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# *Meloidogyne incognita* Infested Soil Amended With Chicken Litter[1]



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*Abstract:* The effects of chicken litter on *Meloidogyne incognita* in cotton, *Gossypium hirsutum* cv. DPL50 were determined in field microplots. Litters (manure and pine-shaving bedding) from a research facility and a commercial broiler house were used. Treatments consisted of 0.25%, 0.5%, and 1% litter by dry weight of soil for each kind of litter. Three control treatments consisted of soil not amended with litter, with and without nematodes, and one treatment to which mineral fertilizer was added at a nitrogen rate equivalent to that of the 0.5% litter rate, with nematodes. Microplots were inoculated at planting with 200 eggs/100 $cm^3$ soil in 1993 and 1,000 eggs/100 $cm^3$ soil in 1994. At 92 and 184 days after planting, nematode population densities decreased linearly with increasing rates of litter. Nematode numbers at midseason were larger in plots treated with mineral fertilizer than in plots treated with a rate of litter equivalent to the 0.5% rate. Fungal and bacterial population densities fluctuated throughout the growing season. Bacterial numbers had a positive linear relationship, with increasing rates of litter only in October 1993; however, significant positive relationships were observed throughout the 1994 growing season. In 1994, nematode population density at 92 days after planting decreased linearly with increasing bacterial numbers 30 days after planting. No other significant relationships between nematode densities and microbial densities were observed. Fungi and bacteria isolated from the litter and litter-amended soil were identified. Fungal genera isolated included *Acremonium, Aspergillus, Eurotium, Paecilomyces, Petriella,* and *Scopulariopsis,* whereas bacteria genera included *Arthrobacter, Bacillus,* and *Pseudomonas.*

*Key words:* bacteria, chicken litter, control, cotton, fungi, *Gossypium hirsutum,* management, manure, *Meloidogyne incognita,* organic amendment, root-knot nematode.

The suspension of several of our most important nematicides has prompted investigation into alternatives for the management of plant-parasitic nematodes. The incorporation of manure into nematode-infested soil suppressed *Globodera pallida* numbers by 96% and *Meloidogyne incognita* numbers by 80% (19,22). Chicken litter previously has been shown to suppress population densities of plant-parasitic nematodes (1,19,22). In addition, chicken litter is a valuable source of plant nutrient because it contains significant quantities of N, P, K, Ca, Mg, and micronutrients (39,41,45). The poultry industry in the southeastern United States accounts for nearly 25% of all agricultural income (31), but a major concern for this industry is the disposal of large quantities of litter and manure. Replacement of inorganic fertilizer with chicken litter would save the farming community $42 million per year (31) and would be an effective method of litter disposal.

Control of plant-parasitic nematodes by soil amendments may result from direct toxic effects and (or) from decomposition of organic matter, resulting in the formation of toxic products (28,29). Changes in microbial populations resulting from the addition of amendments also may impact plant-parasitic nematodes by several possible mechanisms (23,37,38). Many fungi have been reported to parasitize various life stages of plant-parasitic nematodes (9). *Heterodera glycines* females, eggs, and cysts isolated from soil are colonized by a variety of opportunistic fungi (9,10,17), and *Paecilomyces lilacinus* and *Verticillium chlamydosporium* have been shown to parasitize species of *Meloidogyne* (24). Chicken litter also may contain fungi that have the ability to produce metabolites that adversely affect nematodes (26,27,30).

Suppression of plant-parasitic nematodes by chicken litter may result from a combination of microbial effects and con-

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stituent toxicity. Chicken litter contains significant quantities of organic and inorganic nitrogen, which is released from the litter after incorporation into soil and throughout a growing season (4). Nitrogen released in the form of ammonia permeates cell membranes of nematodes and causes death (16,44). In litter, which also contains pine-shaving bedding, the presence of an organic carbon source reduces phytotoxicity caused by accumulation of ammonia and nitrates (21). These nonphytotoxic, but nematode-suppressive constituents stimulate the development of other microbial populations (28,37).

Identification of the fungi and bacteria associated with chicken litter and with litter-amended soil may aid in determining the mode of action of litter on plant-parasitic nematodes. The objectives of this study were to investigate the effects of chicken litter soil amendments on population densities of *M. incognita* race 3 in cotton; monitor fungal, bacterial, and *M. incognita* population changes in relation to applications of chicken litter to soil; and survey and identify fungi and bacteria in the litter and litter-amended soil.

## Materials and Methods

Chicken litter was collected from the University of Georgia Poultry Research Facility (L1) and from a commercial broiler house in Oconee County, Georgia (L2). Both litters (chicken excrement and pine-shaving bedding) were collected from pens that were cleaned monthly. Elemental analysis was performed on the litter by the University of Georgia Cooperative Extension Service and the Agricultural Services Laboratories Soil Testing and Plant Analysis Laboratory. Total N and P were extracted by persulfate digestion, and $NH_4$-N, and $NO_3$-N levels were determined by water extraction and colorimetric analysis (35).

Microplots were established at the Plant Sciences Farm in Oconee County, Georgia. Thirty-six 90-cm fiberglass-enclosed plots containing sandy-loam field soil (76% sand, 10% silt, 14% clay) were fumigated with methyl bromide (1.7 kg a.i./$m^3$) applied under 6-ml polyethylene sheeting. Treatments consisted of two kinds of litter (L1, L2) applied at three rates (0.25%, 0.5%, 1%) based on dry weight of soil (% w/w). Three controls included non-amended soil with and without *M. incognita* and soil amended with mineral fertilizer ($KNO_3$) equivalent to the rate of N in the 0.5% litter treatment with *M. incognita*. The *M. incognita* population was obtained from a cotton field in Johnson County, Georgia, and was maintained on tomato, *Lycopersicon esculentum* Mill. cv. Marglobe, in the greenhouse. Treatments were arranged in a randomized complete-block design with four replications. Each microplot also received 14-7-14 fertilizer at the recommended rate of 560 kg/ha at planting (11).

Cotton, *Gossypium hirsutum* L. cv. DPL50, seeds were planted and microplots were inoculated with *M. incognita* race 3 at 200 eggs/100 $cm^3$ soil on 20 May 1993 and 1,000 eggs/100 $cm^3$ soil on 10 May 1994. One week after planting, seedlings were thinned to six per microplot. Nematode population densities were assayed at 92 and 184 days after planting by collecting 10 2.5-cm-diam., 25-cm-deep soil cores from around the root zones within microplots. Second-stage juveniles (J2) of *M. incognita* were extracted from 500 $cm^3$ soil by elutriation and sucrose centrifugation (2,7). Roots extracted from the soil samples were placed in an intermittent-mist chamber (2), and nematodes were collected after 48 hours and counted. Population densities of *M. incognita* were expressed as the sum of J2 collected from soil and roots per 100 $cm^3$ soil. Soil samples for pH measurements, and fungal and bacterial counts were collected 17, 51, 89, 114, and 146 days after planting in 1993 and 0, 31, 63, 93, 128, 154, and 185 days after planting in 1994. Soil pH was determined in a 50-g soil/50-ml deionized water suspension (5).

Counts of fungi and bacteria were determined by serially plating the soil-litter suspensions and controls onto selective media in 9-cm-diam. petri dishes with a Spiral Plater (Spiral Systems, Bethesda, MD). Two grams of soil were weighed and placed into a whirlpac blender bag with 18 ml of deionized sterile water for the first dilution. Each sample was then placed in a Stomacher blender (Seward Medical, London, England) and agitated for 30 seconds. Further dilutions were made in a laminar flow hood, and the suspensions were plated onto selective media for microbial enumeration.

For fungal isolation, potato dextrose agar (PDA) (Difco, Detroit, MI) amended with streptomycin (0.1 g/liter) and tetracycline (0.01 g/liter) and PDA amended with streptomycin (0.1 g/liter) and chloramphenicol (0.01 g/liter) were used. Nutrient agar with nystatin (0.05 g/liter) was used for isolating bacteria. Serial dilutions were made to $10^{-3}$ for fungi and $10^{-5}$ for bacteria. Fungal colony forming units (CFU) were counted 4 to 5 days after incubation, and bacterial CFU were counted 3 days after incubation at 25°C in the dark.

Fungi isolated from the litter and amended soil serial dilutions were stored on PDA until identified. Approximately 0.1 g of commercial chicken litter from the 1994 season and fresh liter from 1995 were sprinkled evenly onto PDA amended with 0.1 g/liter streptomycin and 0.01 g/liter tetracycline in order to suppress bacterial growth. These plates were incubated at room temperature on a laboratory bench. Six days after plating, fungi were observed and transferred to various media for identification to species (3,6,15,32,33,36,40,42, 43).

After isolation, bacteria were stored at −80 °C for identification of fatty acid methyl-esters (FAME) (14,18,25). Before storing, the bacteria were placed on a shaker at room temperature in 2-ml cryovials with 0.75 ml of trypticase soy broth (TSB) for 24 to 48 hours. Each vial received 0.25 ml of sterilized glycerol and was placed at −80 °C. Bacteria selected for identification based on morphological differences were transferred to a 5% trypticase soy agar (Difco, Detroit, MI) for purification.

Seed cotton was collected from the microplots and weighed at maturity in both years as an estimate of yield. Cotton boll number and shoot height were recorded in the second year.

All differences reported in the results were significant at $P < 0.05$. Regression analysis was used to determine nematode responses to litter application, microbial population dynamics throughout the season, and yield in response to the nematode and to litter amendments. Data were analyzed by ANOVA and means separated by Duncan's multiple-range test (SAS Institute, Cary, NC). Fungal and bacterial counts were transformed with $\log_e (x + 1)$ before analysis.

## Results

Litter L1 contained 5.8% total nitrogen, 0.9% P, with 189 ppm $NO_3$-N and 4,452 ppm of $NH_4$-N, in 1993, and 3.2% total nitrogen, 0.1% P, 1,828 ppm $NO_3$-N, and 2,585 ppm of $NH_4$-N in 1994. Litter L2 contained 3.6% total nitrogen, 1.8% P in 1993 ($NH_4$-N and $NO_3$-N not done) and 5.3% total nitrogen, 0.1% P, 1.3 ppm $NO_3$-N, and 1,456 ppm $NH_4$-N in 1994. Although L1 and L2 litters were from different sources, no interactions were observed between litter source and experimental main effects; therefore, the data were combined. Data were combined across years where no significant interaction with main effects was observed. Phytotoxicity was observed in plots receiving the 1% rate of amendment, and replanting was necessary 1 week after the original planting date.

Population densities of *M. incognita* remained low during the 1993 growing season, so inoculum rates were increased 5-fold in 1994. In both 1993 and in 1994, the J2/100 $cm^3$ soil decreased linearly in response to increasing rates of chicken lit-

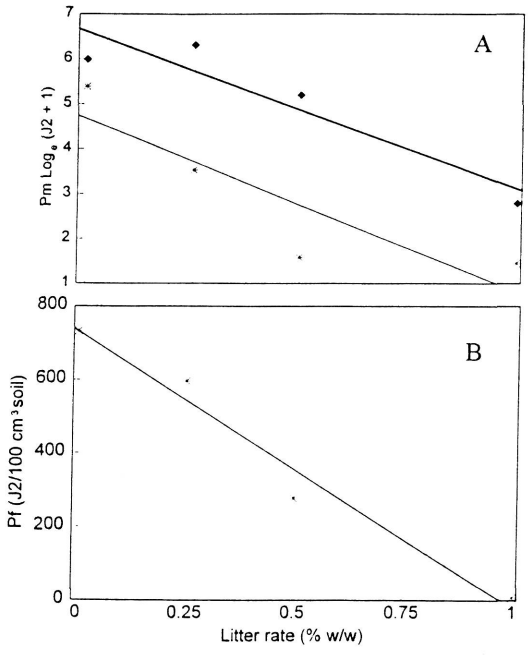


FIG. 1. A) Effects of chicken litter on *Meloidogyne incognita* (J2) population density 92 (Pm) days after planting. Linear regression models, Y = 4.2 − 3.4X, $R^2$ = 0.37, $P$ = 0.0001, and Y = 6.9 − 3.8X, $R^2$ = 0.57, $P$ = 0.001, described the *M. incognita* numbers in relation to quantity of litter in 1993 and 1994, respectively. Data for the control are means of four replicates, and data for the litter treatments are means of eight replicates. B) Effects of chicken litter on population densities of *M. incognita* 184 days after planting (Pf) in 1993. *Meloidogyne incognita* density decreased linearly as litter rates increased, Y = 731.9 − 751.4X, $R^2$ = 0.55, $P$ = 0.0001. Data for the control are means of four replicates, and data for the litter treatments are means of eight replicates.

ter 92 days after nematode inoculation (Fig. 1A). Slopes of the regression lines differed by year, with a 20% decrease in the numbers of *M. incognita* at the 0.5% litter rate compared to controls in 1993 and a 29% decrease at the same rate in 1994. Final nematode numbers (Pf) declined with increasing litter rates for data combined over years (Fig. 1B). Total numbers of J2 extracted from the soil and roots at harvest in 1993 decreased from a mean of 732/100 cm$^3$ soil in the nonamended control to only 6/100 cm$^3$ in the 1% litter treatment, and decreased from a mean of 174 J2/100 cm$^3$ soil in the nonamended control to 78/100 cm$^3$ soil in the 1% treatment in 1994. Regression analysis for combined data indicated that the reproduction rate at midseason and at the end of the season decreased as the litter rate increased (Fig. 2).

In 1994, cotton boll numbers at 184 days after planting increased as litter rate increased (Fig. 3). Boll numbers increased 79% and 102%, respectively, in the 0.5% and 1% litter amendment rates, respectively, compared to the nonamended control. Also in 1994, shoot height and litter rate as well as yield and litter rate (Fig. 3) were described best by quadratic models. Shoot height increased 27% and yield increased 393% from the nonamended control with nematodes to the 1% litter rate.

When data at 92 days after planting were combined for both years, the mineral fertilizer control (F + N +) had larger numbers of J2 (726/100 cm$^3$) compared to the equivalent 0.5% litter rate (166/100 cm$^3$). *Meloidogyne incognita* reduced cotton yields in 1994. However, shoot height and boll numbers in the nonamended control without nematodes (F − N −) were not significantly different from the nonamended control with *M. incognita* (F − N +). Yield in F − N − was greater than the F − N + control (101 g vs. 48 g). In control plots receiving mineral fertilizer equivalent to the

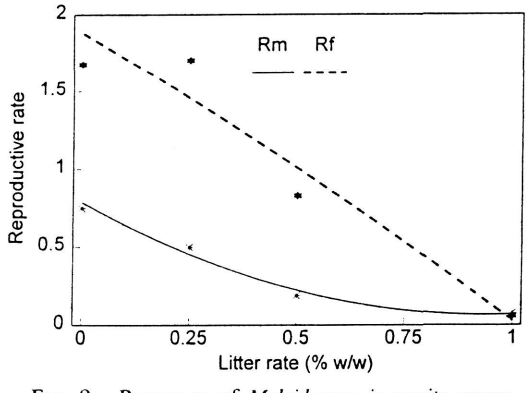


FIG. 2. Response of *Meloidogyne incognita* reproductive rates at 92 days (Rm) and 184 days (Rf) after planting to increasing litter rates. The Rm response to litter was described by the quadratic model, Y = 0.6 − 1.0X + 0.5X$^2$, $R^2$ = 0.20, $P$ = 0.01, and the response of Rf to litter rate is described by the line, Y = 1.9 − 1.9X, $R^2$ = 0.20, $P$ = 0.001. Midseason reproductive rate and Rf for the control are means of eight replicates, and data for the litter treatments are means of 16 replicates. Data combined for both years.

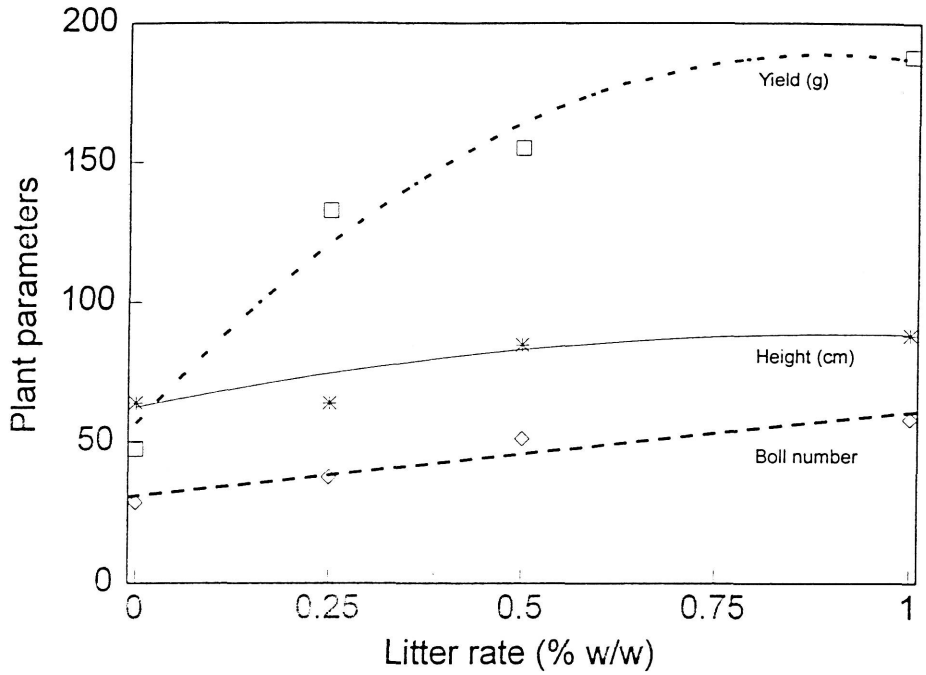


FIG. 3. Effects of increasing rates of chicken litter amendments on cotton boll number in 1994 *Meloidogyne incognita*-infested soil. Regression model, $Y = 31.9 + 28.6X$, $R^2 = 0.36$, $P = 0.001$, indicated a positive response between boll number and quantity of chicken litter. The response of cotton shoot height to litter in *M. incognita*-infested soil was $Y = 62.6 + 57.0X - 31.1X^2$, $R^2 = 0.38$, $P = 0.01$. The response of seed cotton (yield) to litter was described by the quadratic model, $Y = 56.6 + 299.0X - 169.4X^2$, $R^2 = 0.33$, $P = 0.01$. Data for the control are means of four replicates, and data for the litter treatments are means of eight replicates.

nitrogen found in the 0.5% litter rate, plants were taller, boll counts were greater, and yields were greater than in plots receiving only the recommended rate of fertilizer regardless of the *M. incognita* infestation level. Shoots in F−N− were shorter than in F+N+ (74 cm vs. 86 cm), and 62% fewer bolls were produced in F−N− than in the F+N+ control (46 vs. 75). Yield increased by 99% in F+N+ when compared to the yield in the F−N− control (201 g vs. 101 g). Shoot height was greater in F+N+ than in F−N+ (87 cm vs. 64 cm), and boll number production increased 2.6-fold in the F+N+ control when compared to the F−N+ control (75 vs. 28). More seed cotton was produced in the F+N+ control than in the F−N+ (48 g vs. 201 g).

The soil pH in the 2 years differed slightly throughout the season; however, the trends for both years were similar and stabilized near 6.0 by the end of both seasons (Fig. 4). In both years a consistently higher pH was observed in plots with the 1% litter treatment. Rainfall during the 2 years in which the studies were conducted differed. In 1993, cumulative rainfall during the growing season totaled 19 mm, 2.3-fold less than the rainfall in 1994.

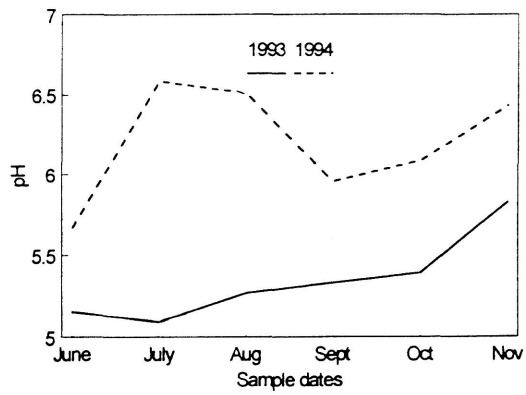


FIG. 4. Average soil pH in microplots during 1993 and 1994 growing seasons.

Bacterial population densities had a positive linear relationship with litter rate at different times throughout the season (Table 1). No relationships between fungal counts and litter rate were observed. Nematode density had a negative linear relationship to bacterial counts only in June 1994 (Fig. 5). No significant relationships between nematode numbers and fungal population densities were observed. Fungal counts were affected only by date of assay and year (Table 2), whereas bacterial counts were affected by rates of litter application, date of assay, and experimental year. There was a significant interaction between rate of litter application and time of assay for bacterial population densities.

Forty-three species of fungi were recovered from the litter and litter-amended soil (Table 3). Fungi isolated from the litter-amended soil consisted primarily of Deuteromycetes, although a few Ascomycetes and Zygomycetes also were identified. Higher numbers of Ascomycetes were isolated from direct plating of the litter (Tables 4,5) as compared to the dilution plating from amended soil. In 1994, *Petriella setifera* was the most common fungus and was found in 95% of the plates. *Eupenicillium brefeldianum* and *Eurotium chevalieri* also were found in 38% and 40% of the replicates, respectively (Table 4). The diversity of isolated fungi was lower in 1995, with only seven species recovered (Table 5). The most common fungal species isolated in 1995 was *Polypaecilum insolitum,* with an incidence of 100%. Bacterial genera identified from the litter-amended soil included *Arthrobacter, Bacillus,* and *Pseudomonas* (Table 6).

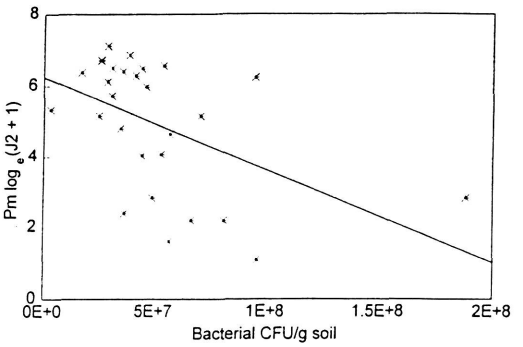


FIG. 5. Response of *Meloidogyne incognita* population density to increasing bacterial colony forming units per gram soil for assay done in June 1994, $Y = 6.5 - 3.1\times10^{-8}X$, $R^2 = 0.35$, $P = 0.001$.

## DISCUSSION

The numbers of *M. incognita* present at the end of the season were lower in the litter-amended plots when compared to the control, and this lower Pf value could translate into a lower Pi value for the next growing season. However, even though litter amendment effects on *M. incognita* were observed in both years, effects on other soil microbes were not consistent from year to year. Differences in rainfall between 1993 and 1994 may have influenced decomposition rate and nutrient release from the litter (39), thus affecting the comparison of treatment effects across

TABLE 1. Regression models for relationships of bacterial colony forming units per gram soil to rates of chicken litter amendment in microplots.

| Month and year | Intercept | Slope[a] | $R^2$ | $P$ |
|---|---|---|---|---|
| October 1993 | $3.88\times10^7$ | $3.61\times10^7$ | 0.17 | 0.05 |
| May 1994 | $1.95\times10^7$ | $1.00\times10^8$ | 0.37 | 0.05 |
| June 1994 | $2.30\times10^7$ | $5.41\times10^7$ | 0.31 | 0.01 |
| July 1994 | $3.89\times10^7$ | $3.18\times10^7$ | 0.17 | 0.05 |
| September 1994 | $2.57\times10^7$ | $2.08\times10^7$ | 0.21 | 0.05 |

[a] Litter was incorporated into microplot soil at rates of 0%, 0.25%, 0.5%, and 1% by dry weight of soil.

TABLE 2. Analysis of variance for effects of litter application, time of assay, and experimental year on bacterial and fungal counts from soil.

| Source | $F$ value | $P > F$ |
|---|---|---|
| Bacterial counts | | |
| Rate of litter application | 8.57 | 0.0001 |
| Month of assay | 4.78 | 0.001 |
| Year | 12.47 | 0.001 |
| Rate × year | 0.80 | NS |
| Rate × month | 1.77 | 0.05 |
| Fungal counts | | |
| Rate of litter application | 0.86 | NS |
| Month of assay | 4.49 | 0.001 |
| Year | 23.62 | 0.01 |
| Rate × year | 1.24 | NS |
| Rate × month | 0.68 | NS |

TABLE 3. Fungal genera and species identified from chicken litter and litter-amended soil.

| Fungal genera and species | Litter-amended soil | Litter 1994 | Litter 1995 |
|---|---|---|---|
| *Acremonium kiliense* Grütz | +[a] | + | – |
| *Alternaria alternata* (Fr.) Keissler | + | – | – |
| *Arthrinium phaeospermum* (Corda) M. B. Ellis | + | – | – |
| *Aspergillus candidus* Link ex Link | + | – | – |
| *Aspergillus flavus* Link ex Gray | + | – | – |
| *Aspergillus fumigatus* Fres. | + | + | – |
| *Aspergillus niger* van Tieghem | + | – | – |
| *Aspergillus wentii* Wehmer | – | + | – |
| *Cladosporium cladosporioides* (Fres.) de Vries | + | – | – |
| *Curvularia inaequalis* (Shear) Boedijn | – | + | – |
| *Emericella rugulosa* (Thom & Raper) C. R. Benjamin | + | – | – |
| *Epicoccum purpurascens* Ehreb. ex Schlecht | – | + | – |
| *Eupenicillium brefeldianum* (Dodge) Stolk & Scott | – | + | – |
| *Eurotium chevalieri* Mangin | + | + | + |
| *Fusarium moniliforme* Sheld. | + | – | – |
| *Fusarium oxysporum* Schlecht | + | – | – |
| *Fusarium semitectum* Berk & Rav. | + | – | – |
| *Gliocladium roseum* Bain. | + | – | – |
| *Gliocladium virens* Miller, Giddens & Foster | + | – | – |
| *Gongronella butleri* (Lender) Peyronel & Dal Vesco | + | – | – |
| *Humicola fuscoatra* Traaen | + | – | – |
| *Monascus ruber* an Tiegh. | – | + | + |
| *Myrothecium verrucarria* (Alb. & Schw.) Ditm. ex Steudel | + | – | – |
| *Paecilomyces inflatus* (Burnside) Carmichael | + | – | – |
| *Paecilomyces lilacinus* (Thom) Samson | + | + | – |
| *Penicillium arenicola* Chalabuda | + | – | – |
| *Penicillium chrysogenum* Thom | + | – | – |
| *Penicillium crustosum* Thom | + | – | – |
| *Penicillium citrinum* Thom | + | – | – |
| *Penicillium decumbens* Thom | + | – | – |
| *Penicillium lividum* Westling | + | – | – |
| *Penicillium miczinskii* Zaleski | + | – | – |
| *Penicillium simplicissimum* (Oudem.) Thom | + | – | + |
| *Petriella setifera* (Schmidt) Curzi | – | + | + |
| *Phoma* spp. | + | – | – |
| *Polypaecilum insolitum* G. Smith | – | – | + |
| *Rhizopus* sp. | + | – | – |
| *Scopulariopsis brevicaulis* (Sacc.) Bain. | + | – | – |
| *Sepedonium* sp. | + | – | – |
| *Trichoderma harzianum* Raifai | + | – | – |
| *Trichoderma koningii* Oudem. | + | – | – |
| *Trichoderma viride* Pers. ex Gray | + | + | + |
| *Verticillium albo-atrum* Reinke & Bertold | – | + | + |

[a] + = Species identified from at least one replicate plating. – = species not recovered from any assay.

years. The higher inoculum rate used in 1994 resulted in higher numbers of nematodes recovered at Pf and caused more damage to the cotton plants. Also, litter mineral constituents differed from year to year and between litters.

Plant growth was enhanced by the addition of litter, as compared to nonamended controls. Chicken litter contains significant quantities of essential nutrients other than NPK that are available to the plant (39,41, 45) when incorporated into the soil. The 0.25%, 0.5%, and 1% amendment rates translate to 10, 21, and 42 tons metric/ha, respectively. If litter is applied at an excessive rate, reduction in germination or emergence can occur because of the combination of high salt, $NH_4$-N, and nitrite-N (39). Replanting of plots receiving the 1% rate was necessary, but no differences in yield would be expected for planting dates falling between 1 April and 25 May (12).

TABLE 4. Incidence and average colony number of fungi isolated from chicken litter in 1994.

| Fungal species | Incidence % | Average number of colonies per plate |
|---|---|---|
| *Petriella setifera* | 95.0 | 4.7 |
| *Eupenicillium brefeldianum* | 40.0 | 0.425 |
| *Eurotium chevalieri* | 37.5 | 0.4 |
| *Monascus ruber* | 17.5 | 0.2 |
| Unknown species | 7.5 | 0.075 |
| *Aspergillus fumigatus* | 5.0 | 0.05 |
| *Trichoderma viride* | 5.0 | 0.05 |
| *Acremonium kiliense* | 2.5 | 0.025 |
| *Aspergillus wentii* | 2.5 | 0.025 |
| *Curvularia inaequalis* | 2.5 | 0.025 |
| *Epicoccum purpurascens* | 2.5 | 0.025 |
| *Paecilomyces lilacinus* | 2.5 | 0.025 |
| *Verticillium albo-atrum* | 2.5 | 0.025 |

Forty replicates consisted of 0.1 g of chicken litter sprinkled evenly on PDA amended with 0.1 g/liter streptomycin and 0.01 g/liter tetracycline.

The environmental impacts of applying high rates of chicken litter are not yet fully understood, but effects on water quality are of concern. Also, long-range transport costs may exceed the nutrient value of the litter, making transport of litter economically impractical based only on its value as fertilizer (31). However, if the value of nematode control is added as well as the costs of alternative methods for disposing of the litter, transport of the litter from production areas may be an economical alternative.

The numbers of nematodes were larger in the treatment with mineral fertilizer than in the treatments with litter at an equivalent percentage nitrogen content. Addition of an organic amendment to the soil stimulates the activity of bacteria and fungi (37), which was consistent with results obtained in this study. The microbe densities in the litter-amended treatments were consistently higher than in the non-amended treatments.

TABLE 5. Incidence and average colony number of fungi isolated from chicken litter in 1995.

| Fungal genera and species | Incidence % | Average number of colonies per plate |
|---|---|---|
| *Polypaecilium insolitum* | 100 | 18.5 |
| *Eurotium herbariorum* | 40 | 0.45 |
| *Petriella setifera* | 20 | 0.5 |
| *Verticillium albo-atrum* | 20 | 0.3 |
| *Monascus ruber* | 10 | 0.1 |
| *Penicillium simplicissimum* | 5 | 0.6 |
| *Trichoderma viride* | 5 | 0.05 |

Twenty replicates consisted of 0.1 g of chicken litter sprinkled evenly on PDA amended with 0.1 g/liter streptomycin and 0.01 g/liter tetracycline.

TABLE 6. Bacterial genera and species identified from litter-amended soil.

| | |
|---|---|
| *Arthrobacter* sp. | *B. megaterium* |
| *A. aurescens* | *B. sphaericus* |
| *A. crystallopoietes* | *B. subtilis* |
| *A. globiformis* | *Cytophaga* sp. |
| *A. ilicis* | *Cytophaga johnsonae* |
| *A. oxydans* | *Micrococcus kristinae* |
| *A. pascens* | *Pseudomonas* sp. |
| *Bacillus* sp. | *P. chlororaphis* |
| *B. brevis* | *P. vesicularis* |
| *B. cereus* | *Rhodococcus equi* |
| *B. laterosporus* | *Xanthomonas* sp. |
| *B. lentus* | *X. campestris* |

Bacterial numbers increased rapidly after the incorporation of the litter, and these rate-dependent increases lasted throughout the growing season. Numbers of nematodes at midseason were lower in plots with higher bacterial densities. This trend may result from the initial release of N in the form of ammonia decreasing the nematode population, whereas other factors increased bacterial densities. Also, incorporation of the litter into the soil may have introduced new bacteria and provided a food source for existing and incoming organisms, hence stimulating the bacterial population (37).

Isolation methods for fungi appeared to influence which species were recovered (8). Most fungi isolated by dilution plating from litter-amended soil were common asexual-stage soil inhabitants. However, all but one of the Ascomycetes were isolated directly from litter. Isolations from the litter-amended soil yielded some of the same genera and species of fungi reported previously from chicken litter (26,27). Genera and species in common with previous reports include *Aspergillus* sp., *A. fumigatus*,

*Cladosporium* sp., *Fusarium* sp., *Penicillium* sp., *Scopulariopsis* sp., and *Sepedonium* sp. Many of the other fungi identified in these studies are commonly associated with feathers, droppings, or pellets of free-living birds (13). These include *Arthrinium phaeospermum, Aspergillus candidus, A. flavus, Humicola fuscoatra, Penicillium chrysogenum, P. citrinum, P. decumbens, P. lividum,* and *Scopulariopsis brevicaulis* (13). *Emericella rugulosa, Epicoccum purpurascens, Paecilomyces lilacinus,* and *Scopulariopsis brevicaulis* have been reported to increase in frequency when NPK or urea fertilizers have been applied to soil (13). Chicken litter is a high-salt medium (39), and the occurrence of *Polypaecilium insolitum* from litter is consistent with its halophilic nature (34).

The bacterial genera *Bacillus* and *Pseudomonas* are ubiquitous in soil and many other habitats. Species of *Bacillus* have the ability to form endospores, which allow long-term survival under adverse conditions (20) and also survival in litter. Bacteria have diverse nutritional requirements and are able to use many different substrates. *Bacillus megaterium,* which was identified from the litter-amended soil, has the ability to use nitrate as a nitrogen source (20) that is found in the litter and also is produced as a result of nitrification.

Further studies are needed to examine the population dynamics of specific microorganisms in response to litter amendments. In addition, these organisms must be screened for possible nematode antagonism. Once antagonistic microorganisms have been identified, they can be tested as a seed treatment or as litter inocula. Investigation of litter rates, which maximize yields and minimize detrimental effects to the environment, are necessary. The practice of incorporation of litter into soil as a nematode-controlled tactic would best be optimized if combined with other management practices. With the addition of litter to nematode-infested soil, smaller quantities of nematicides could be used. Any amount of litter added would aid in soil fertility and enhance root-knot nematode control.

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