Microbial Formicides: Current USDA Research

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ARS biocontrol research has emphasized the study of the specific natural enemies of fire ants, with the goal of establishing a complex of pathogens and arthropods in the United States to effect a permanent amelioration of the fire ant problem. A secondary goal has been to develop a biological formicide. In this report, two investigations we have recently completed in the latter area are presented.

Ingestion of Entomopathogenic Bacteria.

Effective formicides destroy the reproductive capacity (kill or sterilize the queen or queens) of ant colonies. Toxicants which act rapidly kill only worker ants; they reduce the size of colonies temporarily, but do not effectively eliminate them. Therefore, toxicants, including bacterial toxins, must exhibit slow or delayed action to insure distribution through the colony to the queen(s) before the workers begin to die. Furthermore, delayed-action toxicants must remain effective over at least a 10-fold, and preferably greater range of concentrations, for serial dilution occurs through trophallaxis (Banks 1990). These properties are not common to bacterial toxins. Thus, unless cells or spores are actually ingested by the queens, elimination of colonies by non-host specific bacteria is very improbable.

Fire ant queens are fed only highly filtered, regurgitated liquids and (possibly) glandular secretions; bacteria are absent or highly restricted in the gut. Glancey et al. (1981) found that the pharyngeal filters of worker fire ants remove latex microspheres $\geq$ 0.88 $\pm$ 0.02 mu in diameter. Most bacteria, therefore, should be removed from food during trophallaxis.

Several species of bacteria have been tested as formicides against worker ants, but no large-scale screening to identify strains especially virulent for fire ants has been attempted. Moreover, the vulnerability of fire ant queens to bacteria has not been investigated. The purpose of this study was to determine whether selected bacteria are ingested by fire ants, and thereby determine the feasibility and parameters such as size and flagellation for screening strains as microbial formicides.

We created seven small, homogeneous colonies, each composed of 12 queens, approximately 5,000 workers, and 1.5 g of brood, by partitioning a laboratory colony of polygyne *S. invicta*. Daily for 14 days, duplicate colonies were fed either spores of *Bacillus thuringiensis*, spores of *Bacillus sphaericus*, or cells of *Serratia marcescens* mixed with boiled egg yolk to a paste consistency. Daily for the last 10 days of the test, each colony was also fed

one large, living corn earworm larva which had been injected with 0.25 ml of the suspension of bacteria. The ants readily consumed the diet containing bacterial cells or spores, as we observed daily and confirmed by isolating *S. marcescens* from the gut of fourth instar larvae. The seventh colony served as a control.

Ten queens from each colony were surface-sterilized, decapitated, and dissected aseptically in Grace's insect cell culture medium. First, the venom glands were removed, for the venom is antibacterial (Jouvenaz, 1972). The thoraces and gasters were dissected, homogenized and plated separately on differential media (see Appendix for details). All plates were incubated at 28° C and examined at 24 and 48 h. After completing the ingestion tests using queens, we extended this study to include workers that had fed to repletion on egg yolk containing bacteria.

Serratia marcescens was not recovered from the 20 queens or 10 workers fed this bacterium. Cells of *S. marcescens* measure about 0.5 x 0.5-1.0 um and have four peritrichous flagella. Since the smaller cells are below the 0.88 mu filtration limit of fire ant workers, we assume the long flagella become entangled in the filter.

Bacillus sphaericus was recovered from the gaster of only one of 20 queens from two colonies fed spores of this species. The pharyngeal crop of this specimen was sterile; the gaster yielded 10 colonies of *B. sphaericus*. Spores of *B. sphaericus* measure 0.7-1.2 um in diameter. Thus, the smaller spores, if naked, should pass the pharyngeal filter; however, the sporangium adhered strongly to the spores in this preparation, effectively increasing their size. All 10 workers exposed to *B. sphaericus* yielded sterile plates.

Bacillus thuringiensis was not recovered from 20 queens from colonies fed this bacterium. Seven of the workers yielded sterile plates; the remaining three yielded four, six, and 14 colonies of bacteria, all of which were *B. thuringiensis*. The spores of *B. thuringiensis* measure 1.0-1.5 um in diameter and are too large to pass the pharyngeal filters of worker fire ants except rarely.

The suggestion is frequently made that if a large number of strains of *B. thuringiensis* or other entomopathogenic bacteria were screened, a microbial formicide might be found. Indeed, Miller and Brown (1983) reported significant mortality in fire ants fed *S. marcescens*, *Pseudomonas aeruginosa* and two *Enterobacter* spp. under laboratory conditions. However, only workers were used in these tests and death was due to intoxication, for essentially equal mortalities resulted whether the ants ingested food containing living cells or culture medium with cells removed. Fire ant queens are seldom killed by fast-acting toxins. Miller and Brown concluded that fire ants readily ingest bacteria; however, their methods did not differentiate between bacteria from the infrabuccal cavity and those from the gut. In light of the known filtration ability of worker fire ants and our data, we conclude that the

bacteria detected in workers by Miller and Brown were from the infrabuccal cavity.
Only nonflagellated organisms small enough to pass pharyngeal filtration during trophallaxis are candidates for screening as microbial insecticides. Recently we isolated a tiny, slow-growing bacterium (a symbiont?) from the hindgut of apparently healthy fire ant queens. There is no indication that this bacterium is pathogenic; however, biotechnology offers the exciting possibility of making commensals virulent. Other possible candidates for genetic manipulation are Mollicutes (spiroplasmas, mycoplasmas, acholeplasmas). Arthropods, including Hymenoptera, are proving to be a rich source of these microorganisms and potential fire ant-mollicute associations appear worthy of investigation.

Evaluation of nematodes for protection of nursery stock.

Early research by Poole (1976) and Quattlebaum (1980) indicated a potential of steinernematid nematodes for control of fire ants. However, Jouvenaz et al. (1990) did not achieve significant control of fire ants in field trials using nematodes produced by Biosys. Considerable relocation of fire ant nests occurred in our tests. Had we not taken mound movement into consideration, our apparent control in one test would have been 78%. (Poole and Quattlebaum scored all mounds uninhabited after treatment as dead). We subsequently confirmed the aversion of fire ants to nematodes by consistently driving small laboratory colonies from their nests in sandy soil in containers.

The readiness with which fire ants relocated their nests in the field and vacated soil in containers to avoid nematodes prompted us to investigate the potential of these parasites to eliminate fire ants from nursery stock. The early spread of fire ants in the southeastern United States was greatly facilitated by the shipment of infested nursery stock (Lofgren 1986).

Nursery and greenhouse crops ranked third nationally among farm crops in 1990, with approximately 7.6 billion dollars in cash receipts. The states within the Southern Plant Board account for nearly 30% of total U. S. nursery production. In addition to the direct costs (material and labor) of compliance with quarantine regulations, nurseries may also face loss of market opportunities and reduced worker productivity due to fire ants. Currently, federal certification of nursery stock for shipment through quarantine is based solely on incorporation of chlorpyrifos. Granular chlorpyrifos is not as effective as previously believed, drenching poses problems of worker exposure, and both treatments are expensive. According to Mr. Craig Regglebrugge of the American Association of Nurserymen, the improvement of methods for control of fire ants is a critical need of the nursery industry.

We conducted three sequential tests using Biosys nematodes against fire ants nesting in 1 gallon nursery pots containing *Pittosporum* shrubs. The test colonies consisted of five queens, 3,000-5000 workers and about 1.5 g brood. In the first test, 20 pots in separate trays were drenched with an aqueous suspension of 30,000 nematodes (about 1,000 per square inch of soil surface), 20 pots were drenched with 300,000 nematodes, and the remaining 10

pots received water only. An identical container of watered potting soil (but without a plant) was placed in the tray to provide the ants with untreated soil in which to relocate their nests. Without an "escape pot", ants evacuating the treated soil would have clustered around the base of the container, making evaluation difficult. Also, ants would be free to relocate in untreated soil in a nursery. Half of the pots were examined three days, and half 14 days after treatment, by spreading the soil on a white plastic cloth.

The second test was conducted using replanted shrubs, new ant colonies, a new batch of nematodes, and a different brand of potting soil. The same procedures were employed except that the nematodes were sprayed rather than poured onto the soil surface. The third test consisted of 30 pots infested with new colonies, of which 20 were sprayed with a single dose of 75,000 nematodes (about 2,500 nematodes per square inch of soil surface). These pots were arranged and treated in a random pattern on the ground, which was covered with short grass and partially shaded by two oak trees. Thus, the ants were free to relocate in field soil. This test was evaluated four days after treatment.

Our results were, in a word, negative. Of the 80 pots treated in the first two tests, fire ants were not eliminated from a single pot. In the first and second tests, queens and brood remained in all of the treated and control pots; neither was found in the escape pots. The numbers of workers found in the escape pots were generally fewer than 100, and were probably just foragers. In the third test, queens, brood and workers remained in 11 (55%) of the 20 treated colonies and five (50%) of the 10 control colonies. We do not plan further work with steinernematid nematodes.

Literature Cited

Banks, W. A. 1990. Chemical control of the imported fire ants. In R. K. Vander Meer, K. Jaffe, and A. Cedeno (eds.), Applied Myrmecology, A world perspective, pp 596-603. Westview Press, Boulder, CO.

Glancey, B. M., R. K. Vander Meer, A. Glover, C. S. Lofgren, and S. B. Vinson. 1981. Filtration of microparticles from liquids ingested by the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Insect. Soc. 28: 395-401.

Jouvenaz, D. P., C. S. Lofgren, and R. W. Miller. 1990. Steinernematid nematode drenches for control of fire ants, *Solenopsis invicta*, in Florida. Florida Entomol. 73: 190-193.

Lofgren, C. S. 1986. History of the imported fire ants in the United States. In C. S. Lofgren & R. K. Vander Meer (eds.), Fire ants and leaf-cutting ants, biology and management, pp 36-47. Westview Press, Boulder CO.

Miller, S. R. and L. R. Brown. 1983. Studies on microbial fire ant pathogens. Devel. Indus. Microbiol. 24: 443-450.

Poole, M. A. 1976. Survey and control efficacy of endoparasites of *Solenopsis richteri* Forel and *S. invicta* Buren in Mississippi. Ph.D. Dissertation, Mississippi State University, Mississippi State. 92 pp. [Dissert. Abstr. Int. B, 37: 3254].

Quattlebaum, E. C. 1980. Evaluation of fungal and nematode pathogens to control the red imported fire ant, *Solenopsis invicta* Buren. Ph.D. Dissertation, Clemson University, Clemson, S. C. 54 pp. [Dissert. Abstr. Int. B, 41: 16421]

Regelbrugge, C. 1991. The impact of imported fire ants on interstate shipment of nursery stock and sod. Attachment 3, Minutes of the Imported Fire Ant Technical Work Group. Available through Amer. Asso. Nurserymen, 1250 I Street, N.W., Suite 500, Washington, D.C. 20005.

APPENDIX

The extracts of queens from colonies fed *S. marcescens* were plated on mannitol-salt agar, on which this bacterium produces the red pigment prodigiosin in abundance, permitting identification. The extracts of queens from colonies fed bacilli were plated on nutrient yeast salt agar, which stimulates sporulation and thereby aids identification of *Bacillus* spp. The bacterial colonies which grew on the *Bacillus* spp. ingestion test plates were compared with reference colonies of *B. sphaericus* and *B. thuringiensis*. Cells from colonies having morphology similar to those of the reference colonies were examined by phase-contrast microscopy. *Bacillus sphaericus* was tentatively identified by the characteristic swollen, terminal spore, and confirmed by bioassay against highly susceptible, insectary-reared, *Culex quinquefaciatus* mosquitoes. Cells from colonies similar to those of *Bacillus thuringiensis* were examined for the presence of parasporal bodies.