Final Scientific Report

BARD Project Number: US-3353-02

Date Of Submission of Report: November 15, 2006

Project Title: OPTIMIZING NEMATODE MANAGEMENT BY DEFINING
NATURAL CHEMICAL BASES OF BEHAVIOR

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Keywords: attractant, chemotaxis, nematicide, flavonoids

Abbreviations: DAPG, 2,4-diacetylphloroglucinol; cfu, colony-forming units

Budget: IS: \$112,600

US: \$207,400

Total: \$320,000

Principal Investigator (PI)

Institution's Authorizing Official, Principal Institution
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Abstract

This project was based on the hypothesis that nematodes interacting with plants as either parasites or beneficial saprophytes are attracted to their host by natural products. This concept was supported by numerous observations that parasitic nematodes are attracted to root exudates. Our overall goal was to identify nematode sensory compounds from root exudates and to use that information for reducing nematicide applications. We applied skills of the investigators to achieve three specific objectives: 1) Identify nematode behavioral cues (e.g., attractants or repellents) in root exudates; 2) Identify new natural nematicidal compounds; and 3) Combine a natural attractant and a nematicide into a nematode trap. Because saprophytic nematodes benefit plants by mineralizing organic matter, we sought compounds attractive primarily to parasitic nematodes.

The project was constructed on several complementary foundations. First, data from Dr. Spiegel's lab showed that under aseptic conditions *Ditylenchus dipsaci*, a parasite on onion, is attracted to certain fractions of onion root exudates. Second, PI Phillips had a sizeable collection of natural plant products he had identified from previous work on *Rhizobium*-legume interactions, which could be tested "off the shelf". Third, Dr. Ferris had access to aseptic and natural populations of various saprophytic and parasitic nematodes. The project focused on five nematode species: *D. dipsaci*, *Heterodera avenae*, and *Tylenchulus semipenetrans* at ARO, and *Meloidogyne javanica* and *Caenorhabditis elegans* at UCD.

Ten pure plant compounds, mostly flavonoids, were tested on the various nematode species using six different assay systems. Results obtained with assorted test systems and by various scientists in the same test systems were essentially irreproducible. Many convincing, i.e. statistically significant, results in one system or with one investigator could not be repeated with other assays or different people. A recent report from others found that these compounds, plus another 30, were inactive as attractants in three additional parasitic nematode species (Wuyts et al. *Nematology* 8:89-101, 2006). Assays designed to test the hypothesis that several compounds together are required to attract nematodes have thus far failed to find a reproducibly active combination.

In contrast to results using pure plant compounds, complex unfractionated exudates from aseptic onion root reproducibly attracted *D. dipsaci* in both the ARO and UCD labs. Onion root exudate collection, separation into HPLC fractions, assays using *D. dipsaci* and MS-MS experiments proceeded collaboratively between ARO and UCD without any definitive identification of an active compound. The final active fraction contained two major molecules and traces of several other compounds. In the end, analytical studies were limited by the amount of onion root exudate and the complexity of the purification process. These tests showed that aseptic plant roots release attractant molecules, but whether nematodes influence that release, as insects trigger release of attractants from plants, is unknown. Related experiments showed that the saprophyte *C. elegans* stimulates its prey, *Pseudomonas* bacteria, to increase production of 2, 4-diacetylphloroglucinol (DAPG) a compound that promotes amino acid exudation by plant roots. It is thus possible that saprophytic nematodes are attracted primarily to their bacterial or fungal prey and secondarily to effects of those microorganisms on root exudation. These observations offer promising avenues for understanding root-zone interactions, but no direct routes to controlling nematodes in agriculture were evident.

Extracts from two plant sources, *Chrysanthemum coronarium* and *Sequoia sempervirens*, showed nematicidal activity at ARO and UCD, respectively. Attempts to purify an active compound from *S. sempervirens* failed, but preliminary results from *C. coronarium* are judged to form a potential basis for further work at ARO.

These results highlight the problems of studying complex movement patterns in sentient organisms like nematodes and the issues associated with natural product isolation from complex mixtures. Those two difficulties combined with complications now associated with obtaining US visas, slowed and ultimately limited progress on this project. As a result, US investigators expended only 65% of the \$207,400 originally planned for this project. The Israeli side of the project advanced more directly toward its scientific goals and lists its expenditures in the customary financial report.

Achievements

Analysis of the Science: Difficulties encountered while searching for nematode attractants were disappointing, and we see no easy path to explaining how complex root exudates serve as a magnet for parasitic nematodes. Perhaps various nematodes respond to different compounds; or possibly, a mixture of compounds is required for a strong, reproducible attraction. Based on our experience, any favorable plant X nematode system must be pursued in detail at one location with a team containing both nematologists and chemists. In any event, our project produced no significant scientific or agricultural advances toward the goal of attracting nematodes closer to a localized dose of a nematicide.

Six different assay systems were used at various times. Successes and failures associated with five, two-dimensional assays were described in previous reports (The ARO-attraction assay; a center-spot test, racetracks, agar-channel trials, and the arena-assembly assay). A sixth three-dimensional test, which assesses nematode movement into tubes buried in sand, has been used at Davis without convincing results during the fourth (no-cost-extension) year of the project. The ARO-attraction assay, a pair-choice test (Spiegel et al., 2003), showed reproducible attraction of *D. dipsaci* to onion root exudates, in the hands of Ms. Bar-Eyal in both Israel and the US. During her visit to Davis, Ms. Bar-Eyal did not detect movement of *M. javanica* to either onion root exudates or to a variety of flavonoids using this assay. In addition to compounds listed in Table 1, which were used repeatedly by numerous Davis scientists in different assay systems with various nematode species, a variety of plant root exudates were tested unsuccessfully on aseptic *C. elegans* in an effort to find a reproducible positive control saprophytic nematodes.

Table 1. Compounds tested as nematode attractants in concentrations from 10 nM to 100 μ M.

Test Compounds
Coumestrol
4',7-Dihydroxyflavanone
4',7-Dihydroxyflavone
Eridioctyol
Ferulic Acid
Formononetin
Genistein
Luteolin
Naringenin
Quercetin
2',4,4'-Trihydroxychalcone

We also searched for natural nematicides, which might be combined with an attractant. While we showed nematocidal activity in extracts of redwood (Year 2 report) and chrysanthemum (Bar-Eyal et al., 2006), direct use of these plant residues probably is limited by their availability. A more promising avenue to commercialization involves identifying and synthesizing an active compound for agricultural use. Further work with the complex mixture of tannins and other compounds extracted from redwood into methanol lacks promise, but it may be possible to isolate and identify compounds from the aqueous extracts of chrysanthemum that inhibited hatching of *M. javanic* eggs. This project calls for a chemist at ARO.

While the dangers of plant parasitic nematodes were not overcome on this project with diversionary attractants or new nematicides, we have a better understanding of how saprophytic nematodes function in soil food webs and potentially increase plant growth. The classical model claims that saprophytic nematodes prey on bacteria in the soil food web and release N which promotes plant growth. Data collected on this project show that the common bacterial product DAPG triggers increased root exudation of amino acids (Phillips et al., 2004) and that aseptically grown nematodes enhance DAPG production by pure cultures of *P. fluorescens* (See appendix). The concept that bacterial predators manipulate microbial activities to favor plant responses that increase resources available to the food web offers new insight into questions of how plant-growth-promoting bacteria may function. This result thus offers potential avenues for managing soil food webs to promote plant growth.

Details of Cooperation: The lead scientists on this project were personally and professionally compatible. Dr. Spiegel traveled twice to Davis for discussion and planning sessions. The collaboration was facilitated also by email and telephone exchanges. In 2005 one of Dr. Spiegel's research assistants came to Davis for a brief visit where she measured nematode attraction to various HPLC fractions purified by the PI.

Administrative Complications: Administrative difficulties complicated scientific efforts at UCD during the course of this project. In 2003 new US immigration restrictions first slowed and finally precluded the travel and employment of a promising postdoctoral nematologist from The Philippines. No suitable replacement was ever found. Substitute researchers sometimes were diverted for short periods from other projects, and many undergraduates organized chemotaxis experiments under Dr. Ferris' direction. Dr. Phillips personally did all HPLC separations and MS analyses throughout the course of the project. In 2004 Dr. Spiegel graciously offered the assistance of a senior technician who arrived in Davis for a planned 3-month visit in 2005, which

unfortunately was cut short. As a result of these problems, only 65% of the US funds were used on the project. While Phillips and Ferris supplied their own unpaid labor on the project, they both had other duties, which prevented them from functioning as full-time investigators. They remain dismayed by this outcome.

Publications from the Project:

1. Spiegel, Y., P.M. Burrows and M. Bar-Eyal. (2003) A chemo-attractant in onion root exudates recognized by *Ditylenchus dipsaci* in laboratory bioassay. *Phytopathology* 93:127-132.
2. Phillips DA, Fox TC, King MD, Bhuvaneswari TV, Teuber LR. (2004) Microbial products trigger amino acid exudation from plant roots. *Plant Physiology* 136:2887-2894.
3. Bar-Eyal, M., E. Sharon and Y. Spiegel. (2006) Nematicidal activity of *Chrysanthemum coronarium*. *European Journal of Plant Pathology* 114:427-433.
4. Spiegel, Y. Dagan, Z., Bar-Eyal, M. and Y. Kapulnik (2006) Characterization and identification of the citrus nematode, *Tylenchulus semipenetrans*, behavioural cues in root exudates of citrus and non-host plants. *IOBC-OILB Bulletin, Multitrophic Interactions in Soil and Integrated Control*, Wageningen, The Netherlands. 29(2): 147-150.

Appendix

Published Papers: (see following pages)

Unpublished data: (see concluding pages)