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Effects of Chicken-excrement Amendments on *Meloidogyne arenaria*[1]

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Abstract: The effects of chicken litter on *Meloidogyne arenaria* in tomato plants cv. Rutgers were determined in the greenhouse. Tomato seedlings were transplanted into a sandy soil amended with five rates of chicken litter and inoculated with 2,000 *M. arenaria* eggs. After 10 days, total numbers of nematodes in the roots decreased with increasing rates of chicken litter. After 46 days, egg numbers also decreased with increasing litter rates. In another experiment, soil was amended with two litter types, N-P-K fertilizer, and the two primary constituents of chicken litter (manure and pine-shaving bedding). After 10 days, numbers of nematodes in roots were smaller in chicken-excrement treatments as compared to nonexcrement treatments. At 46 days, there were fewer nematode eggs in chicken-excrement treatments compared to nonexcrement treatments. Egg numbers also were smaller for fertilizer and pine-shaving amendments as compared to nonamended controls. Chicken litter and manure amendments suppressed plant growth by 10 days after inoculation but enhanced root weights at 46 days after inoculation. Amendment of soil with chicken litter suppressed *M. arenaria* and may provide practical control of root-knot nematodes as part of an integrated management system.

Key words: amendment, biological control, chicken litter, *Lycopersicon esculentum, Meloidogyne arenaria,* nematode, penetration, root-knot nematode, tomato.

Root-knot nematodes (*Meloidogyne* spp.) are ranked among the most damaging plant pathogens, causing an estimated average annual crop loss of more than 5% worldwide (9). In recent years, many effective and relatively inexpensive nematicides have been withdrawn from the market because of health hazards to production workers or because of their detection at unacceptable levels in ground water (3). Without cost-effective, environmentally acceptable nematicidal compounds, nematode management must change. The addition of many organic soil amendments, in particular those with high nitrogen contents, may be effective alternatives for control of *M. arenaria* (Neal) Chitwood and other plant-parasitic nematodes (4,7). The addition of chicken litter to soils suppressed *Meloidogyne* spp., restricted root galling caused by the nematode, and stimulated plant growth (5).

Previous investigators have limited their research to recording the suppressive effects of chicken litter on *Meloidogyne* spp., based on total numbers of nematode-induced galls per root, and by the use of various root-galling indices (4). It is unknown if any specific growth stage or function in the *M. arenaria* life cycle is disrupted by the addition of chicken-litter amendment. The purpose of this greenhouse study was to determine the effects of chicken-litter amendments and several litter constituents on penetration and growth of *M. arenaria.*

MATERIALS AND METHODS

Chicken litter was collected at the University of Georgia 4-Towers Agricultural Cooperative Extension Service (4T) and Poultry Research Center (PR). The PR litter was from layer hen pens, which were cleaned weekly. The 4T litter was from broiler hen pens, which were cleaned approximately once a month. The primary constituents of the chicken litter were chicken excrement and a pine-shaving bedding. Each batch of chicken litter was prepared so that samples were homogenous by grinding through a soil shredder, passing through a 2-cm sieve, and mixing in a cement mixer. Chicken manure (chicken excrement without bedding) also

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was collected from the Poultry Research Center.

Elemental analysis was done for each litter by the Georgia Cooperative Extension Service Soil Testing and Plant Analysis Laboratory (6). Extractable P and K were determined by the Mehlich-1 extraction method (6). The NO_3-N and NH_4-N analyses were performed using the Kjeltic water and HCl titration method. Total N analysis was performed by potassium chloride extraction and steam distillation after the addition of Devarda's Alloy (6). Total N analysis was also performed by the Chemical Analysis Laboratory (Institute of Ecology, Athens, GA) using a Perkin-Elmer 240C Elemental Analyzer for C-H-N.

Nematode response to increasing rates of chicken litter: A sandy soil (82% sand, 10% silt, 8% clay; pH 6.8) was amended with five levels of chicken litter (0.5, 1.0, 1.5, 2.0, and 2.5% w/w) by thoroughly mixing the soil and litter together in a cement mixer. Nonamended soil was used as a control. Three-week-old tomato seedlings, *Lycopersicon esculentum* Mill. cv. Rutgers, were transplanted into the amended and nonamended soils in 15-cm-d pots. Tomato seedlings were inoculated with 2,000 *M. arenaria* eggs (2) by pipetting 1 ml of a calibrated egg suspension into each of three small holes in the soil near the base of each plant. Control pots receiving no nematodes were treated with 3 ml water in the same manner. Pots were arranged on greenhouse benches in a randomized complete block design with four replicates for each of two sampling dates. The experiment was done twice. PR-chicken litter was used for the first experiment, and 4T-chicken litter was used for the second.

Nematode response to different soil amendments: A sandy soil was amended with inorganic fertilizers, unused chicken pen pine shaving bedding, chicken manure, PR-chicken litter, and 4T-chicken litter. Both the PR litter and the manure were amended to soil at rates of total N equivalent to the total N measured in each of four rates (0.5, 1.0, 1.5, and 2.0% w/w) of the 4T litter. Inorganic N, P, and K, in the form of ammonium nitrate, sodium phosphate, and potassium nitrate, was added to soil at rates equivalent to the N, P, and K found in the 4T-litter treatments. Bedding, consisting of fresh pine shavings, was added to soils in volumes that were equivalent to the volume of each of the four rates of 4T-litter treatments. The bedding and chicken manure treatments were included so that the two constituents of chicken litter could be evaluated separately. The fertilizer treatment was included to determine the effects of equivalent rates of mineral plant nutrients, and most importantly, nitrogen compounds.

Amendments were mixed thoroughly with soil in a cement mixer. Nonamended soil was used as a control. Three-week-old Rutgers tomato seedlings were transplanted into amended and non-amended soils in 15-cm-d pots. Plants were inoculated and arranged as previously described. The experiment was done twice.

Collection of data and data analysis: Ten days after inoculation, tomato stems were cut at the soil line, and root systems were removed from the pots and washed free of soil. Fresh root and oven-dried shoot weights (48 hours at 60 C) were recorded. Roots were cut into 1–2-cm-long pieces, and nematodes in the roots were stained with acid fuchsin (1). Nematodes in the roots were counted and distinguished by growth stages as vermiform or swollen.

At 46 days after inoculation, fresh root and oven dried shoot weights were recorded for the remaining plants. Nematode eggs were collected from each root system with a 1.0% sodium hypochlorite (NaOCl) solution and counted (2).

Data were analyzed by ANOVA and means separated by Duncan's multiple-range test (8). Preplanned single-degree-of-freedom contrasts were used for selected comparisons ($P \leq 0.05$). Data from repeated experiments were combined for presentation where there were no significant interactions among main effects and experimental run. All differences reported in the results were significant at the $P \leq 0.05$ level. Regression analysis was used to determine plant growth and nematode responses to increasing rates of amendments.

Results

Response to increasing rates of chicken litter: Analysis of the litter indicated that the PR litter contained 3.92% total nitrogen, 1.33% phosphorus, and 1.84% potassium, with 2,748 ppm NH_4-N, and 956 ppm NO_3-N. The 4T litter contained 1.36% total nitrogen, 0.45% phosphorus, and 0.74% potassium, with 742 ppm NH_4-N, and 161 ppm NO_3-N. No interaction was observed between experimental run and chicken litter-rate effects, although the chicken litter for the two experiments was collected from different sources. Because there was no interaction, data were combined from the experimental runs.

Ten days after inoculation, total numbers of nematodes per gram fresh root weight decreased in a linear response to increasing litter rates (Fig. 1A). The two growth stages (vermiform and swollen) responded differently to increasing amendment rates. The number of swollen nematodes decreased in a linear response to increasing litter rates (Fig. 1B), whereas a quadratic model best described the relationship between the number of vermiform nematodes per gram fresh root and rate of chicken litter. Numbers of nematodes remaining in the vermiform stage at 10 days were quite small.

After 46 days, numbers of eggs per gram root weight decreased in a linear response to increasing litter rates (Fig. 1C). Decreased numbers of eggs would result from fewer swollen nematodes (females) at the 10-day sampling. The regression equation indicated that after 46 days, the numbers of *M. arenaria* eggs decreased 32.6% for each unit increase in percentage of chicken litter.

Ten days after transplanting Rutgers tomato seedlings in chicken litter and nonamended soils, dry shoot weights and fresh root weights decreased in a linear response to increasing rates of amendment (Y = 1.52 − 0.17X, R^2 = 0.09, P = 0.01, and Y = 12.37 − 1.26X, R^2 = 0.05, P = 0.05, respectively; data not shown). By 46 days, fresh root weights increased in a linear response to increasing rates of litter amendment (Y = 56.2 + 5.5X, R^2 = 0.08, P =

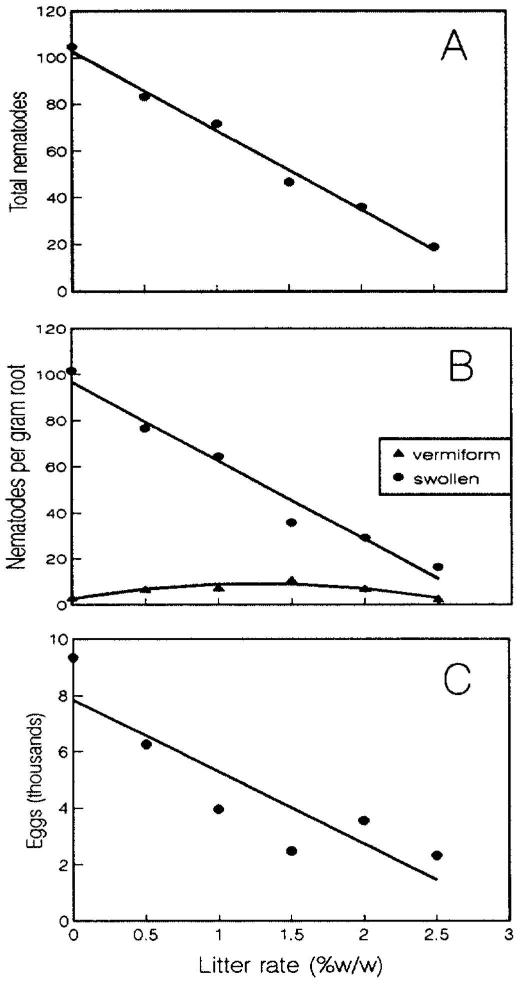

FIG. 1. Effects of increasing rates of chicken litter on *Meloidogyne arenaria* per gram fresh root weight of Rutgers tomato. A) Total nematode numbers 10 days after inoculation. Linear regression indicated a response of Y = 102.8 − 34.1X, R^2 = 0.55, P = 0.0001. B) Nematode numbers by growth stage 10 days after inoculation. A linear regression model, Y = 96.7 − 34.2X, R^2 = 0.57, P = 0.0001, best described the response of swollen nematodes to rates of chicken litter, whereas the quadratic model Y = 2.7 + 10.4X − 4.1X^2, R^2 = 0.26, P = 0.001 best fit the vermiform data. C) Eggs collected 46 days after inoculation. Linear regression indicated a response of Y = 7,844 − 2,558X, R^2 = 0.42, P = 0.0001. Data are means of eight replicates.

0.01; data not shown), and no differences were observed among dry shoot weights. No difference was observed in plant growth between nematode infected and healthy plants on either date.

Nematode response to multiple soil amendments: Analysis of litter and manure indicated that the PR litter contained 4.5% total nitrogen with 1,925 ppm NH_4-N and 310 ppm NO_3-N in the first experimental run, and 3.2% nitrogen with 1,043 ppm NH_4-N and 105 ppm NO_3-N in the second experiment; in contrast, the 4T litter contained 3.3% nitrogen with 2.328 ppm NH_4-N and 75 ppm NO_3-N in the first experiment, and 2.7% nitrogen with 1,666 ppm NH_4-N and 455 ppm NO_3-N in the second experiment. The manure contained 2.6% total nitrogen with 2,499 ppm NH_4-N and 42 ppm NO_3-N in the first experimental run, and 4.1% nitrogen with 1,757 ppm NH_4-N and 63 ppm NO_3-N in the second experiment.

Ten days after inoculation, the numbers of nematodes per gram fresh root weight were greater in nonamended than in chicken-manure-amended soil (Table 1). Contrasts indicated that there were fewer total numbers of nematodes penetrating roots from chicken-excrement treatments (PR, 4T, and manure) than from non-chicken-excrement amended treatments (fertilizer, and pine-shaving bedding). Treatment with 4T-litter reduced the number of nematodes in roots by 67%.

After 46 days, there were fewer eggs per gram fresh root collected from plants grown in litter and manure-amended soils than from plants grown in nonamended soil (Table 1). Corroborating what was observed for the number of nematodes in roots at 10 days, contrasts indicated that fewer eggs per gram root were observed in plants grown in chicken-excrement-amended soil than in those grown in non-chicken-excrement amended soil (Table 1). At 46 days, the pine-shaving bedding and the fertilizer amendment also decreased the number of *M. arenaria* eggs compared to nonamended controls.

Ten days after inoculation, dry shoot weights were less for plants grown in chicken manure and 4T-litter-amended soil than in nonamended controls or in fertilizer-amended treatments (Table 2). Root weights were lower for plants grown in 4T-litter-amended soil than for plants grown in soil amended only with the pine-shaving bedding. Contrasts indicated that both root and shoot weights were lower for chicken-excrement-amended treatments

TABLE 1. Effects of soil amendment on numbers of *Meloidogyne arenaria* in roots of Rutgers tomato 10 days after inoculation, and on numbers of eggs 46 days after inoculation.

Amendment	Number of nematodes 10 days after inoculation: Per gram fresh root	Number of nematodes 10 days after inoculation: Total/plant	Eggs per gram fresh root 46 days after inoculation
Nonamended	31 ab	397 ab	13,241 a
Pine shavings	47 a	446 a	8,888 b
Fertilizer†	37 a	355 ab	8,626 b
PR litter‡	20 bc	212 bc	5,815 c
4T litter§	10 c	102 c	5,513 c
Chicken manure	11 c	125 c	5,045 c
Contrast:			
Chicken excrement vs. non-chicken excrement	0.0001	0.0001	0.0001

Values followed by the same letter within a column are not different ($P \leq 0.05$). Data are means of eight replications for nonamended, 32 replications for amended.

† Inorganic N, P, and K in the form of ammonium nitrate, sodium phosphate, and potassium nitrate.

‡ Chicken litter from University of Georgia Poultry Research Center.

§ Chicken litter from University of Georgia 4-Towers Agricultural Cooperative Extension Service.

TABLE 2. Influence of soil amendment on dry shoot and fresh root weights (g) of Rutgers tomato, 10 and 46 days after inoculation with *Meloidogyne arenaria*.

	10 days after inoculation		46 days after inoculation	
Amendment	Shoot weight	Root weight	Shoot weight	Root weight
Nonamended	1.7 a	10.6 ab	17.9 ab	49.0 ab
Pine shavings	1.6 ab	12.4 a	15.7 ab	49.1 ab
Fertilizer†	1.7 a	10.3 ab	18.7 a	52.4 ab
PR litter‡	1.6 ab	9.7 ab	14.8 bc	54.4 a
4T litter§	1.1 b	7.4 b	11.7 cd	41.0 b
Chicken manure	1.2 b	8.2 ab	11.2 d	43.9 ab
Contrast:				
Chicken excrement vs. non-chicken excrement	0.05	0.05	0.0001	NS

Values followed by the same letter within a column are not different ($P \leq 0.05$). Data are means of eight replications for nonamended, 32 replications for amended.
† Inorganic N, P, and K in the form of ammonium nitrate, sodium phosphate, and potassium nitrate.
‡ Chicken litter from University of Georgia Poultry Research Center.
§ Chicken litter from University of Georgia 4-Towers Agricultural Cooperative Extension Service.

than for non-chicken-excrement treatments.

Forty-six days after inoculation, plants grown in soil amended only with fertilizer had greater dry shoot weights than plants grown in either litter or manure-amended soils (Table 2). The 4T-litter and chicken manure treatments also had lower shoot weights than nonamended controls. Root weights were lower in 4T-litter treatments than in PR-litter treatments. Contrasts indicated that shoot weights were lower for chicken-excrement-amended treatments than for non-chicken-excrement treatments, but this difference was not detected in root weights, since root growth was greatest in PR-litter-amended soils. No difference was observed in plant growth between nematode-infected and healthy plants at either 10 or 46 days after inoculation.

Total numbers of nematodes in roots 10 days after inoculation decreased in a linear response to increasing rates of amendment for 4T-litter and chicken manure, but not for the other amendments (Table 3). By 46 days after inoculation, however, numbers of nematode eggs per gram fresh root weight decreased in response to increasing rates of all amendments. The rate of decrease in nematode eggs observed for the non-nitrogenous pine-shaving bedding was essentially the same as for the 4T-litter. Regression slopes indicated that the organic amendments caused decreases of 5–6,000 eggs per unit increase in amendment rate.

DISCUSSION

The results of these experiments showed that both the total number of

TABLE 3. Regression models for effects of increasing rates of soil amendments on total numbers of *Meloidogyne arenaria* per gram fresh root weight from Rutgers tomato 10 days after inoculation, and on numbers of eggs per gram fresh root weight 46 days after inoculation.

Amendment	Intercept	Slope	R^2	P
Numbers of nematodes				
Pine shavings	—	—	—	NS
Fertilizer†	—	—	—	NS
PR litter‡	—	—	—	NS
4T litter§	28.5	−17.1	0.38	0.0001
Chicken manure	25.8	−14.3	0.35	0.001
Numbers of eggs (in 1,000s)				
Pine shavings	14.0	−5.5	0.31	0.001
Fertilizer	13.1	−3.9	0.23	0.01
PR litter	12.3	−6.1	0.39	0.0001
4T litter	12.4	−5.8	0.33	0.0001
Chicken manure	12.0	−6.0	0.35	0.001

† Inorganic N, P, and K in the form of ammonium nitrate, sodium phosphate, and potassium nitrate.
‡ Chicken litter from University of Georgia Poultry Research Center.
§ Chicken litter from University of Georgia 4-Towers Agricultural Cooperative Extension Service.

nematodes penetrating the roots and those in the swollen growth stage were inversely related to increasing rates of chicken litter. Similarly, the addition of farmyard manure to soil is known to delay the development of *Globodera rostochiensis* (Wollenweber) Behrens in potato roots, compared to fertilizer amended and nonamended soil (10).

Because chicken litter decreased the numbers of *M. arenaria* J2 in tomato roots, associated damage could be restricted in the first growing season after litter application. Egg production also decreased as a result of chicken litter soil amendment, indicating that the final population of *M. arenaria* would be smaller than in nonamended soil, resulting in a smaller Pi for the next season. In this manner, chicken-litter amendment would be useful in combination with crop rotation programs by increasing the efficiency or reducing the length of required rotations.

The nematode suppressive effects of chicken litter have been attributed to nitrogen content, and most importantly to the release of ammoniacal nitrogen from the soil amendment (7). Our study indicated that various chicken-excrement amendments with different percentages of nitrogen and varying ratios of NH_4-N to NO_3-N had similar levels of nematode suppression.

It has also been suggested that organic soil amendments enhance microbial populations that have deleterious affects on plant-parasitic nematodes (7). At 10 days after inoculation, negative effects on *M. arenaria* were observed only for the litter and manure treatments, but by 46 days after inoculation, suppression of egg development also was observed for the pine-shaving bedding. The role of microbes in chicken-litter-amended soil should be investigated further. A dual mechanism of suppression may be operating for chicken litter, with an immediate effect from NH_4 and other toxic compounds, followed by long-term suppression due to the microbial activity associated with organic amendments. A lowering of pH in amended soils due to addition of ammonia may also have contributed to observed decreases in nematode activity. Soil pH levels were not measured for individual treatments in this study.

Chicken litter and manure caused an initial suppression in plant growth. Phytotoxic effects were not evident after 46 days in the rate-response experiments, but in the multiple-amendment experiments shoot growth (weights) was inhibited at 46 days by litter and manure. There was evidence of enhanced root growth at 46 days in both experiments. A previous study indicated that chicken litter promotes plant growth and yield (5). However, before chicken litter can be used safely and effectively, field trials are necessary to better understand the effects of rates and types of chicken litter on crops. Hopefully, future research will provide a basis from which to make chicken-litter-rate recommendations to optimize yield and to avoid phytotoxicity.

Nonphytotoxic rates of the chicken-litter amendments used in this study were equivalent to 10–45 MT/ha (4.4–19.8 tons/acre) mixed to a depth of 15 cm. These large quantities may prevent large-scale field applications. However, the use of chicken-litter amendments by home gardeners, for whom there are no registered chemical nematicides, may provide practical control of root-knot nematodes. The use of early preplant or postharvest applications of chicken litter should aid in minimizing the risk of phytotoxicity.

Literature Cited

1. Byrd, D. W., Jr., T. Kirkpatrick, and K. R. Barker. 1983. An improved technique for clearing and staining plant tissue for detection of nematodes. Journal of Nematology 15:142–143.

2. Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter 57:1025–1028.

3. Johnson, A. W., and J. Feldmesser. 1987. Nematicides—A historical review. Pp. 448–544 *in* J. A. Veech and D. W. Dickson, eds. Vistas on nematology. Society of Nematologists.

4. Mian, I. H., and R. Rodríguez-Kábana. 1982. Soil amendments with oil cakes and chicken litter for

control of *Meloidogyne arenaria*. Nematrópica 12:205–220.

5. Mian, I. H., and R. Rodríguez-Kábana. 1982. Survey of the nematicidal properties of some organic materials available in Alabama as amendments to soil for control of *Meloidogyne arenaria*. Nematrópica 12: 235–246.

6. Plank, C. O., ed. 1989. Soil test handbook for Georgia, pp. 21–22. Cooperative Extension Service, University of Georgia, Athens, Georgia.

7. Rodríguez-Kábana, R. 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. Journal of Nematology 18:129–135.

8. SAS Institute Inc. 1985. SAS user's guide: Statistics, version 5 ed. Cary, NC: SAS Institute, Inc.

9. Sasser, J. N., and C. C. Carter. 1985. An overview of the International *Meloidogyne* Project 1975–1984. Pp. 19–27 *in* J. N. Sasser and C. C. Carter, eds. An advanced treatise on *Meloidogyne*. vol. 1, Biology and control. Raleigh: North Carolina State University Graphics.

10. Van der Laan, P. A. 1956. The influence of organic manuring on the development of the potato root eelworm, *Heterodera rostochiensis*. Nematologica 1: 112–125.