Effect of Organic Amendment Placement and Inoculum Density of *Meloidogyne incognita* on Okra Seedlings

C. H. S. P. Ritzinger*, R. McSorley, and R. N. Gallaher

ABSTRACT

In a greenhouse test, three treatments involving yard-waste compost amendment (applied to the soil surface, mixed with the soil, or no amendment) were evaluated at three inoculum densities of *Meloidogyne incognita* race 1 (0, 250, or 1000 juveniles pot^{-1}). Plant responses were evaluated on the highly susceptible 'Clemson Spineless' okra (*Hibiscus esculentus*) during spring of 1994. There was a significant interaction ($P \leq 0.05$) between organic amendment placement and inoculum density for egg-mass rating, but not for galls, plant height, or top and root dry weights. Egg-mass rating was not affected by placement of the amendment on the surface or incorporation, but was significantly higher ($P \leq 0.05$) at the 1000 inoculum level when no organic amendment was used. Effects of inoculum density were significant ($P \leq 0.05$) for plant height, dry weight, and gall rating, and in some cases for shoot and root weights. Regardless of the inoculum concentration, the shortest plant height and lowest weights of shoots and roots were observed when the amendment was mixed with soil. Gall rating was not affected by organic amendment placement, but was affected by inoculum concentration, showing higher levels at the highest nematode density.

Application of organic amendments has frequently been attempted as a means of managing plant-parasitic nematodes. Nonetheless, the mechanism of organic amendment activity against nematodes is not clear (Muller and Gooch, 1982; Duncan, 1991). Some possible explanations are that the organic amendment may promote good conditions for plant growth, may provide a favorable environment for the development of organisms antagonistic to plant-parasitic nematodes, or may release toxic concentrations of ammonia and phenolic compounds which may then kill plant-parasitic nematodes (Mankau, 1968; Muller and Gooch, 1982; Trivedi and Barker, 1986; Duncan, 1991). Whether compounds such as ammonia released upon organic amendment degradation are nematicidal depends on the amount released, the C/N ratio of the amendment, and environmental conditions, so results may be highly variable (Rodríguez-Kábana, 1986; Stirling, 1991). Organic materials with low C/N ratios are decomposed rapidly by microbes that readily attack water-soluble substances, hemicelluloses, and cellulose. Woody plant materials contain lignin, which is low in plant nutrients and quite resistant to microbial attack. Unless extra N is provided, woody plant material decomposes slowly. Nonetheless, woody residues make excellent mulches for blueberries (*Vaccinium* spp.), fruit trees, ornamentals, and garden crops (Reuszer, 1957). Organic mulches may enable plants to better tolerate nematode damage by increasing soil water-holding capacity (McSorley and Gallaher, 1995).

The root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood is particularly damaging to many vegetable crops including okra (*Hibiscus esculentus* L.; Christie, 1959). Reduction in survival and infectivity of *M. incognita* was obtained in fields treated with organic amendments (Mankau, 1968). Gallaher and McSorley (1994) observed some, but inconsistent, reductions of nematode populations by using yard-waste compost (mainly woodchips) as mulch. In contrast, Ichinohe (1985) reported improved yield of black pepper (*Piper nigrium* L.) by use of mulch, but did not observe reductions in the number of nematodes. The objectives of this study were to compare the effect of organic amendment treatment (placement on the soil surface, mixed in soil, or none) on three different concentrations of root-knot nematodes and on growth of okra under greenhouse conditions.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse during the spring of 1994. Plastic pots 10 cm in diameter and 9 cm high were filled with 700 cm^3 (740 g dry weight) of steam-sterilized field soil (Arredondo fine sand; Grossarenic Paleudults, loamy, siliceous, hyperthermic) mixed with sand, in a 1:1 ratio by volume. Composition of the mixture was 92% sand, 4% clay, and 4% silt. On 14 Feb. 1994, two-week-old seedlings of the okra cultivar 'Clemson Spineless' were transplanted into the pots after applying 0.6 g of granular 6-6-6 (N-P-K) fertilizer. The pots were watered immediately after transplanting. Plants were sprayed two times each week with dilute soap solution to reduce infestation of whiteflies (*Bemisia tabaci*).

The experimental design was a 3 × 3 factorial with three organic amendment treatments and three nematode inoculum densities, replicated five times. The organic amendment treatments consisted of compost applied to the soil surface, mixed into the soil, or a nonamended control. The organic amendment consisted of 190 cm^3 pot^{-1} of yard-waste compost, mainly woodchips, which had been previously heated in an oven at 75°C for 12 hours. The dry organic amendment weight was 45 g pot^{-1}. Subsamples of the compost were ground and screened for nutrient analysis. Nitrogen concentration was determined using an aluminum block digestion method (Gallaher et al., 1975); P was determined by colorimetry; K by flame emission; and Ca, Mg, Cu, Fe, Mn, and Zn by atomic absorption spectrophotometry. Composition of the organic amendment was: 0.39% N, 1049 ppm P, 1497 ppm K, 22 475 ppm Ca, 1323 ppm Mg, 180 ppm Mn, 69 ppm Zn, 25 ppm Cu, and 770 ppm Fe. After 50 days, five soil samples from each of the amendment

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Table 1. F-values from the analysis of variance. April 1994.

Treatment effect	Plant height	Taproot length	Root weight	Top dry weight	Egg-mass rating	Gall rating
Amendment placement (A)	34.97**	2.25 ns	88.29**	17.05**	6.77**	1.69 ns
Inoculum density (B)	3.38*	0.03 ns	0.81 ns	3.42*	9.38**	10.71**
Interaction (A × B)	0.38 ns	1.80 ns	0.32 ns	1.47 ns	3.87*	0.17 ns

*, ** Significant effects at $P \leq 0.05$ and $P \leq 0.01$, respectively; ns = not significant. Data are means of four replications.

treatments were analyzed for pH, organic matter (Walkley and Black, 1934; Walkley, 1947), and extractable soil nutrients (Mehlich, 1953), as described above.

The nematode inoculum consisted of second-stage juveniles (J2) of *Meloidogyne incognita* race 1. Eggs were extracted from roots of 'Rutgers' tomato (*Lycopersicon esculentus* Mill.) plants using 1.05% NaOCl (Hussey and Barker, 1973), and then incubated on Baermann trays (Rodríguez-Kábana and Pope, 1981) for seven days. The nematode densities used were 0, 250, and 1000 J2 pot^{-1}, applied one day after transplanting into three holes in the soil, approximately 2 cm deep.

Plant height was recorded at 30 days after inoculation. At 50 days after inoculation, plants were harvested, and shoot and taproot lengths as well as fresh and dry weights were recorded. Root galls and egg masses were rated according to the root-knot index (0 = none to 5 = severe) of Taylor and Sasser (1978). Second-stage juveniles hatching from egg masses in the okra root system were quantified by extraction in 1.05% NaOCl (Hussey and Barker, 1973), followed by incubation of the eggs on Baermann trays (Rodríguez-Kábana and Pope, 1981) for seven days and counting hatched J2.

Data were statistically analyzed as a completely randomized 3 × 3 factorial to determine main effects and the interaction between organic amendment treatment and nematode levels. When a main effect was significant ($P \leq 0.05$) with no interactions, a separate analysis of variance was carried out, followed by separation of means by Duncan's multiple-range test (Freed et al., 1987).

RESULTS AND DISCUSSION

There were no significant interactions between organic amendment placement and inoculum density for top height and weight, root length and weight, and gall rating. However, there was a significant interaction ($P \leq 0.05$) between organic amendment placement and inoculum density for the egg-mass rating (Table 1). Fewer egg masses were observed for plants receiving either amendment treatment than in unamended plants at an inoculum level of 1000 J2 pot^{-1}, but not at 250 J2 pot^{-1} (Table 2). Gall ratings did not show this trend. There was not enough evidence to determine if organic amendment placement had a significant effect on nematode population levels in soil (Table 3). Overall, organic amendment effects on nematode levels were inconsistent, with some trend toward lower numbers with organic amendment treatment.

Inoculum density had a significant effect ($P \leq 0.05$) on plant height as well as on top dry weight, and on egg and gall ratings. The same effect was observed with respect to organic amendment placement. There were no significant differences among treatments relative to root length (Table 1).

Several plant paramenters were affected by the experimental treatments (Table 1). Top height and weight were affected by organic amendment placement as well as by inoculum density (Table 4). Top growth generally decreased as the nematode inoculum level increased. In general, organic amendment placed on the soil surface provided the highest values of plant height and top weight. Lowest values occurred when the amendment was mixed with the soil. Root fresh weight was affected by organic amendment placement, but not by inoculum density. The lowest root weights were observed when the organic amendment was mixed with the soil (Table 5). Root length was not affected by organic amendment placement or inoculum density (Table 5).

There was evidence that this organic amendment did not consistently benefit plant growth, in contrast to the results of Mankau (1968), Muller and Gooch (1982), and Trivedi and Barker (1986). However, it was

Table 2. Effect of organic amendment placement and inoculum densities of *Meloidogyne incognita* race 1 on egg-mass and gall ratings 50 days after inoculation. April 1994.

Organic amendment placement	250 J2/pot		1000 J2/pot	
	Egg masses†	Galls†	Egg masses	Galls
None	2.0a‡	4.0a	3.2a	4.6a
Surface	1.0a	3.0a	2.2b	4.4a
Incorporated	2.2a	3.8a	2.0b	4.6a

†Egg masses and galls rated on 0 to 5 scale, where 0 = no galls; 1 = 1 to 2 galls; 2 = 3 to 10 galls; 3 = 11 to 30 galls; 4 = 31 to 100 galls; and 5 = > 100 galls per root system.

‡Data are means of five replications. Means followed by the same letter in a column are not significantly different ($P \leq 0.05$), according to Duncan's multiple-range test.

Table 3. Effect of organic amendment placement and inoculum density of *Meloidogyne incognita* race 1 on numbers of juveniles (J2) hatched from egg masses 50 days after inoculation. April 1994.†

Organic amendment placement	250 J2/pot	1000 J2/pot
	------------ J2 per root system	------------
None	425	906
Surface	485	462
Incorporated	1,009	1,185

†Statistical analysis was not performed because data are combined totals of four replications.

Table 4. Effect of organic amendment placement and inoculum density of *Meloidogyne incognita* race 1 on plant height and dry shoot weight of okra. April 1994.

Organic amendment placement	0 J2/pot‡			250 J2/pot			1000 J2/pot		
	Plant height			Plant height			Plant height		
	30 days	50 days	Weight	30 days	50 days	Weight	30 days	50 days	Weight
	cm		g	cm		g	cm		g
None	19.20a†	21.00a	0.85b	19.10a	21.10b	0.83b	17.70a	19.80a	0.68a
Surface	20.10a	24.10a	1.34a	18.60a	23.2a	1.41a	17.60a	21.00a	0.87a
Incorporated	15.50b	16.40b	0.37c	15.90b	17.10c	0.40c	14.20b	15.30b	0.21b

†Data are means of five replications. Means in columns followed by the same letter are not significantly different ($P \leq 0.05$), according to Duncan's multiple-range test.

‡Number of juveniles hatched from the egg masses and used as inoculum.

Table 5. Effect of organic amendment placement and inoculum density of *Meloidogyne incognita* race 1 on taproot length and root dry weight of okra 50 days after inoculation. April 1994.

Organic amendment	0 J2/pot‡		250 J2/pot		1000 J2/pot	
	Taproot length	Weight	Taproot length	Weight	Taproot length	Weight
	cm	g	cm	g	cm	g
None	25.50 a†	0.45 b	21.40 a	0.52 b	20.60 ab	0.47 b
Surface	21.30 a	0.86 a	23.00 a	0.84 a	26.30 a	0.77 a
Incorporated	19.00 a	0.28 c	22.20 a	0.29 c	18.30 b	0.27 c

†Data are means of five replications. Means followed by the same letter in columns are not significantly different ($P \leq 0.05$), according to Duncan's multiple-range test.

‡Number of juveniles hatched from the egg masses and used as inoculum.

evident that mixing the amendment with soil produces a negative growth response compared to amendment applied to the soil surface. According to Reuszer (1957), amendments of this nature, high in lignin and cellulose, may require extra N and P when they are mixed with the soil. Therefore, when incorporated, the amendment will compete for nutrients much more than when simply applied to the soil surface. Research has shown that microorganisms may use great amounts of N to decompose organic amendments and that incorporation of organic matter high in C may create N-deficient soil for higher plants (Gallaher and McSorley, 1994). Soil extractable-nutrient analyses (Table 6) revealed high levels of organic matter and N when yard-waste compost was added to the soil, either on the soil surface or mixed. Nonetheless, in general, higher values of P, Mg, Mn, Cu, and Na were found when the amendment was placed on the soil surface (Table 6), while others (Ca, Zn, Fe) were unaffected. Organic residues with low N content require much more time to be decomposed, producing less humus than organic residues with higher N levels. Therefore, it has been suggested that supplemental N application may increase microbial activity and hasten the decomposition of amendments with high C/N ratios (Holtz and Vandecaveye, 1938).

Rodríguez-Kábana (1986), working with organic amendments having different C/N ratios, reported that nematode control was enhanced when more N was available. These results may have been due to the fact that some organic amendments require much time to be decomposed and release N or other products. According to McSorley and Gallaher (1995), yard-waste compost treatment had no impact on a nematode population even after 4-4.5 months, so a longer time period may be required for this organic amendment to have an appreciable effect.

The manner in which organic amendments affect nematodes is complex and remains poorly understood. Stirling (1991) suggested that amendments which are high in N and have a balanced C:N ratio may act by pro-

Table 6. Organic matter, pH and soil extractable nutrients, after 50 days. April 1994.

Treatments	OM	N	pH	P	K	Mg	Ca	Mn	Zn	Cu	Fe	Na
	%			ppm								
None	0.60b†	0.0054b	7.92a	105b	107b	66c	5328a	3.3c	18.7a	0.32a	4.24a	38.6ab
Surface	1.23a	0.0158ab	7.76b	118a	49c	94a	5408a	5.8a	20.9a	0.21ab	4.24a	41.0a
Incorporated	1.31a	0.0176a	7.72b	95b	254a	78b	5488a	4.8b	28.2a	0.18b	2.64a	33.6b

†Data are means of four replications. Means followed by the same letter in columns are not significantly different ($P \leq 0.05$), according to Duncan's multiple-range test.

ducing nematoxic levels of ammonia. This is not the case with yard-waste compost (mainly woodchips), however (Gallaher and McSorley, 1994; McSorley and Gallaher, 1995). Stirling (1991) suggests that nematicidal activity of wastes with high C:N ratios can be improved by adding urea or other sources of N concurrently.

Under the conditions of this experiment, addition of the organic amendment did not consistently affect development of *Meloidogyne incognita* race 1. However, a beneficial effect on plant growth was observed when the organic amendment was placed on the soil surface, rather than mixed with soil, even at high nematode levels.

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