NOTE

Possible Antagonistic Activity of Two Entomopathogens Infecting Workers of the Red Imported Fire Ant (Hymenoptera: Formicidae)[1]

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Densities of the red imported fire ant, *Solenopsis invicta* (Buren), within its range in the United States are 4× to 7× greater than in its native range in South America (Porter, S.D. et al., Environ. Entomol. 26:373-384, 1997). This is primarily due to a lack of natural enemies in its expanded range in North America (Porter et al. 1997; Jouvenaz, D.P., Florida Entomol. 66:111-121, 1983). Several natural enemies have been studied as to their potential for controlling fire ants in the southern United States. These include the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Pereira, R.M. and J.L. Stimac, Environ. Entomol. 21:1427-1432, 1992; Oi, D.H. et al., J. Econ. Entomol. 87:623-630, 1994) and entomophilic nematodes (Jouvenaz, D.P. et al., Florida Entomol. 73:190-193, 1990; Jouvenaz, D.P. and W.R. Martin, Florida Entomol. 75:148-151, 1992). We are currently evaluating the impact of multiple infections of entomopathogens on the survival of fire ants. Earlier studies have demonstrated that some combinations of entomopathogens within the same insect host can enhance mortality (Fuxa, J.R., J. Invertebr. Pathol. 33:316-323, 1979). The objective of the research reported herein was to determine the compatibility and interaction of *B. bassiana* and *Steinernemia carpocapsae* (Weiser) nematodes within the same fire ant hosts.

Red imported fire ant colonies used in these assays were dug from a pasture located 8 km northwest of The University of Georgia's College of Agricultural and Environmental Sciences Griffin Campus (Griffin, GA). These colonies were transported to the laboratory where they were maintained using procedures described by Banks et al. (USDA, SEA, AATS-21, 1981).

Arenas for the assays were 15 cm diam × 6.5 cm high plastic cylinders. The upper internal surfaces of each were coated with Fluon® (Northern Products Inc., Woonsocket, RI). Sand was sieved (0.83 to 1.75 mm), washed, and autoclaved; 25 ml was

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placed in each arena. This amount was of sufficient volume to cover the bottom of the arena but did not allow tunneling by worker ants.

The *B. bassiana* used in the assays was originally obtained from Ciba-Geigy Corporation, Inc. (Greensboro, NC) and had been designated 'CGEF-12.' The fungus had been maintained in our laboratory on Sabouraud's dextrose agar with periodic passages through susceptible insect hosts. Conidia were harvested from aerial cultures and mixed with sterile distilled water and Tween-80® (Fisher Scientific, Fair Lawn, NJ) (0.05%, v/v) to prepare the conidial suspensions for the treatments. The *S. carpocapsae* nematodes used in the assays were commercially formulated as Ecomask® (BioLogic Co., Willow Hill, PA) and were suspended in water as per label recommendations.

Treatments were applied to the sand in each arena in a total volume of 10 ml, whether the pathogens were applied individually or in combination. Treatments were: (1) *B. bassiana* alone; (2) *S. carpocapsae* alone; (3) *B. bassiana* and *S. carpocapsae* applied simultaneously; (4) *B. bassiana* applied 3 d after the *S. carpocapsae* application; (5) *S. carpocapsae* applied 3 d after the *B. bassiana* application, and; (6) a control (10 ml sterile distilled water).

The concentration of *B. bassiana* added to each arena was 6×10^3 viable colony-forming units (CFUs) per cm^2. The number of viable CFUs was determined by drop-plating serial dilutions of the conidial suspension on agar. The concentration of nematodes added to each arena was 158 active dauers per cm^2 which was determined by microscopic examination of serially-diluted aliquots of the suspension of commercially-formulated nematodes. These concentrations were selected based upon previous reports (Drees et al., J. Econ. Entomol. 85:365-370, 1992; Stimac et al., J. Econ. Entomol. 86:1083-1087, 1993) indicating that these respective concentrations kill approximately 25 to 35% of exposed fire ant workers. Mortality within these ranges resulting from exposure to a single entomopathogenic agent allows for detection of synergistic, additive, independent, or antagonistic interactions between the two entomopathogens when they are applied together, either simultaneously or sequentially (Gardner, W.A., J. Econ. Entomol. 81:463-469, 1988).

Each treatment was replicated five times in a randomized complete block experimental design. A replicate was one arena containing the treated sand, 30 ant workers measuring 3.5 to 5.5 mm in length, and a small vial cap containing honey diluted with water as a food source. These were kept in a room maintained at 24 to 26 C and 42 to 53% relative humidity throughout the bioassay. Sand in each arena was kept moist with the addition of sterile distilled water when needed. Ants in each arena were observed for mortality on a daily basis for 14 d.

Ant cadavers removed from the treatment arenas were either crushed and microscopically examined for nematodes or surface sterilized, plated on Sabouraud's dextrose agar, incubated at 30 C, and observed for external mycelial growth of *B. bassiana.* Cumulative mortality data were corrected for control mortality with Abbott's (J. Econ. Entomol. 18: 265-267, 1925) formula. An expected mortality (derived from the addition of the mortality caused by *B. bassiana* alone to the mortality caused by *S. carpocapsae* alone) was statistically compared to the observed mortality of each of the treatments with the combined agents, either simultaneously of sequentially, using the CATMOD procedure of the Statistical Analysis System (SAS Institute, Inc., SAS/STAT User's Guide, version 6, Cary, NC, 1989). The number of ant cadavers exhibiting external mycelial growth of *B. bassiana* in the combined treatments was statis-

tically compared to the number exhibiting external mycelial growth following treatment with the fungus alone using the general linear models procedure of SAS (1989).

Mean (±SEM) cumulative mortality of ant workers over the 14-d observation period was 35.7% (±7.9) following treatment with *B. bassiana* alone and 21.9% (±11.1) following treatment with *S. carpocapsae* alone. Treatment with both agents yielded cumulative mean (±SEM) mortality levels of 29.1% (±6.3) for the simultaneous treatment, 29.0% (±5.7) for the sequential treatment with *B. bassiana* followed 3 d later by *S. carpocapsae,* and 23.5% (±9.2) for the sequential treatment with *S. carpocapsae* followed 3 d later by *B. bassiana.* While the observed mortality of each of the combined treatments was significantly ($X^2 > 31.49$; df = 1, 13; $P < 0.0001$) lower than the expected mortality derived by the additive effects of the two agents acting alone, the resultant mortality did not differ significantly among the treatments with the agents. Therefore, the effect on ant worker mortality resulting from combining the two agents appeared independent (Gardner, 1988; Fuxa, J.R., J. Invertebr. Pathol. 33: 316-323, 1979).

However, the percentage of ant cadavers exhibiting external growth of *B. bassiana* mycelia was significantly ($F = 9.10$; df = 2, 4; $P = 0.0003$) higher in the treatment with the fungus alone than in the treatments with the combined agents. Mean (±SEM) percentage of cadavers exhibiting the mycelial growth was 86.9% (±5.4) for fungus alone, 54.0% (±6.8) for the simultaneous treatment with both agents, and 49.1% (±7.3) for sequential treatment with the fungus followed by the nematode. None of the cadavers removed from the sequential treatment with nematodes followed 3 d later by the fungus yielded external *B. bassiana* mycelial growth. Reductions in the external growth of *B. bassiana* and, thus, the natural recycling potential of the entomopathogen following death of the ants suggests a potential antagonistic interaction of *S. carpocapsae* with *B. bassiana* in fire ant workers. The effect is especially confounded in sequential treatment of the ants with the nematode followed by treatment with the fungus 3 d later. Bacterial symbionts released by the nematode following successful infection (Akhurst, R.J., J. Gen. Microbiol. 128: 3061-3065, 1982) of the host ants are presumably the cause of the observed effects. This and other factors associated with the interactions of these agents in fire ant workers, as well as their implication on the potential management of fire ants using these agents should be further explored.

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