Evaluation of control of *Meloidogyne incognita* and *Nacobbus aberrans* on tomato by two leguminous plants

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SUMMARY

When the leguminous plant *Concanavalia ensiformis* and tomato were cocultivated in greenhouse pot tests, a reduction in galling caused by *Meloidogyne incognita* and *Nacobbus aberrans* was observed. The leguminous plant *Mucuna deeringiana* generally gave a lesser degree of control of root-knot under the same conditions, as did treatments with powdered, ground beans of the two legumes. The effects of the several treatments on plant growth were inconsistent. The lectin Con A was demonstrated in water in which *C. ensiformis* had been grown. The passage of this lectin from *C. ensiformis* into the soil is proposed as an explanation for the observed reduction in root-knot symptoms on tomato.

RESUMÉ

Évaluation de l'action de deux légumineuses contre Meloidogyne incognita *et* Nacobbus aberrans *parasitant la tomate*

Lors d'expériences en serre, la culture mixte de *Concanavalia ensiformis* (Légumineuse) et de tomate provoque une réduction du nombre de galles causées par *Meloidogyne incognita* et *Nacobbus aberrans*. Dans les mêmes conditions expérimentales, la légumineuse *Mucuna deeringiana* a un effet plus faible, de même que les traitements à l'aide de poudre de graines de ces deux légumineuses. L'effet de plusieurs de ces traitements sur la croissance de la tomate est inconstant. La présence de la lectine Con A dans l'eau où pousse *C. ensiformis* a été démontrée. Le passage de cette lectine dans le sol à partir de *C. ensiformis* pourrait expliquer la diminution du nombre des galles dues aux nématodes sur les racines de tomate.

Novel and environmentally sound measures for the control of plant parasitic nematodes are needed. Contamination of ground water, high residual toxicity, and loss of registration by governmental regulatory agencies have placed serious constraints on chemical control procedures. An active area of research is for biological control agents, the use of green manures and cultural techniques such as interplanting with nematode suppressive plants.

The lectin Con A was shown to bind selectively to mannose residues on the cuticle surface surrounding the cephalic sensilla of *Caenorhabditis elegans* and *Meloidogyne incognita* (McClure & Zuckerman, 1982), and later to these same areas on other plant parasitic nematodes (Robertson *et al.*, in press). A working hypothesis based on these observations stated that these mannose residues could be associated with membrane receptors of chemosensilla neurons. If this were the case then capping of the receptors by Con A would result in a modification of chemotactic behavior (Zuckerman, 1983). Experiments in which chemotactic responses of *C. elegans* to highly attractive *Escherichia coli* extracts were inhibited by Con A provided evidence in support of this hypothesis (Jeyaprakash *et al.*, 1985). Indirect evidence supporting the hypothesis also came from greenhouse, growth chamber and microplot field trials in which significant control of *Meloidogyne incognita* on tomato root-knot by Con A application was obtained (Marban-Mendoza *et al.*, 1987).

The current experiments were undertaken to evaluate the control of root-knot nematodes on host plants grown in the presence of two leguminous plant species.

Materials and methods

ORGANISMS

The nematode species used were the root-knot nematode *M. incognita* Race 3 (from Dr. M. McClure, University of Arizona) and the false root-knot nematode *Nacobbus aberrans* from Chapingo, Mexico. Two tomato varieties were tested, *Lycopersicon esculentum* cv. Rutgers and *L. esculentum* cv. Ace. The trials involving *M. incognita* and the Rutgers tomato were conducted in Amherst, MA. and those with *N. aberrans* and the Ace tomato were in Chapingo, Mexico.

The two leguminous plants assayed for their effects on control of root-knot nematode were *Concanavalia ensiformis*, a plant which is the source from which the lectin Con A is commercially extracted, and *Mucuna deeringiana*.

Control by the leguminous plants was evaluated in two ways. First was the application in the *N. aberrans*

experiments of ground *M. deeringiana* or *C. ensiformis* seed at 2 or 4 g/pot containing 2-3 week old tomato seedlings grown in 250 cm^3 steam sterilized potting soil (1 part potting soil : 1 part sand). The ground seed was applied as a soil drench in 20 ml water. A second approach used in all experiments reported herein (Tabs 1-3), a legume plant and tomato plant were grown in the same pot. Controls were tomatoes with no treatment, tomatoes infested with nematodes alone, and legumes cocultured with tomato plants but with no nematodes to evaluate for fertilizer effects.

The procedure was to transplant 2-3 week old tomatoes, one to each pot in a treatment. Ground legume beans were added one day prior to inoculation with nematodes. *M. incognita* eggs were extracted and collected by the methods of Hussey & Barker (1973). Inoculum levels were 5 000 eggs/pot (Tab. 1) for *M. incognita* and 1 g of nematode galls/pot for *N. aberrans* (Tabs 2, 3). In treatments where the legume and tomato were cocultured, the legume was germinated in vermiculite, then transplanted to a pot containing a tomato plant and inoculated with nematodes one week later. Data were taken on fresh or dry weights of plant roots and tops as indicated in the tables. For *N. aberrans*, nematode damage was assessed by a root gall index where 0 = no galls; 1 = 25 % of root system galled; 2 = 50 %; 3 = 75 % and 4 = 100 %. For *M. incognita* the formula applied to determine the percentage control was *100 % - (Number of galls per treatment/number of galls per infested control* × *100)*. To clarify the effect of crowding on total root growth when the legume and tomato were grown in one pot, two tomatoes were grown in each pot in the *Meloidogyne* experiment (Tab. 1). Soil in which each of the legumes were grown for one week and the legumes then removed, was also tested for reduction of *M. incognita* galling (Tab. 1).

The duration of the *N. aberrans* experiments was 67 days, that of the *M. incognita* experiments 49 days.

Statistics in all trials were performed by ANOVA supplemented by Duncan's multiple range test.

ELISA

Enzyme linked immunoabsorbant assays (ELISA) for Con A were performed against extracts of beans, leaves and roots of the two legumes. In addition, ELISA of water containing root exudates from the legumes was performed to determine if Con A was released by the roots.

The indirect ELISA proceeded using anti-Con A from rabbits as the first antibody (Sigma Chemical Co. Cat N° 67401, St. Louis, MO.), anti-rabbit conjugated with alkaline phosphatase as the second antibody (Sigma N° A802) and p-nitrophenyl phosphate (Sigma N° N9389) as the substrate. Anti-WGA (Sigma N° T4144), anti-PNA (Sigma N° A4404) and Anti-Lotus A (Sigma N° T3517) were also tested against all of the legume plant parts and root exudates. Bovine serum albumin (BSA) was used as a negative control, since it is known not to contain any of the lectins being examined (Bruce Jacobsen, Biochemistry Department, University of Massachusetts, pers. comm.). Two grams of powdered bean, dried roots or dried leaves were added to ten volumes of phosphate buffered saline (0.02 M K_2HP04; 0.9 M NaCl; 0.02 % NaN_3), blended for 1 min every 5 min for 1 h, stirred for 2-3 h and refrigerated overnight at 4°. The homogenate was extracted with ethyl ether to remove lipids, the aqueous layer centrifuged at 10 000 g for 15 min and the supernatant frozen for ELISA. For assay of root exudates, legumes at the one true leaf stage were transferred to 75 ml sterile distilled H_2O held in a flask wrapped in aluminum foil. After 3 days, 400 µl of the water was tested by ELISA for the presence of Con A.

ELISA readings were taken on Dynatech MR 600 microplate reader.

Results

In trials where *C. ensiformis* was cocultured with tomato, a significant reduction of root galling occurred (Tabs 1-3). Under the same condition, *M. deeringiana* was less effective in reducing root-knot symptoms.

The effects of the several treatments on root growth were inconsistent. Results with the most promising treatment (*C. ensiformis* + nematode + tomato) as compared to tomato inoculated with nematodes, showed a significant decrease in root growth. (Tabs 1-3). Since *C. ensiformis* grows much more rapidly than tomato, we interpret these results as reflecting root competition within the small volume of soil held in the pots. The effects of the several treatments on the plant tops were also inconsistent. It is probable that a valid evaluation of the effects of *C. ensiformis* on total plant growth can only be obtained from large microplot or field plot experiments.

Significant increases in both top and root growth of tomato were obtained when the legumes were cocultured with tomato in the absence of *N. aberrans* (Tabs. 2, 3) but not in the comparable *M. incognita* experiment (Tab. 1). Thus, the determination of fertilizer effect associated with the legumes gave inconclusive results under the conditions of these experiments.

Ground seeds of each legume added to soil at 2 g/pot were generally ineffective in reducing the amount of galling due to *N. aberrans* (Tabs 2, 3).

Soil which had been conditioned by one week growth of the legumes had no effect on nematode damage (Tab. 2).

ELISA

Con A, as demonstrated by ELISA, was present at the greatest concentrations in *C. ensiformis* beans, lesser

Table 1

Effect of *Concanavalia ensiformis* and *Mucuna deeringiana* on growth and root galling of tomato by the root-knot nematode *Meloidogyne incognita*

Treatment	Dry weights (x̄) top	root	Number of galls (x̄)	Control (%)
Two tomatoes	4.1	1.0	—	—
Tomato + *C. ensiformis*	2.6	0.7	—	—
Tomato + *M. deeringiana*	3.7	0.9	—	—
Two Tomatoes + *M. incognita*	3.6	1.1	189.2*a*	—
Two Tomatoes in *C. ensiformis* soil + *M. incognita*	3.1	1.2	181.6*a*	4.1
Two Tomatoes in *M. deeringiana* soil + *M. incognita*	3.2	1.3	179.3*a*	5.3
Tomato + *M. deeringiana* + *M. incognita*	2.8	0.9	120.2*b*	37.0
Tomato + *C. ensiformis* + *M. incognita*	1.8	0.54	60.6*c*	68.0

Numbers followed by different letters differ significantly at the $P < 0.05$ level.
Each treatment was replicated five times.

Table 2

Effects of *Concanavalia ensiformis* and *Mucuna deeringiana* on growth and root galling of tomato by the false root knot nematode *Nacobbus aberrans*.

Treatment	Dry weight (g) Top (x̄)	Roots (x̄)	Gall index
Untreated control	7.1	2.0	—
C. ensiformis + sterile soil	5.9	3.7	—
M. deeringiana + sterile soil	4.9	1.9	—
M. deeringiana (2 g) + nematodes	2.6	1.6	4.0*a*
Nematode infected control	2.6	1.4	3.9*a*
M. deeringiana (4 g) + nematodes	4.2	2.0	3.7*a*
C. ensiformis (2 g) + nematodes	3.1	2.5	3.1*a*
M. deeringiana (growing plant) + nematodes	2.1	0.8	2.7*b*
C. ensiformis (growing plant) + nematodes	3.1	0.9	1.5*b*
C. ensiformis (4 g) + nematodes*	—	—	—

Numbers followed by different letters differ significantly at the $P < 0.05$ level.
Each treatment was replicated eight times.
* Phytotoxic.

Table 3

Effects of *Concanavalia ensiformis* and *Mucuna deeringiana* on *Lycopersicon esculentum* var. Ace treated with the false root knot nematode *Nacobbus aberrans*.

Treatment	Wet weight (g) Top (x̄)	Root (x̄)	Gall index
C. ensiformis plant + tomato	58	15	—
M. deeringiana plant + tomato	50	6	—
M. deeringiana seed (2 g) + tomato + nematodes	30	4	5*a*
M. deeringiana plant + tomato + nematodes	40	5	4*a*
C. ensiformis seed (2 g) + tomato + nematodes	30	12	4*a*
C. ensiformis plant + tomato + nematodes	46	13	1*b*

Numbers followed by different letters differ significantly at the $P < 0.05$ level.
Each treatment was replicated eight times.

concentrations in the roots and was not detected in extracts from leaves (Tab. 4). ELISA of water in which *C. ensiformis* had been grown showed small amounts of Con A had passed from the roots into the surrounding medium (Tab. 4). Analysis of plant parts and root extracts of *M. deeringiana* for Con A were negative. ELISA of plant parts of both legumes with anti-WGA, anti-PNA and anti-Lotus A all showed apparent concentrations of lectin at 1 µg/ml as compared to the negative control BSA. This can be explained by the similarity of epitopes of different lectin molecules and these results are interpreted as negative evidence for the presence of the several lectins under assay.

Table 4

ELISA assays of *Concanavalia ensiformis** and *Mucuna deeringiana* plants and root exudates for the lectin Con A.

Sample		Con A µg/ml extract
C. ensiformis	bean	28.96
	root	16.28
	leaf	0.0
	root exudates	4.4
M. deeringiana	bean	0.0
	root	0.0
	leaf	0.0
	root exudates	0.0

* These data represent the average of two ELISA readings.

Discussion

The experiments described here indicate that plant parasitic nematodes can be effectively controlled by interplanting with the leguminous plant *C. ensiformis*. Previous observations suggest that the active principle is the lectin Con A which acts by interfering with host finding mechanisms (Jeyaprakash *et al.*, 1985). A signi cant finding in the current study is that Con A is given off as a root exudate by *C. ensiformis* roots. This strongly suggests that in nature Con A is continuously delivered into the soil, permeating the rhizosphere, thus forming zones which are environmentally unfavorable to plant nematodes. We interpret this observation as explaining the mode of action of our previous report on control of root-knot nematode by Con A (Marban-Mendoza *et al.*, 1987). To our knowledge this is the first report of a lectin as being a component of plant root exudates.

ACKNOWLEDGEMENTS

The authors acknowledge support of this work by Grant No. 1-910-85 from the US-Israel Binational Agricultural Research and Development Fund (BARD) and Massachusetts Centers for Excellence Grant BA-184.

Accepté pour publication le 10 février 1989.

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