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BIOLOGICAL AND MICROBIAL CONTROL

# Field Applications of *Beauveria bassiana* for Control of the Red Imported Fire Ant (Hymenoptera: Formicidae)

DAVID H. OI,[1] ROBERTO M. PEREIRA, JERRY L. STIMAC, AND LOIS A. WOOD

Entomology and Nematology Department, University of Florida, P.O. Box 110620,
Gainesville, FL 32611–0620

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**ABSTRACT** A *Beauveria bassiana* (Balsamo) Vuillemin isolate (Bb447), recovered from the red imported fire ant, *Solenopsis invicta* Buren, in Brazil was applied to fire ant mounds in Florida pastures. Rice with Bb447 applied to the tops of mounds resulted in a maximum infection of 55% of the live ants sampled; 70% of the treated mounds remained active or formed active new mounds within 8 wk. Injections of conidial powder formulations of Bb447 in late fall and early summer resulted in peak infections of 60 and 52% of live ants sampled, respectively. All of the injected mounds remained active or formed active new mounds within 8 wk after treatment. Injection of Bb447 mixed with a hydrophobic silica carrier resulted in a 52% reduction in active mounds. Injection of the silica carrier alone resulted in a 41% reduction. Foraging by the red imported fire ant was reduced significantly in areas within which mounds were injected with fungal formulations, whereas foraging by other ant species increased in these areas.

**KEY WORDS** *Solenopsis invicta*, entomopathogenic fungi, microbial control

MANY METHODS OF CONTROL have been used for the red imported fire ant, *Solenopsis invicta* Buren, since its establishment as a pest in the United States. Types of products currently registered for fire ant control include baits impregnated with slow-acting toxicants or insect growth regulators and various insecticide formulations applied directly to nests (Collins 1992). Natural enemies have been found (Jouvenaz et al. 1981), and various entomogenous bacteria, fungi, nematodes, and mites have been tested against imported fire ants (Quattlebaum 1980, Broome 1974, Drees et al. 1992, Bruce & LeCato 1980), but their use as biological control agents has not been developed.

Stimac et al. (1987), Alves et al. (1988) and Pereira et al. (1993) described the pathogenicity of isolates of the entomogenous fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin, which were obtained from fire ants in the state of Mato Grosso, Brazil. Because *S. invicta* is thought to be a native species in this area (Buren et al. 1974), fungal isolates from fire ants in Mato Grosso may represent strains that coevolved with the fire ants and may be a factor in the control of their populations in this area (Allen & Buren 1974). In the state of São Paulo, Brazil, field applications of the *B. bassiana* strain 447 (ATCC 20872) (Bb447) to the surface of fire ant mounds resulted in colony mortality (Stimac et al. 1989). However, laboratory studies on Bb447 by Pereira & Stimac (1992) showed that inoculation of *S. invicta* nests may not be a viable control strategy because transmission of Bb447 within the nests did not occur. They concluded that conidial contact with a large proportion of the colony is needed to cause substantial mortality. Injections of powder formulations of Bb447 seemed to improve conidial contact with ants in laboratory colonies (Stimac et al. 1993a, b). Here we report the results of a surface application and injections of Bb447 to *S. invicta* mounds under field conditions in Florida.

## Materials and Methods

Fire ant nests may be composed of multiple mounds and underground tunnels; therefore, delimiting the boundaries of a colony in the field with certainty is difficult. Disturbances to colonies can cause the fire ants to either move the entire nest or split into multiple mounds (Williams & Lofgren 1983). Therefore, we defined a fire ant mound as the experimental unit. All mounds were flagged, dated, and mapped. Active mounds present in the experimental plots before treatment were classified as original mounds; mounds forming after the treatment date were classified as new mounds.

We rated a mound as active when ≥30 ants were present within 20 s after the mound was

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[1] Current address: Medical and Veterinary Entomology Research Laboratory, USDA-ARS, P.O. Box 14565, Gainesville, FL 32604.

disturbed; a mound was rated as inactive when <30 ants were observed after disturbance. Mounds were disturbed by shaking vegetation growing out of the mound or blowing on the mound, or both. Activity ratings of original and new mounds were recorded at weekly intervals for the duration of each study.

The percentage of ants infected with *B. bassiana* was determined from live *S. invicta* collected weekly from 10 randomly selected, active mounds per treated or control area. Ants were collected by disturbing a mound, allowing the ants to climb up a spatula or pipe that was placed on the mound surface, and then shaking them into a container. A maximum of 100 ants per mound were killed by freezing, surface sterilized in 95% ethyl alcohol, and either placed on water agar plates (1.5% agar) or in microtiter plates. The ants were held for 7 to 11 d at 25°C and then were examined for evidence of *B. bassiana* infection.

**Surface Application.** An ungrazed pasture with clay soil (0.9 ha) located at the University of Florida in Gainesville was used for the surface application study. The pasture was divided into control and treatment areas, with a 3-m-wide untreated buffer zone separating the two areas. Fifty mounds, each of which were a minimum of 15 cm in basal diameter and contained brood, were used in each area. The mounds were >1 m apart.

Immediately before each mound application in the fungus treatment area, a mound was disturbed to a depth of ≈10 cm with a trowel to expose thousands of *S. invicta*. A rice–fungal formulation was then scattered onto the surface of each of the 50 mounds. The formulation consisted of 200 g of cooked white rice with a sporulating culture of Bb447 containing about 5% conidia by weight (Alves & Pereira 1989). In the control area, 200 g of cooked rice without fungus was applied to the surface of each of 50 mounds. Application to the control area was made in the morning, and the Bb447 formulation was applied in the afternoon of the same day to limit exposure to heat and sunlight. The study was done from 19 April through 14 June 1989, when mean (±SD) maximum and minimum temperatures in the Gainesville area were 32 ± 3 and 16 ± 4°C, respectively, and rainfall totaled 187 mm.

**Injection Applications.** Three studies using injection applications were conducted at the IFAS Horticultural Unit of the University of Florida near Gainesville. An ungrazed pasture (1.5 ha) with sandy loam soil was divided into 10-by-10-m sections to aid in mound location and mapping. Various formulations of Bb447 conidia were injected into mounds through a probe connected to a compressed gas apparatus. $CO_2$ was used as the propellant, with the exception of the first study in which compressed air was used in half of the mounds. The anesthetizing properties of $CO_2$ reduced the hazard of *S. invicta* stings during application. Depending on mound size, 1–10 probe insertions were made per mound. The probe was inserted for its entire length or until the ground was impenetrable (range, 10–60 cm).

Percentages of ants infected with *B. bassiana* were determined for the first two injection studies by using the same procedures described previously, with the following changes: ants were collected from all active mounds, and infections were determined daily for the first week after treatments were applied, and thereafter at 3, 4, or 7 d intervals; and percentage of ants infected was not determined for the third injection study.

Baited traps were set weekly to compare foraging populations of *S. invicta* and other ant species in the treated and control areas. Traps were polyethylene hinge-cap vials (50 ml; 8-cm length, 3-cm diameter) containing a plastic straw (7-cm length, 6-mm diameter), cut lengthwise, and filled with a canned tuna fish and soybean oil mixture (3 g tuna:1 ml oil). All ants could enter and exit the traps until they were collected. Two sets of traps were used. The first set of traps (grid traps) was positioned every 10 m in a grid pattern (16 traps per area). Grid traps were collected after 30 min and capped; a second set of traps (mound traps) also was placed at the base of active mounds for 30 min. Depending on season, both sets of traps were set between 0730 to 1300 h.

*November 1990 Study.* For the first injection study, two 900-$m^2$ sections containing 6 and 10 *S. invicta* mounds were used as the control and treated areas, respectively. A formulation of 10 g Bb447 conidia and 90 g diatomaceous earth (Celatom, Eagle-Picher Minerals, Reno, NV) was injected into each treatment mound. Diatomaceous earth was used as a carrier because ant infection was achieved with a similar formulation in laboratory studies conducted by Stimac et al. (1993a, b). Control mounds were injected with diatomaceous earth. Applications were made in November 1990. Maximum and minimum temperatures during the 8-wk period after treatment averaged (±SD) 23 ± 5 and 11 ± 6°C, respectively, with rainfall totaling 100 mm.

*May 1991 Study.* The second study was initiated in May 1991 to examine the effect of injecting formulations containing >90% conidia by weight. On a volume basis, these formulations consisted of almost pure conidia. Six *S. invicta* mounds, located in a 400-$m^2$ area, were injected with formulations of 1 g diatomaceous earth mixed with either 10, 15, or 20 g of conidia (two mounds per concentration). The diatomaceous earth was added to prevent the conidia from sticking to the injection apparatus. Another application was made to new mounds that were established by ants that moved from three of the originally treated mounds.

Applications were made to seven new mounds because one of the originally treated mounds had split into four separate mounds. This second application was made 1 wk after the first application. The same three concentrations were used in each application. Eight control mounds, located in another 400-m$^2$ area, had a single application of the $CO_2$ propellant. Average (±SD) daily maximum and minimum temperatures were 31 ± 2 and 20 ± 2°C, respectively, with 170 mm of rain during the 4-wk period after treatment.

*July 1992 Study.* The third injection study began in July 1992. A hydrophobic carrier of fumed silica (TS-720, Cabot, Tuscola, IL) was used as a treatment and as an ingredient in the fungal formulation. The hydrophobic properties of the silica seemed to improve the dispersion of the formulation throughout the mound and the adherence of the formulation to the ants (R.M.P. & D.H.O., unpublished observations).

The field was divided into three plots of 3,500, 2,700, and 3,200 m$^2$, with 41, 34, and 37 active mounds, respectively. The mounds within the respective plots received injection treatments of either 10% Bb447 conidia in silica, silica only, or $CO_2$. Unlike the previous injection studies, most of the applications required only one insertion to distribute the formulations throughout a mound. Thorough distribution was indicated by formulation emanating from the mound surface or foraging tunnel openings located around the periphery of the mound, or both. The amount of material injected ranged from 7 to 50 g per mound for the Bb447 + silica treatment and from 3 to 50 g for the silica treatment. $CO_2$ was injected into mounds in the control area until *S. invicta* on mound surfaces were anesthetized. Mound-trap data was collected 2, 3, and 4 wk after treatments were applied. Maximum and minimum temperatures during the 1-mo period after treatment averaged (±SD) 33 ± 4 and 21 ± 1°C, respectively, with rainfall totaling 240 mm.

**Data Analyses.** Comparisons of mean daily *S. invicta* infection levels (2 to 7 d after treatment) between Bb447-treated mounds that received the air or $CO_2$ propellants were made by analysis of variance (PROC ANOVA, SAS Institute 1988). The percentages of the active, treated original mounds and active new mounds were calculated at 1, 4, and 8 wk after application. Percentages were based on the number of active original mounds that received surface application or injections in each area. In some cases, total percentage of active mounds exceeded 100% because more than one new mound appeared for each original mound that became inactive.

Grid-trap data were summarized as the number of traps containing *S. invicta* and as the number of traps containing other ant species. Because of differences in ant species and mound densities in the control and treated areas, we compared changes between counts before and after treatment within each area with chi-square analyses. For the mound traps, the average number of *S. invicta* per trap was compared between treated and control areas by *t*-tests (PROC TTEST, SAS Institute 1988). Average counts from the three areas of the July 1992 study were compared by analysis of variance and Ryan–Einot–Gabriel–Welsch multiple range test (PROC ANOVA, SAS Institute 1988). Counts were transformed [$\log_{10}(x + 1)$ or $\sqrt{(x + 0.5)}$] and averaged over all sampling dates for each trial.

## Results

**Surface Application.** The maximum mean (±SEM) *B. bassiana* infection of live ants sampled from the mounds in the treated area was 54.6 ± 5.5%, whereas infection of ants from mounds in the control area was only 1.8 ± 0.9%. Infection levels in fungus-treated mounds declined to the level in the control area at 6 wk after treatment (Fig. 1a). At 1 wk after treatment, 66% of the 50 mounds that were treated remained active, and 36% were new active mounds (Table 1). For the control area, 92% of the mounds that received rice were active, and 10% were new mounds. At weeks 4 and 8, the percentage of active original mounds in the fungus treatment declined from 68 to 38%, whereas the percentage of new mounds declined slightly to 32%. In the control area, the percentage of active original mounds declined to 74%, and the percentage of new mounds increased to 18% at 8 wk (Table 1). Over the 8-wk period, the total number of active nests in the fungus-treated area decreased by 24% more than was observed in the control area.

**Injection Applications.** *November 1990 Study.* The maximum mean (±SEM) infection of live ants collected from fungus-treated mounds was 59.9 ± 7.0% on the 5th d after treatment (Fig.1b). Infection decreased rapidly thereafter and approached the level in the control area at 5 wk after treatment (Fig.1b).

Mounds treated with Bb447 by using the $CO_2$ as a propellant did not have significantly higher ($F = 4.47$; df = 1, 6; $P = 0.079$) mean (±SEM) daily ant infection (58.4 ± 5.1%) than mounds treated with the compressed air propellant (39.4 ± 7.4%). Differences in mound activity caused by propellant type also were not apparent for treated or control mounds.

Infected ants were obtained from both the treated mounds and new mounds. In the fungus-treated plot, 4 to 8 d after treatment, we observed many *S. invicta* near 9 of the 10 treated mounds to be actively walking on the surface of the grass, not following trails, and exhibiting a behavior similar to the alarm response that is elicited when a nest is disturbed. Mean (±SEM) infection from these ants collected 5 d after treatment

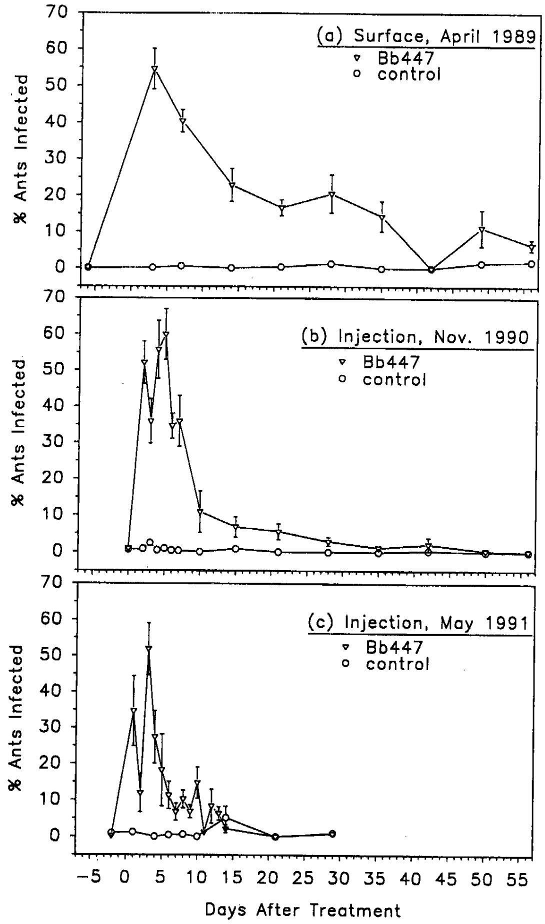

**Fig. 1.** Mean percentages (±SEM) of infected *S. invicta* collected from Bb447-treated and control mounds or new mounds (a) surface application, April 1989; (b) injection, November 1990; and (c) injection, May 1991.

around four mounds was 78.3 ± 6.0%. An average of 55.2 (±8.1)% of the ants collected by disturbing these same mounds were infected for the same sampling date. At 6 d after treatment, piles of dead ants also were observed near treated mounds, and mycelial growth and sporulation from cadavers indicated *B. bassiana* infection. Conidia obtained from such field-collected cadavers were infective in laboratory bioassays (D.H.O., unpublished data).

In the plot treated with fungus, the percentage of original mounds that remained active declined from 87.5% after 1 wk to 25% at 8 wk, whereas the percentage of new mounds increased from 50 to 75% (Table 1). In the control area, the percentage of original mounds that were active declined to 40% in the 1st week and remained low (50%) at week 8. The percentage of new mounds in the control area increased from 40 to 75% (Table 1).

The percentages of grid traps containing *S. invicta* or other species of ants changed significantly over the 8-wk period after treatment, both in the control area ($\chi^2 = 33.9$, df = 8, $P < 0.001$) and the area treated with fungus ($\chi^2 = 74.7$, df = 8, $P < 0.001$) (Fig. 2a). In the control area, the percentage of traps with *S. invicta* remained consistently low throughout the study. The reduction in traps with species other than *S. invicta* probably reflected a seasonal decrease in foraging activity because temperatures became cooler (Porter & Tschinkel 1987). In the area treated with fungus, we observed, in 1 wk, an 89% reduction in the average percentage of traps that contained *S. invicta* before treatment, suggesting a reduction in this species foraging as a result of the Bb447 application. This result contrasted with the more gradual reduction in collections of species other than *S. invicta* in the fungus-treated and control areas. Before treatment, *S. invicta* occupied most of the grid traps, but at

**Table 1. Percentages of mounds that received surface applications or injections of either Bb447 or a control consisting of the carrier without Bb447 that were active at 1, 4, and 8 wk after treatment**

| Application method | Date | Treatment | Week 1 |  | Week 4 |  | Week 8 |  |
|---|---|---|---|---|---|---|---|---|
|  |  |  | Original mounds[a] | New mounds[b] | Original mounds | New mounds | Original mounds | New mounds |
| Surface | Apr. 1989[c] | Bb447 | 66.0 | 36.0 | 68.0 | 32.0 | 38.0 | 32.0 |
|  |  | Control | 92.0 | 10.0 | 84.0 | 14.0 | 74.0 | 18.0 |
| Inject | Nov. 1990[d] | Bb447 | 87.5 | 50.0 | 62.5 | 25.0 | 25.0 | 75.0 |
|  |  | Control | 40.0 | 40.0 | 40.0 | 60.0 | 50.0 | 75.0 |
| Inject | May 1991[e] | Bb447 | 0.0 | 50.0 | 0.0 | 117.0 | — | — |
|  |  | Control | 25.0 | 50.0 | 37.5 | 62.5 | — | — |
| Inject | July 1992[f] | Bb447 + Silica | 4.9 | 41.4 | 0.0 | 34.1 | — | — |
|  |  | Silica | 8.8 | 32.4 | 5.9 | 35.3 | — | — |
|  |  | Control | 21.6 | 54.1 | 18.9 | 51.4 | — | — |

[a] Percentage of original mounds that remained active. (No. of active original mounds/no. of original mounds treated) × 100.
[b] Percentage of original mounds that are active at a new site. (No. of active new mounds/no. of original mounds treated) × 100.
[c] Percentages based on $n = 50$ Bb447-treated and $n = 50$ control (rice) mounds.
[d] Percentages based on $n = 8$ Bb447-treated and $n = 5$ control (diatomaceous earth + $CO_2$ or air) mounds.
[e] Percentages based on $n = 6$ Bb447-treated and $n = 8$ control ($CO_2$) mounds.
[f] Percentages based on $n = 41$ Bb447+silica-treated, $n = 34$ silica-treated, and $n = 37$ control ($CO_2$) mounds.

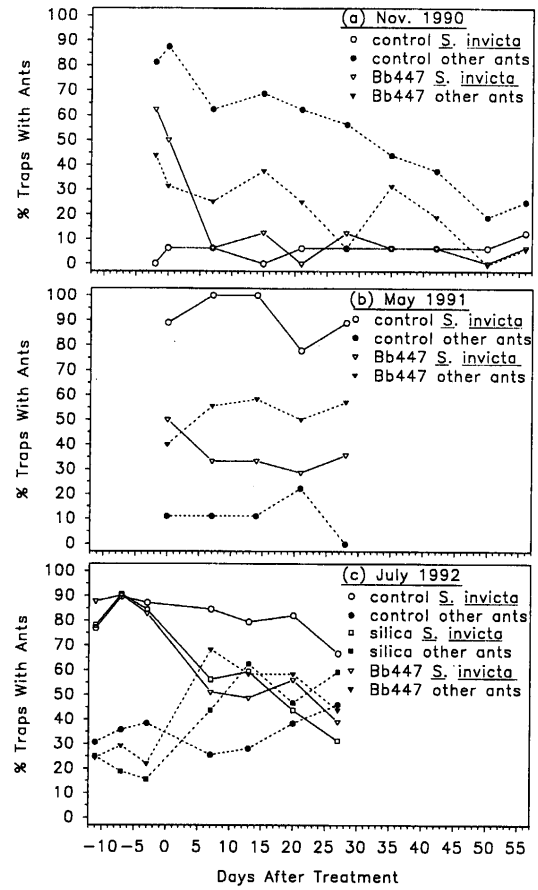

**Fig. 2.** Percentages of grid traps that contained *S. invicta* (open symbols with solid lines) or other ant species (filled symbols with dashed lines) in the control (circles), fungus (triangles), and silica (squares) treated areas for the injection applications in (a) November 1990, (b) May 1991, and (c) July 1992.

1 wk after treatment, other species of ants occupied three times as many traps as *S. invicta*. This pattern of trap occupancy continued throughout the 8-wk observation period. Besides a reduction in *S. invicta* foraging at grid traps in the area treated with fungus, numbers of *S. invicta* collected in mound traps from this area were significantly lower than those collected in the control area (Table 2).

*May 1991 Study.* The maximum mean (±SEM) ant infection of sampled ants for all treatment concentrations was 51.9 ± 7.2% on the 3rd d after treatment. After the second application, the maximum mean ant infection of the retreated mounds was 22.7%. Infection levels decreased to near control levels within 1 wk after each application (Fig. 1c). We detected no proportional increase in infection level with increase in concentration, with maximum infection levels for the 10, 15, and 20 g rates of 34, 73, and 47%, respectively. After 1 wk, none of the original mounds showed ant activity, and new active mounds were associated with half of the treated mounds (Table 1).

All of the colonies that were given a second application established new mounds. Four wk after the first injection, all original mounds remained inactive, and the number of active new mounds was higher than the number of original mounds. Piles of dead ants or infected brood, or both, which later had mycelial growth and in some instances sporulated, were found near all mounds treated with fungus. In the control plot, activity in the original mounds also declined dramatically as new mound formation increased. After 1 wk, only 25% of the original mounds remained active; 50% of the original number of mounds were new mounds. At 4 wk, 37.5% of the original mounds showed activity, and the remaining active mounds (62.5%) were new (Table 1).

**Table 2. Mean ± SEM number of *S. invicta* per trap from baited traps placed at the base of active mounds in treated and control areas of the injection applications**

| Injection trial date | Control | Bb447 | Silica | Test statistics |
|---|---|---|---|---|
| Nov. 1990[a] | 175.1 ± 39.5<br>$n$ = 31 | 15.4 ± 7.3<br>$n$ = 67 | — | $t$ = 5.44; df = 41,[b]<br>$P$ < 0.001 |
| May 1991[c] | 275.0 ± 46.6<br>$n$ = 31 | 76.9 ± 20.4<br>$n$ = 25 | — | $t$ = 2.96; df = 54,<br>$P$ = 0.005 |
| July 1992[d] | 65.2 ± 12.4a[e]<br>$n$ = 84 | 36.9 ± 12.1b<br>$n$ = 43 | 35.5 ± 14.4b<br>$n$ = 37 | F = 6.89; df = 2, 161;<br>$P$ = 0.013 |

[a] Counts (mean ± SEM) before treatment from control (218 ± 56) and fungus-treated (279 ± 58) areas did not differ significantly ($t$ = 0.79, df = 25, $P$ = 0.437). Traps were set 12 and 0 d before treatment application and at weekly intervals after application for 8 wk.

[b] Satterthwaite's approximation because of unequal variances.

[c] Counts before treatment from control (383 ± 107) andf fungus-treated (148 ± 45) areas did not differ significantly ($t$ = 0.71, df = 12, $P$ = 0.489). Traps were set 2 d before the first treatmewnt application and at weekly intervals after application for 4 wk.

[d] Counts before treatment were not available. Traps were set at 2, 3, and 4 wk after treatment application.

[e] Means followed by the same letter within a row were not significantly different ($P$ > 0.05); Ryan-Einot-Gabriel-Welsch multiple range test [SAS Institute 1988]).

Percentages of traps containing *S. invicta* or other ant species did not differ significantly in the control area before and after treatment ($\chi^2 = 2.38$, df = 4 $P > 0.05$) but differed in the area treated with fungus ($\chi^2 = 9.95$, df = 4, $P < 0.05$) (Fig. 2b). In the treated area, average percentages of traps with *S. invicta* decreased by 35% after treatment, and the traps with other ant species increased by 38% relative to percentages before treatment. Further evidence of reduced foraging by *S. invicta* was the significantly lower number of *S. invicta* collected in mound traps in the treated area compared with the control area (Table 2).

*July 1992 Study.* The percentage of active original mounds was reduced to <10% in both treatments at 1 and 4 wk after application, and the formation of new mounds was <42% of the original mounds (Table 1). By week 4, none of the original mounds treated with Bb447 + silica were active, and only 34.1% of the original mounds continued to show activity at new mounds. A similar pattern of ant activity for original and new mounds was observed in the silica treatment. In the control area, 18.9% of the original mounds remained active; 51.4% of the original mounds were active at new mound locations (Table 1).

The percentage of grid traps containing *S. invicta* decreased, while the number of traps with other ants increased significantly in the Bb447 + silica ($\chi^2 = 95.6$, df = 4, $P < 0.001$) and the silica treated areas ($\chi^2 = 98.3$, df = 4, $P < 0.001$) (Fig. 2c). In the control area, percentages after treatment did not differ significantly from percentages before treatment for *S. invicta* and other ants ($\chi^2 = 4.6$, df = 4, $P > 0.05$). The Bb447 and silica-treated areas had an average of 43% less traps with *S. invicta* and a 143% increase in traps containing other ants (Fig. 2c). For the traps placed at active mounds, *S. invicta* counts between the Bb447 + silica and the silica treatments did not differ, but both were significantly lower than the control (Table 2). Thus, based on both methods of bait trapping, we conclude that foraging activity by *S. invicta* was less in the two treated areas than in the control.

## Discussion

Ant infection levels from the first three trials resulted in the same general pattern with peak infection occurring within 7 d of application, followed by a rapid decline. Peak infections levels did not vary greatly among the trials, with a range of from 52–60%. These infection levels were similar to the Bb447 infection level of 55% in *Solenopsis* spp. from a field test done in Brazil (Stimac et al. 1989). Because ant samples were obtained from live ants that climbed to the surface of the soil upon disturbance of the ant mounds, our estimate of percentage infection of live ants is probably lower than the true level of infection in the mound. Although some infected ants were collected in the live ant samples, infected ants that were away from their mounds and ants with advanced stages of mycosis, which probably did not respond to the disturbance, were not sampled. Infection levels were not correlated with mound activity because most mounds remained active at original or new locations. Levels of ant infection thus indicated only that the fungus applications caused infection above naturally occurring levels.

Infection levels from the injection application trials declined more rapidly than in the surface application, probably as a result of greater formation of new mounds with the injection applications. For the first three weekly samples used to estimate infection levels in the surface application study, 90% of the samples were from the original mounds that were treated with fungus. In contrast, in the injection studies only 53% of the samples were from original treated mounds. Samples from new mounds probably showed lower ant infection because only uninfected ants or newly infected ants not exhibiting symptoms of infection moved to new locations. For example Marikovsky (1962) reported the movement of healthy *Tetramorium caespitum* (L.) to a new nest, leaving fungus-infected ants enroute or in the old nest.

More new mounds were formed in the areas treated with Bb447 than in the control areas for all application techniques used. In the surface application trial, 37% of the active mounds in the area treated with Bb447 were new mounds in contrast with only 14% in the control area. For the injection trials, 80% of the active mounds in the areas treated with Bb447 and 65% in the control areas were new mounds. Drees et al. (1992) reported new mound formation after the application of nematodes to fire ant mounds. The disturbance to colonies from probe insertions and injection of compressed gas seemed to have prompted new mound formation within 1 wk (Table 1).

At the conclusion of each trial, the percentage of active mounds in the treated areas relative to controls were reduced in all but the May 1991 trial. These results are consistent with 29.8 and 61.3% decreases in active mounds 60 and 180 d after treatment respectively, as reported by Quattlebaum (1980) for areas treated with *Beauveria*. However, Quattlebaum (1980) only considered treated mounds and not new mounds, which could have increased the estimated percentage of active mounds (Drees et al. 1992). In addition, new mound formation was not monitored closely in the experiment conducted in Brazil, in which a 67 to 100% reduction in active *Solenopsis* spp. mounds treated with Bb447 on rice was observed (Stimac et al. 1989).

The largest reduction in percentage of active mounds for all trials was that with the Bb447 + silica treatment, which had only half as many active mounds as the control after 4 wk. Although less effective than when used in combination with the fungus, the treatment with only silica only was more detrimental to *S. invicta* than fungal treatments in our previous experiments. Because of its hydrophobic properties, the silica, with or without fungus, was well dispersed throughout the nest galleries. In addition, it inhibited the ability of the ants to groom themselves, as indicated by our observation that many of the mouthparts of ant cadavers were clogged with silica particles. Both good dispersal of a control agent and its ability to remain on the ant after contact are desirable characteristics of formulations for the control of fire ants in soil.

Some fungal applications caused a net reduction in the numbers of active mounds in relation to the controls. In addition, the fungus applications also appeared to reduce *S. invicta* populations. For the injection applications, the reduced percentages of traps containing *S. invicta* and the concurrent increase in percentage of traps with other ant species in treated areas were indicative of a reduction in *S. invicta* populations. Markin et al. (1974) reported an increase in the number of species other than *S. invicta* at bait stations after mirex treatment reduced *S. invicta* populations. Further evidence of reduction of numbers of *S. invicta* in treated areas is provided by traps placed at the base of active mounds. The lower number of *S. invicta* in these traps from the fungus-treated areas indicated lower populations or at least a behavioral change toward reduced foraging, perhaps as a result of infection (Oi & Pereira 1993), and imminent death.

Although Bb447 was shown to infect *S. invicta* in the field during the summer and late fall in north central Florida, and conidia collected from field cadavers infected *S. invicta* in the laboratory, we found no evidence that pathogen transmission within and between colonies occurred. The disposal of dead ants in piles outside of the mounds before sporulation and the formation of new mounds suggest that exposure of the ants to new inoculum from cadavers is minimized. These observations concur with laboratory results in which limited transmission of Bb447 (Pereira & Stimac 1992) and no transmission of another strain of *B. bassiana* (Siebeneicher et al. 1992) were reported. These authors attributed lack of transmission to the hygienic behavior of the ants which restricted conidial exposure and transfer. However, a high level of transmission may not be necessary for *B. bassiana* to be a successful microbial control for fire ants. Improvements in application methods and formulations to increase conidial dispersal within the mound and conidial attachment to the ants could overcome the need for extensive transmission.

Results of our field experiments confirm that the Brazilian isolate 447 of *B. bassiana* can infect and kill *S. invicta* and decrease fire ant populations or their foraging activity in Florida pastures. Although further improvements in formulation and application technology are desirable to increase the percentage of mounds that become inactive, our results represent an early step toward the development of *B. bassiana* as a microbial control product for red imported fire ants.

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