Efficacy of the Fungus *Verticillium lecanii* for Suppressing Root-knot Nematode Egg Numbers on Cantaloupe Roots

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SUMMARY. Two strains of the fungus ***Verticillium lecanii*** **(A. Zimmermann) Viégas were studied as potential biocontrol agents for root-knot nematode (*Meloidogyne incognita* (Kofoid & White) Chitwood) on cantaloupe (*Cucumis melo* L.). For the study, pots were filled with soil that had been inoculated with *M. incognita* (inoculum was applied at two levels: 1000 and 5000 eggs/pot). Each fungus strain was applied individually by pouring an aqueous suspension (made from a wettable granule formulation) into the inoculated soil. Controls received water only. One cantaloupe seedling was then transplanted into each pot. Plants were grown for 55 days in the greenhouse, and then harvested and assessed for root and shoot growth and for nematode egg production. In pots inoculated with 1000 eggs/plant, neither fungus strain affected nema-tode egg numbers. At the 5000 eggs/ plant inoculum level, both strains of the fungus suppressed egg numbers (counts were 28% and 31% less than water controls). Neither strain of *V. lecanii* affected the number of eggs embedded in root galls; the fungus suppressed nematode population numbers overall solely by affecting the number of eggs located outside of root tissues. Both fungus strains were also autoclaved and then applied to soil, to test for effects of nonviable fungus. In pots inoculated with 5000 eggs, application of one autoclaved strain resulted in a 35% suppression in egg numbers after 55 days, suggesting that the fungus produced a heat-stable substance deleterious to the nematode.**

The fungus *Verticillium lecanii* has been studied as a potential biocontrol agent for plant-parasitic nematodes, and has been reported to be antagonistic to the cyst nematodes *Heterodera glycines* Ichinohe and *Heterodera schachtii* Schmidt (Hänssler, 1990; Hänssler and Hermanns, 1981; Meyer and Huettel, 1996; Meyer and Meyer, 1995, 1996). Strains of *V. lecanii* applied in alginate prills were effective against *H. glycines* on soybean [*Glycine max* (L.) Merr.] in greenhouse pot tests (Meyer and Huettel, 1996; Meyer and Meyer, 1995, 1996), even though the fungus was not found to be an aggressive colonizer of the soybean rhizosphere (Meyer et al., 1998). However, when *V. lecanii* was applied in alginate prills or as a root drench for management of *Meloidogyne incognita* (root-knot nematode) on to-mato (*Lycopersicon esculentum* Mill.) in the greenhouse, nematode population densities were not affected overall (Meyer, 1994; 1998), even though the fungus reduced egg numbers in some individual trials. There are a number of possible reasons for differences in ability of the fungus to affect the two types of nematodes in the soil. These include greater antagonism of the fungus to soybean cyst nematode than to root-knot nematode, inability of *V. lecanii* to proliferate on or to act in the tomato rhizosphere, and protection of root knot eggs embedded in galls.

Because *V. lecanii* exhibited antagonism to root-knot nematode in some greenhouse trials, effects of the fungus on *M. incognita* were studied with a new host plant. Cantaloupe was selected because many cucurbits are highly susceptible to damage caused by

root-knot nematodes, and *M. incognita* is one of the species capable of causing significant economic losses. Soil fumigation can be employed as a control measure, but may become less frequent in the future due to environmental issues. Planting of resistant cultivars is employed for reduction in nematode-induced damage in many crops, but cucurbits are not highly resistant to root-knot nematode. Reports on *C. melo* vary from some resistance at lower egg inoculum levels (Nugent and Dukes, 1997) to no identified resistance (Fassuliotis, 1967; Fassuliotis and Rau, 1963). Consequently, melons are among the many cucurbits that could be more advantageously produced with additional means for reducing losses caused by root-knot nematode.

Despite the need for added management procedures, only a few biocontrol studies have been conducted for root-knot nematode on melons. Application of the bacterium *Pasteuria penetrans* (Thorne) Sayre and Starr increased melon yields (Eddaoudi and Bourijate, 1998), and the fungus *Paecilomyces lilacinus* (Thom) Samson increased watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] yields compared to untreated controls (Vicente et al., 1991). The mycorrhizal fungus *Glomus intraradices* Schenck & Smith was shown to improve cantaloupe growth in the presence of *M. incognita*, although the effect in that case was postulated to be from enhanced uptake of nutrients, rather than antagonism of the fungus to the nematode (Heald et al., 1989). These studies indicate that further biocontrol research would be beneficial. Consequently, one objective of the current study was to determine whether *V. lecanii* would be effective in suppressing *M. incognita* egg numbers on cantaloupe.

A second objective of the research was to compare effects of the fungus on egg populations embedded in root galls with effects on egg populations in the rhizosphere. Eggs inside females are generally inside roots; eggs in the gelatinous matrix can be inside the root galls, but are often outside root tissues, depending upon the position of the female and the size of the gall. It was expected that eggs inside root tissues would not be subject to fungal attack; *V. lecanii* is not a plant parasite, and although it grew in soybean root cells when the fungus was present in large amounts (Meyer and Wergin, 1998), it

was rarely isolated from cut roots (unpublished). If the hypothesis proved to be accurate that the nematode eggs must be outside the root tissues to be vulnerable to the fungus, counts of total egg populations might mask deleterious effects of the fungus. The egg populations inside and outside the root tissues were consequently examined separately, as well as combined, to determine if *V. lecanii* was active against *M. incognita* on this host plant.

Materials and methods

FUNGI AND NEMATODES. The wild type strain of *Verticillium lecanii* (American Type Culture Collection 58909) was irradiated with ultraviolet light, and strain M2S1 was selected for increased tolerance to the fungicide benomyl (Meyer, 1992). The two fungus strains were individually formulated by Bayer AG in Germany into a wettable granule formulation. The tested strains were fermented to produce 10^9 blastospores per mL, centrifuged at 5000 rpm ($\approx$6000 g_n) for 10 min, and blastospores were then recovered in pellets ($\approx$$1.5 \times 10^{10}$ blastospores per pellet with $\approx$80% to 85% residual water). The pellets were mixed with kaolin (hydrated aluminum silicate) in either a 1:1 or 1:2 pellet to kaolin ratio (by weight). The mixture was run through an extruder and dried to $\approx$7% residual water in a fluid bed dryer with a 50 °C (122 °F) inlet temperature and an outlet temperature of 30 °C (86 °F). The final formulation contained 6×10^9 blastospores/g. No nutrient source for the fungus was incorporated into the granules. To produce nonviable treatments, granules were autoclaved before use in the greenhouse. For inoculating cantaloupe, *Meloidogyne incognita* was collected from cultures maintained on tomato in the greenhouse (Meyer, 1994).

GREENHOUSE PROCEDURES. 'Hearts of Gold' Cantaloupe seeds were planted in flats of Terra-Lite Redi-Earth 3CK Peat-Lite Mix (Grace-Sierra, Horticultural Products Co., Milpitas, Calif.). Seedlings were grown under supplemental lighting (400-W high-pressure sodium bulbs, 16 continuous h·d^{-1}) during September to April. Supplemental lighting was not needed May to August. Two weeks after planting, seedlings were transplanted into sandy loam (73% sand, 17% silt, 10% clay, 3.84% organic matter, pH 6.9, made from 3 parts compost to 1 part sand) that had been mixed with *M. incognita*. Nematode inoculum was

added at two rates: 1000 and 5000 eggs/pot. Before transplanting, a hole was made in the center of each pot for the plant roots, and 60 mL (2.03 fl oz) of water (control) or of water containing 1 g fungus suspension was poured into each hole. One cantaloupe seedling was then transplanted into each pot. Five treatments were tested for each nematode rate: water, viable and nonviable M2S1, and viable and nonviable wild type strain. Each treatment was tested in two trials (10 pots per treatment in each trial), and the results from the trials were combined for analysis, resulting in a total of $N = 20$ per treatment (with a few exceptions due to plant death). Pot diameters were 10.2 cm (4 inches) and volumes were 570 mL (19.3 fl oz); each pot contained $\approx$600 g (21.2 oz) (air-dried weight) of sandy loam. Pots were randomized on one bench, and plants were grown for 55 d. Propagation mats to maintain warm soil temperatures (21 to 27 °C; 70 to 80 °F) were used from September through April. All plants were fertilized with 5 g (0.18 oz) 12N–5.2P–12.4K per pot.

PLANT VIGOR AND EGG COUNTS. Stem heights were measured from the soil line to the tip of the main stem, root lengths from the soil line to the tip of the main root. The combined stem and leaf weights (shoot weights) from each plant were determined after drying the shoots for 48 h at 62 °C (144 °F). Fresh root weights were determined after roots had been washed and patted dry. Dry weights could not be measured because the roots were macerated for egg counts. Root galling index values were as follows (Daulton, 1959): 0 = free from galls; 1 = less than 5 galls; 5 = trace to 25 galls; 10 = 26 to 100 galls; 25 = more than 100 galls (moderate and numerous), mostly discrete; 50 = moderately heavy, numerous galls, many coalesced. Mean values of 15 and 20 (reported herein) indicate gradations between 10 and 25. Following weighing and indexing, eggs from the rhizosphere (referred to as eggs outside roots) were obtained. To do this, the roots were washed for 4 min in 0.525% sodium hypochlorite, and then rinsed with water over nested 60 (pore size 250 μm) and 500 (pore size 25 μm) mesh sieves. The eggs were collected from the 500 mesh sieve and centrifuged at 1700 rpm (350 g_n) for 3 min. The supernatant was poured out, 1 M sucrose solution was added to each tube, and the suspension was centri-

fuged again at the same time and speed. The eggs in the supernatant were washed and collected on a 500 mesh sieve. To collect eggs embedded in the root tissues, referred to as eggs inside roots, the root systems were then macerated, treated with 0.525% sodium hypochlorite solution for 4 min, washed over nested 60 and 500 mesh sieves, and the eggs collected from the 500 mesh sieve. These eggs were centrifuged only if a large amount of debris was present.

ISOLATION OF *Verticillium lecanii*. To test for presence of viable *V. lecanii* at harvest, 0.05 mL (0.002 fl oz) of a 2% aqueous soil suspension was plated onto Petri dishes of semiselective medium and incubated at 25 °C (77 °F). Soil suspension from each pot containing the wild type strain was plated onto 2 petri dishes of Ausher's Medium No. 2 (Ausher et al., 1975) with PCNB replaced by benomyl (Benlate 50 Wettable Powder or DF, E.I. Du Pont de Nemours, Wilmington, Del.). Soil suspension from each pot containing strain M2S1 was plated onto 2 petri dishes of PDA ABE 1000 (Meyer, 1998). PDA ABE 1000 contained 970 mL water, 39 g potato dextrose agar, 2 g Benlate product in 20 mL distilled water, 0.3 g streptomycin sulfate plus 0.3 g of tetracycline in 10 mL sterile water, and 6 mL EtOH. Soil suspension from water controls was plated onto 1 Petri dish of each medium.

DATA ANALYSIS. Treatment and status (nonviable versus viable) were combined into a single factor, treatment, with five levels: water, viable and nonviable wild type, and viable and nonviable M2S1. The variables were analyzed with PROC MIXED (SAS) as two factor general linear models, with treatment and eggs as factors. Variance heterogeneity occurred in all variables except root length and root weight, so treatments were grouped into similar variance groups for analysis. Estimate statements were used to determine means for viable and nonviable treatments at the 1000 and 5000 egg inoculum levels. Contrast statements were used to test the difference between viable and nonviable means. Nine pots lacked egg counts from either inside or outside the root tissues; consequently, the mean number of total eggs per pot is not equal to the mean number of eggs outside roots plus the mean number of eggs inside roots in those treatments. The root galling index values were transformed for analysis to a proportional

ordinal scale; the means were back-transformed and reported after rounding to the nearest five (10, 15, or 20). Means were compared using pairwise contrasts.

Results

Root lengths, root weights, stem heights, and shoot weights were all similar among individual treatments (Table 1), but there was a tendency for stem heights and shoot weights to be greater in plants treated with viable M2S1. When results from a single fungus treatment were combined (regardless of nematode egg inoculum level), plants from the viable M2S1 treatment had significantly greater stem heights and shoot weights than plants from all treatments except nonviable M2S1 (Table 1). Plants treated with viable M2S1 had stems that were 15% taller and shoots that were 31% heavier than stems and shoots from plants treated with a water control. Stems were 28% taller and shoots were 85% to 87% heavier on plants treated with viable M2S1 than on plants treated with the wild type strain (nonviable or viable). Similarly, stems from plants treated with nonviable M2S1 were 22% to 23% taller and shoots were 67% to 69% heavier than stems and shoots from plants treated with either nonviable or viable wild type strain.

Number of root-knot nematode eggs outside the root tissues was affected by *V. lecanii* at high initial egg populations, but not at low initial egg populations (Table 2). When pots were inoculated with 1000 eggs, none of the treatments suppressed the number of eggs recorded from the rhizosphere. However, when pots were inoculated with 5000 eggs, the two strains were both effective at suppressing the number of eggs located outside the roots. Rhizosphere egg numbers were 36% (viable M2S1), 41% (nonviable M2S1), and 33% (viable wild type strain) less than those produced in water-treated controls. While these three treatments were equally effective at suppressing egg population numbers outside root tissues, nonviable wild type strain treatment did not affect egg numbers.

The number of eggs produced inside cantaloupe roots was not affected by any of the treatments (Table 2). However, when counts of eggs inside roots were combined with counts of eggs outside roots (to obtain total number of eggs per pot), three treatments resulted in an overall effect on total egg

population numbers at the 5000-egg inoculum level. The reductions were 28% (viable M2S1), 35% (nonviable M2S1), and 31% (viable wild type strain) compared to water-treated controls, and were due solely to effects on eggs outside of the root tissues.

Root galling indices did not indicate differences in numbers of galls produced on the roots (Table 2). The numbers averaged around 26 to 100 galls per root system, with the means closer to the lower end of the range on plants initially inoculated with 1000 eggs.

Verticillium lecanii strain M2S1 was isolated from seven of the 20 pots that received this strain as a treatment. The wild type strain was not isolated from the soil.

Discussion

This study indicates that *V. lecanii* affects numbers of root-knot nematode eggs that develop in the rhizosphere of cantaloupe roots, but does not suppress the number of eggs produced inside the roots. A similar result was reported with the fungus *Verticillium chlamydosporium* Goddard on tomato. *Verticillium chlamydosporium* did not colonize cells of tomato roots, so *Meloidogyne arenaria* (Neal) Chitwood (root-knot nematode) egg masses that formed inside galls were protected from parasitism by the fungus (de Leij and Kerry, 1991), and roots with smaller galls (and hence more eggs in the rhizosphere) benefited most from fungus application. Further study demonstrated that nematode egg masses inside roots did not contain eggs infected by *V. chlamydosporium,* while egg masses in the rhizosphere all contained the fungus (de Leij et al., 1992a). Despite the inability to affect egg numbers inside roots, the suppression in rhizosphere egg numbers caused by *V. lecanii* was sufficient to significantly reduce total numbers of eggs per pot, compared with control pots.

Efficacy of *V. lecanii* varied with initial egg population. *Verticillium lecanii* was not effective against *M. incognita* on cantaloupe at the low egg inoculum level, but did suppress nematode populations at the high egg inoculum level. The results with *V. lecanii* on cantaloupe are in contrast to studies demonstrating that *V. chlamydosporium* was less effective as *M. incognita* populations increased (de Leij et al., 1992b). When more nematodes are present, galls

Table 1. Effect of the biocontrol fungus *Verticillium lecanii* on root and shoot growth of cantaloupe plants inoculated with *Meloidogyne incognita*.[z]

Treatment	Root length (cm[x]) 1000	5000	1000 and 5000	Root wt (g[w]) 1000	5000	1000 and 5000	Stem ht (cm) 1000	5000	1000 and 5000	Shoot wt (g) 1000	5000	1000 and 5000
Water	24.4	22.7	23.5	7.9	8.5	8.2	36.6	37.4	37.0 bc	10.3	10.3	10.3 b
Wild type												
Viable	21.7	22.3	22.0	6.0	5.9	5.9	31.6	34.4	33.0 c	6.9	7.5	7.2 c
Nonviable	22.0	21.1	21.5	6.8	6.7	6.7	31.9	34.5	33.2 c	7.0	7.6	7.3 c
M2S1												
Viable	24.5	23.9	24.2	8.4	7.2	7.8	43.0	41.7	42.4 a	14.3	12.6	13.5 a
Nonviable	28.4	23.0	25.7	8.5	7.2	7.9	43.1	38.1	40.6 ab	12.7	11.8	12.2 ab

[z]Numbers are means from all pots per treatment. Numbers followed by the same letter are not significantly different at $P \leq 0.05$, based on comparisons of means using pairwise contrasts. Letters are comparable within columns but not between columns. Columns without letters did not have values that were significantly different from each other.
[y]Pots were each initially inoculated with either 1000 or 5000 *M. incognita* eggs. Results are presented for the two egg inoculum levels individually and for the two egg inoculum levels combined.
[x]2.54 cm = 1 inch.
[w]28.35 g = 1 oz.

are larger, and fewer egg masses are present external to the roots (de Leij et al., 1992b; Dropkin, 1954). However, in the current study, root galling indices were not greatly different between the pots inoculated with 1000 eggs and those inoculated with 5000 eggs, and the proportion of eggs inside roots was not greater at the 5000 egg inoculum level. Given the distribution of eggs in the current study, *V. lecanii* would have been expected to affect *M. incognita* populations in a similar fashion at the two egg population levels. This would have been comparable to studies in which *Arthrobotrys irregularis* (Matr.) Mekhtieva reduced galling at three different nematode treatment levels (Vouyoukalou, 1993). The increased efficacy of *V. lecanii* at the higher egg numbers cannot be explained at this time.

All treatments except the *V. lecanii* nonviable wild type strain suppressed egg population numbers outside root tissues, compared to water controls. This differed from results with *V. lecanii* and *H. glycines*, in which strain M2S1 was more effective at suppressing nematode population numbers than was the wild type strain. It is interesting to note that *V. lecanii* was effective on cantaloupe without an added food source. This has also been found with other biocontrol fungi, such as *V. chlamydosporium* and *Monacrosporium ellipsosporum* (Grove) Cooke & Dickinson (de Leij and Kerry, 1991; Dos Santos et al., 1992). Additionally, treatment of cantaloupe roots with autoclaved M2S1 was as effective as treatment with viable M2S1. This suggests that the M2S1 strain produces a heat-stable substance not made by the wild

type strain, or that M2S1 produces greater quantities of a natural compound than the wild type strain. If the kaolin formulation itself affected egg numbers, it would be expected that nonviable formulation containing wild type strain would also have suppressed egg numbers compared to controls, and that was not the case.

Although the viable wild type and mutant strains of *V. lecanii* were equally effective for suppressing nematode egg numbers, stem heights and shoot weights were different between M2S1- and wild type-treated plants. Apparently, the two strains differently affected plant vigor in some way other than suppression of nematode populations. Another interesting difference between the two strains was that M2S1 was still viable at the end of the experiment, as indicated by isolation from pots, while

Table 2. Effect of the biocontrol fungus *Verticillium lecanii* on *Meloidogyne incognita* egg population numbers on cantaloupe. Egg numbers were counted 55 d after inoculation with *M. incognita*. Eggs outside roots protruded into the rhizosphere, eggs inside roots were embedded in root tissues.[z]

Treatment	No. eggs outside roots 1000	5000	No. eggs inside roots 1000	5000	Total no. eggs/pot[y] 1000	5000	Gall index[x] 1000	5000
Water	2092	13,381 a	2751	13,922	4912	27,627 a	10	20
Wild type								
Viable	1915	8992 b	2905	9940	4903	18,932 b	10	15
Nonviable	2910	10,103 ab	3386	14,647	6594	25,456 ab	10	15
M2S1								
Viable	2070	8510 b	4132	11,277	6202	19,787 b	10	20
Nonviable	1620	7947 b	2157	10,068	3777	18,016 b	10	15

[z]Data are presented as mean number of eggs per pot for each egg inoculum level; pots were each initially inoculated with either 1000 or 5000 eggs. Numbers followed by the same letter are not significantly different at $P \leq 0.05$, based on comparisons of means using pairwise contrasts. Letters are comparable within columns but not between columns. Columns without letters did not have values that were significantly different from each other.
[y]Total eggs per pot = eggs outside roots plus eggs inside roots.
[x]Root gall index (Daulton, 1959): 0 = free from galls; 1 = less than five galls; 5 = trace to 25 galls; 10 = 26 to 100 galls; 25 = more than 100 galls (moderate and numerous), mostly discrete; 50 = moderately heavy, numerous galls, many coalesced. Mean values of 15 and 20 indicate gradations between 10 and 25.

the wild type strain could not be isolated from the pots. *Monacrosporium cionopagum* (Drechsler) Subram suppressed nematode populations even though the pellet formulation was destroyed in the soil; the *M. cionopagum* population peaked rapidly but then decreased (Jaffee and Muldoon, 1997). One possible explanation for the nematode-antagonistic effect with *M. cionopagum* and with the *V. lecanii* wild type strain is that nematode suppression occurred soon after application of the fungus to the soil. Although some biocontrol fungi are more effective when applied before addition of root-knot nematode (Cabanillas and Barker, 1989; Mousa et al., 1995; Saikia and Roy, 1994), a fungus that does not survive for prolonged periods in the soil should presumably not be applied far in advance of planting, as populations would tend to decrease rather than build up over time.

This study demonstrates that application of the fungus *V. lecanii* in the tested formulation can suppress the number of root-knot nematode eggs produced on cantaloupe roots. Further research would indicate whether such factors as rate and timing of fungus application affect efficacy of *V. lecanii* as an antagonist to *M. incognita* on this host plant.

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