PATHOGENICITY OF *MELOIDOGYNE JAVANICA* (TREUB) CHITW. TO *ACACIA HOLOSERICEA* (A. CUNN. EX G. DON) AND *A. SEYAL* (DEL.)

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The susceptibility of two acacias (*Acacia holosericea* and *A. seyal*) against the root-knot nematode *Meloiodogyne javanica* has been studied. The nematode was inoculated at different rates (1000, 5000 and 10,000 second stage juveniles per seedling). In both acacias, *M. javanica* inhibited seedling height, shoot and root biomass and the density of root tips per mg of root. In terms of nematode development, the increase of *M. javanica* was greater on *A. holosericea* than on *A. seyal* when the inoculum rate was 1000 J2 per seedling indicating that *A. holosericea* is a better host for *M. javanica* than *A. seyal*.
Keywords: Acacia seyal, Acacia holosericea, Meloidogyne javanica, Rhizobium, Pathogenicity.

A dramatic deforestation has occurred in Sahelian areas of West Africa following ten years of drought and over-exploitation of the natural resources. Consequently reafforestation is now a priority in all Sahelian countries. In Senegal, forestry researchers have tested different leguminous tree species and, in particular, *Acacia* species.

Acacia is the largest mimosoid genus. The 800-900 species are abundant in savanas and arid regions of Australia, Africa, India and the Americas. Some of them serve to prevent wind and rain erosion, control sand dunes, are sources of wood, gum arabic and shade and they provide fodder for browsing livestock. In addition, much of the nitrogen fixed by the *Rhizobium* in the nodules of the acacia roots is returned to the soil with the natural loss of leaves and the resulting humus improves the fertility of the soil and its physical properties. Consequently, acacias are good candidates for soil rehabilitation in arid regions.

Plant-parasitic nematodes are a cosmopolitan and important problem affecting the production of subtropical and tropical crops. In particular, root-knot nematodes (*Meloidogyne* spp.) are major pests of vegetables and four species are of particular importance: *Meloidogyne incognita, M. javanica, M. arenaria* and *M. hapla* (Johnson & Fassuliotis, 1984). Often, these vegetable crops are grown adjacent to leguminous tree species such as *Acacia* species. Little is known about the interactions between *Acacia* species and root-knot nematodes althought it is known that many *Acacia* species are hosts for *Meloidogyne* spp. (Prot, 1986). However, the susceptibility of *Acacia* species to infestation by root-knot nematodes has generally not been assessed.

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This paper reports an investigation of the interactions between *M. javanica* (Treub, 1885) Chitwood, 1949, commonly found in Senegal, and *A. holosericea* (Australian acacia) and *A. seyal* (Sahelian acacia), two species with considerable potential for use in reafforestation in Sahelian areas.

MATERIALS AND METHODS

Seeds of *A. seyal* from provenance Veluigara (Senegal) and *A. holosericea* from provenance MBiddi (Senegal) were scarified for 30 min and 60 min, respectively, with concentrated sulphuric acid. They were washed for 12 h in sterile distilled water before sowing. The germinated seeds were grown for 1 month in 0.5 dm^3 polythene bags (5 cm diam) filled with autoclaved soil (120°C, 90 min). Its physicochemical characteristics were as follows: pH H_2O 7.1; fine silt 0.6%; coarse silt 1.4%; fine sand 61.6%; coarse sand 31.2%; total carbon 0.196%; total nitrogen 0.027%. The seedlings were maintained in a greenhouse (27°C day, 20°C night, 12 h photoperiod) and watered twice weekly without fertiliser.

One month after sowing, when the seedlings had grown to a mean of 5 cm height, they were inoculated with suspensions of 0, 1000, 5000 and 10,000 second stage juveniles (J2) of *M. javanica* to give 0, 2, 10 and 20 J2 per cm^3 of soil, respectively. Each treatment was represented by a block containing 15 seedlings for both tree species spaced at 50 cm from each other in order to avoid any contamination.

The population of *M. javanica* used for the inoculum was cultured for 2 months on tomato (*Lycopersicon esculentum* Mill.) cv. Roma. Then the tomato roots were harvested, cut into short lengths and placed in a mist chamber for 1 week to allow juveniles to hatch from the eggs. The nematode density was determined from 5 ml samples and the required inoculum poured into a hole (5 mm by 100 mm) to one side of each seedling and covered with soil.

The height of the seedlings was measured 4 and 7 weeks after inoculation and the damage associated with the different inoculum densities was observed 7 weeks after inoculation. The seedlings were uprooted and the root systems gently washed. The soil from each polythene bag was mixed, a 250 g subsample was taken and the nematodes were extracted using Seinhorst's (1962) elutriation technique. Mean total stem length and oven-dried weight of shoot (1 week at 65°C) were measured. Galls induced by *M. javanica* were indexed as: 0 = no galls; 1 = 1 to 5 galls; 2 = 6 to 20 galls; 3 = more than 20 galls; 4 = coalescing galls on the entire root system; 5 = rotten root system.

Root nodules induced by indigenous *Rhizobium* were counted and their fresh and dry weights were determined. The nodulation rate per plant was calculated as follows: number of root nodules / (root dry weight - nodule dry weight).

Each root system was then cut into 2-3 cm pieces and placed in a mist chamber for 3 weeks in order to recover J2 from hatched eggs (Seinhorst, 1950). The nematodes that hatched each week were counted. The oven-dried weight of root systems (1 week at 65°C) and the number of root tips per mg of dry root were then measured.

Data were compared with one-way analysis of variance. Means were compared with L.S.D. 5%. For the nodulation rate, the data were transformed by arcsin(sq rt) before statistical analysis whilst for nematode populations data were transformed by log (x + 1) prior to analysis.

RESULTS

Plant growth

One month after inoculation the mean height of the *A. seyal* was significantly decreased (P = <0.05) compared with the control (mean 23.1 cm) by all *M. javanica* inoculum densities (Table I). In contrast, only the 10,000 inoculum had decreased the height of the *A. holosericea* (control mean height 7.2 cm). At harvest, 7 weeks after inoculation, all inoculum densities had significantly (P = <0.05) decreased the height of both *Acacia* spp. (Table I). Seedling height was regressed against inoculum densities and a significant (P = <0.05) negative correlation obtained for both *Acacia* species (r = 0.75 for *A. seyal* and r = 0.66 for *A. holosericea*).

All inoculum densities of *M. javanica* had significantly reduced shoot biomass of *A. seyal* at harvest 7 weeks after inoculation (Table I). The same effect was observed with *A. holosericea* except for 1000 J2 inoculum (Table I). Root weight was decreased by all nematode densities for *A. seyal* but only the 10,000 inoculum significantly (P = <0.05) decreased that of *A. holosericea*. However, the number of root tips was significantly and markedly decreased by all inoculum densities for both species.

Nematode development

The number of juveniles per dm^3 of soil was significantly lower with the 10,000 inoculum for both species (Table I). With *A. holosericea*, fewer new juveniles were recovered from the seedlings inoculated with 10,000 than with 1000 J2 but with *A. seyal*, which grew larger, the opposite was observed (Table I). However, rates of multiplication were much greater at an inoculum of 1000 J2 than at an inoculum of 10,000 J2 (Table II). Overall, *A. holosericea* appeared to be a much better host for *M. javanica* than *A. seyal*. This difference was also reflected in the number of males produced; at the 1000 inoculum density, *A. holosericea* produced more males than *A. seyal*.

Rhizobial symbiosis

Both species of *Acacia* were contaminated by indigenous *Rhizobium* strains but *M. javanica* strongly inhibited nodule development (Table III). All the parameters measured (number of nodules per plant; fresh and dry weight of nodules per plant; nodulation rate) were decreased, with a reduction of 90% in number of nodules in plants with the 10,000 inoculum density.

TABLE I

Effect of Meloidogyne javanica *inoculum density on (i) the number of nematodes per dm³ of soil and per plant and (ii) on the development of* A. holosericea *and* A. seyal *seedlings*

Acacia species	Inoculum	Gall index	J2/l soil	males/plant	J2/plant	Height (cm) 4 wk	Height (cm) 7 wk	Shoot biomass (mg dry weight)	Root biomass (mg dry weight)	Density of root tips (per mg dry weight)
	0 (control)	0 a (*)	0 a	0 a	0 a	7.2 a	10.6 a	406.2 a	187.8 a	12.7 a
A. holosericea	1000	0.2 b	24765.4 b	78.3 b	69959.0 b	8.5 a	8.6 b	301.4 a	171.7 ab	6.2 b
	5000	1.5 b	20770.7 b	511.3 c	88745.4 b	7.2 a	8.3 b	226.4 b	180.7 a	2.9 c
	10000	2.1 c	2396.0 c	544.7 c	18560.0 c	5.4 b	6.0 c	168.6 c	138.2 b	3.4 c
	0 (control)	0 a	0 a	0 a	0 a	23.1 a	38.3 a	942.6 a	1070.5 a	4.5 a
A. seyal	1000	0.2 b	2320.0 b	226.2 b	6081.2 b	20.7 b	27.8 b	752.6 b	489.0 b	2.6 b
	5000	0.8 c	1077.5 c	383.7 b	12900.0 c	17.7 c	25.4 b	586.1 c	457.8 b	1.2 c
	10000	2.1 c	1225.0 c	736.3 b	25487.5 c	18.6 bc	24.6 b	481.8 c	487.4 b	0.8 c

(*): values in the same column followed by the same letter are not significantly different according to one-way analysis of variance (L.S.D. = 0.05)

TABLE II

Effect of Meloidogyne javanica *inoculum density on nematode population development rate (final population/inoculated population)*

Inoculum	*Acacia seyal*	*Acacia holosericea*
1000	8.7 a (*) 1 (**)	94.8 a2
5000	2.9 b1	20.6 b2
10000	2.7 b1	2.2 c1

(*) Values in the same column followed by the same letter are not significantly different according to the one-way analysis of variance (L.S.D. = 0.05). (**) Values in the same line followed by the same number (1 or 2) are not significantly different according to the one-way analysis of variance (L.S.D. = 0.05).

TABLE III

Effect of Meloidogyne javanica *on rhizobial symbiosis*

Acacia species	Inoculum	Number of nodules per plant	Total fresh weight of nodules per plant (mg)	Total dry weight of nodules per plant (mg)	Nodulation rate (nodules per mg of root)
	0 (control)	11.4 a(*)	110.7 a	15.8 a	0.07 a
A. holosiericea	1000	4.0 b	59.8 b	7.8 b	0.02 b
	5000	1.3 c	6.4 c	0.8 c	0.007 c
	10000	1.1 c	11.5 c	1.6 c	0.008 c
	0 (control)	6.7 a	55.9 a	22.3 a	0.06 a
A. seyal	1000	3.9 b	23.3 b	7.7 b	0.05 a
	5000	1.8 c	12.4 c	8.1 b	0.04 b
	10000	0.6 d	2.45 d	2.2 c	0.01 c

(*) Values in the same column followed by the same letter are not significantly different according to the one-way analysis of variance (L.S.D. = 0.05).

DISCUSSION

Our results indicate that *M. javanica* significantly damages both *Acacia* species and markedly decreases root nodulation by *Rhizobium*. Both species are hosts for *M. javanica* but *A. holosericea* (Australian acacia) is a better host for the nematode than *A. seyal* (Sahelian acacia).

With our standard inocula the gall indices were similar for the two *Acacia* spp. and top weight was progressively decreased with increasing inoculum densities. However, our results showed that *A. seyal* from Senegal was marginally more tolerant of *M. javanica* than the Australian species *A. holosericea*.

The mechanism of damage is unclear, but the greatest inoculum densities (10,000 J2 per pot) decreased nodulation by more than 90% in both species, indicating a probably substantial effect of *M. javanica* on the growth and nitrogen fixing ability of both species in the field.

The difference in host status may be even more significant. *A. holosericea* was a particulary good host at the low inoculum density. *A. seyal* was a less good host at the low inoculum density but, perhaps because it grew larger and was more tolerant, at the highest inoculum density (10,000 J2 per pot) the *M. javanica* multiplied almost equally on both species.

We conclude from our investigation that in Africa, *M. javanica* may significantly decrease the potential benefits that may result from growing *Acacia* spp. Tree growth and nitrogen fixation are likely to be greatly decreased and the population of *M. javanica* increased, to the detriment of any adjacent susceptible crops. Imported *A. holosericea* may be more susceptible than the native *A. seyal*. Several research objectives can be indentified in order to minimize the nematode effect: (i) the different tree species used in agroforestry should be screened for their susceptibility to the prevalent nematodes; (ii) if appropriate resistant trees or shrubs cannot be identified, then ways should be sought to minimize nematode damage to susceptible trees. Antagonistic microorganims may be one possibility against nematodes. Nematophagous fungi, the actinomycete *Pasteuria penetrans* and mycorrhizal fungi all have potential. Mycorrhizal associations between plant roots and beneficial soil fungi are formed by the vast majority of woody plants and mycorrhizae enhance nutrient uptake and may protect host plants against pathogenic microorganisms (Harley & Smith, 1983). In addition, when vesicular and arbuscular mycorrhizal fungi (VAM) and *Rhizobium* are inoculated together, the nitrogen fixative process is stimulated (Mosse, 1977; Munns & Mosse, 1980). It has been demonstrated that endomycorrhizal fungi often increase host tolerance to nematode infection. Hence controlled mycorrhization of susceptible trees or shrubs could be a useful technique for improving the tolerance and the growth of forest plantations.

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RÉSUMÉ

Influence de Meloidogyne javanica *(Treub) Chit. sur* Acacia holosericea *(A. Cunn. ex G. Don) et* A. seyal *(Del.)*

La sensibilité de deux acacias (*Acacia holosericea* et *A. seyal*) au nématode *Meloidogyne javanica* a été étudiée. Le nématode a été inoculé à différentes concentrations: 1000, 5000 et 10,000 juvéniles par plantule. Chez les deux acacias le nématode inhibe la croissance en hauteur, les biomasses aériennes et racinaires et la densité des extrémités radiculaires par mg de racine. En ce qui concerne le développement de la population, la capacité de reproduction du nématode est beaucoup plus importante sur *A. holosericea* lorsque l'inoculum est de 1000 J2 par plante. *A. holosericea* peut donc être considéré comme meilleur hôte que *A. seyal* pour *M. javanica*.

Mots clés: Acacia holosericea, Acacia seyal, Meloidogyne javanica, Rhizobium sp., action pathogène.

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