Agroforestry Systems **46**: 123–130, 1999.

Susceptibility of several sahelian *Acacia* to *Meloidogyne javanica* (Treub) Chitw.

R. DUPONNOIS[1,*], K. SENGHOR[2], J. THIOULOUSE[3] and A. M. BÂ[4]
[1] *IRD, Laboratoire de Biopédologie, B.P. 1386, Dakar, Sénégal;* [2] *Université Cheikh Anta Diop, Département de Biologie Animale, Dakar, Sénégal;* [3] *CNRS, UMR 5558, Université Lyon 1, 69622 Villeurbanne Cedex, France;* [4] *ISRA. DRPF. BP. 2312, Route de Hann, Dakar, Sénégal (*Author for correspondence: E-mail: Robin.Duponnois@ird.sn)*

Key words: *Acacia* spp., pathogenicity, *Rhizobium*

Abstract. Four *Acacia* species were tested for their susceptibility to the root-knot nematode, *Meloidogyne javanica*, commonly found in sahelian areas. *Faidherbia albida* and *Acacia senegal* were resistant to this nematode. On the contrary, *A. raddiana*, *A. nilotica* and *A. mangium* were susceptible. Among these three species, the growth of *A. nilotica* and *A. mangium* was inhibited by *M. javanica* but *A. raddiana* was tolerant. The rhizobial symbiosis with *F. albida* and *A. senegal* was stimulated by the nematode. The population build-up of the root-knot nematode induced by tree species in agroforestry systems is discussed.

Introduction

The years of drought and over-exploitation of the natural ressources have involved a dramatic deforestation in all sahelian regions of West Africa. In order to rehabilitate these areas, different solutions have been proposed, and in particular, the cultural practice of agroforestry. The choice of tree species in these agricultural systems must be based on the following properties: (i) good growth on low fertility and arid soils, (ii) source of organic matter for the cultivated soils and, (iii) resistance to the development of pathogenic organisms. Leguminous tree species such as *Acacia* could be good candidates to be associated with annual plants. They are abundant in savanas and arid regions around the world. They can grow in soils very deficient in nitrogen because of their nitrogen fixing property. This nitrogen is returned to the soil by the natural loss of leaves which improves the soil fertility. However the pathogenic microorganisms which can be enhanced by these tree species in agroforestry systems are relatively unknown. In particular, plant-parasitic nematodes are a cosmopolitan and important problem affecting the production of subtropical and tropical crops (Johnson and Fassuliotis, 1984). The root-knot nematodes can parasite a large variety of vegetable crops. Although it is known that *Acacia* species are hosts for *Meloidogyne* spp. (Duponnois et al., 1997a), the susceptibility of these tree species to infestation by root-knot nematodes has been rarely assessed. Some works have been focussed to this problem and it is now well established that some Australian acacias (i.e. *A. holosericea*, *A. mangium*) and an African acacia (*A. seyal*)

are very susceptible to *Meloidogyne javanica* (Duponnois et al., 1995, 1997a). However other *Acacia* species are used in reafforestation in sahelian areas and it is very important to determine their suceptibility to the root-knot nematode *M. javanica*, commonly found in these regions.

This paper reports the study of the susceptibility of four African acacias (*Faidherbia albida*, *A. senegal*, *A. raddiana* and *A. nilotica*) frequently used in agroforestry systems in sahelian areas to *M. javanica*. In this experiment, *A. mangium* (Australian acacia) has also been used as a positive control because of its high susceptibility to *M. javanica* (Duponnois et al., 1997).

Materials and methods

Seeds of *Acacia* species collected in Senegal were scarified for 15 min (*F. albida*, *A. senegal* and *A. raddiana*) and 60 min (*A. nilotica* and *A. mangium*) with concentrated sulphuric acid (H_2SO_4). They were washed for 24 h in sterile distilled water before sowing. Then the seeds were sown in earthenware (40 × 50 cm) filled with an autoclaved sandy soil (140 °C, 40 min). Soil physico-chemical 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 organic carbon 0.54%, total nitrogen 0.15% and soluble phosphorus 30.7 $\mu g\ g^{-1}$. Three weeks after sowing, the germinated seeds were transplanted into 0.5 dm^3 polythene bags (diameter 5 cm) filled with the same autoclaved soil as described above. The seedlings were maintained in a greenhouse (27 °C day, 20 °C night, 12 h photoperiod) and watered twice weekly without fertiliser.

The population of *M. javanica* was reared on tomato plants (*Lycopersicon esculentum* Mill.) cv. Roma. Two month after inoculation, the tomato roots were harvested, cut into short pieces and placed in a mist chamber for 1 week to enable the nematode eggs to hatch (Seinhorst, 1950). The nematode density was determined from 5 ml samples. The required inoculum was poured into a hole (5 mm by 100 mm) to one side of each seedling and covered with soil.

The seedlings were inoculated, after one month culture, with suspensions of 0, 1000, 5000 and 10 000 second stage juveniles (J2) of *M. javanica*. There were 15 replicates per treatment (nematode inoculum level). The pots were arranged in a randomized, complete block design with fifteen replicates.

Three months after the nematode inoculation, the seedling were uprooted and their roots were gently washed. The soil from each polythene bag was mixed and a 250 g sub-sample was taken to extract the nematodes using Seinhorst's (1962) elutriation technique. Mean total stem lenght was measured and the shoots were dried at 65 °C for one week and weighed. Galls induced by the juveniles of M. javanica per plant were indexed as follow: 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 and 5 = rotten root system.

Although the soil was sterile and the seeds were surface desinfected, the

plants have been contaminated with indigenous rhizobial strains. The main explanation was that the spraying water used during this experiment, which was not sterile, contained these bacterial strains. Root nodules were counted on each root system and their dry weights (65 °C, 1 week) were determined.

The roots were then cut into 2–3 cm pieces and placed in a mist chamber for two weeks to recover hatched juveniles (Seinhorst, 1950) which were counted. The roots were oven dried (65 °C, 1 week) and weighed.

Data were treated with a Principal Component Analysis (PCA) (Thioulouse et al., 1997). Multivariate analysis was used complementary to the analysis of variance because it gives a good overall idea of the relationships between *Acacia* species and of their responses to *M. javanica* infection. It also gives interesting insights into the relationships between physiological variables as height (HA), the root and shoot biomass (BR and BA), rhizobial symbiosis as number of nodules per plant (NO), dry weight of nodules per plant (PN) and nematological parameters (J2P, J2G, IDG). These results are much easier to understand than the standard analysis of variance table. For each *Acacia* species, means were compared with one-way analysis of variance ($P < 0.05$).

Results

The PCA showed the relative differences between these tree species. In the PCA correlation circle along F1 (Figure 1a), which describes 51% of the variability, the most important variables are: the number (NO) and the dry weight of nodules per plant (PN), the root biomass (BR) for the positive values and for the negative values, the gall index (IDG), the number of juveniles per plant (J2P) and per gram of root weight (J2G) and the number of males per plant (MAL). Along F2 which describes 16% of the variability, the main important parameters are the shoot biomass (BA) and the height of the plants (HA). In the factorial plane (Figure 1b), for each *Acacia* species, the data corresponding to the inoculum levels are ranged from the right to the left along F1 from *F. albida* (Al), *A. senegal* (Se), *A. raddiana* (Ra) to *A. nilotica* (Ni) and *A. mangium* (Ma). This arrangment is correlated to the negative range of nodule dry weight (PN), the number of nodules per plant NO) (*A. mangium*, *A. nilotica*) and to a positive range of the gall index (IDG), the number of juveniles per plant (J2P) and per gram of root (J2G) (*F. albida*, *A. senegal* and *A. raddiana*). The data obtained for each *Acacia* species are closely grouped together for *F. albida*, *A. senegal* and *A. raddiana*. On the contrary, the data of the control treatments are relatively distant from the data of the treatments with nematodes for *A. nilotica* and *A. mangium*.

The one way analysis of variance showed the differences within these tree species for their susceptibility against *M. javanica* and confirmed the dispersion of the data obtained by the PCA (Tables 1 and 2). For *F. albida*, the gall index significantly decreased when the number of inoculated juveniles increased. No significant differences have been detected with the other species

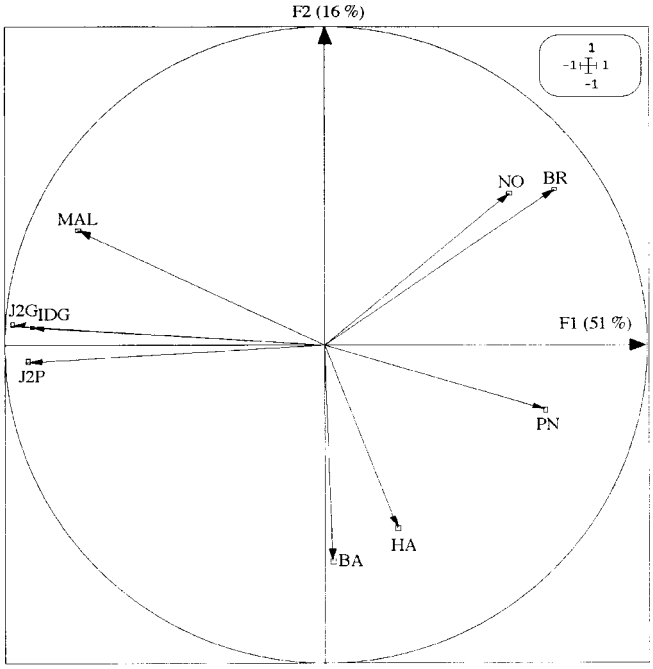

Figure 1a. PCA correlation circle
HA: height; BA: Shoot biomass; BR: Root biomass; NO: Number of nodules per plant; PN: dry weight of the nodules per plant; MAL: Number of males per plant; IDG: Gall index; J2P: number of juveniles per plant; J2G: number of juveniles per gram (dry weight) of root.

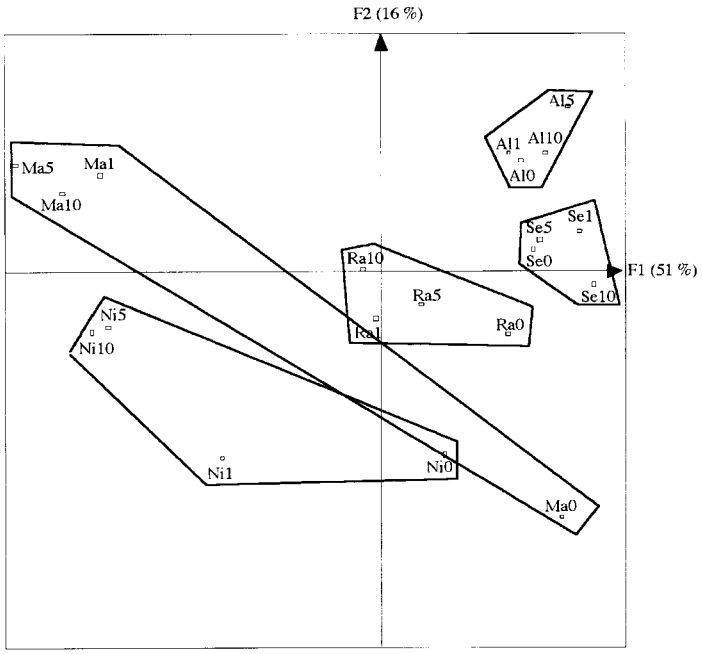

Figure 1b. F1 × F2 factor map.
1: inoculum 1000; 5: inoculum 5000; 10: inoculum 10000. Ma: *Acacia mangium*; Ni: *A. nilotica*; Ra: *A. raddiana*; Se: *A. senegal*; Al: *Faidherbia albida*.

Figure 1. Study of the plant growth of the *Acacia* species infected or not by *Meloidogyne javanica* in Senegal. The data are analysed with Principal Component Analysis.

Table 1. Effect of *M. javanica* inoculated at different rates on the nematode development in Senegal.

Acacia species	Inoculum	Gall index	Males/plant	J2/dm^3 of soil	J2/plant	J2/g of root	Mult. rate
F. albida	0 (control)	0[a]	0	0	0	0	0
	1000	1.4 a[b]	20 a	1669 a	4023 a	1.2 a	4.4 a
	5000	0.9 b	39 a	549 a	3178 a	1.1 a	3.4 a
	10000	0.14 b	5 a	47 a	1426 a	0.5 a	1.5 a
A. senegal	0 (control)	0	0	0	0	0	0
	1000	0.5 a	0.7 b	468 a	320 b	0.1 a	0.6 b
	5000	0.4 a	35 a	684 a	2434 a	0.8 a	2.8 a
	10000	0.1 a	2.1 b	40 a	571 b	0.2 a	0.6 b
A. raddiana	0 (control)	0	0	0	0	0	0
	1000	2.0 a	42 a	8948 a	29552 a	13 a	33 a
	5000	1.5 a	10 b	5566 b	10966 b	4.7 b	14 b
	10000	2.5 a	20 b	3760 b	29171 a	13.6 a	31 a
A. nilotica	0 (control)	0	0	0	0	0	0
	1000	2.0 a	39 ab	304 b	31746 a	24.1 a	32 a
	5000	3.1 a	58 a	350 b	50834 a	47.1 a	51 a
	10000	3.6 a	35 b	1262 a	51227 a	50.9 a	52 a
A. mangium	0 (control)	0	0	0	0	0	0
	1000	2.9 a	235 a	46 a	29704 a	39.5 a	23 a
	5000	3.0 a	290 a	16 b	41020 a	51.5 a	41 a
	10000	3.0 a	201 a	46 a	35530 a	50 a	36 a

[a] The data from the control treatment have been excluded fom analysis.

[b] For each column and for each *Acacia* species, the data followed by the same letter are not significantly different according the one-way analysis of variance ($P < 0.05$).

for this parameter (Table 1). For all the inoculum levels, the multiplication rates were not different except for *A. senegal* (significantly higher with the inoculum 5000) and *A. raddiana* (significantly lower with the inoculum 5000). The development (height and shoot biomass) of *A. senegal* and *A. raddiana* has not been modified by the nematodes (Table 2). On the contrary, the growth of *A. mangium* and *A. raddiana* was significantly decreased by *M. javanica* (Table 2). A better growth of *F. albida* has been measured when these seedlings were inoculated with 1000 and 5000 juveniles (Table 2). The nitrogen fixative process has been inhibited by *M. javanica* for *A. mangium* and *A. nilotica* and stimulated for *F. albida* (Table 2). No effect has been recorded with *A. senegal* and *A. raddiana*.

Discussion

The infection of root systems by phytoparasitic nematodes of the genus *Meloidogyne* is easily recognised by formation of galls. These galls are descended from the development of the giant cells and the females inside the

Table 2. Effect of *M. javanica* inoculated at different rates on the plant growth and the rhizobial symbiosis in Senegal.

Acacia species	Inoculum	Height (cm)	Shoot biomass (g)	Root biomass (g)	Nodules/ plant	Nodules weight (mg)
F. albida	0 (control)	72.6 c[1]	8.3 b	2.9 a	13.1 b	14 b
	1000	84.5 ab	1.6 a	3.2 a	25.5 b	30 ab
	5000	92.1 a	2.0 a	2.9 a	52.3 a	35 a
	10000	80.8 b	1.3 b	2.8 a	27.5 b	25 ab
A. senegal	0 (control)	77.9 a	2.3 a	3.3 ab	6.3 a	28 b
	1000	80.8 a	2.3 a	3.7 a	12.6 a	67 ab
	5000	76.7 a	1.9 a	3.1 ab	8.2 a	71 ab
	10 000	79.0 a	1.9 a	2.7 b	7.9 a	112 a
A. raddiana	0 (control)	100.8 a	2.6 a	1.8 b	6.1 a	46 a
	1000	96.2 a	2.7 a	2.3 ab	5.6 a	73 a
	5000	95.4 a	2.3 a	1.8 b	7.1 a	40 a
	10000	90.2 a	2.1 a	2.4 a	9.4 a	54 a
A. nilotica	0 (control)	113 b	4.2 b	0.6 b	1.9 a	23 a
	1000	117.8 a	5.2 a	1.3 a	0.07 b	2 b
	5000	92.7 c	2.6 c	1.0 a	0.07 b	5 b
	10000	91.0 c	2.6 c	1.0 a	0.4 b	5 b
A. mangium	0 (control)	74.1 a	1.5 a	0.6 a	13.4 a	82 a
	1000	62.8 b	0.8 b	0.7 a	2.0 b	10 b
	5000	65.2 a	0.9 b	0.8 a	0.2 b	1 b
	10000	62.4 b	0.9 b	0.7 a	0.4 b	0.5 b

[1] For each column and for each *Acacia* species, the data followed by the same letter are not significantly different according the one-way analysis of variance ($P < 0.05$).

roots (De Guiran and Netscher, 1970). Consequently, every *Acacia* species tested in this study can be considered as host plant for *M. javanica* as already suspected by Duponnois et al. (1997b).

However the intensity of the nematode infection varied according to the *Acacia* species. These species can be split into two groups: (i) susceptible species: *A. mangium*, *A. nilotica* and *A. raddiana* and (ii) resistant species: *F. albida*, *A. senegal*. The susceptibility of the three species is also shown by the high development of the males in the roots. Although the development of males in high number is indicative of resistance, their multiplication can be increased when the environmental conditions are unfavourable for the life cycle of *Meloidogyne* (Davide and Triantaphyllou, 1967a,b). In this experiment, the high multiplication of the males is probably caused by the lack of nutrients due to the large nematode infection (Taylor and Sasser, 1978).

Among the susceptible species, *A. mangium* and *A. nilotica* are not tolerant to the nematodes. Their height, shoot biomass and the rhizobial symbiosis have been significantly decreased by *M. javanica*. No negative effects have been recorded with *A. raddiana* which is tolerant to *M. javanica*.

Among the resistant species, no effect of the nematodes has been observed with *A. senegal* on the plant growth except for the root biomass with the inoculum level of 1000. On the contrary, the nematodes have stimulated the growth of the aerial parts (height and biomass) of *F. albida*. Two hypothesis could be proposed to explain this phenomena. A low intensity of the nematode infection may induce a better development of the short roots which enhance the mineral nutrition of the plant. The second explanation could be the stimulation of the rhizobial symbiosis. The nematodes can caused some injuries on the roots thus facilitating the penetration of the bacteria inside the roots. It is also well known that nematodes can modify the quantity and the quality of the root exudates which are implicated in the processes of the root nodulation. The stimulation of the rhizobial symbiosis expressed by the number of nodules per plant and the total dry weight of nodules per plant has also been observed with *A. senegal* but without plant growth stimulation. The inoculation of rhizobia is not controlled in this experiment. Consequently the bacterial strains which infected the *A. senegal* plants may differed from those infecting *F. albida* and consequently less efficient on the plant growth.

From a pratical point of view, this experiment has been realised over three months in a glasshouse. This cultural length can be compared to that usually recommended in the tropical nurseries. As the substrates used in these nurseries are generally not disinfected and can be infested by phytoparasitic nematodes, the results obtained in this experiment can be easily transferred to the tropical forestry practices. The growth of the two susceptible species (*A. mangium* and *A. nilotica*) can be inhibited by the root-knot nematodes during the nursery period or, if this substrate is without nematodes, their growth in the field can be depressed by the indigenous nematode population. On the contrary, the resistant species such as *F. albida* and *A. senegal* can be used without pathogenic problems.

Acacia raddiana has to be differently considered. It is a good host for *M. javanica* and tolerant for the nematode pathogenic effect. If it is planted in association with vegetables in agroforestry systems, this tree can maintain the reproduction of *M. javanica* which can suppress the associated culture. This situation can result in a highly infested field where no crop can be cultivated.

In conclusion, prior to the selection of tree species to be used in agroforestry programs, each species has to be tested for its susceptibility to phytoparasitic nematodes. In cases where susceptible species have to be planted, following cultural practices have to be followed: (i) the nursery substrate must be disinfested to eliminate the nematodes, (ii) the substrate can be inoculated with microorganisms which can enhance the growth of the seedlings and/or act as antagonisms against pathogenic nematodes (selected mycorrhizal fungi and rhizobial strains) (Munns and Mosse, 1980; Duponnois and Cadet, 1994).

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