Red Imported Fire Ant (Hymenoptera: Formicidae) Control in Nursery Pots Treated with *Beauveria bassiana* and Bifenthrin[1]

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Abstract *Beauveria bassiana* (Balsamo) Vuillemin GHA strain was tested alone and in combination with different rates of bifenthrin for control of red imported fire ant, *Solenopsis invicta* Buren, in potted nursery medium. The fungus killed fire ant workers in the potting medium, but was not effective at eliminating infestations in all containers. Bifenthrin at a rate of 1.18 kg Talstar™ (nursery granular, 0.2% AI) per m^3 soil alone performed better than *B. bassiana* alone; however, bifenthrin was also inconsistent in eliminating fire ants from all pots over the 4 yrs of the study. When *B. bassiana* was combined with reduced rates (1/4 and 1/2) of bifenthrin in 2001, infestations were eliminated from treated pots within 96 h each time workers were added to the pots for the 7-wk test period. The number of infested bifenthrin + *B. bassiana*-treated pots was significantly lower than the number of infested untreated pots on all 19 sampling dates in 2001. Use of bifenthrin + *B. bassiana* was as effective or more effective than use of bifenthrin alone and could save growers as much as 15 to 40%.

Key Words *Solenopsis invicta, Beauveria bassiana,* Hyphomycetes, Moniliales, horticultural nurseries

Red imported fire ants, *Solenopsis invicta* Buren, pose serious problems for horticultural nursery managers because of the expense associated with chemical control, the threat to worker safety, and the potential for fire ants to spread to uninfested areas in transported nursery stock (Collins 1999). Container stock in quarantine areas must be treated before shipping, and fire ant infestations in containers can result in delay or rejection of shipments (Costa et al. 2001) as well as punitive fines for nurseries. Oi and Williams (1996) stated that granular formulations of the pyrethroids bifenthrin and tefluthrin were the only insecticides that were approved by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service Plant Pest Quarantine for incorporation into potting soil before planting; however, the cost of these products was much higher than formerly-approved insecticides. Only one other granular insecticide (fipronil) has since been approved for incorporation into potting media (Callcott 2003). Additional inexpensive and safe fire ant control methods are needed. The fungus *Beauveria bassiana* (Balsamo) Vuillemin is naturally occurring, cosmopolitan, and infects many different insect species. Fire ants were susceptible to *B. bassiana* infection in laboratory tests conducted by Stimac et al. (1993b) and Brinkman and Gardner (2000, 2001). Champlin et al. (1981) found that pecan weevils, *Curculio*

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caryae (Horn), became infected as they burrowed into soil through surface layers containing *B. bassiana* conidia. However, it is not known whether the agent can effectively eliminate fire ant infestations in potted soil. The objective of this study was to evaluate *B. bassiana* alone and in combination with bifenthrin for control of the red imported fire ant in soil within nursery containers.

Materials and Methods

Fire ants used in these studies were obtained from field populations in Spalding Co., GA, and were removed from soil using procedures described by Jouvenaz et al. (1977). These colonies were maintained in the laboratory in plastic trays (58.2 × 43.2 × 18.5 cm) containing artificial nests consisting of 150-mm diam plastic Petri dishes with dental plaster on the bottom to maintain moisture (Stimac et al. 1993a). Ants were fed foods including sugar water, tuna in oil, and yellow mealworm, *Tenebrio molitor* L., larvae.

In each year of the study, the Center for Applied Nursery Research in Dearing, GA, provided 80 nursery containers that were prepared with untreated pine bark soil mix or pine bark soil mix with bifenthrin (Talstar™ nursery granular 0.2% AI, FMC, Philadelphia, PA). Pots that were prepared with the standard rate of bifenthrin received 1.18 kg Talstar per m^3 soil. These containers were then transported to the University of Georgia Research and Education Garden on the College of Agricultural and Environmental Sciences Griffin Campus where they were placed in an open area exposed to full sun. Existing fire ant mounds in the area were not eliminated because of the potential for interference with the treatments being tested.

A small metal spatula was used to scoop ants from laboratory colonies to add a mean (±SE) of 116 ± 6 workers into each nursery container. Groups of ants were randomly collected from laboratory colonies and contained a mixture of minor, media, and major workers. The number of workers placed in pots was determined by adding similar volumes of ants to five empty containers, freezing and killing the ants, and then counting ants in each container.

Following treatment and addition of ants, containers were checked by lightly tapping a pot and visually inspecting the soil surface and inside of the pot for live fire ants. A pot was considered infested if there was one or more live fire ants inside the pot. After tapping the pot, workers would often quickly exit tunnel nests to search for the source of the disturbance.

Each year, soil in containers was treated once and the addition of ants was considered to be the start of each test. In 1999 and 2000, fire ants were added to pots one time and containers were checked for 14 d. In 2001 and 2002, fire ants were added to pots, and pots were inspected daily until ants were eliminated from all treated containers. Thereafter, ants were added to all uninfested pots every 7 d until day-time temperatures became too high for ants to survive in any pots.

1999. *Ilex glabra* (L.) Gray 'Compacta' liners were potted in 26.6-L containers. Treatments were an untreated control, bifenthrin, and *B. bassiana* (BotaniGard ES, emulsifiable suspension, GHA strain, Emerald BioAgriculture Corp., Butte, MT). Bifenthrin was mixed with soil using the standard rate before potting on 10 May. *Beauveria bassiana* was applied at 1.8×10^{11} conidia per m^3 of soil to the soil surface of pots on 01 June. A gardening tool was used to incorporate *B. bassiana* treatments to about a 6-cm depth. On 02 June, fire ant workers were added to each container. Soil samples about 64 cm^3 in volume were taken from all *B. bassiana* treated containers for 8 d following treatment. These samples were collected from within 4-cm of the soil surface with a small metal spatula. A 0.5 g subsample of this soil was mixed with 4.5 mL sterile distilled water (SDW), serially diluted, and drop-plated on oatmeal-dodine agar, which is selective for *B. bassiana* (Storey and Gardner 1988). These samples were incubated at 25°C and colony forming units (CFUs) were counted about 48 h after plating.

Blocks were replicated four times in a randomized complete block design (RCBD). There were a total of eight containers per treatment, and these pots were blocked in pairs. The number of days on which live fire ants were observed in each container was pooled and transformed by square root (x + 0.5) before analysis. PROC GLM (SAS Institute 1996) was used to conduct analysis of variance (ANOVA), and means were separated by the least significant differences test (LSD, $P = 0.05$).

2000. *Ilex glabra* liners were potted in 19.0-L containers. Treatments were an untreated control, bifenthrin standard rate, *B. bassiana* ES at 2×10^{13} conidia per m^3 soil and *B. bassiana* formulated as BotaniGard 22 WP (wettable powder) at 1×10^{14} conidia per m^3 soil. Soil was mixed with bifenthrin treatments on 29 May. Fungus-treated containers received *B. bassiana* ES or WP on 01 June. Fire ant workers were added on 02 June. Four containers were used per treatment in each block. Blocks were replicated four times in a RCBD. The number of days on which live fire ants were observed in each container was pooled and transformed by square root before analysis with ANOVA and LSD.

Soil samples were taken from two of the *B. bassiana* ES and WP-treated containers each day for 14 d following treatment. This soil was tested for viable fungal fragments as previously described. Ten days following addition of ants, efforts were made to collect all dead fire ants from the soil surface of containers treated with ES and WP. These ants were washed with 10% sodium hypochlorite bleach and rinsed with SDW. They were then plated on nutrient agar, incubated at 25°C, and inspected daily for external growth of *B. bassiana*.

2001. Treatments were an untreated control, bifenthrin standard rate, bifenthrin at 0.59 kg Talstar (1/2 rate) plus *B. bassiana* WP at 2.44×10^{14} conidia per m^3 soil, and bifenthrin at 0.29 kg Talstar (1/4 rate) plus *B. bassiana* WP at 2.44×10^{14} conidia per m^3 soil. Bifenthrin and *B. bassiana* treatments were mixed with soil in 11.4-L containers on 25 May. Four containers were used per treatment in each block. Blocks were replicated five times in a RCBD. Fire ants were added to pots on 28 May. Live ants were added to uninfested containers once a week until 09 July. The number of infested pots in each replication was transformed by square root (x + 0.5) before analysis with ANOVA by sampling date. Means were separated with LSD tests in SAS.

One day after addition of workers, efforts were made to collect all dead individuals from the soil surface of containers treated with bifenthrin 1/2 rate plus *B. bassiana,* and bifenthrin 1/4 rate plus *B. bassiana.* These ants were washed with 10% bleach, rinsed with SDW, and plated on nutrient agar. Ants on agar plates were inspected daily for external growth of *B. bassiana*.

2002. Treatments were an untreated control, bifenthrin standard rate, 1/2 rate of bifenthrin plus *B. bassiana* WP at 2.44×10^{14} conidia per m^3 soil, 1/2 rate of bifenthrin plus *B. bassiana* WP at 2.78×10^{12} conidia per m^3, and 1/4 rate of bifenthrin plus *B. bassiana* WP at 2.78×10^{12} conidia per m^3. Treatments were mixed with soil prior to potting in 3.37-L containers on 10 May. Fire ants were added to each pot on 13 May. Ants were added to uninfested containers every 7 d until 03 June. There were four containers per treatment in each block. Blocks were replicated four times in a RCBD for a total of 16 containers per treatment. The number of infested pots in each replication was transformed by square root (x + 0.5) before analysis with ANOVA by sampling date. Means were separated using LSD.

Results and Discussion

In 1999, bifenthrin eliminated fire ants from pots within 2 d following addition of workers, and live ants were observed in those pots on significantly ($F = 14.62$; df = 2, 3; $P = 0.0002$) fewer sampling days compared with the other treatments (Table 1). Live fire ants were present in six of eight untreated pots 22 d following addition of ants to containers. Dead workers were observed on the soil surface of containers treated with *B. bassiana,* but fire ant infestations were not eliminated from all pots. Oi et al. (1994) applied a *B. bassiana* and rice formulation to fire ant mounds and observed a maximum infection rate of 55% of live ants. Relatively high levels of fire ant mortality due to *B. bassiana* have been observed in laboratory tests (Brinkman and Gardner 2001); however, 100% mortality is difficult to achieve, even under controlled conditions. Pathogenicity of *B. bassiana* to insects is dependent on many factors including persistence of conidia, number of conidia coming into contact with the individual (Pereira et al. 1993), and immune response of individuals to fungal infection (Bidochka and Khachatourians 1987).

The number of *B. bassiana* CFUs in pots treated with *B. bassiana* was reduced by a factor of 3.6× after 2 d (Fig. 1). There was a rebound in CFUs on the third day, but the downward trend in CFU viability continued on the subsequent sampling dates. Solar radiation and dessication are detrimental to *B. bassiana,* and Gardner et al. (1977) found a rapid loss in *B. bassiana* viability on plant foliage following application. Soil provides protection for *B. bassiana* conidia from photodeactivation and dessication (Gaugler et al. 1989), but our soil samples were taken from within 4-cm of the surface where those stressors may have still played a role in deactivating spores. Irrigation of nursery pots in our study may have caused some downward movement of CFUs over time. Storey and Gardner (1987) studied the vertical movement of *B. bassiana* in soil and found that a majority of CFUs were recovered in the upper 10 to 15 cm, but there were differences due to soil type. In a subsequent study, Storey and

Table 1. Mean number of sampling days with active red imported fire ant infestation in control, bifenthrin at standard rate, or *B. bassiana* formulated as BotaniGard ES treatments, 1999

Treatment	Rate amount per m^3 of soil	Mean no. (±SEM) of days of active infestation
Control	Untreated	7.13 ± 1.39a
B. bassiana ES*	1.8×10^{11} conidia	8.38 ± 1.07a
Bifenthrin**	1.18 kg Talstar	1.88 ± 0.13b

* Applied to soil surface in pots and incorporated into media using a gardening tool.
** 0.2% (AI) by weight, granular mixed with soil before potting.
Within a column means followed by the same letter are not significantly different ($P > 0.05$, LSD).

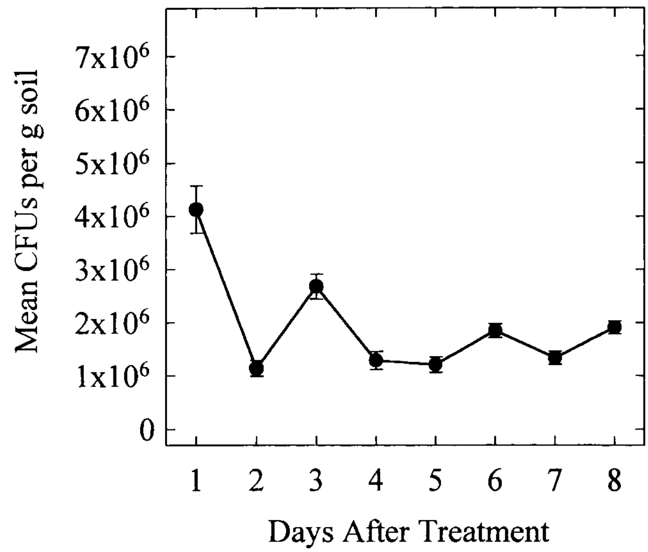

Fig. 1. Mean number of *B. bassiana* colony forming units (CFUs) in potting soil treated with BotaniGard ES at a rate of 1.8 × 10^{11} conidia per m^3 soil, 1999.

Gardner (1988) observed that vertical movement of *B. bassiana* was greater in soils with higher water infiltration rates. *Beauveria bassiana* activity can also be inhibited by antagonistic factors in nonsterile soil (Pereira et al. 1993) and by fire ant venom alkaloids (Storey et al. 1990).

In 2000, live fire ants were observed in containers treated with bifenthrin on significantly (F = 13.59; df = 3, 3; P = 0.0001) fewer sampling days compared with the other treatments (Table 2). However, fire ants were not totally eliminated from those containers. Live fire ants were observed in two bifenthrin-treated pots 14 d following

Table 2. Mean number of sampling days with active red imported fire ant infestation in control, bifenthrin at standard rate, *B. bassiana* formulated as BotaniGard ES, or BotaniGard 22 WP treatments, 2000

Treatment	Rate amount per m^3 of soil	Mean no. of days of active infestation
Control	Untreated	9.19 ± 1.10a
B. bassiana ES*	2.1 × 10^{13} conidia	10.63 ± 0.89a
B. bassiana WP*	1.3 × 10^{14} conidia	9.13 ± 1.06a
bifenthrin**	1.18 kg Talstar	3.50 ± 0.67b

* Applied to soil surface in pots and incorporated into soil using a gardening tool.

** 0.2% (AI) by weight, granular mixed with soil before potting.

Within a column means followed by the same letter are not significantly different (P > 0.05, LSD).

treatment. Dead workers were found on the soil surface of containers treated with *B. bassiana* (ES and WP), but infestations were not eliminated from all pots. The mean number of days of fire ant infestation in containers did not differ significantly between ES or WP treatments. External growth of *B. bassiana* developed on 18.0% of fire ants from ES-treated pots and 74.7% of ants collected from pots treated with WP. More dead ants in WP-treated pots developed external growth of the fungus than dead ants from ES-treated pots. This may have been due to formulation or because a slightly higher number of conidia in WP was added to pots at treatment. In a study by Stimac et al. (1993b), a powder formulation of *B. bassiana* was not repelled by fire ant cuticle, as was a conidial suspension in water. This difference was attributed to the hydrophobic nature of the fire ant cuticle. Stimac et al. (1993b) theorized that fewer conidia in the aqueous suspensions came in contact with workers as a result.

Soil in pots treated with ES or WP contained similar numbers of CFUs at 1 d after treatment (Fig. 2). There was a slight decline in CFU counts in ES-treated pots thereafter until day 8. On that sampling day, the number of CFUs was 5.9 to 34.7× higher than the previous or subsequent sampling day. In WP-treated pots, there was a 6.8× increase in CFU counts on day 3 compared to day 1, but the decrease in CFUs the next sampling day was of a similar magnitude. On day 14, *B. bassiana* was not detected in ES-treated pots. There were 11.8×10^4 CFUs per g soil in WP-treated pots on that day. We observed increases in numbers of CFUs following treatment in 1999 and 2000, but these were only temporary. An increase in CFUs within 72 h of application of *B. bassiana* to Georgia and Florida soils were observed by Storey and Gardner (1988) and McCoy (1986), respectively. McCoy (1986) attributed the increase in CFUs to propagation of the fungus in the soil.

On the day following addition of ants to pots in week one of the 2001 test, all untreated pots were infested and all treated pots were devoid of ants (Fig. 3). Because such dramatic results were observed in week one, we repeated the test in subsequent weeks by adding ants to uninfested containers and inspecting pots daily for ant activity. Bifenthrin and bifenthrin combined with *B. bassiana* treatments continued to eliminate infestations within 96 h each time ants were added to containers during the 7-wk period of the study. The number of infested pots treated with bifenthrin 1/2 rate + *B. bassiana* and bifenthrin 1/4 rate + *B. bassiana* was significantly lower than the number of infested untreated pots for every sampling period over the duration of the study. In week four, no fire ant activity was detected in any treated or untreated pots 72 h following addition of ants to containers. Bifenthrin 1/2 rate + *B. bassiana* eliminated fire ants within 24 to 48 h each time ants were added to containers. Bifenthrin 1/4 rate + *B. bassiana* eliminated fire ants within 24 to 72 h each time ants were added to containers. On four sampling dates, there were no significant differences between the number of infested untreated pots and the number of infested pots treated with the standard rate of bifenthrin only.

On 30 May, external growth of *B. bassiana* developed on 28.6% of dead ants removed from containers treated with bifenthrin 1/2 rate + *B. bassiana* and 28.0% of dead ants in the bifenthrin 1/4 rate + *B. bassiana* treatment. External growth of *B. bassiana* developed on 4.4% of dead ants removed from containers treated with bifenthrin 1/2 rate + *B. bassiana* and 6.7% of dead ants in the bifenthrin 1/4 rate + *B. bassiana* treatment on 05 June. None of the dead ants collected from pots between 12 June and 10 July 2001 developed external growth of *B. bassiana*. Although those dead ants may have been killed by *B. bassiana*, the fungus inside the ants may have been deactivated later by high temperatures and dessication due to exposure of ants

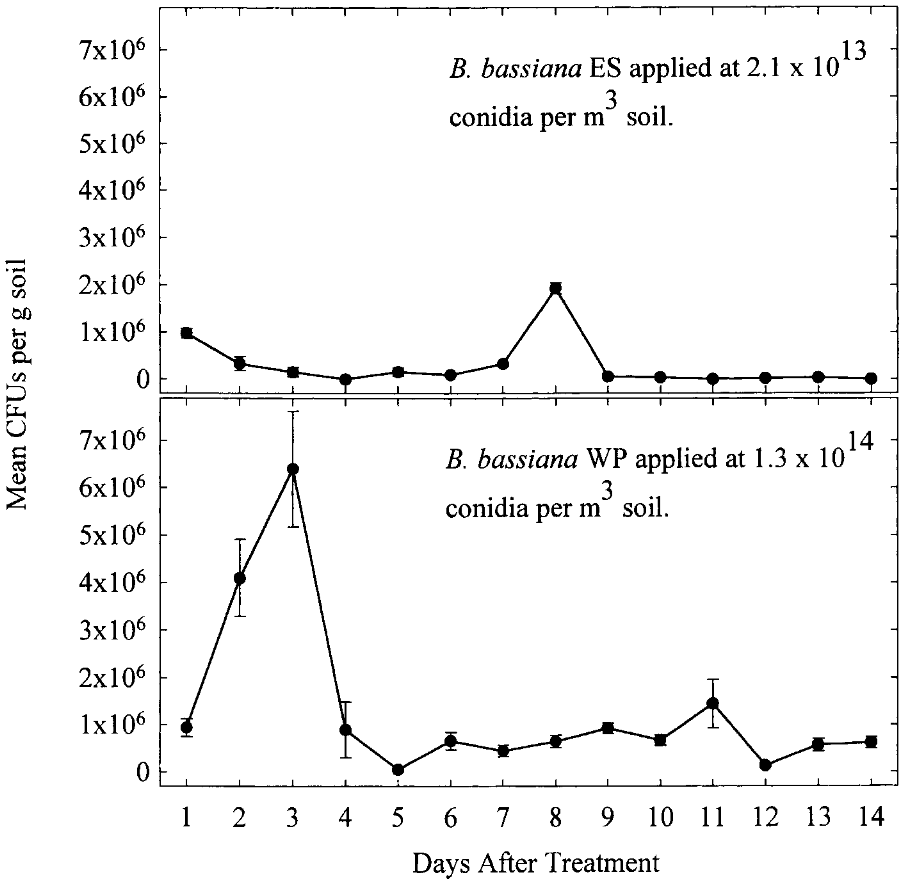

Fig. 2. Mean number of *B. bassiana* colony forming units (CFUs) in potting soil treated with the fungus formulated as BotaniGard ES or BotaniGard 22 WP, 2000.

on the soil surface. Also, Marcandier and Khachatourians (1987) treated grasshoppers with *B. bassiana* and infection occurred independently of relative humidity (RH), but external mycelial growth on cadavers did not occur below 100% RH.

Each time ants were added to pots in 2002, live fire ants were observed in containers treated with bifenthrin 1/4 rate + *B. bassiana* at 2.78 × 10^{12} conidia per m^3 over a longer period compared with the other treatments (Fig. 4). However, all treatments effectively eliminated fire ants from pots within 72 h following addition of ants. In the first two weeks of the study, fire ants were not found in any containers treated with the standard rate of bifenthrin, bifenthrin 1/2 rate + *B. bassiana* at 2.44 × 10^{14} conidia per m^3, or bifenthrin 1/2 rate + *B. bassiana* at 2.78 × 10^{12} conidia per m^3 the day following addition of ants. All treated and untreated containers were devoid of live fire ants 48 h following addition of ants in the third and fourth week of the study.

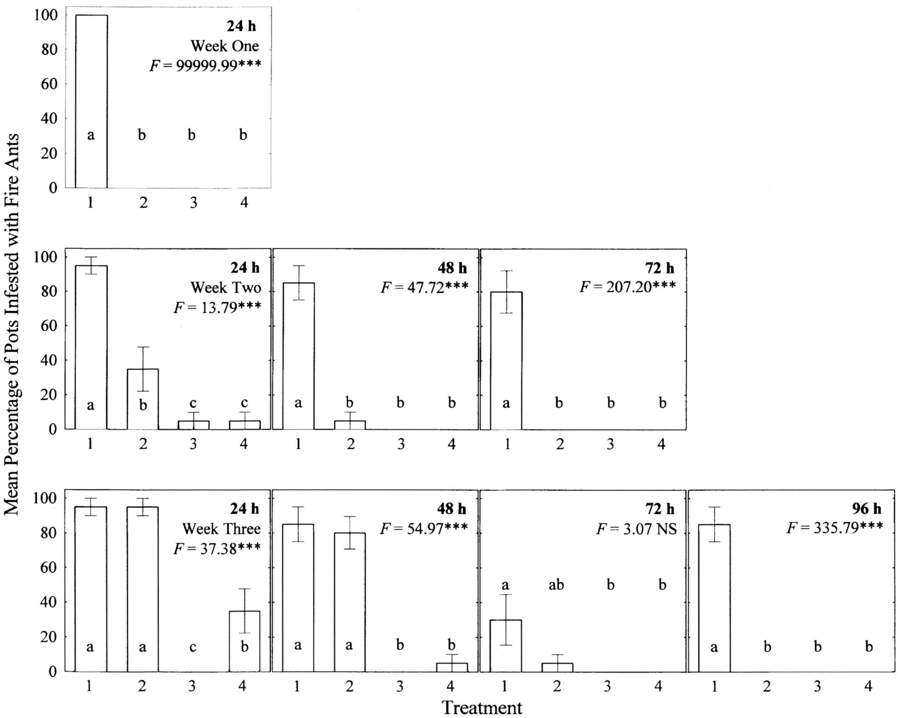

Fig. 3. Mean percentage of nursery pots with fire ant activity following treatment with (1) nothing (untreated), (2) standard rate of bifenthrin (Talstar 0.2 G), (3) bifenthrin 1/2 rate plus *B. bassiana* (BotaniGard 22 WP) at 2.44×10^{14} conidia per m^3 soil, or (4) bifenthrin 1/4 rate plus *B. bassiana* at 2.44×10^{14} conidia per m^3. Treatments were mixed with soil before potting on 25 May. Live fire ants were added to pots on the day before each of the first sampling periods in each row of graphs. Bars with the same letter within a graph are not significantly different ($P > 0.05$; LSD mean separation was done on data transformed by square root [x + 0.5]; df = 3, 4; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant).

Over the 4 years of this study, the standard rate of bifenthrin eliminated infestations from more pots and killed fire ants more quickly than *B. bassiana* regardless of formulation. *Beauveria bassiana* killed fire ants, but was not effective at eliminating infestations from all nursery containers when used alone. When reduced rates of bifenthrin were combined with *B. bassiana* in 2001 and 2002, these combination treatments eliminated fire ants from pots as quickly or more quickly than the standard rate of bifenthrin. These results suggest that there may be additive or synergistic effects from combining the fungus with bifenthrin. Cossentine and Lewis (1984) defined additive mortality as being equal to the sum of the effects of two pathogens, and synergistic mortality as greater than the sum of the effects of two pathogens. It is not known whether bifenthrin reduced the activity of microbial competitors of *B. bassiana*

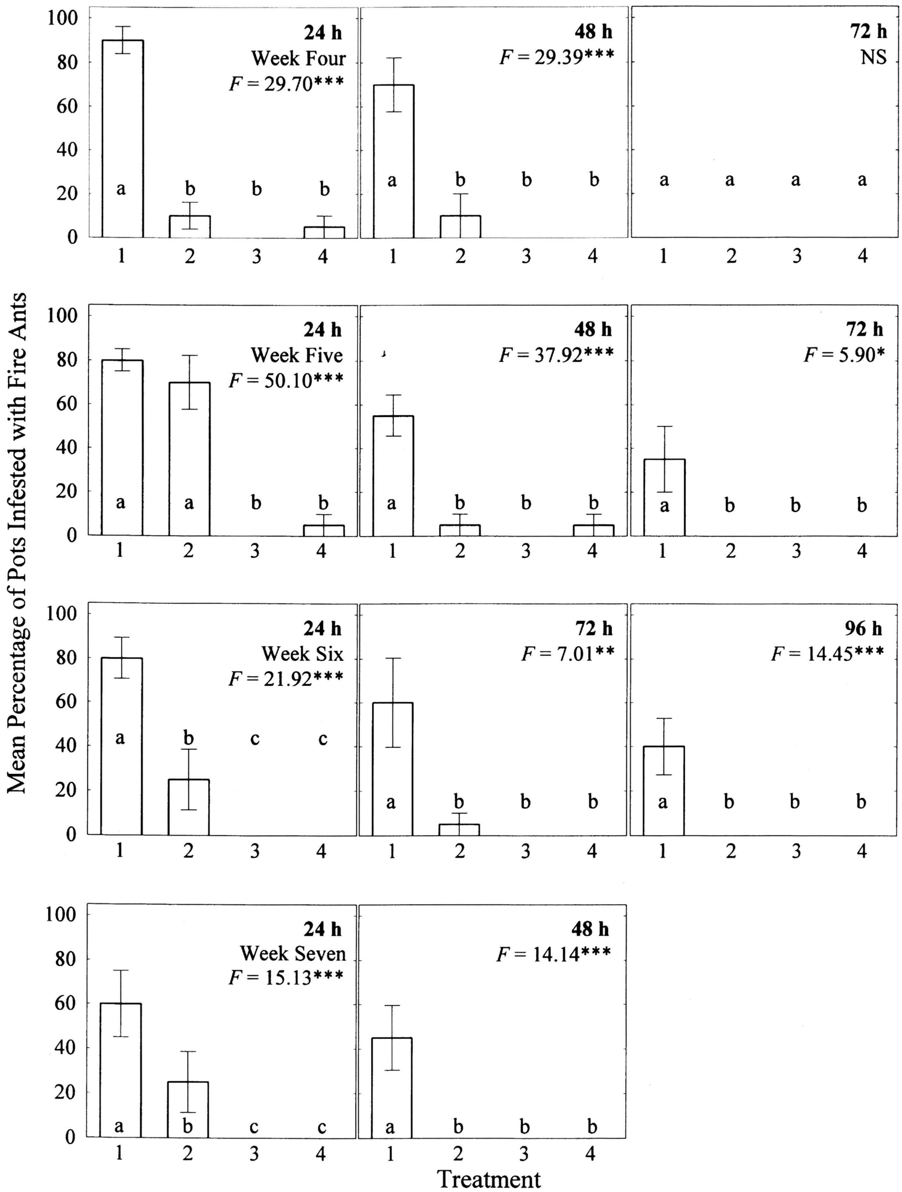

Fig. 3. Continued

in the soil. In the early stages of infection (<24 h), *B. bassiana* produces cuticle-degrading enzymes (St. Leger et al. 1986) and toxins (Dresner 1950, West and Briggs 1968) that are harmful to the host. However, red imported fire ants were treated with *B. bassiana* in the laboratory and noticeable increases in mortality were typically not

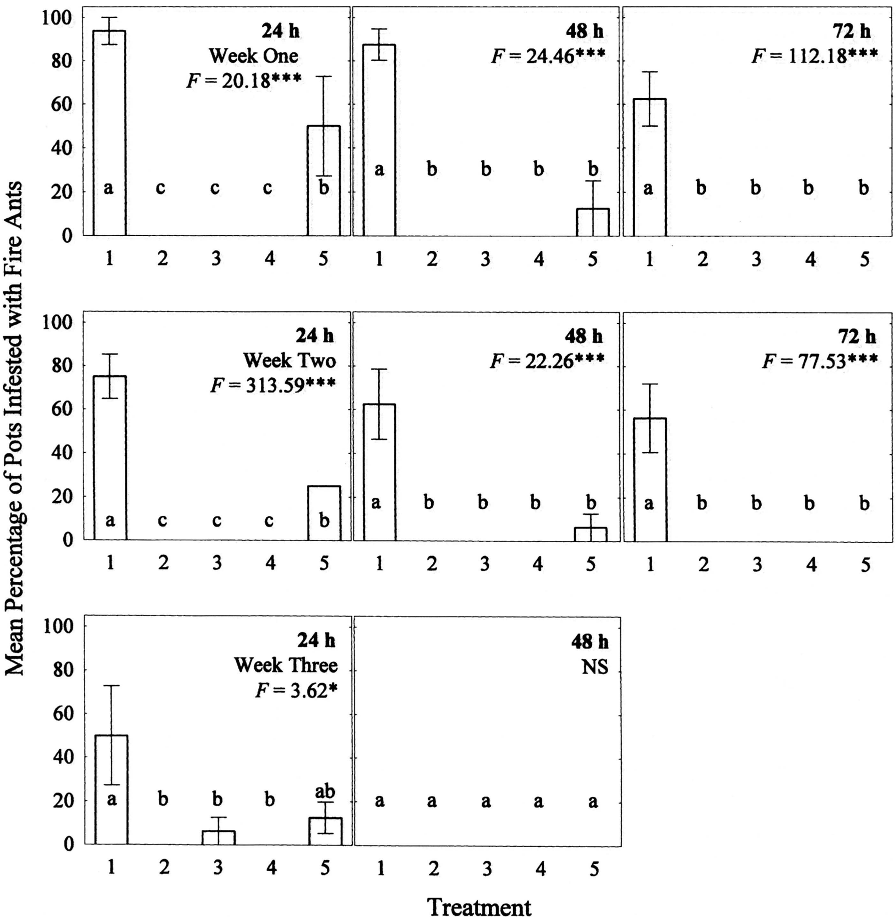

Fig. 4. Mean percentage of nursery pots with fire ant activity following treatment with (1) nothing (untreated), (2) standard rate of bifenthrin (Talstar 0.2 G), (3) bifenthrin 1/2 rate + *B. bassiana* (BotaniGard 22 WP) at 2.44 × 10^{14} conidia per m^3 soil, (4) bifenthrin 1/2 rate + *B. bassiana* at 2.78 × 10^{12} conidia per m^3 soil, (5) bifenthrin 1/4 rate + *B. bassiana* at 2.78 × 10^{12} conidia per m^3 soil. Treatments were mixed with soil before potting. Live fire ants were added to pots on the day before each of the first sampling periods in each row of graphs. Bars with the same letter within a graph are not significantly different ($P > 0.05$; LSD mean separation was done on data transformed by square root [x + 0.5]; df = 3, 4; *, $P < 0.05$; ***, $P < 0.001$).

observed until 3 to 5 d following treatment (Stimac et al. 1993b, Brinkman and Gardner 2001). A similar trend was observed following treatment with *B. bassiana* for migratory grasshoppers, *Melanoplus sanguinipes* (F.) (Brinkman et al. 1997a), and alfalfa leafcutting bees, *Megachile rotundata* (F.) (Brinkman et al. 1997b). Breakdown of the host cuticle by fungal enzymes may have enhanced the entry and action of bifenthrin and toxins in fire ants.

Another possible explanation for the quick elimination of fire ants from treated containers is that bifenthrin and *B. bassiana* acted as repellents. However, when fire ant workers were placed onto the soil surface of pots, many began nest construction, and we did not observe large numbers of ants evacuating containers. On the days following addition of ants, dead and debilitated fire ants were observed in many of the treated containers, suggesting that the treatments killed the ants. Foraging workers were seen on the ground in the area of the pots, but it is not known whether they had exited pots or were from existing mounds in the general area.

Use of lower rates of bifenthrin in combination with *B. bassiana* against fire ants in potted nursery soil may provide monetary savings for nursery managers. Cost analysis was done comparing current prices for the rates of bifenthrin and *B. bassiana* used in 2002 with the standard rate of bifenthrin only. Based on $38.00 for 22.68 kg (50 lb) of Talstar and $48.00 for 0.45 kg (1 lb) of BotaniGard 22WP, there were no financial savings for the high rate of *B. bassiana* plus either rate of bifenthrin when compared with the standard rate of bifenthrin only. Bifenthrin 1/4 rate + the low rate of *B. bassiana* costs $1.19 to treat 1 m^3 of soil, which was 40% less than for the standard rate of bifenthrin only. Use of bifenthrin 1/2 rate + the low rate of *B. bassiana* cost $1.69 to treat 1 m^3 of soil, which was 15% less than for the standard rate of bifenthrin only. Although this combination treatment costs more to implement than the bifenthrin 1/4 rate + the low rate of *B. bassiana* treatment, it provided quicker elimination of fire ants from containers. Labor was not included in calculation of costs, but the additional effort required to prepare the two products for mixture with soil was minimal. Also, it would be difficult to determine the financial benefits from reduced exposure of nursery workers to insecticide active ingredient. However, improved worker safety would be another positive aspect associated with use of the combination treatments.

In addition to treating potting soil for fire ants, nurseries also need to eliminate fire ant colonies on premises in order to ship products outside of quarantine areas (Collins 1999, Callcott 2003). Insecticidal broadcast baits and mound treatments are used for this purpose. Our study addressed alternatives to use of the standard rate of bifenthrin, which could lead to safer, more cost-effective control of fire ants in nursery containers. Continued baiting and mound treatments for fire ant management on nursery grounds would aid in prevention of infestations in potted nursery soil.

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