Effect of Soil Amendments on Hatching of *Meloidogyne incognita* Eggs

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ABSTRACT

Eggs of *Meloidogyne incognita* were placed in soil amended with various plant materials. Hatching was determined periodically with an agar-slide technique. Hatching was inhibited in alfalfa- or soybean-amended soils, but not in soils amended with other plant materials. Progressively more inhibition of hatching occurred as the concentration of alfalfa was increased from 1% to 8% (w/w). In soil containing 4% alfalfa, ammonium-nitrogen rose to 380 μg/g, a concentration previously found to reduce nematode populations in the retention zones in fields fertilized with anhydrous ammonia. Inorganic and organic compounds containing nitrogen were added to soils in quantities such that the nitrogen concentrations were equivalent to that of soils containing 4% alfalfa. Egg hatching was inhibited in such soils amended with inorganic ammonium compounds, amino acids, or protein hydrolysate.

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Certain organic materials added to soil reduce severity of root knot caused by *Meloidogyne incognita* (Kofoid & White) Chitwood (5, 10, 12, 16, 17). Disease severity was reduced proportionally by increased concentrations of the amendments. Susceptible plants grown in soils to which plant materials had been added at the rate of 4% (w/w) were completely free of the disease (8). Reduction in severity of root knot appears to be influenced by the nature of the amendment (5, 10, 16), incubation period (time between amendment incorporation and nematode assay) (5), environmental conditions during incubation (6), and nitrogen content of the soil (7). Attempts to relate reduction brought about by adding organic amendments with activities of predaceous organisms have been unsuccessful (3, 13). Nematicidal substances have been extracted directly from undecomposed plant materials (9), decomposing plant segments recovered from soil (15), and from soil amended with plant materials (9); therefore, reduction in infectivity or viability of eggs and larvae may be due partly to the presence of toxins. Since the mechanism has not been clearly elucidated, the present study was made to determine the effect of different kinds of soil amendments on egg hatching of *M. incognita*.

MATERIALS AND METHODS.—Mature, dry plant materials were chopped into 1- to 4-mm fragments in a Wiley mill. The particles were mixed thoroughly with surface samples of Etowah silt loam. The final concentration of plant material to soil was 4% for most of the tests. Amended soil samples were moistened to 50% of field capacity by atomizing with water while being stirred. Inorganic or organic chemicals containing nitrogen which were to be used as amendments were first dissolved in the water necessary to bring the soil samples to 50% of field capacity. The solutions were then atomized onto the samples while they were being stirred. Amounts of the compounds added were adjusted so that the final concentration of nitrogen was equivalent to that in soil containing 4% alfalfa. Inorganic nitrogen concentrations in amended and unamended soil were determined with the methods outlined by Jackson (4). Determination of ammonium-nitrogen and nitrate-nitrogen were made with Nessler's reagent and phenoldisulfonic reagent, respectively.

Egg hatching of *M. incognita* in amended and unamended soil was determined with a modified agar-slide technique (11). Egg masses picked from infested tomato roots were shaken for 4 minutes in vials containing 10% Clorox (5.25% solution of sodium hypochlorite) to separate them from the gelatinous material. The Clorox was diluted with water and the eggs were concentrated by centrifuging and decanting. To remove larvae and extraneous material, the eggs were washed through a 47-μm (300-mesh) screen and again were concentrated by centrifuging and decanting. They were then added to 1.7% melted agar held in a water bath at 45 C. Two drops of the egg-agar suspension were placed on a cleaned microscope slide and spread over a 2.54 cm^2 area with a glass rod. Slides thus prepared with hardened agar films were placed vertically in 250-ml beakers (three to four slides per beaker). The beakers were then filled with soil and were rapped sharply on a table top to ensure close contact of soil particles with the agar films. Moisture was retained by covering the beakers with polyethylene film.

To determine hatching after periods of incubation of 7, 14, and 21 days at 25 C, slides were removed gently from the beakers, and soil adhering to the films was removed with a camel's hair brush under a gentle stream of water. Cleaned films were stained for 10 minutes with 0.02% crystal violet in 5% aqueous $NaHCO_3$. Numbers of unhatched eggs and hatched eggs (empty egg shells) were counted in at least 10 microscope fields (× 100). The percentage of eggs that hatched on each slide during incubation was calculated for each soil treatment. An average count on five slides was used for the determinations for each treatment-incubation interval.

RESULTS AND DISCUSSION.—*Plant material amemdments.*—Considerable inhibition of hatching occurred in alfalfa-amended soil (Fig. 1-A). Hatching in oat straw-amended soil was similar to that in unamended soil. It appears that hatching inhibition in alfalfa-amended soil was proportional to the concentration of the amendment (Fig. 1-B); fewer eggs hatched as the concentration was increased. In additional tests, inhibition of hatching, similar to that in alfalfa-amended soil, occurred in soil amended with soybean stems and

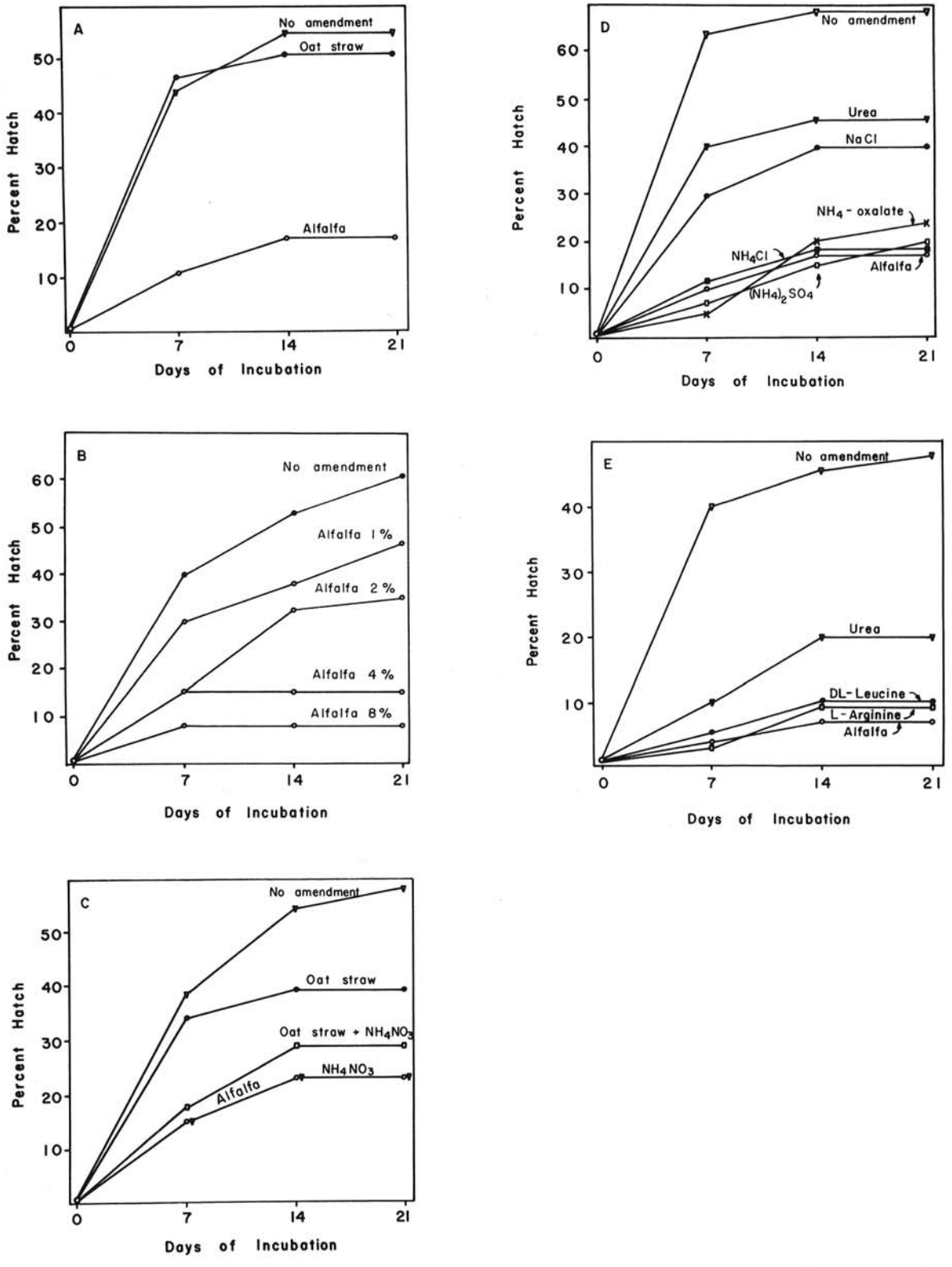

Fig. 1-(A to E). Effect of various soil amendments on egg hatching of *Meloidogyne incognita*; **A)** Oat straw and alfalfa; **B)** Concentration of alfalfa; **C)** Oat straw, alfalfa, and NH_4NO_3; **D)** Ammonium salts, NaCl, urea, and alfalfa; **E)** Urea, amino acids, and alfalfa.

TABLE 1. Inorganic nitrogen content (μg/g) and pH of soil samples after amendment with various plant materials (4%, w/w) and incubation for 3-28 days

Measurement and admendment	Days after adding admendment				
	3 days	7 days	14 days	21 days	28 days
Ammonium nitrogen (NH_4-N)					
None	5	8	5	10	6
Oat straw	10	10	5	10	13
Alfalfa	75	350	380	225	44
Lespedeza	25	40	22	8	10
Sericea lespedeza	28	45	20	10	8
Nitrate nitrogen (NO_3-N)					
None	30	25	35	37	40
Oat straw	0	0	0	0	0
Alfalfa	0	3	28	325	465
Lespedeza	0	0	5	0	0
Sericea lespedeza	0	0	6	0	0
Soil reaction (pH)					
None	5.8	5.6	5.6	5.6	5.5
Oat straw	6.1	6.3	6.1	6.3	6.0
Alfalfa	7.9	8.4	8.2	6.2	5.3
Lespedeza	6.4	6.4	6.3	6.4	6.4
Sericea lespedeza	6.5	6.5	6.4	6.5	6.5

leaves. No significant inhibition occurred in soils which contained the following materials (leaves and stems) at concentrations of 4% (w/w): Johnsongrass, corn, barley, flax, timothy, celery, onion bulbs, tomato, marigold, common lespedeza, *Sericea lespedeza*, and Birdsfoot trefoil.

Inorganic nitrogen in amended soils.—To determine if there were differences in ammonification and nitrification in the variously amended soils, samples were amended at 4% by weight with oat straw, alfalfa, common lespedeza, and *Sericea lespedeza*. Concentrations of NH_4-N and NO_3-N were determined after 3, 7, 14, 21, and 28 days of incubation. NH_4-N increased to 380μg/g in alfalfa-amended soil after 14 days of incubation (Table 1). This increase was reflected in a corresponding rise in pH. Much smaller increases of NH_4-N occurred in soils amended with lespedeza or *Sericea lespedeza*; no increase was detected in oat straw-amended soil. Nitrification was detected after 14 days in alfalfa-amended soil, then NO_3-N increased during the next 2 weeks to a level of 465 μg/g. Significant amounts of NO_3-N were not detected in soils amended with oat straw, lespedeza, or *Sericea lespedeza*. Since moderate amounts of NO_3-N (25-40 μg/g) occurred throughout the incubation period in unamended soil, the absence or very low levels of nitrate in these amended soils probably was caused by nitrate assimilation by the microflora.

The total protein nitrogen content of lespedeza is similar to that of alfalfa (14), but in the present study, very little NH_4-N or NO_3-N was formed from lespedeza particles in soil. Perhaps the nitrogen content of these particular lespedeza samples was abnormally low, or they were more resistant than alfalfa to decomposition by the soil microflora.

Soil amendments with N-containing chemical compounds.—Nitrogen could be related to inhibition of hatching in alfalfa-amended soil. Experiments were performed to determine if chemical compounds containing nitrogen would produce a similar effect. Average samples of alfalfa hay and oat straw contain 2.35% and 0.64% total nitrogen, respectively (14). The quantities of chemicals added to soil were adjusted so that final concentrations of nitrogen were the same as that in soil containing 4% alfalfa. More inhibition of hatching occurred in alfalfa and in NH_4NO_3-amended soils than in soils amended with oat straw or oat straw plus NH_4NO_3 (Fig. 1-C). Hatching may have been inhibited in NH_4NO_3-amended soil because of the higher osmotic concentration. The amount of NH_4NO_3 added was 2.7 g/kg soil, and this was dissolved in the soil solution. The soil moisture content was about 20%, and thus the concentration of NH_4NO_3 in solution was 0.16 molar. When eggs of *M. javanica* or *M. arenaria* were incubated in solutions of 0.2 molar NaCl, hatching was delayed (1), but the eggs incubated in salt solutions up to 1 molar concentration would hatch when transferred to distilled water. In the present study, additional hatching could not be obtained when slides containing eggs were transferred from amended soil to fresh unamended soil. In another experiment, three ammonium compounds, NaCl, and urea were tested. NaCl was added in quantities sufficient to make the soil solution 0.16 molar. Ammonium oxalate, NH_4Cl, and $(NH_4)_2SO_4$ were as effective as alfalfa (Fig. 1-D). Urea and NaCl inhibited hatching, but were not as effective as the other compounds. In other similar tests, the amino acids dl-leucine and l-arginine were as effective as alfalfa, but urea was not quite as effective (Fig. 1-E). Other compounds that inhibited hatching (Johnson and Shamiyeh, *unpublished*) in a manner similar to alfalfa were glycine, l-glutamic acid, l-cystine, and casein hydrolysate.

In previous studies (7), high concentrations of NH_4NO_3 (0.1 - 0.15%) in soil reduced severity of root knot of tomatoes. Severity was not affected by similar concentrations of KCl. This, together with results of the present study, indicates that nitrogen in inorganic and

organic compounds affects hatching. Furthermore, decomposition of certain legume amendments results in concentrations of NH_4-N sufficient to inhibit hatching. This reduction in hatching is similar to nematode population reductions obtained with 300 μg/g or higher of NH_4-N (2). Almost complete control in soils was obtained with 608 μg/g (2), a level of N that occurs often in the retention zone when anhydrous ammonia is added to soil in the field. High concentrations of NH_4-N occurring in soil amended with certain legumes apparently inhibit hatching of eggs, and could be related to a corresponding reduction in severity of root knot.

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