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Control of *Meloidogyne incognita* on tomato by two leguminous plants

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Summary — Root galling of tomato due to *Meloidogyne incognita* was significantly reduced when the tropical legumes *Pueraria phaseoloides* and *Arachis pintoi* were co-cultured with tomato. Four other legumes tested had no effect on root galling. Soluble lectin homologs which reacted with antisera to Con A and PHA were detected by ELISA in root exudates of *A. pintoi*. Antisera to root exudates of *P. phaseoloides* reacted only with lectin homologs to PHA. The presence of these soluble lectins in root exudates is postulated as being responsible for the anti-nematodal properties of these legumes. The current experiments effectively eliminate the possibility that the described effects on root-knot suppression accrue from leakage of lectins from damaged legume roots. Molecules homologous to *Phaseolus vulgaris* lectin were most abundant in plant parts and seeds of both *P. phaseoloides* and *A. pintoi*.

Résumé — ***Contrôle de* Meloidogyne incognita *sur tomate à l'aide de légumineuses*** — Les galles racinaires des tomates causées par *Meloidogyne incognita* sont réduites de manière significative quand ces tomates sont cultivées en association étroite avec deux légumineuses tropicales, *Pueraria phaseoloides* et *Arachis pintoi*. Les essais concernant quatre autres légumineuses n'ont pas donné le même résultat. De faibles taux de lectines solubles réagissant avec l'antisérum à Con A et PHA ont été détectés, grâce au test ELISA, dans les exsudats radiculaires de *A. pintoi*. L'antisérum aux exsudats radiculaires de *P. phaseoloides* réagit avec les lectines homologues de PHA. Il est supposé que la présence de ces lectines solubles dans les exsudats radiculaires est responsable des propriétés anti-nématodes de ces légumineuses. Une molécule semblable à la lectine de *Phaseolus vulgaris* est présente en abondance dans les graines et dans certaines parties de deux légumineuses *P. phaseoloides* et *A. pintoi*.

Key-words : *Meloidogyne*, tomato, leguminous plants, biological control, lectins.

Since the widespread ban on nematicides, leguminous plants are increasingly being evaluated for their efficacy in reducing damage by plant-parasitic nematodes. Previously, we reported that cocultivation of tomato with jackbean, *Canavalia ensiformis*, resulted in a significant reduction of tomato root galling by *Meloidogyne incognita* and *Nacobbus aberrans* (Marban-Mendoza *et al.*, 1989). Jackbean and a number of other tropical legumes (i.e. *Mucuna deeringiana*) are recorded as immune to attack by root-knot nematodes (Tapica-A., 1971) and do not support buildup of *M. arenaria* and *Heterodera glycines* (Rodriguez-Kabana *et al.*, 1990). Cover crops of *Pueraria* reduced populations of *Meloidogyne* (Caveness, 1982). A practical drawback to using jackbean for interplanting with field crops, not at first recognized, is that this species grows extremely rapidly, so that in field trials it overgrew the target crop plants (Marban-Mendoza & Zuckerman, unpubl.). The results indicated limited potential of jackbeans as a biocontrol agent, since field use could require continued pruning or limiting application to tree crops, such as coffee.

Based on these observations several slower growing, tropical legumes were evaluated for their potential in nematode control. This paper reports on the results of these studies.

Materials and methods

ORGANISMS

The nematode species in our experiments was *Meloidogyne incognita* from Turrialba, Costa Rica and Amherst, MA. The tomato variety tested was *Lycopersicon esculentum* cv. Tropic.

The leguminous plants assayed in these trials were *Centrosema macrocarpum, C. acutifolium, C. pubescens, Desmodium ovalifolium, Pueraria phaseoloides* and *Arachis pintoi*.

GREENHOUSE TRIALS

The protocol for the experiment reported in Table 1 was as follows : Legumes were grown from seed in pots containing 1 dm^3 of soil (potting soil/sand : 1/1); Pots were fertilized weekly with Hoagland's solution and seedlings were thinned to one plant/pot. A 3 week-old tomato seedling was added to each pot when the legumes were 70 days old. At 90 days, where appropriate, 5000 J2 and eggs of *M. incognita* were added to each pot. Forty-two days later the experiment was terminated and data taken on the dry weight of tomato roots and tops, and the degree of galling. The gall index applied was 0 = no galls; 1 = 20 % galling; 2 = 40 % galling; 3 =

Table 1. Effects of six species of legume on growth and root-knot due to *Meloidogyne incognita* on tomato.

Treatment	Dry weight (g)		Root gall index**
	tops	roots	
two tomatoes	4.8[a]	1.3	---
tomato + *Centrosema macrocarpum*	5.5[b]	1.4	---
tomato + *C. acutifolium*	5.4[b]	1.5	---
tomato + *C. pubescens*	5.3[b]	1.5	---
tomato + *Desmodium ovalifolium*	5.3[b]	1.3	---
tomato + *Pueraria phaseoloides*	5.4[b]	1.3	---
tomato + *Arachis pintoi*	5.4[b]	1.3	---
two tomatoes + Mi*	4.4[ad]	1.3	4.7[a]
tomato + *Centrosema macrocarpum* + Mi*	4.3[cd]	1.4	4.5[a]
tomato + *C. acutifolium* + Mi*	4.1[c]	1.4	4.9[a]
tomato + *C. pubescens* + Mi*	4.6[ad]	1.5	4.7[a]
tomato + *Desmodium ovalifolium* + Mi*	4.6[ad]	1.5	4.8[a]
tomato + *Pueraria phaseoloides* + Mi*	5.3[b]	1.4	2.6[c]
tomato + *Arachis pintoi* + Mi*	4.6[ad]	1.3	2.6[c]

(Figures represent averages of eight replicates. Numbers followed by different letters are significantly different at $P < 0.05$ %.)

* Mi = *Meloidogyne incognita* — ** For root gall index see methods.

60 % galling; 4 = 80 % galling; 5 = 100 % galling. The experiment was performed twice. Controls were tomatoes with no treatments, tomatoes infested with *M. incognita* alone and legumes co-cultivated with tomato plants but without nematodes to evaluate for fertilizer effects.

One of two experiments designed to demonstrate that root-knot suppression was not due to leakage of lectins from damaged roots of either tomato or legume plants, was a greenhouse pot trial. For this test, seeds of *A. pintoi* and *P. phaseoloides* were germinated on moistened filter paper and transplanted separately to pots as soon as the seed coat had broken. A 2-3 week old tomato seedling was planted in each pot, then 10 000 *M. incognita* eggs added at the time legume seedlings were 1-2 cm tall. Pots containing two tomato seedlings and to which *M. incognita* were added, served as controls. Each treatment was replicated five times. After 42 days plants were harvested and data taken on root and top growth and a gall index taken as before. The results of this trial are shown in Table 2.

Statistical analyses were by ANOVA supplemented by paired T-tests.

ELISA

Enzyme linked immunoabsorbant assays (ELISA) for several lectins (or homologous lectins) were performed on root exudates, seeds, leaves and roots of *A. pintoi* and *P. phaseoloides* (Table 3). The indirect ELISA proceeded

Table 2. Effects of *Arachis pintoi* and *Pueraria phaseoloides* on suppression of root-knot of tomato.

Treatment	Root gall index**	Control (%)
two tomatoes + Mi*	4.2[b]	
one tomato + *P. phaseoloides* + Mi	3.0[a]	29
one tomato + *A. pintoi* + Mi	2.7[a]	37

(Figures represent averages of eight replicates — Numbers followed by different letters are significantly different at $P < 0.05$ %.)

* Mi = *Meloidogyne incognita* — ** For root gall index see methods.

using rabbit anti-lectin as the first antibody, goat anti-rabbit conjugated with alkaline phosphatase as the second antibody (Sigma No. A 802) and p-nitrophenyl phosphate (Sigma No. N 9389) as the substrate. The antisera tested were anti-Con A (Sigma Chemical Co. No. C 7401); anti-WGA (Sigma No. T 4144); anti-PNA (Sigma No. A 4404); and anti-PHA (Sigma No. T 0526). The carbohydrate binding specifities for the lectins were for *Canavalia ensiformis* lectin (Con A) - mannose, glucose; wheat germ agglutinin (WGA) - n-acetylglucosamine; *Arachis hypogaea* lectin (PNA) - galactose; *Phaseolus vulgaris* lectin (PHA) - n-acetylygalactosamine.

Bovine serum albumin (BSA) served as a negative control. For each species 2 g of powdered seed, dried root or dried leaves were added to phosphate buffered saline (PBS 0.02 M K_2HPO_4; 0.9 M NaCl; 0.02 % NaN_3; volume of plant material to PBS = 1:10), blended for 1 min every 5 min for 1 h, stirred for 2-3 h and held overnight at 4 °C. The homogenate was extracted with ethyl ether to remove lipid, the aqueous layer removed and centrifuged at 10 000 *g* for 15 min and the supernatant frozen for ELISA (Falasca *et al.*, 1979).

A second experiment to examine for lectins in root exudates of *A. pintoi* and *P. phaseoloides* was also designed to avoid damage to the roots, with the objective of proving that lectins comprise normal constituents of exudates. In this protocol, seeds of each species were germinated on moist filter paper within a sealed container. The roots of a seedling were immersed in 1 ml water for 72 h and the entire water sample tested by ELISA for the presence of lectins. The data given in Table 3 represents the total lectin which appeared in the root exudates during the 72 h test period.

ELISA readings were taken on a Dynatech MR600 microplate reader.

Results

GREENHOUSE TRIALS

Significant reductions in galling due to *M. incognita* resulted when *P. phaseoloides* or *A. pintoi* were co-cultivated with tomato. The other legumes did not affect

Table 3. ELISA assays of four lectins from plant and root extracts of *Arachis pintoi* and *Pueraria phaseoloides.*

Treatment	Con A	PNA	PHA	WGA	Total µg/ml lectin activity
A. pintoi seed*	0.028	0.117	0.450	0.000	362.3
A. pintoi leaf*	0.000	0.000	0.569	0.000	273.9
A. pintoi root*	0.044	0.052	0.638	0.000	316.6
A. pintoi exudate**	0.062	0.000	0.211	0.000	122.5
P. phaseoloides seed*	0.038	0.000	0.523	0.085	398.2
P. phaseoloides leaf*	0.027	0.000	0.540	0.000	276.2
P. phaseoloides root*	0.027	0.000	0.529	0.000	271.3
P. phaseoloides exudate**	0.000	0.000	0.135	0.000	155.4

* Average of six replicates minus BSA control (Background).

** Average of three replicates minus BSA control (Background).

the incidence of root galling. Enhanced vegetative growth of tomatoes co-cultivated with legumes in controls not inoculated with *M. incognita* indicated that the legumes fertilize the tomatoes (Table 1).

Significant reductions in root-knot galling also occurred when germinated seedlings of *A. pinto* or *P. phaseoloides* were co-cultivated with tomato seedling (Table 2). The roots of these two legume species showed no galling following exposure to *M. incognita* for the test period, thus indicating that these legumes were immune or highly resistant to root-knot attack.

ELISA RESULTS

Lectin homologs which reacted with anti-PHA were more prevalent in roots, stems and seeds of *A. pintoi* and *P. phaseoloides* than were lectin homologs to Con A and PNA (Table 3). For the most part homologs to WGA were absent, with the notable exception of the *P. phaseoloides* seed.

The revised experimental protocol for detecting lectin homologs in root exudates, allowed for the demonstration that lectin can pass from undamaged roots into the rhizosphere. Homologs to PHA appeared in the exudates from both legume species (Table 3). A small amount of lectin which reacted with Con A antisera was also detected in root exudaes of *A. pintoi.*

Discussion

Previously, we reported that the lectin Con A is given off into the rhizosphere by the jackbean *Canavalia ensiformis* (Marban-Mendoza *et al.*, 1989). Based on this observation, we proposed that root exudation of Con A provided the mechanism underlying the reported control of the root-knot nematode (Marban-Mendoza *et al.*, 1987). In the 1989 paper we stated that, " To our knowledge this is the first report of a lectin as being a component of plant root exudates ". A broader review of the literature proved the latter statement incorrect, for lectins had previously been reported from the rhizosphere of germinating seeds and young plants. For example, Gade *et al.* (1981) found substantial amounts of lectin released from soybean roots. Mishkind *et al.* (1980) observed that WGA was given off into the rhizosphere of young wheat plants and presented evidence that WGA in the rhizosphere acted as a fungistatic agent. Barondes (1984) advanced the hypothesis that soluble lectins in cellular slime molds and vertebrates appear to play a role in shaping extracellular environments. Other investigators found that root extracts from the legume *Mucuna deeringiana* caused a significant reduction in reproduction of *M. incognita* on tomato (Vicente & Acosta, 1987), though the active principle in the root exudates was not identified. These, and other research reports, suggest that soluble plant lectins may function as an active defense mechanism between a wide range of rhizosphere organisms and plant roots (see review by Etzler, 1986). The current studies provide proof that lectins can pass from undamaged legume roots, thus supporting previous reports and theories on the presence and role of lectins in the rhizosphere.

Lectins, particularly those from closely-related cultivars, appear to be highly conserved. Stinissen *et al.* (1983) reported that a survey of seeds from 100 species of Tritaceae demonstrated that all species contain immunochemically identical lectins. Etzler (1986) cites several examples of lectins from related plant species where carbohydrate specificities were identical but for which isoelectric focusing revealed molecular differences. The results of the current immunological studies, which show cross-reactivity between PNA and PHA with lectins from *A. pintoi* and *P. phaseoloides*, support our initial premise that a common mechanism exists whereby soluble lectins from certain legume species deploy from the roots and reduce root-knot incidence of co-cultivated tomato plants. The low incidence of molecules reacting with anti-WGA, a lectin derived from a plant species of a family unrelated to the legumes, supports this conclusion. In addition, the lack of control with the other 4 legumes tested suggests that the nematode depressing principal is not universally distributed in the Leguminosae. In the current study we postulated that closely related plant species would display lectin homologs which are functionally similar, though probably having demonstrable molecular differences. Lectins detected in root exudates of legumes are important, for these represent a continuous delivery of soluble lectins to the rhizosphere, a mechanism which we postulate is responsible for the antinematodal characteristics of *A. pintoi* and *P. phaseoloides.*

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