Effects of climatic factors on native arbuscular mycorrhizae and Meloidogyne exigua in a Brazilian rubber tree (Hevea brasiliensis) plantation

Isabelle Laffont-Schwob, M. Ducher, A Coudret

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I. Schwob*, M. Ducher and A. Coudret

Laboratoire de Physiologie et Biotechnologies Végétales – Université Blaise Pascal, 63177 Aubière Cedex, France

The root-knot nematode *Meloidogyne exigua* and arbuscular mycorrhizal (AM) fungi may both occur in the roots of Brazilian rubber trees (*Hevea brasiliensis*). AM fungi may stimulate plant growth whereas nematodes usually reduce it. Variations of native AM fungi and *M. exigua* populations in soil and roots of rubber trees were studied for one year in a Brazilian plantation. The number of AM spores in the soil was generally greater in the rainy season than in the dry season, although AM colonization of roots was unaffected by season. During the dry season, numbers of juveniles and eggs of *M. exigua* in roots were lower than in the rainy season. A site without nematodes in the soil or roots showed the greatest numbers of AM spores in soil and highest AM colonization of roots. A negative correlation was observed between the percentage of AM colonization and the number of second-stage juveniles in soil and second-stage juveniles and eggs in roots. Microscope observations revealed (i) tissue specificity for each of the microorganisms in the roots, with a cortical location of mycorrhizae and a mainly vascular cylinder location of nematodes, and (ii) that *Gigaspora* was the most abundant AM genus in the plantation soil.

Keywords: antagonistic microorganisms, endomycorrhizae, *Hevea brasiliensis* (rubber tree), *Meloidogyne exigua*, root-knot nematode

Introduction

The tropics are characterized by climatic factors that favour high plant biomass productivity throughout the year. Despite these climatic conditions, most soils are poor in plant nutrients, phosphorus being the most deficient (Read, 1991; Diederichs & Moawad, 1993). Under these conditions, no latency occurs in pathogenic microorganism cycles during the year and these microorganisms may cause serious economic losses in tropical crops. In this context, arbuscular mycorrhizae (AM) play a key role, stimulating plant growth (Azcon-Aguilar *et al*., 1992; Cui & Caldwell, 1996; Schwob *et al*., 1998) and protecting plants against pathogens (Hussey & Roncadori, 1982; Cooper & Grandison, 1987).

Native plants such as rubber (*Hevea brasiliensis*) are being reintroduced in Brazil, but this species is susceptible to the root-knot nematode *Meloidogyne exigua*, which causes severe damage (Santos, 1992; Santos *et al*., 1992). D'Angremond & Van Hell (1939) first reported the presence of arbuscular mycorrhizal (AM) fungi in roots rubber trees. Because plant-parasitic nematodes and AM fungi can be intimately associated in these roots, it is reasonable to suspect an interaction between these two microorganisms. There are, however, wide gaps in the knowledge of the interactions that AM fungi have with other soil microorganisms, especially in tropical soils (Gianinazzi-Pearson & Diem, 1982).

The purpose of this study was to determine (i) the variations of native AM fungal and nematode populations during one year, and (ii) whether the AM fungal populations could affect reproduction of *M. exigua* in the roots in the field. To achieve this, the occurrence of nematodes and AM fungi in the roots and surrounding soil were monitored during one year, and their dependence on climatic and edaphic conditions (rainfall, temperature and relative humidity, and chemical and physical characteristics of the soil) were studied. Possible correlation between the populations and seasonal fluctuations are discussed.

As rubber trees are usually intercropped with cover crops, mycorrhizal and nematode infectiveness in the roots of some of these crops were examined.

Materials and methods

Sampling

Samples of the roots of rubber trees and of the surrounding soil were collected at five sites (parcels of 100 ha) in a plantation (10 000 ha) in Mato Grosso, Brazil, during one year. At the beginning of the study, four samples of soil from each site were chemically and

*To whom correspondence should be addressed.

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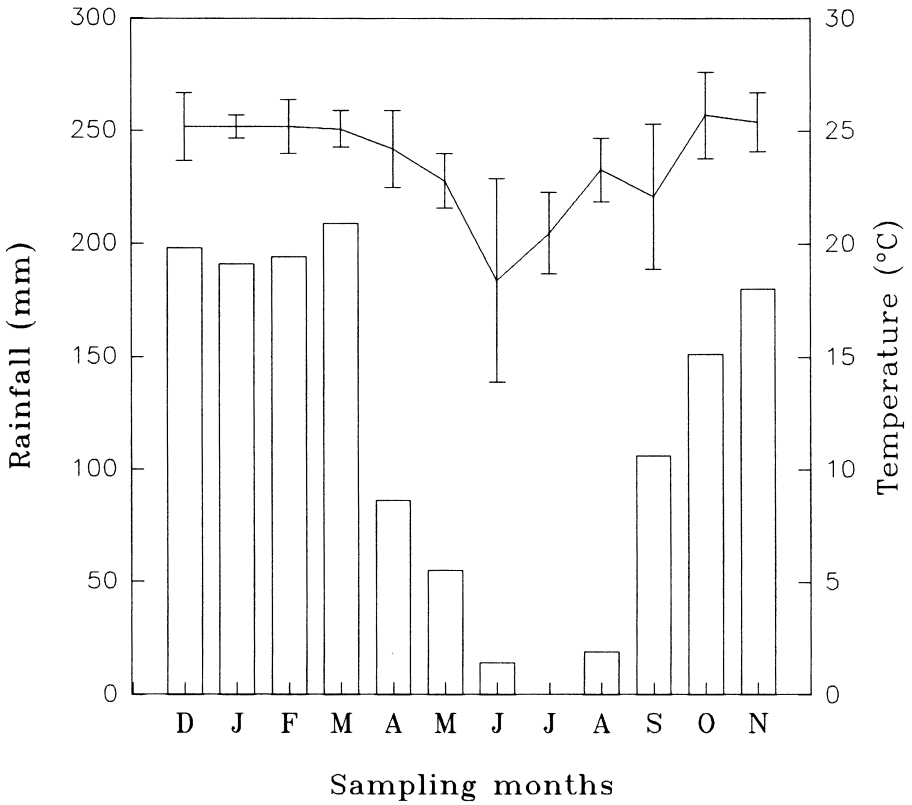

Figure 1 Monthly temperature (line graph) and rainfall measurements in a rubber tree plantation in the Mato Grosso, over the year of the experiment. Standard errors are represented by bars.

physically analysed. The pH was measured by the $CaCl_2$ method (Schofield & Taylor, 1955). Total P, K^+, Ca^{2+}, Mg^{2+} and Al^{3+} contents, cation exchange capacity, exchangeable base content, base saturation percentage, organic matter content and soil texture were determined by the Pirasolo laboratory, Rondonopolis, Brazil. All the soil samples were taken from the top 15–20 cm of the soil. Rainfall, relative humidity in air, and temperature were measured daily, and monthly means were calculated for these last two parameters.

Soil (400 g) and roots (60 g) were collected at 4 positions chosen at random at each sampling site, each month. Samples were stored at 4°C and processed 2 weeks after collection. Four 50-g subsamples of soil were used for nematode extraction and four 50-g subsamples for mycorrhizal spore extraction. Four 10-g subsamples of root were removed for nematode assay and 10 g for mycorrhizal infection. Ten grams of roots of tropical legumes and other cover crops (*Canavalia* sp., *Crotalaria spectabilis*, *Stylosanthes guianensis*, *Glycine max*, *Zea mays*, *Brachiaria decumbens*, *Pueraria phaseoloides* and *Vernonia* sp.) were assessed for mycorrhizal colonization and nematode susceptibility 2 months after germination in the plantation soil.

Nematode extraction from soil and roots

Nematode second-stage juveniles were recovered from soil by differential sieving and sugar flotation (Jenkins, 1964). Second-stage juveniles and eggs were collected from the roots according to the procedure of Hussey & Barker (1973). Following extraction, second-stage juveniles and eggs were quantified under a stereoscopic microscope at ×100 magnification.

The species of *Meloidogyne* were identified according to the method described by Santos & Maia (1996). The predominant species was *M. exigua* (99%), with 1% *M. incognita* and *M. javanica*.

Assay for AM fungi

AM spores in soil were collected using the wet sieving and decanting technique (Gerdemann & Nicolson, 1963) modified by the sucrose gradient centrifuging technique (Daniels & Skipper, 1982). The number of spores of *Gigaspora* sp. was then estimated under a stereoscopic microscope at ×200 magnification.

For assessing AM infection of roots, samples were washed to remove soil, clarified and stained with 0·05% trypan blue in lactic acid (Phillips & Hayman, 1970). The percentage root colonization was determined using the grid line intersect method under a stereoscopic microscope at ×100 magnification (Giovanetti & Mosse, 1980).

Table 1 Total AM colonization percentage, spore number in soil, second-stage juvenile and egg number in rubber root, and second-stage juvenile number in soil, at five sites

Sites	Season	AM colonization percentage	Spore number/200 g soil	Second-stage juvenile and eggs number/g root	Second-stage juvenile number/200 g soil
1	Rainy	84 b (0·159)	3139 b (3·497)	0	0
	Dry	82 b (0·157)	2367 ab (3·374)	0	0
2	Rainy	43 a (0·114)	2551 ab (3·407)	11644 a (4·066)	56 a (1·756)
	Dry	53 a (0·128)	1875 a (3·273)	12449 a (4·095)	56 a (1·756)
3	Rainy	40 a (0·110)	2672 ab (3·427)	17958 b (4·254)	58 a (1·771)
	Dry	44 a (0·116)	1663 a (3·221)	8451 a (3·927)	58 a (1·771)
4	Rainy	38 a (0·107)	2744 ab (3·439)	17861 b (4·252)	66 a (1·826)
	Dry	43 a (0·114)	1569 a (3·196)	10355 a (4·015)	85 b (1·934)
5	Rainy	44 a (0·116)	2612 ab (3·417)	21848 b (4·339)	57 a (1·763)
	Dry	40 a (0·110)	2018 a (3·305)	9643 a (3·984)	130b (2·117)
ANOVA results: main factors and interactive effects					
sites		$F_{4\text{-}710} = 27$**	$F_{4\text{-}710} = 3$*	$F_{4\text{-}566} = 3514$**	$F_{4\text{-}566} = 534$**
season		NS	$F_{1\text{-}710} = 47$**	$F_{1\text{-}566} = 34$**	$F_{1\text{-}566} = 21$**
sites×season		NS	NS	$F_{4\text{-}566} = 56$**	$F_{4\text{-}566} = 6$**

NS, not significant; * $P < 0{\cdot}01$; ** $P < 0{\cdot}001$.
Values are the means of the observations performed each month, for each season (dry: April to September; rainy: October to March).
Means followed by the same letter in the same column are not different according to Tukey's multiple range test ($P < 0{\cdot}05$) performed on data tranformed to $\log_{10}(x+1)$ values for spore number in soil, second-stage juvenile and egg number in rubber root, and second-stage juvenile number in soil and transformed to arcsin $\sqrt{x}$ values for AM colonization percentage.
Data in parenthesis are log10 $(x+1)$ or arcsin transformed.

Microscopic observations of hand-cut root sections

Longitudinal and cross sections of pieces of rubber tree roots with and without galls were hand-cut and stained with trypan blue (Merck) to enable the fungal hyphae and nematodes structures to be viewed.

Statistical analysis

To test the main and interactive effects of season and location in the plantation on AM colonization, spore number in soil, juvenile and egg number in root and juvenile number in soil, data were submitted to an analysis of variance (ANOVA) and Tukey's multiple range test ($P \leq 0{\cdot}05$). Nematode and AM spores numbers were transformed to $\log(x+1)$, and AM colonization percentages to arcsin x, to reduce the association between means and variances. Possible correlations between the AM fungal and nematode populations were tested using Pearson's correlation coefficients.

Results

Soil and climate analysis

The chemical and physical soil analysis data obtained were not significantly different among the five sites. Soil samples revealed a clay-sandy soil with a low pH (4·2) typical for tropical soil. This low pH caused soil desaturation (base saturation percentage = 9%) and allowed free Al^{3+} ions in soil solution. Aluminium binds with phosphorus, resulting in low P availability (a mean of only 5 ppm found in the soil samples) and/or aluminium toxicity for plants. Concentrations of K^{+}, Ca^{2+} and Mg^{2+} were deficient. Only organic matter was not limiting, with a percentage of 1·9.

Temperature averaged above 25°C from October to March and below 25°C from April to September (Fig. 1). Relative humidity was quite uniform, with an average of 80%. Monthly rainfall was lower than 110 mm from April to September and exceeded 150 mm from October to March (Fig. 1). Thus, a dry season occurred from April to September and a rainy season from October to March. Comparison with monthly averages of the previous 15 years was performed for temperature, relative humidity and rainfall measurements. No significant differences were found between the year of the study and the 10 previous years.

Nematodes

No second-stage juveniles were found in the soil at site 1 during the year. As shown in Table 1, sites 4 and 5 were the most infested and sites 2 and 3 were less but similarly infested. Numbers of second-stage juveniles at sites 4 and 5 were greater during the dry season than during the rainy season ($P < 0{\cdot}05$).

Neither second-stage juveniles nor eggs were found in roots of rubber trees at site 1. Overall, numbers of both were generally greater in the rainy season ($P < 0{\cdot}01$). This was also the case for sites 3, 4 and 5 ($P < 0{\cdot}05$).

Nematodes parasitized the roots of all species of cover

Table 2 Percentage of mycorrhizal infection and susceptibility to *Meloidogyne exigua* infection of cover plants grown in the soil of a rubber plantation, 2 months after germination

Cover plants	AM infection percentage	*Meloidogyne exigua* susceptibility
Crotalaria spectabilis	40 ± 8	poor-host
Stylosanthes guianensis	43 ± 8	poor-host
Canavalia sp.	63 ± 10	host
Zea mays	63 ± 10	host
Glycine max	70 ± 8	host
Pueraria phaseoloides	75 ± 13	nonhost
Brachiaria decumbens	98 ± 5	nonhost
Vernonia sp.	98 ± 5	nonhost

The values are means ±†SE of 10 replications of 10 1-cm pieces of root.

crops inspected, except *B. decumbens*, *Vernonia* sp. and *P. phaseoloides* (Table 2). However, infection of *S. guianensis* and *C. spectabilis* was minor.

Fungi

No site without mycorrhizae was found.

For all the sites taken together, spore numbers in soil was greater in the rainy season than in the dry season ($P < 0{\cdot}01$) but for each site taken independently, seasonal differences were not significant (Table 1). Observation of the spores showed that the most abundant genus was *Gigaspora*. This identification was confirmed by scanning electronic microscopy by Santos (1995, personal communication).

In the roots of rubber trees, AM colonization percentage was highest at site 1 ($P < 0{\cdot}05$). At the other sites the roots were similarly colonized, to a lesser degree (Table 1). No seasonal effect on AM colonization was observed at any site.

The root hairs of plants of all the cover crops were colonized by mycorrhizae (Table 2). *Brachiaria decumbens* and *Vernonia* sp. were the most heavily colonized (98%). On *Pueraria phaseoloides* the infection rate was 75% and on *Glycine max* 70%. *Canavalia sp.* and *Zea mays* were less colonized (63%). *Stylosanthes guianensis* and *Crotalaria spectabilis* had the least infection (43% and 40%, respectively).

Correlations

Nematode populations in the soil and in the rubber roots were negatively correlated with the percentage of AM colonization in roots (Table 3). Number of second-stage juveniles in soil was positively correlated with number of second-stage juveniles and eggs in roots. However, variations in the population of nematodes in soil or in roots, and in the percentage of AM colonization, were not correlated with the number of AM fungal spores in soil.

Microscopic observations

Cross sections of roots of rubber trees without nematodes showed vascular cylinders with intact xylem vessels, phloem and medullar parenchyma (Fig. 2A). All tissue structures were conserved. Mycorrhizal mycelium and arbuscules, stained blue, were present only in the cortical parenchyma and never penetrated the vascular cylinder (Fig. 2B). Figure 2(C.a) longitudinal section shows arbuscules inside cells of the cortical parenchyma and mycelium running along the cell walls. A cross section of a nematode-infected root (Fig. 2D) showed loss of root structure in the vascular cylinder and cortical parenchyma. A female was present in contact with the vascular cylinder and, on the opposite side of the root section, AM mycelium was present in the cortex (Fig. 2D). The nematode females laid their eggs in a gelatinous matrix (Fig. 2E) inside the root tissue and not outside. A longitudinal section of a gall showed the shape of a female with its eggs and the giant cells in the vascular cylinder (Fig. 2F). The second-stage juveniles penetrated the root to a final site in the vascular cylinder where they became sedentary. AM mycelium was present only on the side opposite to that of the female site.

Discussion

Soil composition and the effect of climatic conditions on AM and nematode populations in the plantation soil and in roots of rubber trees were examined. As there was no difference in the chemical and physical components of the clay–sandy tropical soil at the five sites, variations in mycorrhizal and nematode populations at these sites could not be accounted for by edaphic conditions. However, seasonal fluctuations (rainy or dry) had some effect on AM and nematode populations. Gemma & Koske (1988) found that the abundance of spores of *Gigaspora gigantea* varied seasonally in a sandy dune soil, but the distinct fluctuations in spore density and mycorrhizal colonization at a Singapore tropical site, where there were no

Table 3 Pearson correlation coefficients between mycorrhizal colonization (arcsin-transformed data), spores in soil and nematodes in soil and in roots (log transformed data)

	Spore in soil	AM colonization percentage	Nematode in soil
AM colonization percentage	$r = 0{\cdot}002$		
Nematode in soil	$r = -0{\cdot}139$	$r = -0{\cdot}542$*	
Nematode in roots	$r = -0{\cdot}094$	$r = -0{\cdot}556$*	$r = 0{\cdot}921$*

*$P < 0{\cdot}01$.

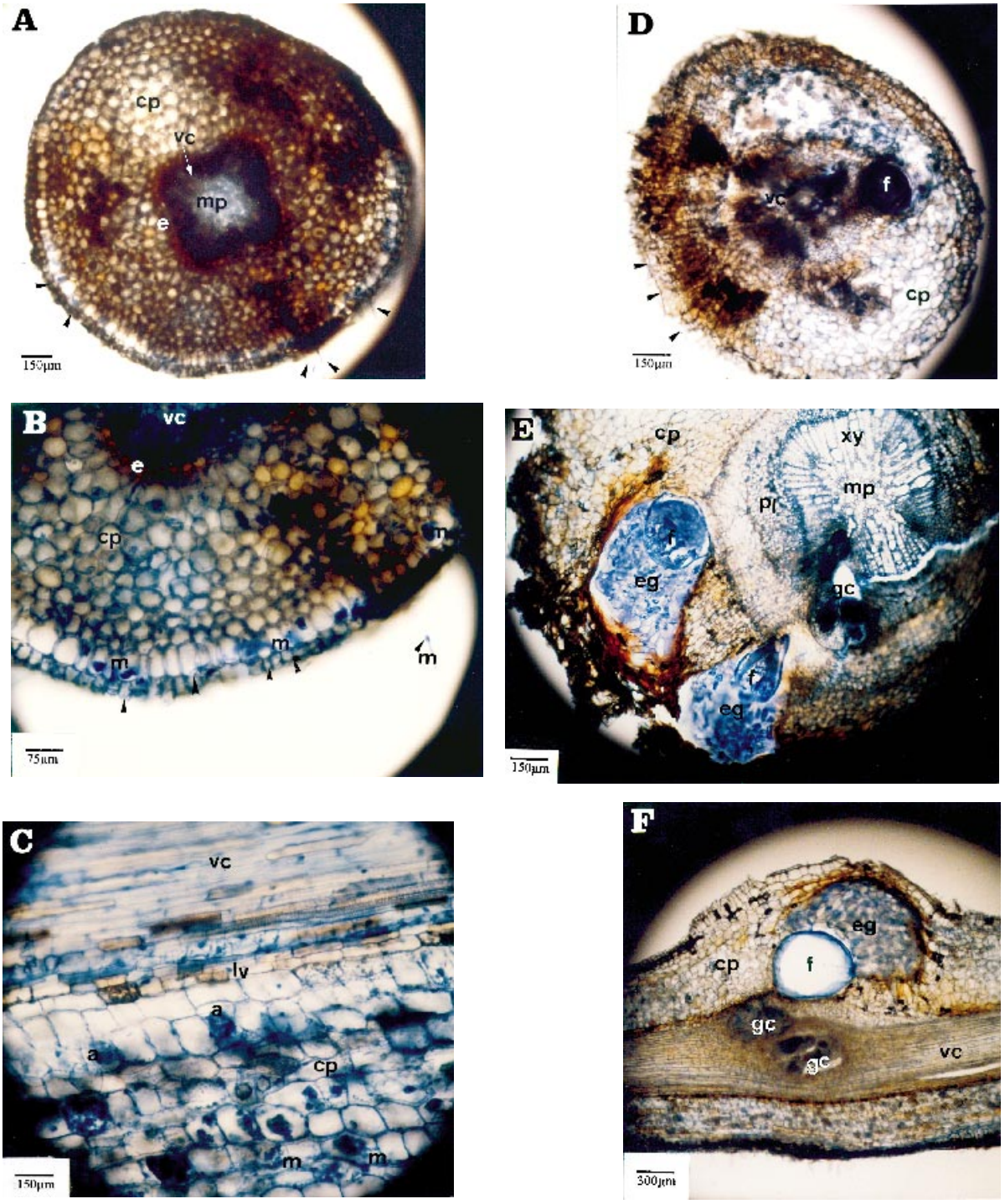

Figure 2 (A) Cross section of a rubber tree root without nematode infection. The structure of the bark with the cortical parenchyma and endoderm, and the vascular cylinder with the phloem, xylem, and medullar parenchyma intact. Mycorrhizal infection (in blue) is localized in the cortical cells (arrows). (B) Higher magnification of the cross section of the root shows the mycelium (arrows) colonizing the cortical parenchyma and the mycelium outside the root. (C) Longitudinal section of a root: AM colonization is limited to the cortical parenchyma. No colonization is present in the vascular cylinder. (D) Cross section of a *Meloidogyne exigua* colonized root. AM mycelium occurs only on the opposite side of the root section (arrows), (E) *M. exigua* female with eggs and giant cells. (F) Longitudinal section of a gall in the root, showing root deformation. (cortical parenchyma: cp; endoderm: e; vascular cylinder: vc; phloem: pl; xylem: xy; medullar parenchyma: mp; mycelium: m, arbuscule: a; nematode female: f; nematode eggs: e.g. ; giant cells: gc; latex vessel: lv).

marked seasons, could not have been caused by seasonal variations (Louis & Lim, 1987).

Spore numbers in soil were not correlated with AM colonization in roots. Furthermore, the occurrence in soil of the native AM fungal spores, mainly *Gigaspora* during the dry season (although their numbers were lower than during the rainy season), showed that the fungi survived under low humidity, as observed in a dark red latosol by Miranda (1981). Brundrett & Abbot (1994) found no substantial seasonal fluctuations in mycorrhizal formation in clover roots and the capacity of root colonization of AM propagules was maintained throughout the year, as in the present work. Mendonça *et al*. (1992) reported that numbers of spores were not correlated with AM colonization of *Eucalyptus viminalis* in southern Brazil; however, *E. viminalis* is able to form a dual mycorrhizal system. Competition between ecto- and endomycorrhizae does not occur in *Hevea brasiliensis*.

Several sites in addition to site 1 in the plantation were found without any second stage juveniles or eggs (data not shown), suggesting that *M. exigua* was not widespread in the plantation soil. Second-stage juveniles and eggs in roots and second-stage juveniles in soil were affected by seasonal variation. During the dry season, fewer nematodes were found in roots than during the rainy season and more juveniles were found in soil. At the end of the study, the numbers of second-stage juveniles in soil had increased considerably compared with the initial numbers (180 vs. 30 per 200 g soil).

There was an inverse relationship between AM fungi and nematode populations in roots and soil: higher AM fungal colonization in roots was associated with fewer second-stage juveniles and eggs in these roots and fewer second-stage juveniles in the soil. Moreover, microscopic observations of roots showed mutual exclusion with histological specificity of the two organisms. AM fungi were found on the unaltered, opposite side of nematode-infected roots as suggested by Kellam & Schenck (1980). A prophylactic role of AM fungi is postulated. Mycorrhizal rubber tree roots are more lignified than non-mycorrhizal ones (results not published). When AM fungi penetrate into roots, they increase cell wall lignification and protect the roots from penetration by other pathogens (Morandi, 1996). However, no curative effect was shown and nematode life cycles appeared to be complete (eggs in roots and second-stage juveniles in soil were observed at infected sites throughout the year). No toxic component affecting the nematode reproduction appeared to be produced by the native AM fungi. The two organisms may compete for the same host. Antagonist effects of *Gigaspora* sp. against *Meloidogyne* spp. have been reported (Roncadori & Hussey, 1977; Hussey & Roncadori, 1982). Sharma & Triverdi (1994) also found interference between occurrence of AM fungi and nematodes in the same root system and showed that AM fungi decreased the amount of damage caused by the nematode. Pinochet *et al*. (1997) found that mycorrhizal colonization did not affect *Meloidogyne javanica* build-up in the roots of banana, but mycorrhizal association compensated for the damage caused by the pathogen on plant growth. Similar effects were demonstrated on peanut (Carling *et al*., 1996).

Examination of AM spore numbers in soil in relation to cover crops showed that *Brachiaria decumbens* and *Vernonia* sp. were the best. Because they were not hosts for *M. exigua* they could be used for increasing the AM inoculum potential without increasing the nematode population. *Stylosanthes guianensis* and *Crotalaria spectabilis* were the least AM-colonized plants in the present study. These plants have nematicidal properties, producing root exudates that prevent *Meloidogyne* sp. development and/or reproduction in the roots (Cayrol *et al*., 1992). The nematodes are thus unable to complete their life cycle. However, the low AM infection observed may also be caused by these exudates. *S. guianensis* and *C. spectabilis* may be useful in maintaining a low but effective AM fungal inoculum potential and preventing nematode multiplication. Exclusive cultivation of rubber would decrease the inoculum potential of fungi while increasing the nematode infestation. Sieverding (1991) reported that intercropping stimulates AM propagule density because AM fungi depend on photosynthetic assimilates from the host plants. Contact occurring between roots of cover crops and rubber trees may enhance rubber tree colonization by AM fungi. Cover crops that are potential hosts for root-knot nematodes should be removed or excluded from the plantation, since they constitute a source of infestation.

The present study showed the importance of maintaining mycorrhizal colonization of rubber tree roots because of a possible prophylactic (but not curative) effect of the native AM fungi on *M. exigua* infection.

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