Chapter 6.

Effect of Exotic Tree Plantations on Free Living and Plant Parasitic Soil Nematodes and Population Changes with Eucalypt Hybrids and Plantation Age

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Introduction

The effect of changing environment on microfauna in plantations compared to savanna was studied for free-living and plant parasitic nematode populations. The first are of importance in the micro-food web, and although little is known about it, change in organic matter quality is assumed to affect their populations. The populations of plant parasitic nematodes, which are bound to one or several host plants, are expected to be influenced by the new planted and undergrowth species and by the disappearance of savanna species. Thus if planted eucalypts were host-plants for one of the native plant parasitic nematode species, population densities of this parasite could increase and their possible pathogenic effect on the crop should be considered.

Knowledge of nematodes associated with the eucalypts' rhizosphere in natural environments and in plantations is very limited, according to Majer *et al.* (1997), who mentioned nematodes as the most numerous animal class in the soil but did not quote any references on this topic. In eucalypt plantations in the Congo, a qualitative approach showed two species of plant parasitic nematodes belonging to the genus *Xiphinema* (Family: Longidoridae) as parasites of eucalypt roots: *X. parasetariae* and *X. souchaudi* (Baujard *et al.* 1998).

The words "nematode" and "eucalypt" can be found linked very often in bibliographic data bases. This is related to the permanent quest for nematicidal properties of natural substances among plants, including eucalypts, and the need to reduce the use of synthetic chemical nematicides for environmental considerations. However, such studies were not concerned with nematode parasites of eucalypt roots.

The study had two aims: (i) to assess the changes in species distribution and population densities of nematodes associated with changes in plant cover; (ii) to study a possible pathogenic effect of these changes on the eucalypt crop.

Studied Plots and Methods

Field studies

The study of nematodes was first carried out in the age series of the Pointe-Noire savanna and experimental plantations (Sa, Ea, Ed, Ee, Ef) and the Loudima savanna and plantations (SL, El, AL, PL, Table 0.2). Three to five composite soil samples were extracted along one or two transects across each plot. Composite samples resulted from five soil cores (0 to 15-20 cm depth) taken 15 cm away from the trunk of every other planted eucalypt tree. A second sampling series in the commercial plantations (second project, Table 0.3)

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aimed to study the distribution pattern of nematodes in savanna (Sb), in *E.* PF1 plots (Ep, Er, Et, Eu) and in the 8-9-year-old multiclonal plot where *E.* urograndis was studied (Ev). Due to the transect method, in this last plot, the sampled transect crossed several clones and hybrids. Soil cores were made on tree lines at every other tree. This resulted in 10 to 20 samples on each sampled tree line, beginning on the plot edge. Samples were taken 1 m away from the trunks, and cores were 15 cm depth, with a soil volume of 424 ml. Nematodes were extracted from soil by the two-flask technique (Seinhorst 1955) and from roots by the mistifier extraction technique (Seinhorst 1950). All individuals of each species of plant parasitic nematodes were counted, and neither genera nor species were determined for free-living nematodes. Plant parasitic nematodes extracted from roots were very scarce, whereas free-living nematodes extracted from roots were sometimes very numerous. According to the short life-cycle of free-living nematodes (4-7 days), and the time required for an efficient recovery of plant parasitic nematodes from roots through the mistifier (2 weeks), these free-living nematodes were assumed to multiply on the roots during mistifying. Therefore data on free-living nematodes extracted from roots were not included in the results. The frequency was calculated as the ratio of the number of samples containing nematodes to the total number of samples expressed as a percentage. Root weight was measured for each sample.

Laboratory study of eucalypt clone susceptibility to *Xiphinema parasetariae*

The susceptibility of eucalypts to *Xiphinema parasetariae*, the major plant parasitic nematode found in the eucalypt plantations, was assessed in the laboratory. Young cuttings were planted in PVC vessels (one cutting per vessel) with 500 ml of heat-sterilised savanna soil (maintained for 10 hours at 75°C). Each plant was inoculated with 10 nematodes and the vessels were kept at 30°C in a temperature-controlled box. Nematodes were extracted from each vessel after three months. The final population was obtained by addition of the *Xiphinema* extracted from soil and from roots, and the reproduction rate was calculated as the ratio final population/initial inoculum. The experiment comprised four replications for each clone.

Results and Discussion

Two great groups of soil nematodes were considered, free-living and plant parasitic, because of the different ways in which their populations could change. The second group is linked to the occurrence of host plants although these plants are not always known. The mean number of individuals of the two groups of soil nematodes was calculated (Table 6.1), and eventually compared to the vegetation characteristics.

Free living nematodes

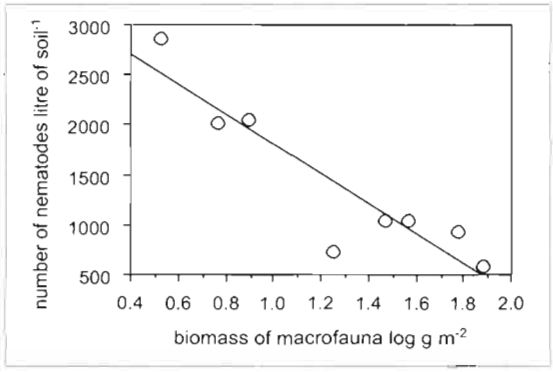
Free-living nematodes were more numerous in savannas than in plantations. In plantations a significant correlation ($r = 0.733$ for 12 plots) was observed with the percentage of savanna plant species (as defined in chapter 1) in the vegetation which is an index of environment change. It suggested that the savanna populations of nematodes were disappearing while those of the forest had not yet stabilised, and indicated that there were no differences due to the planted tree species. Consequently, although an increase of nematode number with increasing carbon content is generally reported (Yeates 1979; Pradhan *et al.* 1988), no significant relationship occurred in the studied plantations. Taking into account the data of the first project, a negative relationship between the number of free-living nematodes and the macrofauna biomass (expressed as log, $r = -0.915$ $p = 0.1\%$ Fig. 6.1), which is largely dominated by earthworms, could indicate an antagonistic effect. However this hypothesis deserves more study because this relationship could also result from the effect of litter or organic matter quality. The density of free-living nematodes was lower in the second sampling series in commercial eucalypt plantations than observed in the experimental plots, and in the commercial plantations, their density was higher in the old *E.* PF1 plots than in the younger plots ($p=0.02$). There was no significant difference between the first rotation crop and coppice. The comparison between the sandy soil (Pointe-Noire) and the clay soil (Loudima), showed a trend towards a higher density of free-living nematodes in sandy soil under savanna (Table 6.1), whereas this was less obvious under eucalypt and acacia plantations

Table 6.1 Average density (number of individuals litre^{-1} of soil) of free-living and plant parasitic nematodes in savanna and eucalypt plantations at Pointe-Noire and Loudima

Sample period*		Plant code	Plot age (yr)	Free-living nematodes	Species of plant parasitic nematodes				
					Xiphinema parasetariae	*Helicotylenchus dihystera*	*Pratylenchus brachyurus*	*Meloidogyne* sp.	*Tylench -orynchus* sp.
Pointe Noire site					Average density (number of individuals litre^{-1} of soil)				
1	Savanna	Sa	-	2876	42	842	58	0	0
2	Savanna	Sb	-	2940	15	2089	0	0	0
1	*E.* PF 1	Ea	6	737	148	136	4	0	0
1	*E.* PF 1	Ed	16	571	95	9	4	22	0
1	*E.* PF 1	Ee	18	762	47	38	0	0	0
1	*E.* PF 1	Ef	20	1050	77	206	0	4	0
1	Acacia	A	12	949	202	64	124	915	0
2	*E.* PF 1	Ep	6	191	2	<1	21	0	1
2	*E.* PF 1	Er	13	201	7	<1	<1	0	0
2	*E.* PF 1	Et	19	305	42	0	0	0	0
2	*E.* PF 1	Eu	19	263	67	0	0	0	0
2	Multi-clone	Ev	8	251	132	0	0	0	0
Loudima site									
1	Savanna	SL	-	2032	3	224	75	132	0
1	*E.* PF 1	El	26	589	0	0	0	0	0
1	Acacia	AL	12	1043	0	0	34	5742	0
1	Pine	PL	27	745	29	20	0	10	0

*Sampling period: 1=1st project 1996, 2= 2nd project 1998.

Figure 6.1 Relationship between free living nematode density and macrofauna fresh biomass in plantations and savanna



because of other constraints. The chemical effect (inactivation of organic compounds) and physical effect (porosity) of clay were assumed to affect nematode populations (Fargette 1987; Groethals 1987).

The drastic decrease in free-living nematode density in the soils of plantations compared to savanna could be explained by the effect of the cover-crop tillage, which was made to 20 cm depth, and might result in a more exhaustive drying than nematodes were able to survive, especially if the plot was tilled during the dry season. However, tillage did not affect the density of bacterial-feeding nematodes in a semi-arid Mediterranean agrosystem (Lopez-Fando and Bello 1995). Life-cycles of microbivorous nematodes, which are the main part of the free-living nematode populations, are rather short (4-7 days) and the reproduction rate is high (Nicholas 1975). Therefore the recovery of the initial population density could be easily achieved within one rainy season (8-9 months). The decrease in density could be better explained by a change in the microbial status of soil resulting from the disappearance of root exudates from grasses and the input of eucalypt litter essential oils (Li and Madden 1995) or polyphenols (Conde *et al.* 1997) which have antibiotic activity. A decrease in microbial biomass could result in a shortage of food for free-living nematodes, since most of them are microbivorous. *Acacia auriculiformis* litter was poorer in phenolic compounds than eucalypt litter (Bernhard-Reversat 1999) and more free-living nematodes were found in soil under acacias than in soil under eucalypts. Moreover, some chemical compounds of eucalypt litter could exhibit a direct antinutritional effect on nematodes as has been already observed on soil detritivore arthropods (Maity and Joy 1999).

Whatever the origin of the decrease in density of free-living nematodes, it affected soil functioning because nematodes are known to have an active part in several soil processes, as microbial activity, organic matter decomposition and nitrogen mineralisation (Pradhan *et al.* 1988; Ferris *et al.* 1998). However the status of soil environment would be assessed more accurately if free-living nematode populations were studied at the generic level (Porazinska *et al.* 1999).

Field study of plant parasitic nematodes

Eucalypt plantations

Eucalypts were planted as cuttings grown in artificial substrate so that the cuttings could not have been infected with nematodes. Infection was assumed to spread from nematodes living on savanna host plants and to develop when they found a host plant in the plantations. Six species of plant parasitic nematodes were found in eucalypt plantations: *Xiphinema parasetariae*, *X. souchaudi*, *Helicotylenchus dihystera*, *Pratylenchus brachyurus* and *Tylenchorhynchus* sp. The only abundant species was *X. parasetariae*, for which eucalypt is a host plant, as was demonstrated by experimental inoculation in the laboratory. *Xiphinema souchaudi* also parasitises eucalypts but remained scarce in the plantations (Baujard *et al.* 1998). *Xiphinema parasetariae* and *X. souchaudi* were located in the 0-30 cm layer of soil and during the dry season, from June to September, they survived soil desiccation. Both species were shown to be able to enter in anhydrobiosis (Reversat 1996). The other three species were scarce and were assumed to parasitise undergrowth plant species. *Helicotylenchus* sp. alone was more abundant in savanna than in plantations. Its host plant was assumed to be a savanna species and to disappear with the aging of plantations (Huttel and Loumeto, chapter 1), resulting in the decrease or disappearance of *H. dihystera* in plantations (Goede *et al.* 1993).

Xiphinema parasetariae predominated in the plantations studied (Table 6.1). However the density of *X. parasetariae* in the young experimental plantations was higher than that of the commercial areas and this may be due to different silvicultural practices. The numerous samples made in the commercial plantations

Figure 6.2 Density of free-living nematodes in savanna and eucalypt plantation plots

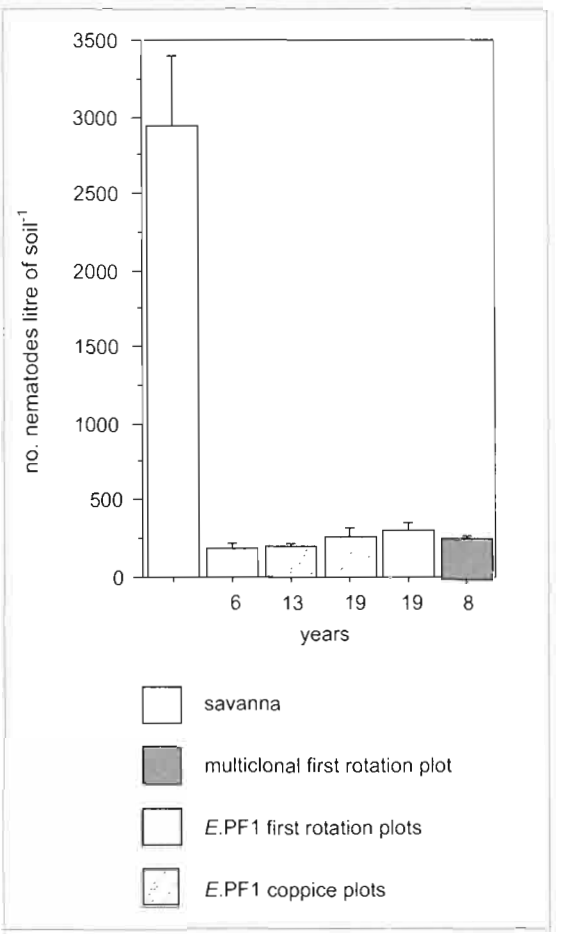
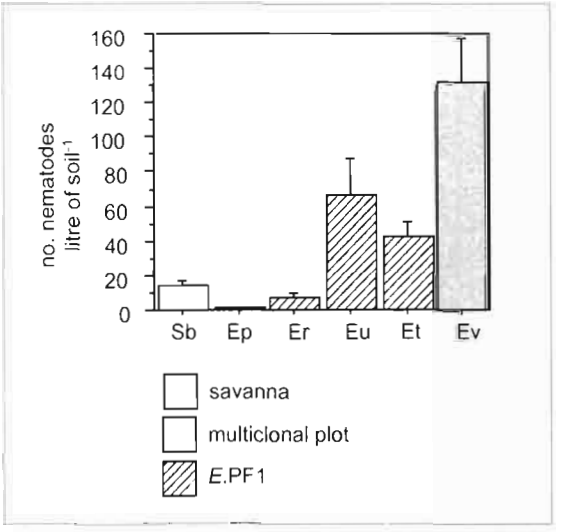


Figure 6.3 Density of *Xiphinema parasetariae* in savanna and eucalypt plantation plots



during the second project allowed the study of the distribution of *X. parasetariae* in the *E.* PF1 plots. Differences among plots in density and frequency of *X. parasetariae* were highly significant (p=0.0001, Fig. 6.3). Along the sampled transect, patches of samples with zero nematodes separated patches of positive samples. The estimation of the average length of positive patches was significantly correlated (p=0.001) with plot age (Loubana and Reversat, in preparation).

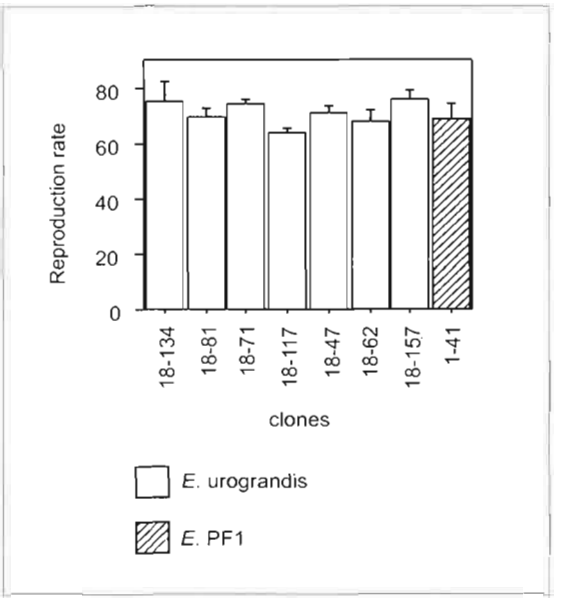
Another distribution pattern of *X. parasetariae* was observed in the 8-year-old multiclonal plantation, where it was widely spread, with a higher density than in the 6-year-old *E.* PF1 plot. *Xiphinema* development is dependent on the number of root apices, and root density in the sampled cores was significantly higher in the multiclonal plot (0.26 g sample^{-1}, standard error 0.01) than in the 6-year-old *E.* PF1 plot (0.11 g sample^{-1}, standard error 0.005), due to closes spacing (666 trees ha^{-1} and 532 trees ha^{-1} respectively). The hypothesis of the control of *Xiphinema* by root apex density will be investigated in further studies.

The high densities of *X. parasetariae* found in old eucalypt plantations could be high enough to affect the trees, and especially the seedlings at the time of replanting. This nematode attacks the root apex and might prevent a normal root growth (Wallace 1973). The resulting wounds enable the infestation of the plant by bacteria and fungi and could be responsible for low vigour in young plantations . However few data are available on the specific effect of *Xiphinema* spp. on eucalypt growth, although this nematode genus was found in South African eucalypt plantations by Marais and Buckley (1993). It was also reported on young *Pinus caribaea* in Nigerian plantations (Gbadegesin 1993). *Xiphinema elongatum* was shown to be harmful for sugar cane crops planted on infested soil previously under eucalypt plantation (Spaull 1998).

Acacia plantations

Meloidogyne spp. were the most abundant plant parasitic nematodes encountered under acacia, which is a host plant for them. *Meloidogyne* did not occur in savanna around Pointe-Noire, and this might be related to the inability of this genus to withstand desiccation during the dry season.

Figure 6.4 Comparison of the reproduction rates of *Xiphinema parasetariae* on eight eucalypt clones



Acacia plants might have been infested by *Meloidogyne* in the nursery from soils from vegetable crop areas used as a potting mix, most of which are highly contaminated with *Meloidogyne* (Loubana 1996). *Xiphinema* spp. were also abundant under acacia which is a good host plant, and the contamination might have occurred from the savanna populations, as in eucalypt plantations.

Laboratory study on clone susceptibility to Xiphinema parasetariae

The test for clone susceptibility showed the reproduction rates of *Xiphinema parasetariae* were very similar among the eight studied clones (Fig. 6.4), and the ANOVA was not significant. However when the ANOVA was made without the three most variable clones, it was significant for the five other clones (p=0.04) and some clones appear to be less sensitive. It could be interesting to test a greater number of clones. However, the low genetic variability of eucalypt hybrids suggested that resistant clones could not be found, and plant resistance to the genus *Xiphinema* was very rarely found (Luc and Reversat 1985).

Conclusions

Dramatic changes in nematode populations occurred when eucalypt trees were planted on savanna. Population densities of free-living nematodes decreased drastically to one tenth of their initial values, from several thousand individuals per litre of soil in savanna to several hundred of individuals per litre of soil in 6-year-old eucalypt plots. During the same time, the biodiversity of plant parasitic nematodes was reduced from savanna (five genera) to eucalypt plots (only one genus, *Xiphinema*) and the density of the main species, *X. parasetariae,* increased in the eucalypts.

Much remains to be done concerning the functional groups of free-living nematodes and their role in soil functioning. A more careful approach during the early stages (between the beginning and 5 years) of eucalypt plantation establishment is needed. The investigations should focus on the variation of the free-living nematode population and an evaluation of the soil microbial biomass, of which a part is consumed by free-living nematodes.

The plant parasitic nematode populations are better known, because their taxonomical status is very simple. Until now, only two species have been demonstrated as parasites of eucalypt roots in Congo: *Xiphinema parasetariae* and *X. souchaudi*. Their densities were found to be as high as 150 individuals per litre of soil in eucalypt plots, and the possible detrimental consequence of this infestation on the functional status of roots and the growth of trees deserves further study.