Final Report to the Elvenia J. Slosson Endowment Fund

Title: Root-knot nematode-destroying microorganisms for home garden and landscape use

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INTRODUCTION

Plant-parasitic nematodes are inconspicuous pests because of their small size, hidden activities and often non-specific damage to their hosts. Root-knot nematodes (RKN), *Meloidogyne* sp., are perhaps the best known of these pests attacking well over 2000 different plant species including many vegetable crops and ornamentals grown in home gardens. In California, four species of RKN are of main concern, *M. incognita, M. javanica, M. arenaria* and *M. hapla*. RKN spend only a short part of their life in soil either as eggs or as second stage larvae. The latter then enter the roots and establish feeding sites in susceptible hosts. They undergo three more molts during which the roots become swollen and have a characteristic "knotty" appearance (galling). Each female can produce several hundreds of eggs that under favorable conditions continue to develop into the next generation. Normal functions of water and nutrient uptake as well as transport are severely limited by the root galling. Secondary root tissue infections by fungi and bacteria can accelerate the damage and ultimately may lead to root death. Limitation of the root function is typically expressed with symptoms of malnutrition, chlorosis and stunting. Consequently, vigor and production capacity of diseased crops are noticeably reduced.

RKN management in production agriculture and horticulture includes soil fumigation, application of non-fumigant nematicides, soil pasteurization, and rotations with non-host crops or the use of resistant varieties. For gardeners the choices are more limited, as no nematicides are allowed for home use in California. A limited number of RKN resistant vegetable cultivars are available. Currently no biological control product against plant parasitic nematodes is commercially available in the US. For large agrochemical companies, the expected return on investment for such products is perceived as too small

to pursue R&D of nematode antagonists. Small companies typically do not have the technical expertise and/or the financial stamina to research and develop such a product.

In some soils nematode population development is suppressed. Typically, their population density remains low despite cropping to a susceptible host plant and favorable environmental conditions for the activity and development of the nematode population. Such nematode-suppressive soils present instances in which biotic factors influence the relationship between a pest and its host in favor of the latter. In recent years, we have identified and investigated such soils in Southern California (Pyrowolakis *et al*., 2001, Westphal and Becker, 2001). We have isolated strains of nematode-destroying fungi from various nematode-suppressive soils. The objective of this project was to evaluate the efficacy of these strains against root-knot nematodes and develop procedures to enhance their potential utilization in home gardens. While the use of such microorganisms is most likely too expensive in production agriculture because of relatively high application rates, they might be appropriate in home gardens or landscape sites where the control effort is likely to be concentrated in a small area.

MATERIALS AND METHODS

Greenhouse screening of nematophagous fungi against root-knot nematodes

Several nematophagous fungal strains previously isolated from nematode-suppressive soils were evaluated for their efficacy on RKN population reduction and mitigation of RKN damage. These tests were conducted under greenhouse conditions with tomato as a plant species susceptible to RKN. All fungal strains were stored in sand cultures (long-term) and on agar slants (short-term). The fungal inoculum was grown on autoclaved millet seeds in Erlenmeyer flasks. Three-week-old cultures were air-dried in a laminar flow hood and were then stored at 4 C in the dark. RKN (*M. incognita*) were reared on tomato in greenhouse pot cultures. Nematode eggs were extracted and used to infest pasteurized sandy soil at approximately 4000 eggs per 1000 cc. The fungal inoculum was mixed into the soil at 1% v/v. Nematode-infested soil amended with fungal-colonized but autoclaved millet (1% w/v) served as a control. Fenamiphos (5 kg a.i./ha) was included as a chemical standard. *Pasteuria penetrans*, an obligate bacterial parasite of root-knot nematodes was used as a biological control standard. The pots were incubated in the greenhouse at ambient temperatures for five days before three-week old tomato seedlings were transplanted into the infested soil. Each pot received slow-release fertilizer recommended for tomato production. The pots were arranged in the greenhouse in a randomized complete block design at 24±2 C and ambient light. After eight weeks, the fresh weight of the plant foliage was determined. Root galling was rated on a scale of 0-10 (0 = no galling). The stained egg masses of the RKN were counted. Soil subsamples were extracted on a modified Baermann funnel at 26 C for 4 days. Second-stage juveniles were collected and counted under a dissecting scope.

Fungal inoculum culture

Preliminary tests had suggested an improved rearing of the fungal inoculum by using autoclavable culture bags with microfilters instead of Erlenmeyer flasks. *Verticillium chlamydosporium* was chosen as a test strain for comparing the culture methods. It produces both conidia and chlamydospores that can serve as a quantitative indicator for growing conditions. Both the Erlenmeyer flasks and culture bags were filled with 150 g moist millet. After autoclaving and cooling to

room temperature, five replicates per treatment were infested with agar pieces from a growing culture of *V. chlamydosporium*. Both flasks and bags were incubated for 2 weeks at 25C in the dark. The containers were shaken to ensure even colonization of the millet. The inoculum was then air dried in a laminar flow hood. Five samples (0.5 g) were taken from each culture and were vigorously agitated in a water-filled tissue homogenizer for one minute. Chlamydospores and conidia of the fungus were enumerated with a counting chamber under 400x magnification.

Efficacy tests of two egg-parasitic fungi against root-knot nematodes

The egg-parasitic fungi *Verticillium clamydosporium* and *Dactylella oviparasitica* were each further assayed for their potential to suppress *M. incognita* in a greenhouse trial. The inoculum was produced in culture bags as previously described. The fungal inoculum was produced on millet as previously described. It was mixed with 2000 cc pasteurized sandy soil at 0, 0.1, 0.5, 1.0, 2.0 and 5.0% (w/v) and planted with 3 wk-old tomato seedlings (cv. tropic). Five percent of Verticillium-colonized but autoclaved millet served as another control treatment. Ten days later each pot was inoculated with 10,000 *M. incognita* eggs. Each trial was arranged in a greenhouse at 25°C in a randomized complete block with 5 replications. Each pot received slow-release fertilizer recommended for tomato production. After 8 weeks the trial was terminated and evaluated as previously described.

Statistical analysis.

All the data were subjected to ANOVA and, if appropriate, mean separation with Fisher's protected LSD ($P \leq 0.05$).

RESULTS AND DISCUSSION

All tested biocontrol agents significantly reduced the population of *M. incognita* as determined by enumeration of egg masses and soil-dwelling second-stage juveniles at the termination of the trial (Fig 1). Galling was significantly reduced by six strains and all but one increased plant top weight compared to the non-treated control. It should be noted that the inoculum production was not optimized for each biocontrol strain. Consequently, infestation density is likely to vary greatly among strains. For example, *Hirsutella rhossilliensis* spores are only infective if freshly produced and other nematophagous fungi might require special nutritional conditions to produce their traps. However, the purpose of this initial screen was to select two fungal strains that are good candidates for potential product development. *Dactylella oviparasitica* and *Verticillium clamydosporium* were chosen because of their overall good performance, their mode of action as egg parasites and consequent efficacy potential.

The comparison of conidia and chlamydospores production by *V. chlamydosporium* showed no statistical difference between the two culture methods (data not shown). Culturing fungi in Erlenmeyer flasks on autoclaved cereals such as oats or millet is a standard plant pathology technique. Typically, it serves the purpose of rearing sufficient amounts of inoculum for greenhouse pot tests. However, it is cumbersome if larger quantities are needed. Flasks can only be filled with a shallow layer of millet to avoid growth inhibition by volatile metabolite build-up and/or oxygen deficient culture conditions. Consequently, depending on the amount of inoculum needed, flask cultures require considerable space during autoclaving, culturing and storing. The millet often compacts during the culture of certain fungi which might affect oxygen supply. Frequently the fungus-colonized millet is

difficult to retrieve from the flasks. Flask cultures notoriously attract mites that feed on and contaminate the cultured fungus with bacteria. In contrast, the culture bags are autoclavable and allow air exchange through 0.2 micron filters. After inoculation, the bags can be aseptically sealed and stored with minimum space requirements. The flexible polypropylene bags also allow better mixing and separation of the inoculum without compromising aseptic conditions (Fig 2).

The good efficacy of both *V. chlamydosporium* and *D. oviparasitica* against root-knot nematodes was confirmed in another greenhouse trial (Tables 1a, 1b). For both fungi, a clear dose response was established. With increasing application rates of the biocontrol agents, galling, number of second-stage juveniles, number of egg masses and number of eggs decreased. At the high application rates, more than a third of the root-knot eggs were parasitized. The reduction in egg masses suggests that the fungi also destroy or at least damage the females. Shoot dry weight was significantly higher in the 2% *D. oviparasitica* and 5% *V. chlamydosporium* treatment compared to the non-treated control.

CONCLUSIONS

The results confirm our hypothesis that use of nematophagous fungi may help gardeners to suppress root-knot nematode populations when the biocontrol agent is applied at fairly high application rates. Both *D. oviparasitica* and *V. chlamydosporium* have been previously described as egg and/or female parasites of root-knot nematodes (Stirling and Mankau, 1978, Godoy *et al*., 1983, De Leij and Kerry, 1991). In our tests, they reduced the root-knot nematode egg population by about 90% with application rates between 2% and 5%. Of the remaining eggs, more than a third were infested with the fungi. It is expected that such large population reductions will be reflected in increased overall health of the crop and its yield or quality. In home gardens, the application rate of biological control products is of lesser importance than in commercial agriculture because the planting sites are typically fairly small. The culture bags are a promising tool for the production of quality inoculum and also provide several additional production and storage advantages. The next step in this research program will be to evaluate this technology in root-knot nematode infested garden sites.

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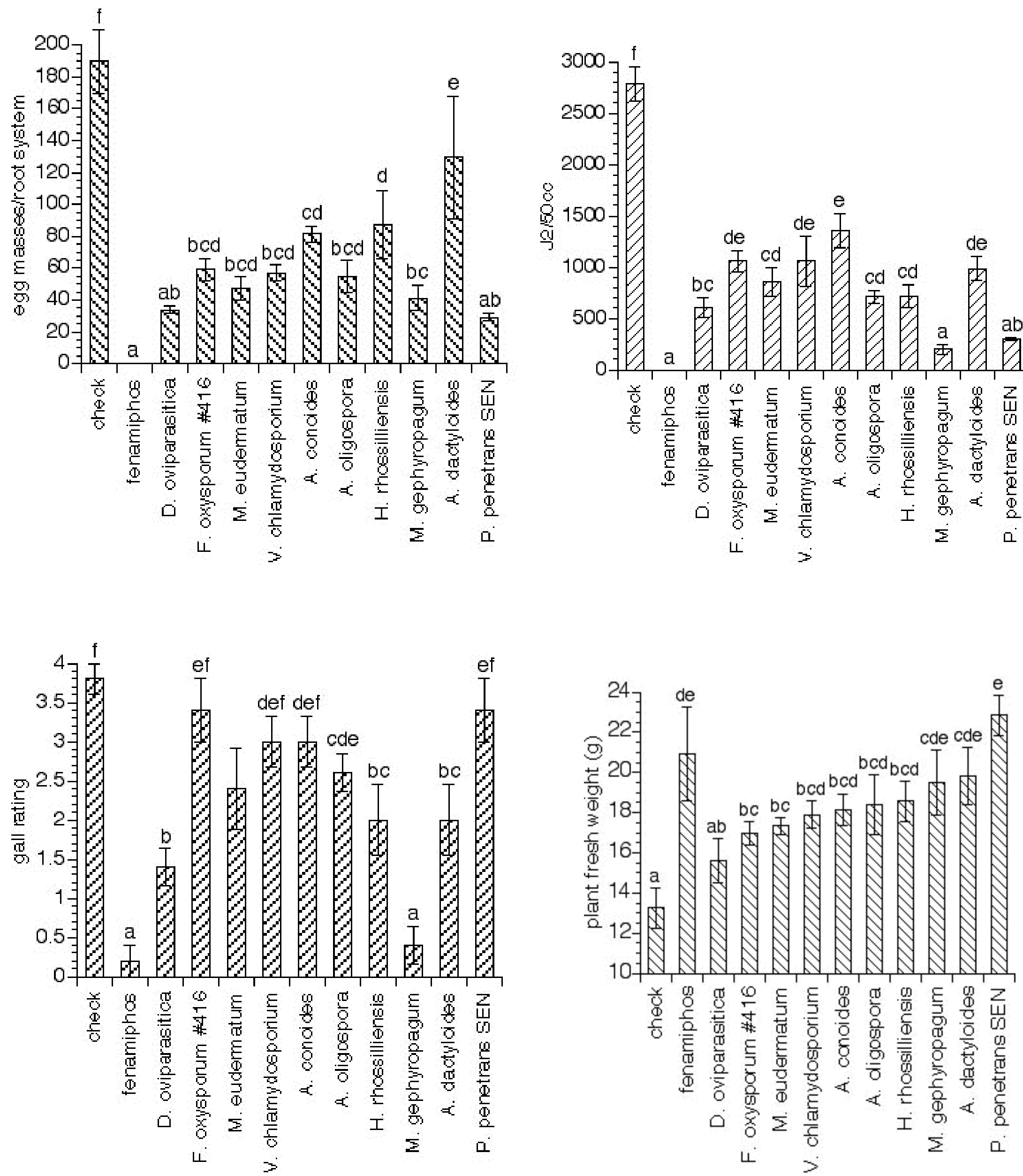


Fig 1. Effect of various nematophagous fungi on root-knot nematode population, root galling, and plant weight after a eight-week tomato crop in a greenhouse trial.

Table 1a. Effects of the application rate of *Verticillium chlamydosporium* and *Dactylella oviparasitica* on root galling and shoot dry weight of tomato caused by *Meloidogyne incognita* in an 8-week greenhouse trial.

Application rate	Gall rating (0-10)*	Shoot dry weight (g)
non-infested check	0	35.2 c
autoclaved 5%	4.6 f	26.2 ab
0.1% *V. chlamydosporium*	4.6 f	22.5 a
0.5% *V. chlamydosporium*	4.2 f	25.9 ab
1.0% *V. chlamydosporium*	3.4 e	25.8 ab
2.0% *V. chlamydosporium*	2.2 cd	26.3 ab
5.0% *V. chlamydosporium*	1.4 b	30.3 bc
0.1% *D. oviparasitica*	4.6 f	23.6 a
0.5% *D. oviparasitica*	4.2 f	24.3 a
1.0% *D. oviparasitica*	2.8 de	28.5 ab
2.0% *D. oviparasitica*	1.6 bc	31.2 bc
5.0% *D. oviparasitica*	1.4 b	25.9 ab

Table 1b. Effects of the application rate of *V. chlamydosporium* and *D. oviparasitica* on the population of *M. incognita* after an 8-week tomato greenhouse trial.

Application rate	J2/ 100 cc soil	# egg masses/ root
non-infested check	0 a	0 a
autoclaved 5%	2696 e	373 i
0.1% *V. chlamydosporium*	1496 d	341 hi
0.5% *V. chlamydosporium*	976 cd	283 gh
1.0% *V. chlamydosporium*	528 abc	211 ef
2.0% *V. chlamydosporium*	248 ab	102 cd
5.0% *V. chlamydosporium*	70 a	26 ab
0.1% *D. oviparasitica*	1304 d	365 i
0.5% *D. oviparasitica*	826 bcd	272 fg
1.0% *D. oviparasitica*	464 abc	156 de
2.0% *D. oviparasitica*	244 ab	74 bc
5.0% *D. oviparasitica*	154 ab	21 ab

* Within columns, values followed by the same letter are not significantly different according to Fisher's protected LSD test ($P \leq 0.05$).

Fig 2. Fungal inoculum production in a culture bag and an Erlenmeyer flask.

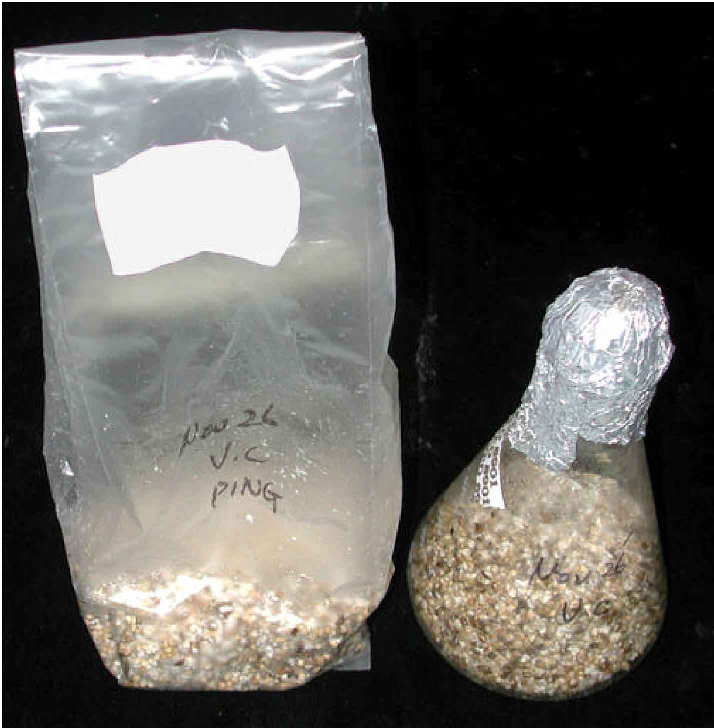
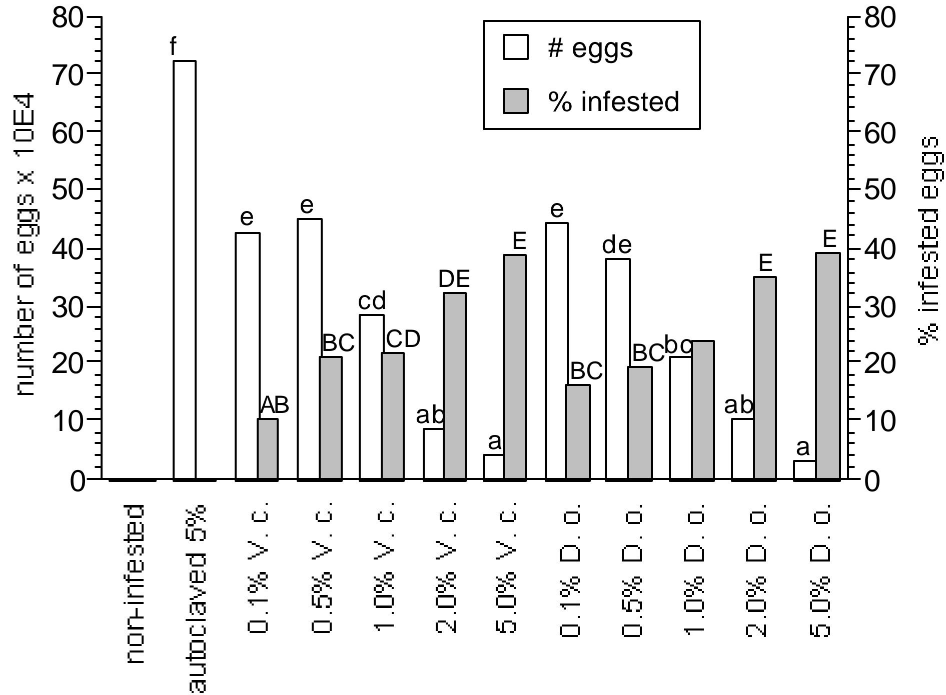


Fig 3. Effects of the application rate of *Verticillium chlamydosporium* and *Dactylella oviparasitica* on root-knot nematode reproduction and percentage of parasitized eggs after an 8-week tomato greenhouse trial.



Within a series, bars with the same letter are not significantly different according to Fisher's protected LSD test (($P \leq 0.05$).