Evaluation of *Beauveria bassiana* Formulations for Red Imported Fire Ant Control in Nursery Plant Containers

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NATURE OF WORK:

Beauveria bassiana formulated as BotaniGard ES has been shown to kill fire ants in potted soil, but control was poor compared to the insecticide Talstar®. Different formulations of *B. bassiana* are needed to enhance the persistence of the fungal conidia in the soil and improve its efficacy against fire ants. The objectives of this study were to evaluate different formulations of *B. bassiana* for persistence in soil media and their efficacy against fire ants. Information gained from this study may lead to improved safety conditions in nurseries by preventing fire ant colonizations and reduced need for expensive soil insecticides.

Greenhouse study Two formulations provided by Mycotech Corporation (Butte, MT) were an experimental granular preparation (726, 20-30 mesh) of *B. bassiana* and BotaniGard ES® (emulsifiable suspension). The amount of ES used was 0.0092 ml per 6-inch azalea pot. Granular *B. bassiana* was applied at 15 g per pot. There were five pots in each treatment, and treatments were replicated six times in a randomized complete block design. Treatments were mixed with soil, and 100–200 worker red imported fire ants were placed on the surface of each experimental unit. Soil samples were taken from two pots in each treatment daily for 12 days, and fire ant activity was monitored for 20 days. Soil from pots treated with ES and granular *B. bassiana* formulations was tested for viable fungal fragments by plating on oatmeal-dodine agar and incubating at 25° C.

Nursery study *Ilex glabra* (L.) Gray 'Compacta' liners were potted in 80, 5-gallon containers with standard soil mix. All containers were transported to the University of Georgia Research and Education Garden on the College of Agriculture and Environmental Sciences Griffin Campus. Containers were arranged in an open area in the Garden with treatments assigned in a randomized complete block design. Treatments were untreated controls, Talstar (bifenthrin, FMC Corporation, Philadelphia, PA) at 0.004 lb [AI] per cubic yard of soil, two rates of BotaniGard ES, and BotaniGard 22 WP® (wettable powder). On 1 June, each treatment was applied to two containers, and the test was replicated eight times in a randomized complete block design. Pots that were treated with ES treatments each received 19.8 ml of material. Pots treated with WP received 56.7 g per container. On 5 July, 16 of the pots treated earlier with ES received 13.2 ml additional BotaniGard ES material. The other 16 pots originally treated with ES were retreated with 26.4 ml of the material. Following application of treatments, fire ants from laboratory colonies were introduced to containers on 2 June and 5 July. Pots were inspected daily for presence of live fire ants for 14 days in the June test and 14 days in the July test. Ant activity data for the June and July tests were pooled prior to statistical analysis. In the June and July tests, dead ants in pots treated with ES and WP were removed 7-11 days following treatment and were plated on oatmeal-dodine agar. The dead ants on agar plates were checked daily

for external growth of *B. bassiana*. In June, soil samples were taken daily for 14 days from containers treated with ES and WP and tested for viable fungal elements by plating on oatmeal-dodine agar. Soil samples were not taken in July.

RESULTS AND DISCUSSION:

Greenhouse study The mean number of *B. bassiana* colony forming units (CFUs) in pots treated with the granular formulation was 7.0×10^6 per g of soil one day after treatment (Fig. 1). On day two, the mean number of CFUs dropped to 1.0×10^6. In the days following, mean CFUs in granular-treated pots fluctuated and then declined to 2.7×10^5 per g of soil on day 12.

One day after treatment, the mean number of CFUs in pots treated with ES was 7.5×10^4 per g of soil (Fig. 2). The number of CFUs increased to 1.6×10^5 on day two and then cycled between 2.0×10^3 and 2.5×10^5 every three days. *Beauveria bassiana* was not detected in ES-treated pots on day nine, but peaked at 2.5×10^5 on day ten. There were 20 times fewer CFUs in ES treated soil on day 12 compared to potted soil treated with granular *B. bassiana*.

Dead ants were observed on the soil surface of pots treated with ES and granular *B. bassiana*. However, ant activity as measured by the number of days live ants were observed in pots, was not significantly ($F = 1.02$; df = 2,5; $P = 0.3649$) different between treated and untreated containers.

Nursery study On the first day following treatments, there was a mean of 3.2×10^4 CFUs per g of soil in pots treated with ES (Fig. 2). The number of CFUs rose to 4.4×10^6 on day two and then cycled every two days between 0.0 and 9.2×10^6 until day seven. After day seven, the mean number of CFUs in ES-treated pots was $\leq 5.6 \times 10^4$.

There was a mean of 9.4×10^5 CFUs per g of soil in pots treated with WP one day after treatment (Fig. 2). The following day, mean CFUs counts increased and then peaked at 6.4×10^6 on day three. Thereafter, mean CFUs in pots treated with WP were $\leq 1.4 \times 10^6$ per g of soil. Although WP-treated pots initially received about 3 times more conidia than ES-treated pots, there were 75 times more CFUs in pots treated with WP compared to ES on day 14.

The number of days of the 14-day sampling periods on which live fire ants were observed in treated pots is presented in Table 1. Live fire ants were present in Talstar-treated pots on significantly ($F = 7.99$; df = 4,155; $P < 0.0001$) fewer days than for the other treatments. However, in the June test, live workers were observed in two Talstar-treated pots 14 days following the start of the test. In July, live fire ants were observed in three pots treated with Talstar 13-14 days after they were placed in containers. There was no significant ($P > 0.05$) difference in the number of days that fire ants were observed in untreated, BotaniGard WP, or BotaniGard ES (high rate) treated pots. In June, 22% of dead ants from pots treated with ES developed external growth of *B. bassiana*. The percentage of dead ants from WP-treated pots that developed external growth of *B. bassiana* was 75%. In July, the percentage of dead ants from pots treated with the low and high rates of ES and WP ranged from 10-17%.

SIGNIFICANCE TO INDUSTRY:

In the greenhouse and the nursery study, *B. bassiana* formulated on solid carrier (granular and WP) was more persistent in potted soil than the liquid BotaniGard ES. External growth of *B. bassiana* was found on dead ants taken from pots treated with ES and WP, demonstrating that the treatments killed fire ants. However, ES and WP treatments did not totally eliminate fire ants from containers. Talstar provided better fire ant suppression than the BotaniGard formulations, but did not totally eliminate workers from containers either. This contrasts with the results of our similar study in 1999, in which fire ants were totally eliminated from Talstar-treated pots within 2 days of addition of ants. By itself, *B. bassiana* may not be a viable alternative to Talstar for fire ant control in nursery pots. However, potential exists for combining the fungus with very low rates of insecticide for that purpose.

Table 1. Days on which live fire ants were observed in treated pots in nursery study.

Treatment	Days* (± SE)
Untreated Control	7.2 ± 0.9a
Talstar	3.9 ± 0.5b
BotaniGard ES Low	9.6 ± 0.7c
BotaniGard ES High	8.0 ± 0.8ac
BotaniGard WP	7.4 ± 0.7ac

*Data from June and July tests were pooled and represent the means (± SE) for the 14 day sampling period. Means followed by the same letter are not significantly different (LSD, $P > 0.05$).