Survival and Reproduction of Some Nematodes as Affected by Muck and Organic Acids[1]

ISMAIL A. ELMILIGY and DON C. NORTON[2]

Abstract: Fulvic, humic, acetic, N-butyric, formic, lactic, and propionic acids were inhibitory to the survival or reproduction of *Aphelenchus avenae, Aphelenchoides goodeyi, Helicotylenchus pseudorobustus, Meloidogyne hapla* or *Xiphinema americanum*. Reproduction of *H. pseudorobustus* and *M. hapla* significantly increased with increasing amounts of muck added to sand, and with the initial amount of nematode inoculum. All acids except humic and fulvic were lethal, *in vitro*, to all nematode species tested. When *A. goodeyi* was treated with fulvic acid, reproduction was reduced significantly when compared with sodium humate or water treatments. Treatment of *H. pseudorobustus* with fulvic acid (pH 3.5) resulted in a greater reduction in reproduction in soil than did treatment with humic acid (pH 3.5). *Key word:* toxicity.

Reductions in plant-parasitic nematode populations occur after the addition of various organic materials to soil (2, 6, 10, 11, 12, 19). A frequent explanation is that organic manures increase the concentration of organisms that are parasitic or predacious on plant-parasitic nematodes. There also is evidence that fatty acids produced during decomposition of organic matter have nematicidal activities (1, 7, 8, 14, 16, 17, 18). Ponchillia (15) concluded that the fulvic acid fraction of muck soil had a deleterious effect on *Xiphinema americanum* Cobb. Humic compounds are widely distributed natural products in soil, but little is known of their chemical structure. The main humic fractions are humic and fulvic acids and contain carboxyl, phenolic, alcoholic hydroxyl and carbonyl groups (4). The chemical structure of these acids differ with the source of organic matter. Because of the nature of their functional groups, it is possible that these acids may likewise have an effect on soil nematodes.

The objectives of these investigations were to determine the effect of muck on reproduction of *H. pseudorobustus* (Steiner) Golden and *Meloidogyne hapla* Chitwood, and to measure the toxicity of some organic acids on survival and reproduction of *Aphelenchus avenae* Bastian, *Aphelenchoides goodeyi* Siddiqi and Franklin, *H. pseudorbustus* and *Xiphinema americanum*.

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[2] Department of Botany and Plant Pathology, Iowa State University, Ames 50010. Present address of senior author: Buckman Laboratories, Wondelgemkaai 156, B-9000 Ghent, Belgium.

MATERIALS AND METHODS

Pots containing 800 g of steam-sterilized sand plus muck to make a concentration of 0, 3, 6, 9 or 12% muck were planted with soybean, *Glycine max* (L.) Merr. 'Amsoy'. The plants were thinned to one plant/pot 10 days later. Aliquots of either 1000 (level I) or 2000 (level II) larvae of *M. hapla*, or 2000 (level I) or 4000 (level II) individuals of different stages of *H. pseudorobustus*, were pipetted around the roots of each remaining plant 2 weeks after planting. Five replicates of each treatment were used. Plants were kept in a greenhouse maintained at 23-27 C. The roots were dissected 60 days after inoculation, and females and males of *M. hapla* were counted. *H. pseudorobustus* were extracted from soil (5), counted and expressed as number/pot.

Humic and fulvic acids were extracted by treatment of muck with 0.1 N NaOH. The alkali extract was separated into humic and fulvic fractions by acidification to *p*H 3.0 with 0.1 N H_2SO_4 (9). The humic acid fraction was separated from the fulvic fraction by centrifugation and filtration. Humic and fulvic acids were purified by dialysis. Humic acid was dissolved in 0.01 N $NaHCO_3$. Two hundred individuals, in 0.5 ml water, of each nematode species to be tested were placed in each of four vials in given acid solutions to bring the final concentration of the acid to 0.5% humic, 0.1% fulvic or 0.005 M acetic, N-butyric, formic, lactic or propionic acids. The different percentages of humic and fulvic acids were used because the quantity of humic acid released in the soil during composition of organic matter is about five times that of fulvic acid. The percentage of nematodes alive after 0, 24 and 48 hr was determined. A nematode was termed dead when it did not move after two probings. Procedures for testing the effect of *p*H on

nematicidal activity of formic and fulvic acids were the same as in the bioassay except that citric acid-phosphate buffer was added to provide a range from *p*H 3.0 to *p*H 8.0. These tests were repeated three times for each nematode species.

In tests measuring the effect of humic and fulvic acids on reproduction of *A. goodeyi*, five females were placed in solutions of either 0.5% sodium humate (*p*H 6.0) or 0.1% fulvic acid (*p*H 5.8) for 24 hr, then transferred to colonies of *Rhizoctonia solani* growing on cornmeal agar in petri dishes (3). Seven dishes were used for each treatment. Dishes were maintained at 25 C for either 2 or 4 weeks, and the amount of reproduction was measured. This experiment was repeated three times.

Soybean seedlings growing in pots containing 800 g of loamy sand were inoculated with 560 nematodes previously treated for 24 hr with either 0.5% humic acid (*p*H 3.5), 0.5% sodium humate (*p*H 6.0) or 0.1% fulvic acid (*p*H 3.5), or with 0.1% fulvic acid adjusted to *p*H 5.8 by addition of citric acid-phosphate buffer. Each treatment was replicated four times. The pots were kept in a greenhouse maintained at 23-27 C for 60 days after inoculation. Nematodes were extracted from the soil by centrifugal flotation (5) and counted.

RESULTS

Numbers of *H. pseudorobustus* (Fig. 1A) and *M. hapla* (Fig. 1B) increased significantly (P = .01) as the percentage of muck and the inoculum level increased. The dry weight of roots and tops of plants increased significantly (P = .05) with an increase in muck. Reduction in plant growth due to nematode infection was significant (P = .05) in plants infected with *M. hapla* at both inoculum levels at all levels of muck and *H. pseudorobustus* at inoculum level II with 12% muck.

All organic acids except humic and fulvic were nematicidal against at least one of the four nematodes tested (Table 1). *Aphelenchus avenae* and *A. goodeyi* were most tolerant to the organic acids, whereas *X. americanum* was killed within 24 hr in all acid solutions except humic and fulvic acids. Formic acid was the most toxic. The nematicidal activity of formic acid against *H. pseudorobustus* (Fig. 2A) and *X. americanum* (Fig. 2B) was reduced at *p*H 6.0 and above. The hydrogen-ion-concentration at *p*H 3.0 was toxic to *X. americanum* in all solutions. Fulvic acid was not nematicidal from

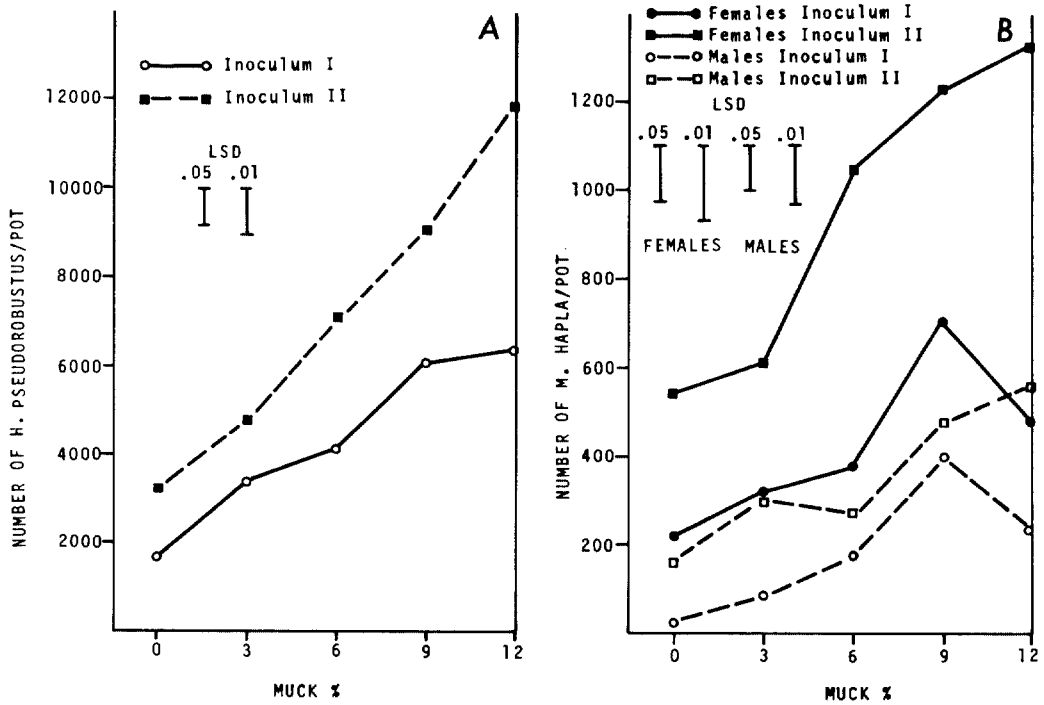

FIG. 1. Reproduction of A. *Helicotylenchus pseudorobustus* and B. *Meloidogyne hapla* in sand with different concentrations of muck.

TABLE 1. Percentage survival of 200 individuals of *Aphelenchus avenae, Aphelenchoides goodeyi, Helicotylenchus pseudorobustus* and *Xiphinema americanum* in organic acids at *p*H 3.5.

	A. avenae hr		*A. goodeyi* hr		*H. pseudorobustus* hr		*X. americanum* hr	
Organic acid	24	48	24	48	24	48	24	48
	%	%	%	%	%	%	%	%
Fulvic (0.1%)	99	98	98	97	99	98	99	99
Humic (0.5%)	99	99	99	99	100	99	100	98
Acetic (0.005 M)	98	97	98	97	65	64	00	00
N-Butyric (0.005 M)	83	73	75	56	35	30	00	00
Formic (0.005 M)	46	16	36	20	00	00	00	00
Lactic (0.005 M)	97	90	98	93	91	89	00	00
Propionic (0.005 M)	94	81	58	41	10	5	00	00
Water (distilled)	98	97	98	97	97	96	98	98

*p*H 4.0 to *p*H 8.0 against either nematode.

Reproduction of *A. goodeyi* treated with fulvic acid before a culturing on *R. solani* was significantly (P = .01) lower than with nematodes treated with sodium humate or water (Fig. 3). The difference between the treatments was greater (P = .01) after 4 than after 2 weeks' incubation.

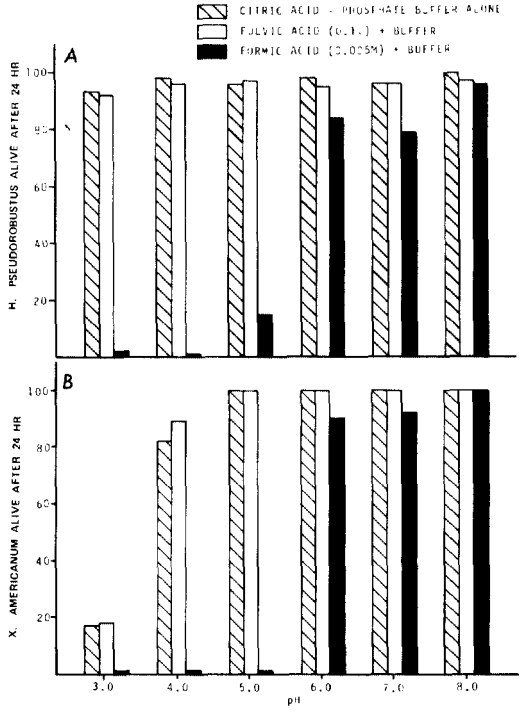

FIG. 2. Numbers of nematodes alive after 24-hr exposure to organic acids or a citric acid-phosphate buffer at different *p*H levels. **A.** *Helicotylenchus pseudorobustus* **B.** *Xiphinema americanum.*

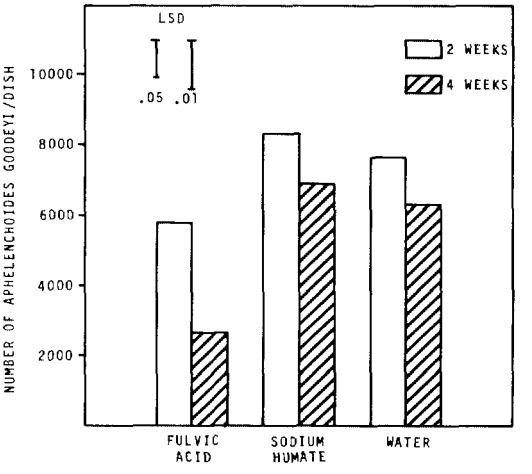

FIG. 3. Reproduction of *Aphelenchoides goodeyi* treated for 24 hr with fulvic acid, sodium humate or water prior to a culturing on *Rhizoctonia solani* for 2 or 4 weeks.

Reproduction of *H. pseudorobustus* treated with either fulvic or humic acids before inoculation of plants in soil was significantly (P = .01) reduced as compared with sodium humate or water treatments. Fulvic acid (*p*H 3.5) caused the greatest reduction in reproduction of *H. pseudorobustus* (Table 2).

DISCUSSION

The increase in reproduction of *H. pseudorobustus* and *M. hapla* with the increase of percentage of muck added to sand agrees with results of O'Bannon (13) for *Tylenchulus semipenetrans* Cobb. This could be explained in terms of changing the physical environment in the soil by raising the field capacity, resulting in

TABLE 2. Effect of 24 hour pretreatment of *Helicotylenchus pseudorobustus* with fulvic or humic acids on subsequent nematode reproduction in soil.

Treatment		
Organic acid	*p*H	Nematodes/pot after 60 days[a]
Fulvic (0.1%)	3.5	2190
Fulvic (0.1%) citric acid - phosphate buffer	5.8	5400
Humic (0.5%)	3.5	4750
Sodium humate (0.5%)	6.0	9700
Water	6.1	9820
		L.S.D. .05 = 1260
		.01 = 1766

[a]Original population was 560 nematodes/pot

a balance between moisture and air in the soil creating favorable conditions for nematode reproduction. The increase in reproduction with increase in muck might also partly result from the significant (P = .05) increase in plant dry matter with increasing peat.

Nematode species differ in their sensitivity to fatty acids. The nematicidal activity of acetic, butyric, formic, lactic and propionic acids against *A. avenae, A. goodeyi, H. pseudorobustus* and *X. americanum* agrees with other reports for other nematodes (7, 8, 14, 16, 17). Although humic and fulvic acid showed no effect on 48-hr survival of any nematode tested (Table 1), they had indirect effects on the reproduction of nematodes. This effect was evident in the reduction of reproduction of *A. goodeyi* treated with fulvic acid and *H. pseudorobustus* treated with fulvic or humic acids. These acids are most active at low *p*H. The explanation of Albert (1) and Sayre et al. (17) that the degree of ionization is important in toxicity can be applied to our results.

These results suggest that applying organic matter as a means of controlling plant parasitic nematodes may depend, in part, on the nematode species present, the organic amendment used and the soil conditions. They also suggest that fulvic and humic acids, which are produced in the soil during the decomposition of organic matter (9), might have an effect in reducing reproduction of some nematodes.

LITERATURE CITED

1. ALBERT, A. 1960. Selective toxicity. John Wiley & Sons, Inc., New York. 233 p.
2. DUDDINGTON, C. L. 1957. The friendly fungi. Faber and Faber, London. 188 p.
3. GOODEY, J. B. 1963. Laboratory methods for work with plant and soil nematodes. Tech. Bull. 2, Ministry Agr. London. 72 p.
4. HANSEN, E. H., and M. SCHNITZER. 1969. Zn-dust distillation and fusion of a soil humic and fulvic acid. Proc. Soil Sci. Soc. Amer. 33:29-36.
5. JENKINS, W. R. 1964. A rapid centrifugal technique for separating nematodes from soil. Plant Dis. Rep. 49:692.
6. JOHNSON, L. F. 1962. Effect of the addition of organic amendments to soil on root knot of tomatoes. II. Relation of soil temperature, moisture, and *p*H. Phytopathology 52:410-413.
7. JOHNSTON, T. M. 1957. Further studies on microbiological reduction of nematode population in water-saturated soils. Phytopathology 47:525-526 (Abstr.).
8. JOHNSTON, T. M. 1959. Effect of fatty acid mixtures on the rice stylet nematode (*Tylenchorhynchus martini* Fielding, 1956). Nature (London) 183:1392.
9. KONONOVA, M. M. 1966. Soil organic matter, its nature, its role in soil formation and in soil fertility. (Transl. from Russian). Pergamon Press, New York. 450 p.
10. LEAR, B. 1959. Application of castor pomace and cropping of castor beans to soil to reduce nematode populations. Plant Dis. Rep. 43:459-460.
11. LINFORD, M. B., F. YAP and J. M. OLIVEIRA. 1939. Reduction of soil populations of the root-knot nematode during decomposition of organic matter. Soil Sci. 45:127-141.
12. MANKAU, R., and R. J. MINTEER. 1962. Reduction of soil populations of the citrus nematode by the addition of organic materials. Plant Dis. Rep. 46:375-378.
13. O'BANNON, J. H. 1968. The influence of an organic soil amendment on infectivity and reproduction of *Tylenchulus semipenetrans* on two citrus rootstocks. Phytopathology 58:597-601.
14. PATRICK, Z. A., R. M. SAYRE and H. J. THORPE. 1965. Nematocidal substances selective for plant-parasitic nematodes in extracts of decomposing rye. Phytopathology 55:702-704.
15. PONCHILLIA, P. E. 1972. *Xiphinema americanum* as affected by soil organic matter and porosity. J. Nematol. 4:189-193.
16. SAYRE, R. M., Z. A. PATRICK and H. THORPE. 1964. Substances toxic to plant-parasitic nematodes in decomposing plant residue. Phytopathology 54:905 (Abstr.).
17. SAYRE, R. M., Z. A. PATRICK and H. J. THORPE. 1965. Identification of a selective nematicidal component in extracts of plant residues decomposing in soil. Nematologica 11:263-268.
18. TARJAN, A. C., and P. C. CHEO. 1956. Nematocidal value of some fatty acids. Bull. 332. Rhode Island Agr. Exp. Sta., Kingston. 41 p.

19. 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.