Extraction of Oat Straw, Flax, and Amended Soil to Detect Substances Toxic to the Root-Knot Nematode

Leander F. Johnson

Professor of Plant Pathology, Department of Agricultural Biology, The University of Tennessee Institute of Agriculture, Knoxville, Tennessee 37916.

ABSTRACT

A substance toxic to eggs and larvae of the root-knot nematode was extracted from mature, dried oat straw and leaves and stem segments of flax. A toxin was extracted also, but in lower concns, from soil amended and incubated 10 wk with similar amounts of these organic materials. Oat-straw-amended soil contained more toxin than did flax-amended soil.

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It has been demonstrated that many kinds of organic materials added to soil will reduce severity of root knot caused by *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (2, 5, 6, 10, 11). Factors that appear to influence degree of nematode control include nature and amount of amendment (2, 5, 10), incubation period (time between amendment incorporation and nematode assay) (2), environmental conditions during incubation (3), and nitrogen content of the soil (4). The mechanism of nematode control has not been clearly elucidated. Attempts to relate control brought about by adding organic amendments with activities of predaceous organisms have been unsuccessful (1, 7). A toxin, extracted with water from decomposing segments of rye, was found to immobilize larvae (8). One of the nematicidal components of the toxin was identified as butyric acid (9). The present study was made to determine

TABLE 1. Effect of extracts of plant material, soil, and amended soil on viability or infectivity of eggs and larvae of *Meloidogyne incognita*. Bioassay for viability and infectivity was on 6-wk-old tomato seedlings

Nematode forms treated	Sources of extracts used	Gall production (%)[a] following treatment of eggs or larvae with extracts obtained with:		
		Water	Alcohol	Ether
Eggs	1 kg soil	96	93	82
	1 kg soil + 20 g oat straw	100	36	43
	1 kg soil + 20 g flax	94	100	66
	20 g oat straw or flax	...	0	...
Larvae	1 kg soil	100	88	32
	1 kg soil + 20 g oat straw	100	82	15
	1 kg soil + 20 g flax	100	71	24
	20 g oat straw or flax	...	0	...

[a]Compared to the number of galls produced by eggs or larvae not treated with extract (water control).

whether mature plant materials contain toxic substances, and if toxins can be extracted from a soil mass containing these decomposing plant material-amendments.

Mature, dry oat straw and flax stems and leaves were chopped into 1-4 mm fragments in a Wiley mill. Samples, 20 g each, were extracted directly with 95% ethyl alcohol or were mixed with 1-kg samples of moist soil previously amended with 60 μg/g N, 120 μg/g K, and 120 μg/g P in the form of 6-12-12 fertilizer. Such amended soils were incubated in the laboratory in stoppered 2-liter Erlenmeyer flasks for 10 wk at room temp. One-liter quantities of the extractants (water, 95% ethyl alcohol, or ethyl ether) were added to each flask. After 24 h at room temp with occasional shaking and stirring, the extractants were filtered through paper and then evaporated almost to dryness in a Buchler flash evaporator under reduced pressure at 50 C. The residue from each flask was brought up to 25 ml with distilled water.

Eggs for testing were obtained from egg masses picked from galled tomato roots. The masses were shaken for 4 min in 10% Clorox to separate them from the gelatinous material. The Clorox was diluted with water and the eggs were concd by centrifuging and decanting. To remove larvae and extraneous material, the eggs were washed through a 44-μm (325-mesh) screen and again were concentrated by centrifuging and decanting. Larvae were obtained from galled tomato roots placed on a screen in a mist chamber. Water under the screen containing larvae was collected periodically and passed through a 74-μm (200-mesh) screen. The residue on the screen was discarded and the liquid was passed through a 37-μm (400-mesh) screen. Larvae caught on the screen were washed off and concd to a small volume by centrifuging and decanting.

Eggs or larvae, thus obtained, were added to the extracts previously described and incubated at 5 C (eggs) or 15 C (larvae) for 48 h. For controls, eggs or larvae were incubated in similar quantities of distilled water. Numbers of eggs or larvae were previously adjusted so that approximately equal numbers were treated with extracts of plant material, soil, amended soil, and water control. To determine viability or infectivity, 1-ml samples of the extracts containing eggs or larvae were injected into the soil about 1.3 cm below the soil surface adjacent to the stems of 6-wk-old tomato seedlings. After 5 wk of incubation in the greenhouse, the roots were washed free of soil and numbers of galls per plant were determined. Each test was repeated at least four times.

No galls were produced on tomato roots inoculated with either eggs or larvae treated with alcohol extracts of 20-g samples of undecomposed oat straw or flax (Table 1). Repeated tests yielded similar results. Material toxic to eggs was extracted with alcohol and ether from oat straw-amended soil but with only ether from flax-amended soil. Ether extracts of oat straw and flax-amended soils also contained material toxic to larvae. No toxins were demonstrated in water extracts of amended soil. A substance toxic to larvae, but less toxic to eggs, was extracted with ether from soil without an organic amendment.

These results indicate that a substance or substances toxic to nematodes occurs naturally in oat straw and flax. The toxin is apparently degraded in amended soil or strongly adsorbed on soil particles since smaller quantities were extracted from soil amended with similar amounts of oat straw or flax. It is possible that this toxin has an adverse effect on survival and infectivity of nematodes in soil amended with organic plant material.

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