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Invasion of Tomato Roots and Reproduction of *Meloidogyne incognita* as Affected by Raw Sewage Sludge

PHILIPPE CASTAGNONE-SERENO AND ALAIN KERMARREC[1]

Abstract: The antagonistic effects of raw sewage sludge on infection of tomato by *Meloidogyne incognita* were tested in greenhouse pot experiments. Sludge was mixed with the soil or added on its surface before and after inoculation of tomato plants with nematode eggs. Juvenile penetration was determined 1 and 10 days after inoculation, and 6 weeks later root systems were assessed for nematode reproduction. Fewer juveniles penetrated roots in pots with sludge added to the soil than in unamended control pots. In both experiments, roots were severely galled despite a significant reduction in gall ratings in amended relative to unamended soils. Egg production in treated soil was less ($P = 0.05$) than in control pots, regardless of whether sludge was incorporated or added 1 day before or after inoculation. In treated pots, RF values (final egg number/inoculation egg number) were strongly reduced. The toxic effects observed on the parasite may result from the ammoniacal nitrogen released in the soil within 7 days after treatment, associated with possible poor host suitability of tomatoes grown in amended substrate and short-lasting compound(s) active after root invasion.

Key words: *Lycopersicon esculentum*, *Meloidogyne incognita*, nematode, organic amendment, root-knot nematode, tomato, urban waste.

A reduction of plant-parasitic nematode population densities is frequently observed after the application of organic material as a soil amendment. Linford et al. (8) first reported a decrease of root-knot nematode population densities during the decomposition of organic matter added to soil. Other reports (13,15) indicate that oil-cakes are the most frequent organic amendment used, but results obtained with crop residues, manure, sawdust, and compost of crop and agro-industrial wastes appear to be promising. Composted sewage sludge from urban areas sometimes is used as a soil conditioner and fertilizer (4), but few studies have been conducted on its possible nematicidal properties. Habicht (3) described the effects of varied amounts of both raw and composted sewage sludge on the galling and dry weight of tomato plants inoculated with root-knot nematodes. *Tylenchus* sp. population densities were reduced by sewage sludge treatments, even though the opposite effect occurred with *Pratylenchus* sp. and *Tylenchorhynchus* sp. (6).

Reduction of nematode population densities has been attributed to direct toxicity of the decomposition products of organic matter, an increase of natural antagonists in the soil, and the alteration of plant-nematode relationships (16). A modification of the abiotic parameters of the soil environment has also been suggested (14). The objectives of these studies were to determine the efficacy of raw sewage sludge in controlling root-knot nematodes on tomato roots and to provide new information on the possible mode(s) of action of this organic material.

MATERIALS AND METHODS

Raw sewage sludge for this work was obtained from the treatment station of Jarry (Guadeloupe, French West Indies), which collects the waste water of Pointe-à-Pitre/Abymes, the largest urban center of the island. Its average chemical and biochemical composition was analyzed (Table 1).

Meloidogyne incognita (Kofoid & White) Chitwood were collected from infested fields, and populations were increased in the greenhouse on *Lycopersicon esculentum* Mill. cv. Caraïbo. The same tomato culti-

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[1] INRA, Centre des Antilles-Guyane, Station de Zoologie et de Lutte Biologique, BP 1232, 97185, Pointe-à-Pitre Cédex, Guadeloupe, FWI.
Present address of first author: INRA, Station de Nématologie et de Génétique Moléculaire des Invertébrés, BP 2078, 06606, Antibes Cédex, France.
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var was used as host in all the trials. Excised egg masses from infested roots were shaken for 3 minutes in a 5% solution of sodium hypochlorite to separate eggs from the gelatinous matrix. They were washed with water through a 45-μm-pore sieve to eliminate the sodium hypochlorite and concentrated by centrifuging at 1,000 *g*. All experiments were performed in a greenhouse at 25–27 C. Two-week-old tomato seedlings grown in a steam-sterilized silty clay with pH 5.7 and organic matter <3% (w/w) were transplanted (one per pot) into 1-liter cylindrical plastic pots containing 1,000–1,200 g of the different soil mixtures studied.

Experiment 1: Sludge was thoroughly mixed with the steam-sterilized soil to obtain a final substrate with 33% (v/v) sludge. Pots containing only sterilized soil served as controls. Seventy pots each of treated and untreated soil were prepared. After 3 weeks, each plant was inoculated by pipetting a suspension of 10,000 eggs in 2 ml water directly onto the soil surface around the stem base, followed with light watering. Root penetration was determined 1 and 10 days after inoculation on 20 pots of both substrates, each time using a centrifugal flotation technique. Roots were washed and cut in pieces about 1 cm long, which were crushed for 15 seconds with an electric blender. They were centrifuged for 4 minutes at 1,800 *g* in 500 ml water containing 5 g kaolin. The pellet was homogenized in a sucrose solution (sp. gr. = 1.18) and centrifuged at 700 *g* for 1 minute. The supernatant was quickly decanted onto a 5-μm-pore sieve, from which the retained larvae were washed and recovered in distilled water for counting. The remaining pots were analyzed for nematode reproduction. Six weeks after inoculation, roots were carefully washed and rated for gall severity on an index of 0–5, where 0 = no galls, 1 = 1–3 galls, 2 = 4–10 galls, 3 = 11–30 galls, 4 = 31–100 galls, and 5 = > 100 galls. Whole root systems were placed in cold eosin yellow (0.1 g/liter in distilled water) and stirred for 30 minutes to stain egg masses. Numbers of egg masses per root system were counted. Thirty egg masses per root system were dissolved in chlorine bleach (0.8% NaOCl), and numbers of eggs per egg mass were determined. A reproductive factor (RF) was calculated according to the following ratio:

$$RF = (\text{no. egg masses} \times \text{no. eggs/egg mass})/10{,}000.$$

Experiment 2: This study was designed to determine the possible influence of the time interval between sludge addition and inoculation on nematode reproduction. Tomato plants were transplanted, one per pot, into soil sterilized as mentioned above, and the soil was covered with a 2-cm-thick bed of sludge as follows: (a) no sludge (control); (b) sludge added 7 days before inoculation; (c) sludge added 1 day before inoculation; (d) sludge added 1 day after inoculation; and (e) sludge added 7 days after inoculation. Two milliliters of water containing 10,000 eggs were injected into the soil around the root system with a syringe to

TABLE 1. Average characteristics of raw sewage sludge from the treatment station of Jarry (Guadeloupe, FWI).

Component	Measure
Principal parameters	
Total solids	13.15%
Organic matter	71.15%
Ashes	28.85%
C/N ratio	5.82
pH (water)	6.18
Components of total solids	
C	37.90%
N	6.52%
P	1.54%
K	0.28%
Ca	1.84%
Mg	0.41%
Mn	0.10%
S	0.78%
Fe	1.92%
Cu	184.00 ppm
Cd	0.90 ppm
Ni	6.30 ppm
Pb	26.80 ppm
Microbial components (no./g dry matter)	
Bacteria	4.0×10^9
Fungi	2.6×10^7
Actinomycetes	1.4×10^7

TABLE 2. Penetration, galling, and reproduction of *Meloidogyne incognita* on tomatoes growing in soil treated with raw sewage sludge.

Quantity evaluated	Sterilized soil (control)	Sterilized soil with sewage sludge (33% v/v)
Juveniles/root system after 1 day	0.5	0.0
Juveniles/root system after 10 days	52.9	2.3**
Gall index†	4.87	4.27**
Egg masses/root system	443	117**
Eggs/egg mass	392	243*
Reproductive factor‡	17.4	2.8

Data on juveniles/root system are means of 20 replicates; others (except RF) are means of 30 replicates. *, ** indicate significant differences from controls at $P = 0.05$ and $P = 0.01$, respectively, according to the Mann and Whitney *U*-test.

† Gall index 6 weeks after inoculation: 0 = no galls; 1 = 1–3 galls; 2 = 4–10 galls; 3 = 11–30 galls; 4 = 31–100 galls; 5 = > 100 galls.

‡ 6 weeks after inoculation.

TABLE 3. Reproduction of *Meloidogyne incognita* (Mi) on tomato growing in soil to which raw sewage sludge had been added before or after inoculation with 10,000 eggs.

Sludge treatment	Gall index†	No. egg masses/ g root	No. eggs/ egg mass	RF‡
None	4.75 a	80 a	543 a	90.2
7 days before Mi	3.20 b	61 b	990 b	128.5
1 day before Mi	3.50 b	21 c	391 a	15.7
1 day after Mi	3.85 b	28 c	413 a	27.4
7 days after Mi	4.35 b	78 ab	453 a	91.4

Values (except RF) are the means of 20 replicates. Means within a column followed by the same letter are not significantly different according to the Mann and Whitney *U*-test ($P = 0.05$).

† Gall index 6 weeks after inoculation: 0 = no galls; 1 = 1–3 galls; 2 = 4–10 galls; 3 = 11–30 galls; 4 = 31–100 galls; 5 = > 100 galls.

‡ Reproductive factor 6 weeks after inoculation.

avoid direct contact between eggs and the organic matter. Nematode reproduction was calculated as described for Experiment 1, except that numbers of egg masses were expressed per gram of root. Each treatment was replicated 20 times for a total of 100 pots for the experiment.

All data (except the RF values) were compared statistically with the Mann and Whitney *U*-test to test for significant differences between treated and control pots.

RESULTS

In both experiments, fresh weights of shoots were higher ($P = 0.05$) in amended than in unamended soil. In contrast, no differences were observed between root weights.

Experiment 1: Ten days after inoculation, root invasion in the amended pots was strongly reduced relative to the controls (Table 2). Although all roots were severely galled, differences were significant between gall indices of treated and control plants ($P = 0.01$). Egg mass production was suppressed on the roots of plants grown in sludge-amended soil, and there was a small decrease in the number of eggs per egg mass (Table 2). RF ranged from 2.8 for amended to 17.4 for unamended pots.

Experiment 2: Gall indices were reduced by sewage sludge treatments, although roots once again were attacked quite severely, with gall indices > 3 in all pots (Table 3). Sludge applications 1 day before or 1 day after inoculation appeared to be the most efficient in reducing the number of egg masses per gram of root and the RF values relative to the two other sludge treatments and the control. Except when sludge was added 7 days before inoculation, no significant differences were observed among treatments in the numbers of eggs per egg mass (Table 3).

DISCUSSION

Raw sewage sludge used in our experiments suppressed invasion of tomato roots by *M. incognita* juveniles, at least within 10 days after inoculation. Egg mass production by females and eggs per egg mass were reduced significantly, especially when sludge was mixed with the soil.

Organic matter can be directly toxic to eggs and juveniles of *Meloidogyne* species because of its chemical or biochemical composition (3,5,11). The microorganisms in the rhizosphere also may be involved in the reduction of nematode invasion by producing proteolytic enzymes (12), modifying root exudation patterns (7), or affecting egg hatch (2). In addition, a reduction in severity of root knot can be related to a modification of the recognition of the roots by the nematodes (9,18). Chemical analyses conducted under our conditions indicated a strong release of ammoniacal nitrogen (about 150 mg NH_4^+/kg soil) in soil within 7 days after addition of this kind of amendment (Clairon, pers. comm.; 10). This toxic flush, related to the chemical and microbiological composition of the sludge (6.52% total N and large microbial population) may result in both reduction of egg hatch and direct toxicity to juveniles, and consequently may account for the lack of penetration observed in the roots (Table 2). The high rate of ammonia release lasted only a week, however, and the egg hatch may have spread beyond this time, allowing some juveniles to avoid the toxic activity of ammonia. This may account for the high gall indices on plants in treated pots at 6 weeks, but periodic observations throughout the experiment could provide more detailed information. This toxic effect does not explain the decrease in reproduction observed in both experiments. The lack of a strong relation between root galling and female fecundity may be related to an event occurring after the penetration of juveniles in the roots and their development into females. Van der Laan (17) proposed and Alam et al. (1) later demonstrated that the chemical composition of roots is affected by several organic amendments. Thus, it is possible that tomatoes grown under our conditions in soil amended with sewage sludge have become less suitable hosts for *M. incognita*. Comparative analysis of the chemical composition of the root tissues before and after application of the amendment would provide useful information about this hypothesis that, if verified, could account for the poorer reproduction observed with treatments in both experiments. The RF values also suggest that the amendment is most efficient when contact between the sludge and the parasite is close in space (sludge mixed with the soil) or time (short time between amendment and inoculation). In Experiment 2, when the period of time between inoculation and sludge addition was short (1 day), reproduction was significantly lower than in the other treatments and the control. This may be related to a short-lasting active compound from the sludge other than NH_4^+. Such a transitory process has already been described for lectins (9). Even if the ammoniacal nitrogen flush detected in the soil within 7 days after amendment application is the main reason for the lower infection observed in the treated pots, another factor is needed to fully explain the specific mode(s) of short-term action of this sewage sludge on root-knot nematodes. In this connection, we propose further testing of two complementary hypotheses: poor host suitability of plants grown in amended soil and existence of a short-lasting compound active after root penetration by juveniles.

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