible alternative to the use of traditional insecticides.

Materials and Methods

Source of Insects for Greenhouse Studies. Citrus thrips were collected in Riverside County, Riverside, CA, from wild laurel sumac, *Malsoma* (=*Rhus*) *laurina* (Nuttall), a suspected major host for this species before citrus was introduced into the state (Morse 1995). Thrips were collected via aspiration the morning of the bioassay and held in 15-dram (55-ml) plastic aspiration vials with a copper mesh-screened lid. A small sumac leaf, just large enough to fit in the vial, was included to allow the insects to settle on the leaf and feed.

In experiments where late second instar thrips were needed, that is, thrips that were close to pupation, selected thrips were large and had darkened in color. Their abdomens appeared plump, and the overall color of the thrips was a deep yellow with almost no opalescence. Early to mid-second instar thrips show limited abdomen distention and have an overall pearlescent hue. When adult females were used, selected females were of unknown age.

Location of Citrus Thrips Pupation in Potted Blueberries. Because of the complex arrangement and number of blueberry canes (typically 3–8) arising from the rhizome of commercial blueberry plants, we first measured movement of second instar citrus thrips on potted single cane blueberry plants. Known numbers of late second instar citrus thrips were released onto the leaves of potted blueberry plants in the laboratory. Paper sprayed with Tangle Trap sticky coating (BioQuip Products, Rancho Dominguez, CA) was placed 1) at the base of the plants with a ring of sticky tape around the base of the stem and partially on the stem of the plant (0–0.1 cm in height) to capture any insects crawling down, and 2) extending from the base of the plant horizontally outward above the pot surface to ensure complete coverage of the area covered by the plant canopy (thrips numbers were measured at a radius of 0.1–12.7 and 12.8–25.4 cm to split the area under the plant canopy in half). This experiment was replicated on a single potted plant over time on seven dates (total of 231 thrips, 30–38 insects released per pot per date; a different plant was used each time). Data were analyzed using Fisher exact test using SAS 9.2 (SAS Institute 2008).

Field Sampling of Thrips Pupation Sites. At our field trial site (Delano, CA) that would later be used in the *B. bassiana* trial, pupation emergence cages were used to sample insects moving off foliage toward pupation sites and later emerging out of the soil after pupation. Cages were made from Schedule 40 white PVC pipe (Powell Pipe & Supply Co., Riverside, CA) with a diameter of 10.2 cm and with cages cut to a height of 5.1 cm. The cage was then topped with a double-sided sticky card cut to fit, which was fixed into place with two elastic bands. Four lines of four cages (16 total per plant) were pushed into the soil to a depth of ≈1 cm immediately adjacent to each other at the base of a blueberry plant and oriented in a cardinal plane (north, south, east, and west) to determine which direction showed the most thrips activity. The four adjacent cages in a particular plane were used to assess thrips movement in the understory of the blueberry plant in each direction. The study was replicated on five plants on a single date and conducted just before the commencement of the field trial. Data were analyzed with a nested ANOVA using SAS 9.2.

Fungal Formulation and Timing of Application. In a greenhouse trial, Mycotrol O (BioWorks, Inc., Victor, NY), a commercially available organic formulation of the GHA strain of *B. bassiana*, was applied directly to the soil surface as conidia and compared with the same GHA strain colonized onto millet seed (hulled pear millet, *Pennisetum glaucum* L., Arrowhead Mills, Boulder, CO), also using soil application. The Mycotrol O label (10.9% *B. bassiana* strain GHA) states there are 2.11×10^{10} conidia/ml of formulated product and the maximum label use rate is 7.015 liters/ha. Mycotrol O was applied in the lab study at a rate of 7.5 ml/liter of water (equivalent to 7.015 liters per 935.3 liters/ha).

Colonized millet seed, when allowed to imbibe water and incubate in the laboratory, can support 1.0×10^6 conidia per seed (Stanghellini and El-Hamalawi 2005). Based on Stanghellini and El-Hamalawi (2005) with modification, we held the GHA-colonized millet seed in containers such that the seed mat was at a depth of no >2.54 cm. The seeds were wet (but not submerged in water) with the consistency of a thin slurry and were gently stirred three times per day for 4 d to ensure they imbibed water properly so that mycelial growth and sporulation would occur. Sporulation was confirmed by slide mounting random sections of mycelia and checking for conidia formation under the microscope. Once conidia were initially observed (on the fourth day of incubation), the seed was held an additional 3 d so that sporulation could

continue before use of the colonized seed in lab studies.

Thrips Avoidance of Colonized Millet Seed. The colonized millet seed was tested in the greenhouse (maintained at 27°C, 30% relative humidity [RH], and a photoperiod of 16:8 [L:D] h) to determine whether late second instar citrus thrips (i.e., those ready to seek a pupation site) would become infected if they crawled over or through the colonized seed when it was placed at the base of a laurel sumac seedling. A single small laurel sumac seedling, ≈10 cm tall, was placed into each of ten, 9.5 by 9.5 by 18 (height) cm styrene cages with 6 cm diameter air holes on all four sides that were covered with ultra-fine mesh screening (0.015 by 0.0059 mm, Catalog no. 7261, BioQuip Products). Small holes were made in the bottom of the container and covered with pebbles to allow for drainage, then soil was added to a depth of 7.62 cm, and the top of the container was covered with a removable lid. The base of each plant was completely surrounded by either *B. bassiana*-colonized millet seed or with uncolonized seed (as a control). A minimum of 20 late second instar thrips were released onto the leaves of each plant, and were left until enough time (average of 5 d) had passed for the thrips to molt to the propupal stage. The seedling was then cut at the soil line and examined for pupating thrips; the soil was not examined, as it would have been difficult to find dead thrips, which dehydrate quickly following death. The removable lid of the cage was sprayed with Tangle Trap sticky coating to collect any emerging adults after 5 d so infection could be measured. The study was replicated on five dates (i.e., millet seed with or without *B. bassiana* × five replicate plants per date with ≥20 thrips per plant × 5 dates). Data were analyzed using one-way ANOVA with time as a factor, and means were separated using Tukey's Least Significant Difference (LSD) test using SAS 9.2.

Density of Colonized Millet Seed to Use. To determine the optimum number of colonized millet seeds needed for close to 100% infection when thrips were seeking pupal refuges off the plant, varying amounts of colonized seed were measured in a greenhouse trial based on the size of the seed once it had imbibed water and sporulation had occurred. After incubation, nine seeds completely filled 1 cm^2 of soil surface. A laurel sumac seedling (≈10 cm in height) was placed into each of 12 styrene cages (same as above, cage 9.5 by 9.5 by 18 cm high, filled with soil to 7.62 cm) per block. There was a 0.5-cm buffer area around all sides of the cage, which was kept clear of seed to provide a 9 by 9 cm grid of seed on the soil surface below the plant. All but two similar size leaves were removed from each seedling. Small holes were made in the bottom of the container, which was covered with pebbles to allow for drainage. The 9 by 9 cm^2 grid was created from wire screen, and differing amounts of sporulated seed (0.5, 1, and 2 seeds/cm^2) or seed alone (control) were placed on the light imprint made from the wire screen on the soil surface. Two replicate seedlings per treatment were set up on five dates in a complete block design (2 replicate plants × colonized or uncolonized millet seed × 0.5, 1, or 2 seeds/cm^2 × 5 dates = 60 plants total). Plants were watered every third day. A minimum of 20 late second instar thrips were placed onto the leaves of each plant, and were left until enough degree-days had passed for the thrips to molt to the propupal stage, typically ≈5 d. The seedling was then cut at the soil line and examined for pupating thrips on the plant; the soil was not examined, as it would have been difficult to find dead thrips, which dehydrate quickly following death. The removable lid was sprayed with Tangle Trap sticky coating to collect any emerging adults after another 5 d. Data were analyzed using a three-way ANOVA with density of seed (four levels including the uncolonized seed control), application of *B. bassiana* (with vs. without), and date as factors using SAS 9.2. Unrecovered insects counted as missing data and were not included in the analysis.

Field Measurement of *B. bassiana* for Citrus Thrips Management. The commercial blueberry test site was located north of Bakersfield in Delano, CA. The trial began in August of 2008 and was conducted after harvest was complete. The *V. corymbosum* varieties contained within the test area were 'Santa Fe,' 'Jewel,' and 'Star' (see Fig. 1 for details). The most susceptible variety of blueberry to citrus thrips damage grown at the test site was the Star variety (Haviland et al. 2009), and Star was used consistently for measurement of thrips numbers for all aspects of the trial (i.e., pupation cage data, measured shoot growth, and thrips beating samples). Our grower cooperator was interested in alternatives to traditional pesticides, as the farm regularly was dealing with high citrus thrips populations. For example, during the summer of 2008, while our study was ongoing, the grower sprayed 5–10 times per field (depending on thrips pressure) in the other blueberry fields at this ranch, rotating between available traditional chemicals to reduce the impact of thrips feeding on the subsequent year's fruit set.

Irrigation in all fields took place via drip irrigation with one emitter per line servicing each plant, and two lines of irrigation tubing per row, one line located on either side of the row of plants. We chose to work in a field where the center portion of the field was also equipped with 360° overhead irrigation sprinklers. The grower had installed these overhead sprinklers several months before our study started, only in the center portion of this one field, hoping the overhead irrigation would 1) reduce heat stress on the blueberry bushes during the heat of the summer and 2) lead to reduced populations of citrus thrips. These overhead sprinklers were turned on for an average of 5 h every day throughout the study and for 3 wk before, during, and after treatment applications. The water output from these sprinklers ranged from 281 to 295 liters per day per sprinkler. Despite being forced to use pseudoreplication (Hurlbert 1984; see Fig. 1) with respect to the location of overhead irrigation plots, this field provided the ideal situation to test *B. bassiana* under two irrigation regimes, that is, the entire field was irrigated via the drip emitters but only the center of the field had additional overhead irrigation (rows

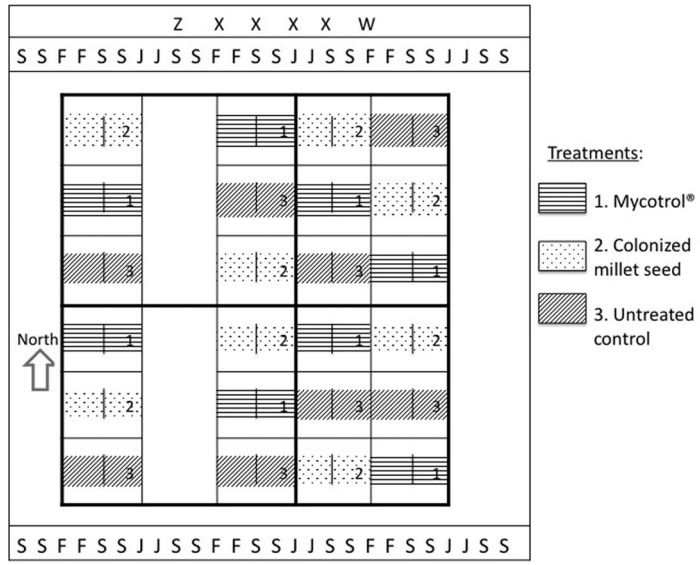

Fig. 1. Map of the blueberry field site in Delano. Rows ran north–south and the letters S ('Star'), F ('Santa Fe'), and J ('Jewel') at the top and bottom of the map indicate the variety of blueberry planted in each row; experiments were conducted in the center Star variety row of each plot, in each case with a Santa Fe row to the west and a second Star row to the east. The solid black lines delineate the four replicate blocks, each containing six plots. Rows bearing an X (top of the map) indicate rows with overhead sprinklers spaced every 7 m (Z indicates overhead sprinklers turned off; W indicates overhead sprinklers were adjusted to water only 180 degrees to the west so study plots to the east of the sprinkler did not receive overhead irrigation). Each plot was 30 plants in length (north to south) but only the center 16 plants (solid horizontal line) were treated with either Mycotrol O or colonized millet seed and is where data plants were located.

marked with X, Z, or W in Fig. 1). Because *B. bassiana* conidia are highly subject to desiccation, we hypothesized that the supplemental overhead irrigation might improve citrus thrips control with the fungus.

The commercially available GHA strain (Mycotrol O) is formulated to be mixed with water and for application via chemigation or as a foliar spray. The product label states that no surfactant is needed to keep the spores in suspension. However, we observed that spray tank agitation alone was insufficient to keep the material from precipitating; therefore, Silwet L-77 (GE Silicones, Friendly, WV) was added to the tank mix at the maximum label rate of 0.25% vol:vol. Mycotrol O was applied directly to the soil surface with a gas-powered sprayer with a hand spray gun equipped with an adjustable flow meter. The dimensions of the plots were used to calculate the amount of material needed for both *B. bassiana* formulations (raw spore soil application and colonized millet seed; see below).

Plants in the test field were spaced every 0.914 m down each row, with 3.35 m between rows, and each row was ≈165 m in length. We used a 20-row section (0.95 ha) of this 4.04-ha field of blueberries. The overhead sprinklers were spaced every 7 m in the row, and six rows of such sprinklers were located every other row for 12 center rows in the section of the field we used; the outer two overhead sprinklers (marked Z or W in Fig. 1) were turned off or adjusted so they did not water the study rows (see Fig. 1 for details). Each of the four replicate blocks contained six treatment plots laid out in a 3 by 2 factorial design consisting of three fungal treatments (Mycotrol O sprayed on the soil, the same fungus applied via colonized millet seed, and an untreated control) and two irrigation levels (drip line irrigation versus drip line + overhead irrigation). Each plot consisted of most of five rows of blueberries (both sides of the inner three rows and only the inner half of the two outer rows), each being 27.4 m long (30 plants) (Fig. 1). We treated only the 16 center plants (14.6 m of each row), leaving the seven plants at the N and S of each of the five rows untreated. Owing to the location of the overhead sprinklers, we were constrained in our experimental design. The outer two study rows (i.e., the six plots to the west and six to the east) were the drip line irrigation plots and the inner two study rows (inner 12 plots) were the drip line + overhead irrigation plots (Fig. 1).

The berm (raised soil bed) used to grow blueberries at the commercial farm was 1.22 m wide with 3.35 m between rows. Previous research indicated second instar thrips do not move much away from the base of the plant when choosing where to pupate in the soil (Grout et al. 1986, Schweizer and Morse 1996; this was confirmed by the field pupation study results, see below) and thus, we chose to concentrate soil treatments only in the area of the berm. Each treated plot was 1,569.7 m^2 in size (the equivalent of 4 rows × 3.35 m between rows; 14.63 m in length), but by treating only the berm, only 36.4% of the soil surface area in each plot was treated. The Mycotrol O label

states that the maximum field rate is 7.01 liters/ha or 137.6 ml/plot. This amount of Mycotrol O in 378.5 liters of water per hectare was applied directly to the berm on 15 August 2008 with no application between the rows. Our field trial was intended to determine the extent to which *B. bassiana* might fit into a program projected to both control citrus thrips effectively and provide rotation among available chemistries so as to reduce thrips resistance evolution. Thus, we felt it was important to operate under the best possible conditions for thrips infection by Mycotrol O, regardless of financial considerations, that is, application of product at the maximum label rate in the area where thrips were most likely to be active.

The amount of millet seed used in the field trial was calculated based on the surface area of the berm, that is, as with the Mycotrol O, only 36.4% of the surface area in each plot was treated. The amount of seed used was one colonized seed per 2 cm^2 (0.5 seeds/cm^2 determined from the results of greenhouse trials; see Results below) over an area of 71.3 m^2 (16 plants per treated row × 0.914 m between plants × 4 rows wide [3 full rows, the inner portion of the two outer rows] × 1.22 m wide berm treated per row). A kilogram of dry colonized millet contained ≈1,75,412 seeds and thus, 3.35 kg of seed was needed to treat the eight plots (four receiving overhead irrigation and four not).

Every other plant within the middle 10 plants of the middle row of each plot (Star variety) was sampled with a single pupation emergence cage in each plot (see details above: 10.2 cm diameter Schedule 40 white PVC pipe cut to a height of 5.1 cm, fitted with a double-sided sticky card on the top, pushed into the soil to a depth of 1 cm). On 15 August 2008, the cages were placed tight against the base of each set of canes on the east side (see the Results of "Field sampling of thrips pupation sites"—this was the direction where the most thrips were found). With five cages per block and four replicate blocks per treatment, a total of 20 cages sampled thrips pupation per treatment over each of two sampling periods, that is, for two consecutive 3-d periods after the Mycotrol O soil application. The treatments were as follows: (1, 2) no *B. bassiana* with and without overhead sprinkler irrigation; (3, 4) colonized millet seed with and without overhead irrigation; and (5, 6) soil application of Mycotrol O with and without overhead irrigation (Fig. 1). The colonized millet seed was allowed to imbibe water and incubate for 3 d before application and was applied 11 August 2008 using a hand fertilizer applicator (Scott's Handy Green, model# 71133, Lowe's, Moreno Valley, CA). We chose 3 d of sporulation before millet seed field application because the previous lab study showed that conidia formation started on day 4 and colonized seed was effective when exposed to thrips in the lab on day 7. Because of this delay, the soil application of Mycotrol O was applied 4 d after the millet seed (15 August) and pupation emergence cages were placed in the field and left out for 3 d (sample period 1 = 15–18 August). After 3 d, the sticky cards from each emergence cage were collected and replaced with new cards and the traps were switched to the next plant (moving north) on the east side. These traps were left in the field to sample thrips for another 3 d (sample period 2 = 18–21 August). Because the traps were placed on every other plant, this ensured that all of the middle 10 plants were sampled over the two, 3-d sampling periods (i.e., blocked through time).

For 3 wks before the Mycotrol spray through 3 wk after that application (nine samples total), counts were taken of thrips levels on 10 central "data plants" in each of the 24 treatment plots at a frequency of 1–2 times per week (25, 28 July; 8, 15, 19, 22, 26, 29 August; 4 September). Beat samples were taken by beating random canes of flush foliage such that the thrips (adults + larvae) would fall onto a 12 by 12 cm black acrylic beat tray. One beat sample (leaves beat onto trays, counting larvae and adults) was taken from each of the 10 sampled plants.

Results

Location of Citrus Thrips Pupation in Potted Blueberries. Figure 2 shows the location of late second instar citrus thrips at death in the greenhouse study as well as those that located pupal refuges on the plant (were alive or died as adults on the plant). Based on where they dropped off the plant or were stuck on the ring of sticky tape at the base of the stem, >92% of the thrips would have pupated off the plant, likely in the soil near the base of the plant. Less than 8% of the thrips pupated on the plant. Numbers did not vary significantly by location over the seven dates this study was run ($F = 95.4$; df = 9; $P = 0.587$); therefore, data were pooled. Note that the proportion of second instar thrips crawling down the base of the plant was higher (Fig. 2, 0–0.1 cm) than the proportion dropping off the plant at distances measured past the base of the plant (i.e., in the shadow of the plant at a distance of 0.1–25.4 cm from the base) (Fisher Exact Test, $F = 96.0$; df = 9; $P = 0.0162$).

Field Sampling of Thrips Pupation Sites. The four emergence cages placed under the field blueberry plants in each cardinal direction (16 cages total) provided estimates of the number of late second instar citrus thrips moving toward the soil to pupate (Fig. 3A) versus levels of adults emerging out of the soil following pupation (Fig. 3B). Data from the nested ANOVA generated P values for direction ($F = 4.69$; df = 3; $P = 0.0217$), as well as distance grouping from the base of the plant ($P < 0.0001$). The cage closest to the base of the plant had significantly higher numbers of thrips emerging from the soil ($F = 16.92$; df = 3; $P < 0.0001$). Total numbers of thrips collected were pooled for the four traps in each direction for each plant to determine which cardinal direction showed the most activity, and therefore was the most appropriate location to sample for citrus thrips levels in the field trial. Numbers of thrips trapped in the eastern direction were significantly higher for both mean numbers of thrips moving to and from the soil ($F = 41.85$; df = 3; $P < 0.0001$). Results of the two analyses

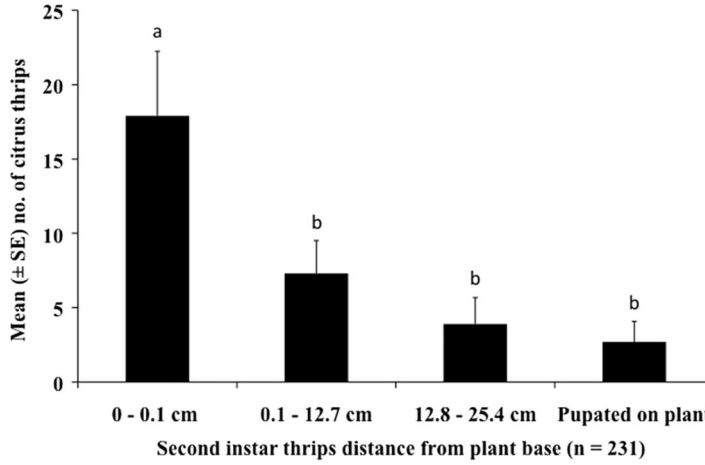

Fig. 2. Location of citrus thrips (most were second instars) in potted blueberries at the termination of the laboratory study; 0–0.1 cm indicates thrips were found dead on the ring of sticky tape at the base of the blueberry stem. The far right bar (Pupated on plant) indicates thrips that were able to complete development by pupating on the blueberry plant and were discovered alive or were found dead (as adults) on the sticky sheet below the plant. Means (SE bars) followed by the same letter are not significantly different.

suggested that for the field trial, emergence cages should be placed directly adjacent to the base of the plant on the east.

Density of Colonized Millet Seed to Use. In the lab study, mycelial growth and sporulation occurred readily and conidia were first observed on day 4 of incubation. Second instar citrus thrips did not appear to avoid the GHA colonized millet seed and they were observed (at irregular intervals) actively walking through it. Of the proportion of thrips not finding pupal refuge on the plants (<10%) with colonized seed (as opposed to controls with seed alone), 100% infection was seen with each of the different quantities of seed, that is, each of 0.5, 1, or 2 seeds/cm^2 was a sufficient density to infect and kill all late second instar thrips in the greenhouse study. Control mortality using uncolonized millet seed varied from 0 to 8% over the five dates this trial was run. Because all three densities tested were effective, we chose to use the most economical density in the field trial, that is, 0.5 seeds/cm^2.

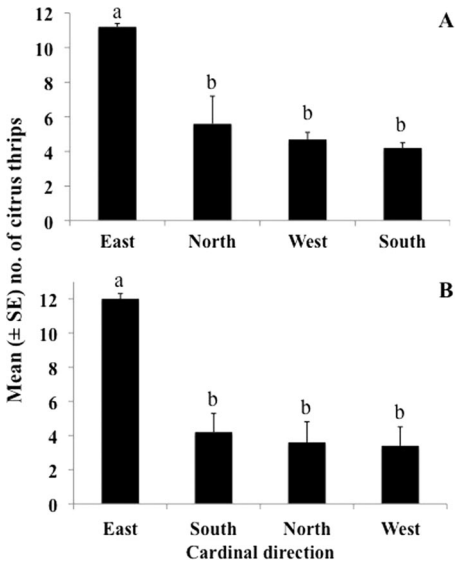

Fig. 3. Results of the pretrial field emergence cage study showing the mean number of (A) late second instar citrus thrips moving off the blueberry plant to pupate and (B) adults emerging out of the soil in each cardinal direction below sampled plants. Means followed by the same letter are not significantly different.

Field Measurement of *B. bassiana* for Citrus Thrips Management. In the split-plot design model, the whole plot factor was water and the split-plot factor was fungus treatment in a type three analysis of variance (ANOVA) (Table 1). Water, time, and treatment were the main effects in the full model, and the impacts of all three were significant. Thrips levels measured on pupation traps at 3 d after Mycotrol O treatment were lowest with colonized millet seed, intermediate with Mycotrol O, and highest in the untreated control (Tables 2 and 3). However, at time two (emergence cages out for days 3–6 after Mycotrol O treatment), thrips levels in Mycotrol O plots were

Table 1. Statistical model for the blueberry field trial pupation cage data showing results from type 3 tests of fixed effects

Effect	Numerator df	Denominator df	*F* value	$P > F$
Water	1	3	28.52	0.0128
Treatment	2	222	54.23	<0.0001
Time	1	222	68.39	<0.0001
Time × water	1	222	14.95	0.0001
Time × treatment	2	222	2.00	0.1377
Water × treatment	2	222	3.58	0.0294
Time × water × treatment	2	222	0.90	0.1789

Table 2. Mean number of citrus thrips emerging from the soil collected on sticky cards in emergence cages over sampling time 1 (0–3 d posttreatment) and sampling time 2 (3–6 d posttreatment); means were separated using Tukey's test

Mean no. citrus thrips over two sampling periods	
Sample	Mean no. citrus thrips (SE)[a]
Sampling time 1	
Colonized millet seed	7.4 (1.1)a
Mycotrol O	10.8 (1.2)b
Control	13.7 (1.1)c
Sampling time 2	
Colonized millet seed	3.78 (0.54)a
Mycotrol O	8.08 (0.90)b
Control	8.78 (0.52)b

[a] Means followed by the same letter are not significantly different ($P < 0.05$; Tukey's test).

no longer less than observed in untreated control plots.

While thrips levels measured using pupation traps were generally less in fungus-treated versus control plots (Tables 2 and 3), thrips levels on plants measured using beat samples were similar across fungal treatments if viewed separately for the two overhead water treatments (Fig. 4). It appeared that presence or absence of over irrigation had a much greater impact on foliar thrips levels than did our fungal treatments.

Discussion

The ultimate goal of this work was to determine whether the GHA strain of *B. bassiana* could be used effectively as an alternative to traditional insecticides in commercial blueberries in California. Laboratory and greenhouse trials with *B. bassiana* have shown variable success in controlling thrips and several other insect species (Frantz and Mellinger 1998, Murphy et al. 1998, Jacobson et al. 2001, Azaizeh et al. 2002, Stanghellini and El-Hamalawi 2005, Ugine et al. 2005), whereas field trials have shown limited overall success. However, few previous field trials included Thysanoptera (Saito 1991; Maniania et al. 2002, 2003). Improved results observed under laboratory and greenhouse conditions results from the fact that climatic conditions are stable and more humid (conditions optimal for *B. bassiana*, Charnley and Collins 2007) than the ambient field environment in arid areas like most of California. Unfavorable environmental surroundings, including low humidity, high temperature, and intense solar radiation are commonly referred to as the principal constraints to the field performance of *B. bassiana* (Glare and Milner 1991, Hajek 1997, Goettel et al. 2000, Inglis et al. 2002, Ugine et al. 2007). Conidia of *B. bassiana* are prone to desiccation and death if they do not contact a host shortly after spray application (Ignoffo 1992, Hajek and St. Leger 1994, Charnley and Collins 2007). The microclimate around the spore is thought to be primarily responsible for maintaining spore integrity (Fargues and Remaudiere 1977, Goettel and Inglis 1997); temperature, sunlight, and ultraviolet light affect spore integrity but humidity, especially the immediate local humidity around the spore, dictates the spore's persistence and germination, particularly when ambient temperatures are high (Ignoffo 1992, Hajek and St. Leger 1994, Azaizeh et al. 2002, Charnley and Collins 2007). Perhaps it is for these reasons, that the water-saturated seed was able to provide a suitable microclimate in which strain GHA could better sporulate and persist in comparison with the Mycotrol O spray despite more spores being present/m^2 in the latter. The Mycotrol O label states there are 2.11×10^{10} conidia/ml. Based on our application of 136.7 ml of Mycotrol O over the 71.3 m^2 of each plot, the spore density was 40.7×10^9 conidia/m^2. In contrast, the colonized millet seed supported 1×10^6 conidia per seed (Stanghellini and El-Hamalawi 2005) and was applied at a density of 0.5 seed/cm^2. Thus, spore density was 5.0×10^9 conidia/m^2 or 12.3% of the Mycotrol O density.

Table 3. Treatment interactions at sampling time 1 (0–3 d posttreatment) and sampling time 2 (3–6 d posttreatment) with significance based on $\alpha = 0.05$

Interactions among field trial treatments during two sampling periods	
Sample	*P*
Sampling time 1	
Colonized millet seed vs Mycotrol O	0.0011
Colonized millet seed vs control	<0.0001
Mycotrol O vs control	0.0056
Sampling time 2	
Colonized millet seed vs Mycotrol O	<0.0001
Colonized millet seed vs control	<0.0001
Mycotrol O vs control	0.3995

Determining methods of applying the GHA strain of *B. bassiana* so as to optimize field efficacy was one of the most interesting parts of this research. We took advantage of the observation that late second instar larval citrus thrips did not avoid the colonized seed and were able to inoculate themselves by either walking through or over the colonized seed. Following the Stanghellini and El-Hamalawi (2005) protocol proved to be an effective method of applying and sustaining GHA in the field. Whereas this system is experimental, it exhibits potential and provided a more persistent level of citrus thrips control than did the soil application of conidia using the commercial product.

Currently, there is no IPM program in place for citrus thrips pests of blueberries in California. The development of economic injury levels, economic thresholds, and optimal timing and rotation of registered insecticides are all essential portions of an IPM program, and this information will form the basis of whether or not the application of *B. bassiana* in any form, for example, conidia or colonized seed, would be an effective alternative to rotate with traditional insecticides. There is the possibility of mixing entomopathogenic fungi and insecticide applications, and several studies showed a synergistic relationship between the use of insecticides and fungi (Olmert and Kenneth 1974, Anderson et al. 1989, Neves et al. 2001). Possible synergism of strain GHA and insecticides registered for citrus thrips management in blueberries may be worthy of future study.

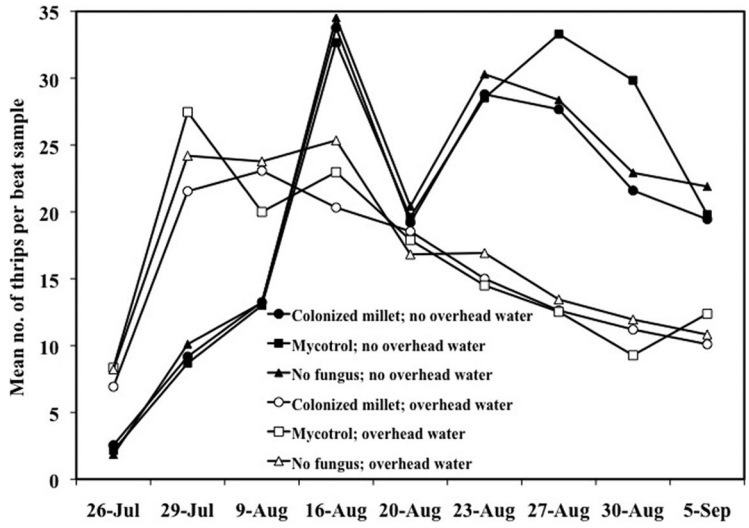

Fig. 4. Foliar counts of immature plus adult citrus thrips taken from beat samples prefungal application (25 July, 28 July, 8 Aug. 2008), during the study (15 Aug., 19 Aug. 2008), and poststudy (22 Aug., 26 Aug., 29 Aug., 4 Sept. 2008).

The cost of Mycotrol O, not including application costs, ranges roughly from US$90–110/liter, and as mentioned above, the maximum application rate per hectare is ≈7.015 liters of formulated product. The cost of Mycotrol O at the maximum application rate therefore would be approximately US$631–772/ha. The similar nonorganic BotaniGard ES is typically US$55–70/liter and would be US$386–491/ha at the same application rate. Alternatively, the colonized seed would be considerably less expensive to produce and has a long shelf life in the dry state (Stanghellini and El-Hamalawi 2005). Generally, biopesticides, such as entomopathogenic fungus, are higher in price than insecticides because they cost more to produce, are not in widespread use, and thus, are not produced on as large a scale as traditional insecticides. The fermentation process, that is, submerged liquid fermentation (Romback 1989) or solid state fermentation for production of aerial conidia (Rousson et al. 1983), propagation requirements, and storage and shelf life are all important considerations and steps for mass production of entomopathogenic fungi and their successful use. Fungal strain sporulation failure under mass production settings is often the limiting factor to strain availability and usage, and it is currently not well understood why this occurs.

Our results suggest that *B. bassiana* strain GHA can be used against citrus thrips on blueberries, but is unlikely to be adopted by traditional (nonorganic) blueberry growers. The foliar beat samples taken during and just after the trial did not show significant differences in thrips numbers on plants subjected to either of the *Beauveria* treatments. However, emergence trap data showed that over 0–3 d posttreatment, mean thrips numbers were decreased by 50% in both fungal treatment plots, that is, with both Mycotrol O and colonized seed. Although this reduction is significant, it is not economically prudent to spend US$350–968/ha, plus application costs, to achieve 50% reduction in the number of thrips that emerge from the soil for 3 d. This is in contrast to conventional insecticides that can achieve >50% reduction in thrips density on plant foliage for 2–3 wk after application for <US$100/ha (Haviland 2007; Haviland and Rill 2008b, 2009). However, blueberries are a high-value crop in the San Joaquin Valley, with an average yield of ≈11,000 kg/ha valued at a season-long average of US$6.60/kg (Jimenez et al. 2009). This is an average crop value of US$72,600/ha, which means that even a 1.4% reduction in crop value exceeds the upper estimate of the cost of an application of Mycotrol O.

This information, when coupled with the need for insecticide resistance management, indicates that using entomopathogenic fungi could be worthwhile for insecticide resistance management of citrus thrips, as there are repeated documented cases of pesticide resistance in citrus thrips populations (Morse and Brawner 1986, Immaraju et al. 1989, Khan and Morse 1998, Morse and Hoddle 2006, Morse and Grafton–Cardwell 2012). This is particularly true for producers of organic blueberries; to date the only registered organic insecticides that provide significant control of citrus thrips are based on one active ingredient, spinosad (Haviland and Rill 2008a). For resistance management purposes, and because Mycotrol O is approved for organic use, its use might be of interest to organic growers as an alternative to traditional insecticides.

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