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Meloidogyne incognita Survival in Soil Infested with *Paecilomyces lilacinus* and *Verticillium chlamydosporium*[1]

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Abstract: *Meloidogyne incognita*-infected tomato seedlings were transplanted into sterilized soil or unsterilized soil collected from 20 California tomato fields to measure suppression caused by *Paecilomyces lilacinus, Verticillium chlamydosporium,* and other naturally occurring antagonists. Unsterilized soils Q, A, and H contained 35, 39, and 55% fewer *M. incognita* second-stage juveniles (J2) than did sterilized soil 1 month after infected tomato seedlings were transplanted to these soils and placed in a greenhouse. Three months after infected seedlings were transplanted to unsterilized or sterilized soil, unsterilized soils K, L, and Q had 97, 62, and 86% fewer J2 than the corresponding sterilized soils. Unsterilized soils of *M. incognita*-infected seedlings that were maintained 1 month in a greenhouse followed by 1 or 2 months of post-harvest incubation contained J2 numbers equal to, or greater than, numbers in the corresponding sterilized soil. The most suppressive of the unsterilized soils, K and Q, were not infested with *V. chlamydosporium. Paecilomyces lilacinus* and *V. chlamydosporium* increased in colony forming units in unsterilized soil of all bioassays, but they were not associated with lower numbers of J2.

Key words: bioassay, biological control, *Meloidogyne incognita, Paecilomyces lilacinus, Verticillium chlamydosporium.*

The nematophagous fungi *Paecilomyces lilacinus* (Thom) Samson and *Verticillium chlamydosporium* Goddard are associated with soils suppressive to *Meloidogyne* spp. and *Heterodera* spp. (12,14). Introduction of *P. lilacinus* to *Meloidogyne incognita* (Kofoid and White) Chitwood infested microplots significantly reduced the final population of *M. incognita* and increased the yield of tomato (4), but other studies with soil artificially infested with *P. lilacinus* or *V. chlamydosporium* have resulted in little or no reduction in *Meloidogyne* numbers (6,8,10,15). The factors determining the success or failure of egg parasites and other nematode antagonists might be elucidated by understanding their role in naturally infested soil.

In a study of 20 *M. incognita*-infested soils from tomato fields in California, *P. lilacinus* and *V. chlamydosporium* were consistently recovered from some fields but not others (9). All field isolates of *P. lilacinus* and *V. chlamydosporium* parasitized *Meloidogyne* eggs in vitro and surprisingly, *V. chlamydosporium* was positively correlated with *Meloidogyne* population densities in soil. This study was undertaken to measure the reproduction and survival of *M. incognita* in these 20 soils.

Materials and Methods

Soils used in four bioassays to measure *M. incognita* reproduction and survival were collected from 20 central California tomato fields that were currently or previously infested with *M. incognita* and have been previously described (9). Tomato (*Lycopersicon esculentum* Mill. cv. UC82) seedlings infected with *M. incognita* were planted to these soils and maintained in a greenhouse. Infected tomato seedlings in sterilized field soils were the controls. Tomato plants of bioassay 1 were maintained in a greenhouse for 500 degree days (1,16), the shoots were removed and weighed, and *M. incognita, P. lilacinus,* and *V. chlamydosporium* population densities were determined. Tomato plants of bioassays 2 and 3 were also maintained in a greenhouse for 500 degree days, but after removing and weighing the shoots, the plant containers with soil and roots were placed in polyeth-

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ylene bags and incubated for an additional 1 (bioassay 2) or 2 (bioassay 3) months at 20–30 C. Soils were moist and well drained (ca. 50–60% field capacity) at the start of the incubation period, but soil moisture was not measured. Plants of bioassay 4 were maintained 3 months in a greenhouse before the shoots were removed and weighed and *M. incognita, P. lilacinus,* and *V. chlamydosporium* numbers were determined.

Soil collection: Soils for bioassays 1, 2, and 3 were collected in April 1986 from 20 tomato fields, sieved (1-cm pore size sieve), and stored in 20-liter closed plastic containers at 10 C from 3 to 9 months before being used in the bioassays. Field soils for bioassay 4 were collected in April 1987 and were used within 2 weeks of collection (9).

Seedling preparation: Three thousand second-stage juveniles (J2) of *M. incognita* were added to 2–3-week-old tomato seedlings in 172-cm^3 plastic containers filled with fine sand (9). A group of plants in excess of the number sufficient for one replication was inoculated. Four days after inoculation, root systems from 5% of the plants were stained (3) and nematodes were counted. If the coefficient of variation of juveniles in stained roots exceeded 20%, the plants in that group were discarded and the inoculation repeated. One group of these infected plants was used for each replicate of the bioassays. The average number of *M. incognita* in each group of infected seedlings ranged from 800 to 1,500 juveniles per root system. In bioassays 1, 2, and 3, infected tomato seedlings were transplanted into 400 cm^3 sterilized or unsterilized soil in 10-cm-d polyvinyl chloride containers. *Meloidogyne incognita*-infected seedlings of bioassay 4 were transplanted into 915 cm^3 sterilized or unsterilized soil in plastic containers. All plants were maintained in a greenhouse at 20–30 C.

Soil sterilization: In bioassays 1, 2, and 3, sterilized soil refers to soil irradiated with a minimum of 3 Mrad of gamma radiation from Co^{60}, and unsterilized soil refers to nonirradiated soil. Irradiation was used to minimize chemical and physical changes that can occur with other methods of disinfestation (13). A subsample of irradiated soil was incubated in nutrient broth to test sterility. In bioassay 4, soil was disinfested by autoclaving twice for 1 hour at 1.05 kg/cm^2 pressure and 120 C. The autoclaved soil was stored in open containers for 2 weeks before use.

Fungal and nematode population determination: After removing the containers from the greenhouse or from incubation, a 20-g soil subsample was removed from each container and dispersed in 200 ml water by shaking 5 minutes with a wrist-action shaker. A 1-ml aliquot of the soil suspension was diluted in a 10-fold serial dilution series using 9-ml sterile water blanks. A 0.5-ml aliquot was removed from each dilution level (10^{-2} to 10^{-6}) and spread onto Rose Bengal chitin agar medium amended with 50 mg/liter of iprodione to isolate *P. lilacinus* or 50 mg/liter of benomyl to isolate *V. chlamydosporium* (9).

After removing the 20-g subsample, the remaining soil in the container was elutriated (2), and nematodes retained on a 0.038-mm-pore sieve were centrifuged in a sugar solution and counted (2). Root systems were removed during elutriation, and 30 egg masses from each root system were transferred to *P. lilacinus* semi-selective medium, *V. chlamydosporium* semi-selective medium, or 1.5% water agar in 9-cm-d petri dishes (10 egg masses per medium per root system). After removing egg masses for fungal isolation, the roots were placed on Baermann funnels in a mist chamber. *Meloidogyne incognita* J2 were collected after 7 days and counted (2). The petri dishes with egg masses were incubated 7 days at 24–27 C before the numbers of egg masses infested with *P. lilacinus* or *V. chlamydosporium* from semi-selective media or nematode-trapping fungi from water agar were determined.

Statistical analysis: A randomized complete block design was used in all bioassays. Bioassays 1, 2, and 3 were replicated five times, and each replication was initiated at a different time. Bioassay 4 was replicated four times, and all replications were initiated at the same time. A paired *t*-test anal-

TABLE 1. *Meloidogyne incognita* second-stage juveniles (no./g soil) as affected by the presence of *Verticillium chlamydosporium* and *Paecilomyces lilacinus* (VcPl) in four bioassays.

	VcPl soil		Non-VcPl soil		
Bioassay†	Sterilized	Unsterilized‡	Sterilized	Unsterilized‡	LSD
1	393	266	417	275	79
2	126	48	101	92	50
3	11	15	12	25	8
4	208	329	362	303	179

† In bioassay 1 *Meloidogyne incognita*-infected tomato plants were grown for 1 month, in bioassays 2 and 3, infected plants were grown for 1 month followed by 1 month or 2 months of postharvest incubation, respectively, and in bioassay 4 infected plants were grown for 3 months.

‡ Eight soils infested with both *P. lilacinus* and *V. chlamydosporium* (VcPl) were compared with eight soils in which *V. chlamydosporium* was absent (Non-PlVc). *P. lilacinus* was present in two of the eight non-PlVc soils.

ysis of *M. incognita* in unsterilized soil compared to *M. incognita* in sterilized soil was used to determine if final numbers were different for each soil. Final *M. incognita*, *P. lilacinus*, and *V. chlamydosporium* numbers were analyzed by analysis of variance. Final *M. incognita* numbers in soils infested with *P. lilacinus* and *V. chlamydosporium* were compared to soils in which these fungi were absent. Tukey's studentized range test was used to determine differences between means at $P = 0.05$.

RESULTS

The number of J2 in unsterilized soil was greater than, or equal to, the number of J2 in sterilized soil for all soils of bioassays 2 and 3. Unsterilized soils A, H, and Q of bioassay 1 contained 39, 55, and 35% fewer J2, respectively, than did corresponding sterilized controls. Unsterilized soils K, L, and Q of bioassay 4 contained 97, 62,and 86% fewer J2, respectively, than corresponding sterilized controls. Unsterilized soils K and Q of bioassay 4 were the most suppressive of *M. incognita* reproduction, but *P. lilacinus*, and *V. chlamydosporium* were not recovered from those soils (Figs. 1, 2). Although soils A, H, K, L, and Q had fewer J2 than corresponding sterilized controls, the shoot weights of these soils were also

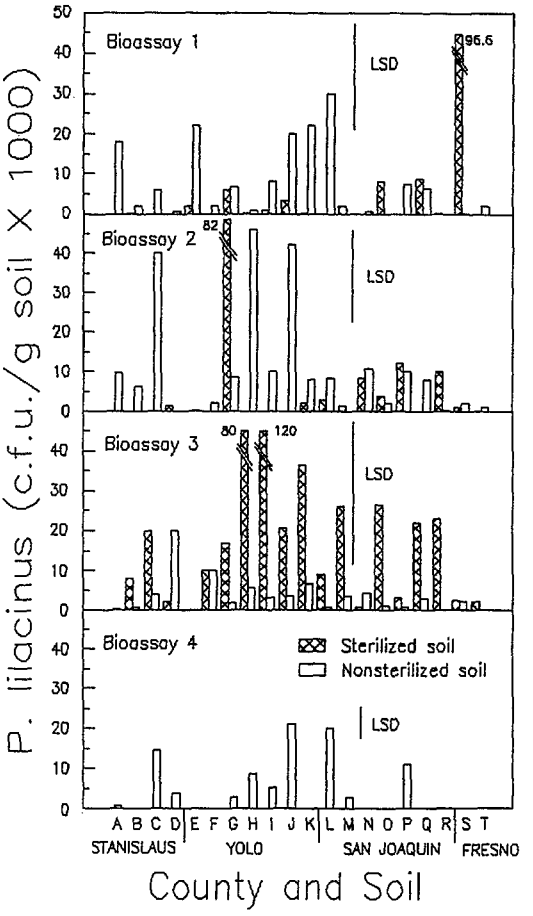

Fig. 1. *Paecilomyces lilacinus* population colony-forming units (c.f.u.) per gram soil from 20 field soils (A–T, sterilized and unsterilized soil) in which *Meloidogyne incognita*-infected tomato plants were grown for 1 month (bioassay 1); infected plants were grown for 1 month followed by 1 or 2 months of postharvest incubation (bioassays 2 and 3, respectively); or infected plants were grown for 3 months (bioassay 4). LSD is Tukey's least significant difference for all means ($P = 0.05$).

lower than corresponding sterilized controls. Final *M. incognita* numbers (Pf) in soil infested with *P. lilacinus*, and *V. chlamydosporium* did not differ from soils without *V. chlamydosporium* (Table 1).

Paecilomyces lilacinus was recovered from both sterilized and unsterilized soil of bioassays 1, 2, and 3 and from unsterilized soil of bioassay 4 (Fig. 1). Sterilized soil of bioassay 4 was not evaluated for *P. lilacinus*. Numbers of *P. lilacinus* colony-forming units were higher in sterilized soils than in

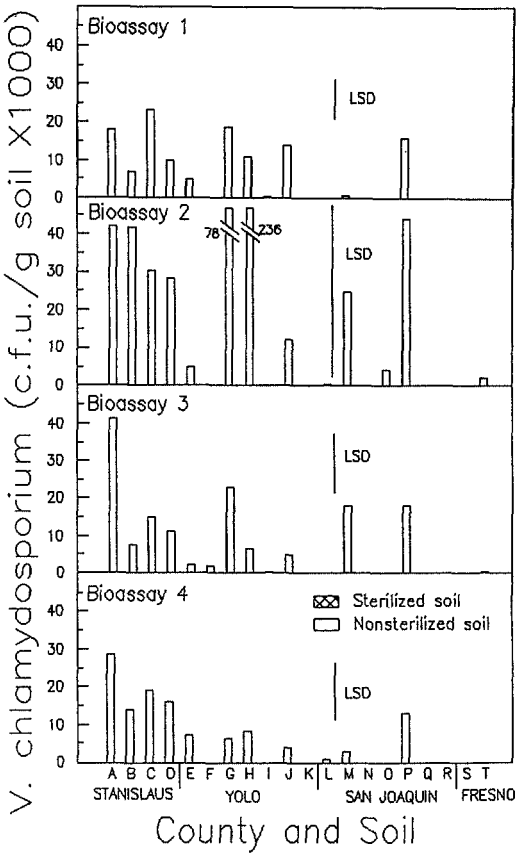

Fig. 2. *Verticillium chlamydosporium* population densities (colony-forming units per gram soil) from 20 field soils (A–T, sterilized and unsterilized soil) in which *Meloidogyne incognita*-infected tomato plants were grown for 1 month (bioassay 1); infected plants were grown for 1 month followed by 1 or 2 months of postharvest incubation (bioassays 2 and 3, respectively); or infected plants were grown for 3 months (bioassay 4). LSD is Tukey's least significant difference for all means ($P = 0.05$).

unsterilized soils of bioassay 3. In each bioassay, *P. lilacinus* densities were higher at the end of the bioassay than at the beginning.

Verticillium chlamydosporium was recovered only from unsterilized soil (Fig. 2). *Verticillium chlamydosporium* numbers differed among many of the soils in bioassays 1, 3, and 4 but not in bioassay 2 (Fig. 2). *V. chlamydosporium* Pf was higher than the initial population (Pi) in all soils where detected.

Meloidogyne incognita egg masses collected from roots of bioassay 1 and incubated on agar were infested with *P. lilacinus*, and *V. chlamydosporium* (Table 2). Nematode-trapping fungi, principally *Monacrosporium* spp. and *Arthrobotrys* spp. as well as *P. lilacinus* were present in egg masses from both unsterilized and sterilized soil, but *V. chlamydosporium* was present in egg masses from unsterilized soil only.

TABLE 2. Incidence of nematode-trapping fungi, *Paecilomyces lilacinus*, or *Verticillium chlamydosporium* infesting egg masses (percentage) from *Meloidogyne incognita*-infected tomato plants grown for 1 month in sterilized or unsterilized soil from 20 California tomato fields.

Field	Trapping fungi†		*P. lilacinus*		*V. chlamydosporium*	
	Unsterile	Sterile	Unsterile	Sterile	Unsterile	Sterile
Stanislaus County						
A	10	2	0	0	8	0
B	6	6	4	2	4	0
C	10	12	0	2	4	0
D	4	4	0	0	2	0
Yolo County						
E	12	8	0	0	4	0
F	6	12	0	0	0	0
G	12	12	4	0	14	0
H	8	2	2	0	2	0
I	14	24	2	0	0	0
J	6	16	6	6	6	0
San Joaquin County						
K	4	16	0	2	0	0
L	6	6	2	0	0	0
M	12	8	0	6	4	0
N	0	14	0	0	0	0
O	12	6	4	0	0	0
P	4	14	4	0	14	0
Q	0	4	0	0	0	0
R	8	4	0	0	0	0
Fresno County						
S	0	14	0	10	0	0
T	0	2	0	0	0	0

Percentage of egg masses infested is based on 50 observations.

† Principally *Monacrosporium* spp. and *Arthrobotrys* spp.

DISCUSSION

Suppressive soils are well known in plant pathology and to a lesser degree in nematology. When antagonists responsible for suppression in soil are eliminated by physical or chemical treatment, pathogen reproduction and survival should increase

(14). We expected the sterilized control soils from which *P. lilacinus, V. chlamydosporium,* and other antagonists were initially eliminated to have greater *M. incognita* numbers than soils where the antagonists were present. Contrary to expectations, however, the majority of soils showed little or no reduction in *M. incognita* reproduction and survival. Soils A, H, K, L, and Q associated with reduced reproduction and survival in one or more of the bioassays were also associated with lower shoot weights. The existence of biological control in California tomato fields cannot be ruled out by the results of the bioassays; however, it is unlikely *P. lilacinus* or *V. chlamydosporium* had a significant role in reducing *M. incognita* reproduction in the soils tested.

Numbers of J2 were not lower in soils infested with *P. lilacinus* and *V. chlamydosporium,* even though both fungi increased in propagule density and were recovered from both soil and egg masses. The use of *P. lilacinus* and *V. chlamydosporium* for biological control has been based on the assumption that increases in inoculum densities will increase parasitism. In cereal monoculture, 300 chlamydospores of *V. chlamydosporium* per gram of soil are required for suppression of *Heterodera avena* populations (15). In our study more than 10^5 propagules of *V. chlamydosporium* per gram of soil were recovered without suppression of *M. incognita,* but chlamydospore densities were not known. Results from this study suggest that factors in addition to length of exposure during the growth of the host (bioassays 1, 4), postharvest incubation (bioassays 2, 3), and propagule density (8–10) influence the parasitic activity of these fungi.

Meloidogyne incognita Pf in the 20 soils were compared with Pf in the same soils in which antagonists had been destroyed to indirectly measure antagonism. However, the sterilized soils were reinfested with nematode-trapping fungi and *P. lilacinus,* and this may have influenced the results. The presence of nematode-trapping fungi and *P. lilacinus* in the sterilized soil might be of concern if both soils contained low numbers of *M. incognita*; however, relatively large numbers of J2 were present in all treatments. In Florida, *P. lilacinus* was present in high densities in microplots but did not reduce *M. javanica* numbers (10). At the end of that study, *P. lilacinus* had spread to virtually all plots and was recovered from roots and soil.

Although *V. chlamydosporium* was present only in unsterilized soil and propagule densities increased in all bioassays *V. chlamydosporium* was not associated with significant suppression of J2.

Isolates of *P. lilacinus* and *V. chlamydosporium* vary in parasitic activity (5,6,11,15). All isolates of *P. lilacinus* and *V. chlamydosporium* used in this study parasitized less than 50% of *M. incognita* eggs in vitro (9). Other studies have reported that parasitism is lower in soil than in vitro (7,8,15). Our investigations suggest that naturally occurring *P. lilacinus* and *V. chlamydosporium* have only a minimal role in regulating *M. incognita* in California tomato fields. However, the activity of *P. lilacinus* and *V. chlamydosporium* might be increased through selection of virulent isolates, altering the soil environment, or by adding amendments that stimulate antagonism (4,15). Further research is needed to determine the biological attributes and soil characteristics that promote biological control of nematodes.

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