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The development of plant-parasitic nematode infestations on micro-propagated banana plants following field control measures in Côte d'Ivoire

By THIERRY MATEILLE[1], PATRICK QUÉNÉHERVÉ[2] and RÉMI HUGON*[3]

ORSTOM, 01 BP V51, Abidjan 01, Côte d'Ivoire
**IRFA, 01 BP 1740, Abidjan 01, Côte d'Ivoire*

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Summary

Of the weeds that were found in banana production areas, only *Asystasia gangetica* was parasitised by *Radopholus similis; Helicotylenchus multicinctus* and *Hoplolaimus pararobustus* were able to parasitise all weed species. Field trials were carried out in Côte d'Ivoire to assess the potential for using nematode-free micro-propagated banana plants following cultural and chemical methods for nematode control. Banana (*Musa acuminata*) cv. Poyo was examined for nematodes after weed fallow, flooding and chemical treatment. Before replanting bananas, nematode assays showed that: i) all nematode species declined but were not eliminated after a 1,3-dichloropropene soil treatment; ii) *H. multicinctus, H. pararobustus* and *Cephalenchus emarginatus* were still present after either a 2-year weed fallow (dominated by *Chromolaena odorata* or *Asystasia gangetica*) or a 10-week flooding; iii) *R. similis* did not persist after fallowing or flooding. All nematode species were found in plots treated with 1,3-dichloropropene and that had been planted with bullheads, suckers or nematode-free micropropagated plants. After both fallow and flooding, *R. similis* was reintroduced on infested planting materials (bullheads and suckers) even when they were pre-treated with a nematicide. When fallow and flooded plots were planted with nematode-free materials (*vitro*-plants), *R. similis* did not appear in the roots for two vegetative cycles. The other species, still present in the soil, invaded the roots and increased slowly in numbers. These results were confirmed in commercial banana plantations.

Key words: Banana, micro-propagated plant, fallow, flooding, phytoparasitic nematodes

Introduction

Numerous studies have been conducted to determine the feasibility of different cultural management strategies for the control of the burrowing nematode, *Radopholus similis* and to assess their efficacy (Loos, 1961; Keetch, Reynolds & Mitchell, 1975; Salas, Oyuela & Stover, 1976; Zem & Alves, 1983). Cultural management can often exacerbate problems when, as in the tropics, nematode communities are polyspecific, because weeds or cover

Present addresses:
[1]ORSTOM, BP 1386, Dakar, Sénégal
[2]ORSTOM, BP 8006, 97259 Fort de France Cedex, Martinique, F.W.I.
[3]CIRAD, BP 5035, 34032 Montpellier Cedex 1, France

crops can be non-hosts for one nematode species but good hosts for another (Colbran, 1964; Edmunds, 1970; Swaine, 1971; Stoyanov, 1973). Fallowing or intercropping can modify the equilibrium among the different nematode populations in the soil, and species formerly considered as secondary parasites can achieve damaging levels (Minz, Ziv & Strich-Harari, 1960). Flooding can reduce nematode populations by starvation (Loos, 1961; Mateille, Foncelle & Ferrer, 1988), anaerobiosis (Van Gundy, Stolzy, Szuszkiewicz & Rackham, 1962), or indirect toxicity (Hollis & Rodriguez-Kabana, 1966; Jacq & Fortuner, 1978, 1979). In bananas, the efficacy of flooding depends on the length of submersion (Loos, 1961; Maas, 1969; Rajendran, Naganathan & Vadivelu, 1979; Sarah, Lassoudière & Guérout, 1983), and results vary according to the nematode species: only *R. similis* is eradicated (Mateille *et al.*, 1988). Weed fallowing is now usually employed in banana culture in Côte d'Ivoire and flooding can be practised in banana areas set on polders. If weeds are left to grow after a banana crop for about two years, usually only one or two become predominant. The objectives of this study were: i) to compare the banana-parasitic nematode populations on weeds under bananas or in fallows; ii) to observe nematode population changes under bananas after weed fallowing, flooding and chemical treatment; and iii) to see how any advantage of planting nematode-free micro-propagated *vitro*-plants depends on the degree of soil disinfection.

Materials and Methods

Weed survey

Weeds associated with bananas (*Musa acuminata* AAA cv. Poyo) were collected from three areas in the south-east of Côte d'Ivoire, located on three different soil types: the lagoon valley of Nieky on peat and clay soils; the regions of Azaguié on ferralitic soils and on sandy-silts; the region of Aboisso on silty soils. From two to four banana plots of 0.5 ha in each of these locations, weeds were sampled and identified from within 2 m diameter circles around 10 banana plants randomly chosen. Endoparasitic nematodes were extracted from roots of the prevalent weeds using a mist chamber (Seinhorst, 1950).

Field trials

Two trials were conducted in which bananas were planted after a two-year weed fallow period. The first trial was done in the Nieky region on a pure peat soil (pH 3.8), where the dominant weed species was *Asystasia gangetica* The second was done in the region of Azaguié on a sandy-silt soil (61.5% sand, 24.8% silt, 8.7% clay, 3% organic matter, 2% water, pH 6.3), with the fallow dominated by *Chromolaena odorata*. A 10-week flooding trial was done, as described by Mateille *et al.* (1988), in the Nieky region on a clay-peat soil (50.7% clay, 10.2% silt, 5.8% sand, 27% organic matter, 6.3% water, pH 5.0). In all trials, soil was sampled prior to planting and nematodes were extracted by elutriation (Seinhorst, 1962) from 30 replicates at each site. The endoparasitic nematodes *Radopholus similis, Helicotylenchus multicinctus* and *Hoplolaimus pararobustus* and the ectoparasitic species *Cephalenchus emarginatus* are the most important nematode parasites of banana in Côte d'Ivoire (Fargette & Quénéhervé, 1988), and their frequency and abundance were determined according to the method of Fortuner & Merny (1973).

For the fallowing trials, four situations were compared:

i) no fallow because bananas planted; soil fumigated with 1,3-dichloropropene 92% (150 litre/ha); bullheads planted (BDB).
ii) no fallow because bananas planted; soil fumigated with 1,3-dichloropropene 92% (150 litre/ha); *vitro*-plants planted (BDV).

iii) fallow; bullheads planted (FB).
iv) fallow; *vitro*-plants planted (FV).

For the flooding trial, three types of planting material were compared: i) suckers (S); ii) suckers coated with 1.2 g a.i. of liquid fenamiphos (CS); iii) *vitro*-plants (V). Treatments were arranged in randomised complete block designs with six replicates for each pre-treatment and planting material combination. Experimental plots were separated from one another by 50–60 cm deep by 50 cm wide trenches. Each month during the first vegetative cycle and at the end of the second cycle, soil and roots of the fruit-bearing plants were sampled according to the method described by Quénéhervé & Cadet (1986) and nematodes extracted as described above. Numbers of the ectoparasitic species *C. emarginatus* were followed in the soil, and of the endoparasites *H. multicinctus, H. pararobustus* and *R. similis* in the roots. Statistical analysis was conducted on the nematode fluctuations by a Friedman two-way analysis of variance followed by the Mann Whitney U test in order to classify the treatments according to their efficacy in reducing the nematode populations, considering BDB (in fallow trials) or S (in the flooding trial) treatments as controls.

Tests in plantations

In the three locations described above and in a fourth location in the east-central region of Côte d'Ivoire, a total of 120 one-year weed fallow plots were replanted, 91 with bullheads coated with 1 g a.i. of granular fenamiphos and 29 with *vitro*-plants. In the Nieky region, nine plots were replanted with nematode free *vitro*-plants after a six week flooding period. Each month during three vegetative cycles, banana roots were sampled and *R. similis* were extracted by the centrifugal-flotation technique (Coolen & D'Herde, 1972). All plots where banana infestation reached 4000 *R. similis*/100 g of roots (an arbitrary threshold level above which a control method is performed by farmers) were recorded.

Table 1. *Survey of the weeds occurring in three banana producing areas in Côte d'Ivoire*

Weeds		Nieky		Azaguié		Aboisso
Family	Species	Clay	Peat	Ferralitic	Sandy silt	Silty clay
Acanthaceae	*Asystasia gangetica*	++	++	+	+	+
Asteraceae	*Chromolaena odorata*	–	–	–	++	–
Amaranthaceae	*Alternanthera sessilis*	+	–	+	–	–
	Amaranthus viridis	+	+	+	+	+
Capparidaceae	*Cleome ciliata*	–	–	–	+	–
Commelinaceae	*Commelina benghalensis*	+	–	+	–	–
Euphorbiaceae	*Euphorbia prostata*	–	–	–	–	+
	Phyllanthus amarus	+	+	++	++	+
Labieae	*Solenostemon* spp.	–	–	+	+	–
Loganiaceae	*Spigelia anthelmia*	–	–	–	–	+
Piperaceae	*Peperomia pellucida*	–	–	+	+	+
Portulacaceae	*Portulaca oleracea*	–	++	+	++	+
	Talinum triangulare	+	+	+	+	+
Rubiaceae	*Borreria chaetocephala*	–	+	–	+	–
Urticaceae	*Fleurya aestuans*	+	–	+	+	+
Convolvulaceae		+	–	+	+	–
Cyperaceae		+	+	+	+	+
Gramineae		+	++	+	++	++
Rosaceae		+	–	–	–	–

– = absent or rare; + = present; ++ = frequent (>30% of the weeds).

Results

Weed survey

In the banana fields surveyed, 15 weed species from 12 families were identified but representatives of the Convolvulaceae, Cyperaceae, Gramineae and Rosaceae that were found were not identified to species (Table 1). Cyperaceae and Gramineae were present in all banana fields whatever the soil type. Convolvulaceae were absent from peat and silty clay soils. Numerous Rosaceae were found only on clay soils. Some species, such as *Amaranthus viridis* (Amaranthaceae), *Asystasia gangetica* (Acanthaceae), *Phyllanthus amarus* (Euphorbiaceae) and *Talinum triangulare* (Portulacaceae) were ubiquitous with varying degrees of prevalence, but *Portulaca oleracea* (Portulacaceae) was not recorded on clay soils. In contrast, some species were very rare, depending on the region or on the soil type: *Chromolaena odorata* (Asteraceae) and *Cleome ciliata* (Capparidaceae) were confined to sandy soils; *Euphorbia prostata* (Euphorbiaceae) and *Spigelia anthelmia* (Longaniaceae) were found only on silty-clay soils in the region of Aboisso.

Assessments of nematode infections of roots revealed that *Helicotylenchus multicinctus, Hoplolaimus pararobustus* and *Radopholus similis* were frequently associated with prevalent weeds, except that *C. odorata* and the Convolvulaceae species were free of *R. similis* (Table 2). Generally, *H. multicinctus* and *H. pararobustus* were more common than *R. similis*, whose infestation levels were both low and heterogeneous.

Nematode populations in soil after fallowing or flooding (Fig.1)

Radopholus similis was not detected in the soil after a two-year weed fallow, but *H. multicinctus* and *H. pararobustus* were still present. These nematodes were less abundant and frequent on a peaty soil (Nieky) colonised by *A. gangetica* than sandy-silt soil (Azaguié) colonised by *C. odorata*. Population levels of *C. emarginatus* were fairly similar on these two sites, but less frequent on the second.

Radopholus similis was not found after the fifth week of flooding, but low populations of *H. multicinctus, H. pararobustus* and *C. emarginatus* remained after a 10-week flooding period.

Table 2. *Average population densities of* Helicotylenchus multicinctus, Hoplolaimus pararobustus *and* Radopholus similis *in weed roots (nematodes/g), and frequency of infested samples (%). Data in brackets are minima and maxima*

	Helicotylenchus multicinctus		*Hoplolaimus pararobustus*		*Radopholus similis*	
Weeds	Nematodes/g	Infested plants (%)	Nematodes/g	Infested plants (%)	Nematodes/g	Infested plants (%)
Amaranthus viridis	22 (2–51)	37.5	43 (1–105)	37.5	3 (3–3)	12.5
Asystasia gangetica	25 (1–54)	57.1	5 (3–8)	14.3	9 (2–11)	28.6
Chromolaena odorata	15 (6–30)	60.0	15 (15–15)	20.0	0	0.0
Phyllanthus amarus	66 (1–275)	50.0	5 (5–5)	7.1	100 (100–100)	7.1
Portulaca oleracea	87 (18–200)	66.7	8 (6–13)	50.0	1 (1–1)	16.7
Talinum triangulare	25 (3–76)	100	4 (4–4)	14.3	6 (6–6)	14.3
Convolvulaceae	32 (4–80)	50.0	20 (20–20)	16.7	0	0.0
Cyperaceae	67 (1–250)	75.0	109 (3–236)	75.0	10 (4–21)	25.0
Gramineae	44 (1–178)	72.7	52 (3–181)	72.7	27 (13–72)	45.5

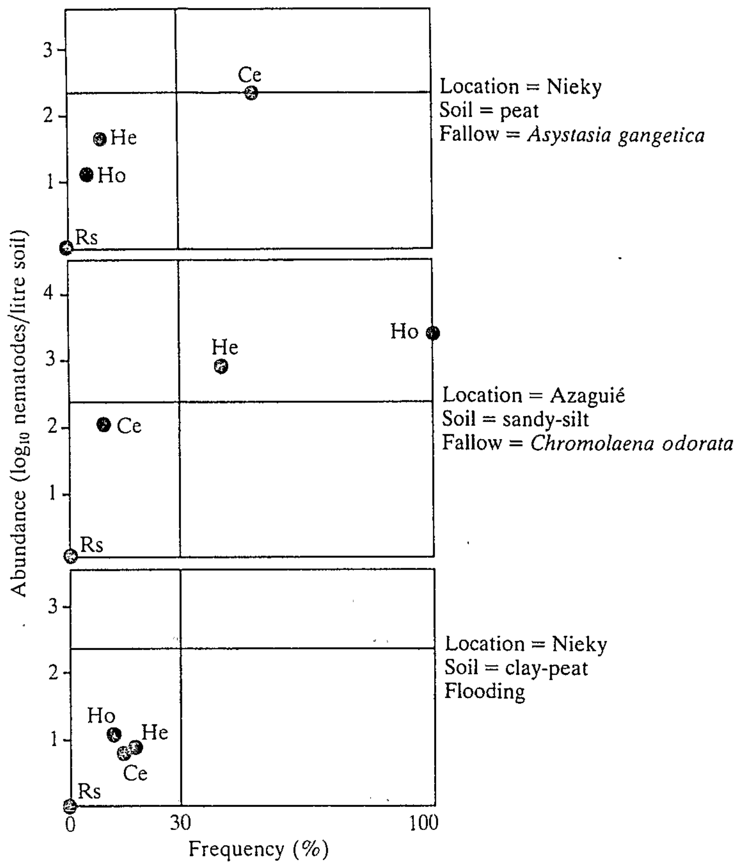

Fig. 1. Abundance and frequency of *Helicotylenchus multicinctus* (He), *Hoplolaimus pararobustus* (Ho), *Radopholus similis* (Rs) and *Cephalenchus emarginatus* (Ce) populations in peat or sandy-silt soils after fallowing, and in a clay-peat soil after flooding in banana plots.

Nematode infestations of banana plants after fallowing

First trial, in the Nieky region (Fig. 2; Tables 3 & 4)

During the first seven months of cultivation, soil population densities of *C. emarginatus* remained low, then increased to high levels after fallowing with more in plots planted with *vitro*-plants than those planted with bullheads. Among the endoparasites, numbers of *H. pararobustus* in the roots remained low regardless of the control method. The numbers of *H. multicinctus* also remained low, with the exception of those in *vitro*-plants planted after a 1,3-dichloropropene pre-treatment. In such treated plots, *vitro*-plants were less infested by *R. similis* than plants derived from bullheads. In fallow plots, *R. similis* were not found in roots of *vitro*-plants, even at the end of the second vegetative cycle.

Second trial, in the region of Azaguié (Fig. 3; Tables 3 & 4)

The ectoparasite *C. emarginatus* increased after all treatments but only very slightly in FV plots. The population densities of *H. multicinctus* in roots remained at low levels but

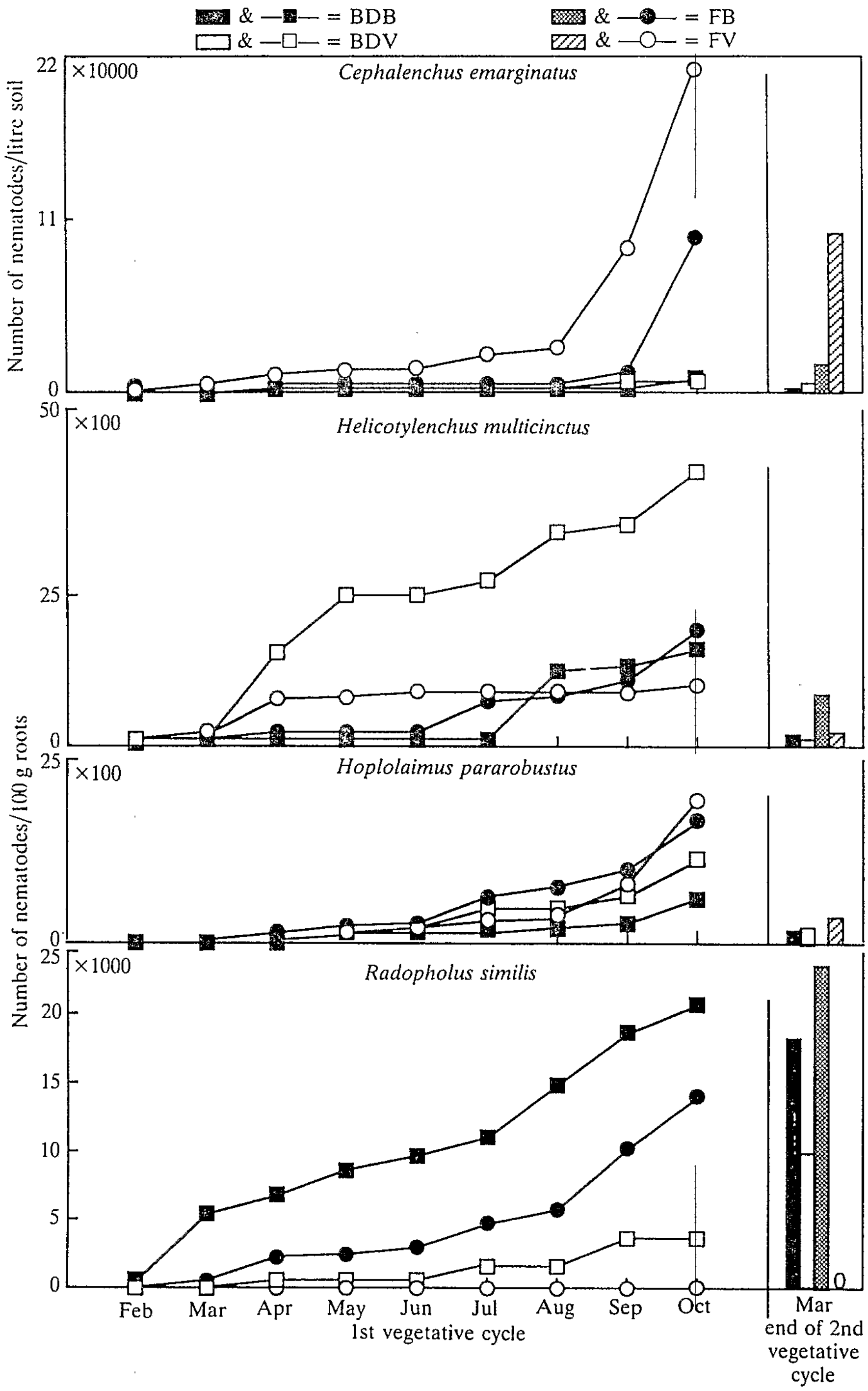

Fig. 2. Nematode populations in roots and soil under bananas cultivated on a peaty soil in the Nieky region. Treatments: i) no fallow because bananas planted; soil fumigated with 1,3-dichloropropene 92% (150 litres/ha); bullheads planted (BDB). ii) no fallow because bananas planted; soil fumigated with 1,3-dichloropropene 92% (150 litres/ha); *vitro*-plants planted (BDV). iii) 2 year-fallow period with *Asystasia gangetica*; bullheads planted (FB). iv) 2 year-fallow period with *Asystasia gangetica*; *vitro*-plants planted (FV).

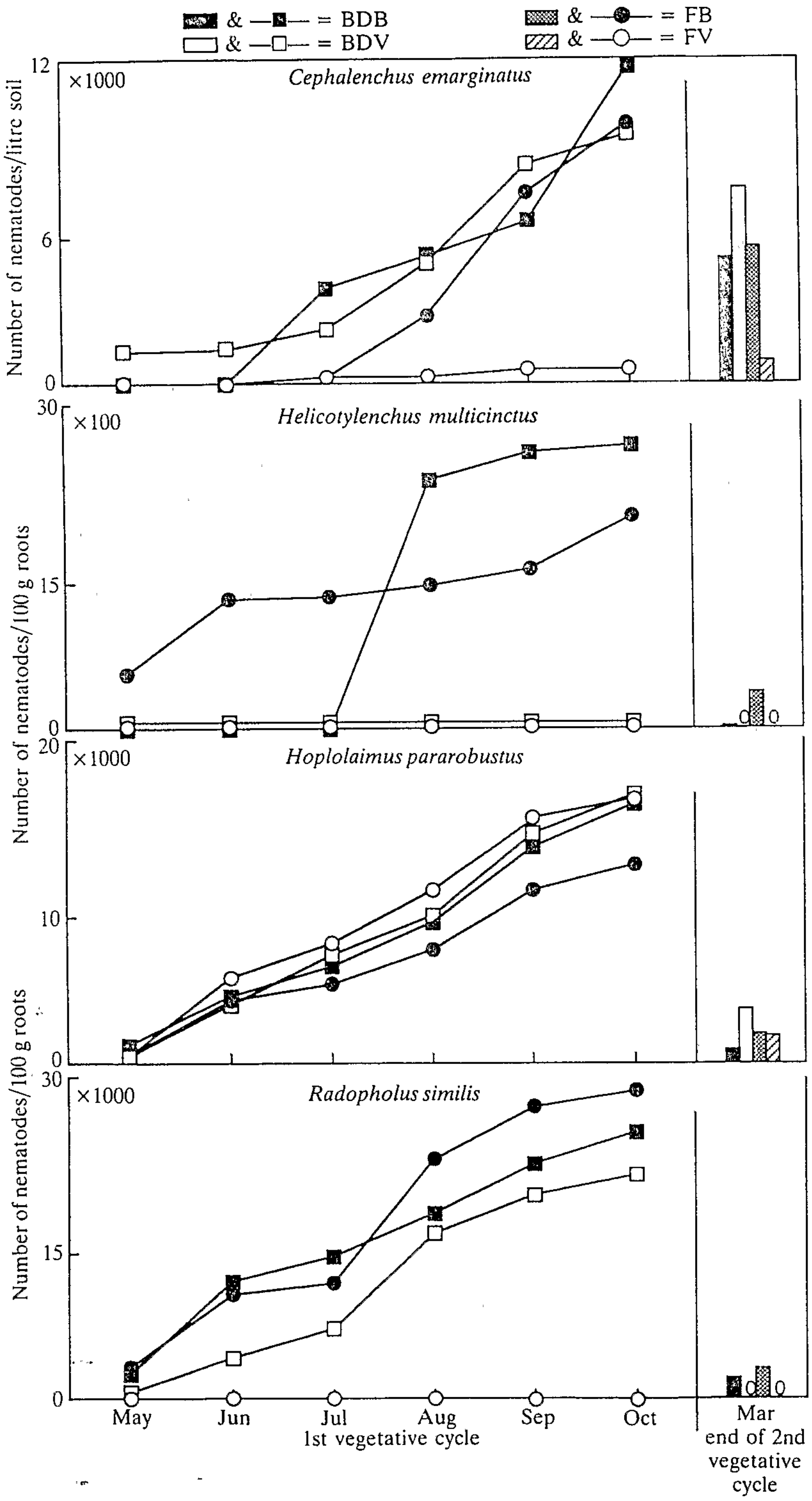

Fig. 3. Nematode populations in roots and soil under bananas cultivated on a sandy-silt soil in the region of Azaguié. Abbreviations used for treatments are defined in Fig. 2 but with a *Chromolaena odorata* fallow.

Table 3. *Percentage changes, relative to the fallow or flooding experiments, of the nematode populations in soil and banana roots at the end of the first vegetative cycle. Abbreviations used for treatments are defined in Figs 2 and 4*

	Soil	Roots		
Treatments	*Cephalenchus emarginatus*	*Helicotylenchus multicinctus*	*Hoplolaimus pararobustus*	*Radopholus similis*
Location = Nieky: Soil = peat; Fallow = *Asystasia gangetica*				
BDV	−17.0	+191.0	+97.8	+82.7
FB	+774.1	+22.1	+191.5	−94.3
FV	+1773.5	+35.1	+247.9	−32.4
Location = Azaguié: Soil = sandy-silt; Fallow = *Chromolaena odorata*				
BDV	−27.7	−97.4	+2.0	−16.0
FB	−24.5	−24.5	−24.2	+16.0
FV	−96.3	−99.7	+1.4	−98.8
Location = Nieky: Soil = clay-peat; Flooding				
CS	−8.0	+92.6	−21.2	−35.0
V	−78.6	−96.1	+4.9	−100

were higher in plants derived from bullheads than in *vitro*-plants. *H. pararobustus* infestations in roots were relatively high and were unaffected by soil treatment or planting material. *R. similis* increased in all situations except when *vitro*-plants were planted after fallow.

Nematode infestations of banana plants after flooding (Fig. 4; Tables 3 & 4)

In plots planted with *vitro*-plants, even though *C. emarginatus* were present during the first vegetative cycle, they could not be detected after the second cycle. Populations of *H. multicinctus* were smaller in the roots of the *vitro*-plants than in roots of plants derived from suckers and the chemical treatment had no depressive effect. By the end of the second vegetative cycle, populations were low in all treatments. *H. pararobustus* was rare in the roots throughout both cycles. The *vitro*-plants were free of *R. similis* throughout the observation period, while in plants derived from suckers, even those coated with fenamiphos, *R. similis* increased.

Reinfestation of banana fields by R. similis *after fallowing or flooding*

According to Mateille, Adjovi & Hugon (1992), five months after planting fallowed plots, the infestation levels of *R. similis* began to exceed 4000 nematodes/100 g of roots and, from this time, increased more rapidly in plots planted with coated bullheads than with *vitro*-plants (Fig. 5). After three vegetative cycles, *R. similis* infestations were higher than 4000/100 g roots in 56% of the plots planted with bullheads compared with 28% of the plots planted with *vitro*-plants.

The number of flooded plots included in these observations was too small (nine replicates) for statistical significance to be detected. However, the 4000 nematodes/100 g roots infestation level was reached after four months of cultivation in plots where immersion had been imperfect. About half of the flooded plots had reached this infestation level at the end of the first vegetative cycle (eight months after planting), and 80% at the end of the second cycle.

Table 4. *Classification of the treatments according to their efficacy in reducing the nematode populations in soil and banana roots during the first vegetative cycle. Abbreviations used for treatments are defined in Figs 2 and 4. Data in brackets represent the sum of the ranks. Sums of ranks followed by the same letter are not significantly different* ($P > 0.05$)

	Soil	Roots		
Treatments	*Cephalenchus emarginatus*	*Helicotylenchus multicinctus*	*Hoplolaimus pararobustus*	*Radopholus similis*
Location = Nieky; Soil = peat; Fallow = *Asystasia gangetica*				
	BDV (10) a	FB (14) a	BDV (15.5) a	FV (10.5) a
	BDB (22) ab	FV (19) a	FV (17.5) a	BDV (16.5) a
	FB (23) ab	BDB (23) a	BDB (22) ab	FB (27) b
	FV (35) c	BDV (34) b	FB (35) b	BDB (36) c
Location = Azaguié; Soil = sandy-silt; Fallow = *Chromolaena odorata*				
	FV (8.5) a	FV (6) a	FB (9) a	FV (6) a
	FB (11.5) ab	BDV (13.5) b	BDV (10.5) a	BDV (12) b
	BDV (17) b	BDB (19.5) b	FV (16.5) a	FB (18) cd
	BDB (23) b	FB (21) b	BDB (24) b	BDB (24) d
Location = Nieky; Soil = clay-peat; Flooding				
	Not significant	V (7.5) a	V (10.5) a	V (7.5) a
		S (16) ab	CS (11.5) a	CS (13.5) b
		CS (18.5) b	S (20) b	S (21) c

Discussion

Effects of the control measures on nematode populations

Different weed communities developed when different banana plots were left fallow. In established bananas, the most frequent weeds were Convolvulaceae, Cyperaceae, Gramineae and Rosacae but, during fallowing, these weeds were overrun by formerly less common weeds such as *A. gangetica* or *C. odorata*. Fallows dominated by *A. gangetica* or *C. odorata* eradicated *R. similis* from banana soils, even when the *A. gangetica* was infected by *R. similis* under banana culture. This indicates that *A. gangetica* is only a poor, 'temporary' host for *R. similis*, which is known as a strict endoparasite of bananas and which cannot persist in the absence of this host. However, *A. gangetica* and *C. odorata* maintain populations of *H. multicinctus* and *H. pararobustus* (endoparasites) and *C. emarginatus* (ectoparasite) in the absence of bananas. In the literature, references to these weeds mention only parasitism by *Meloidogyne* and *Pratylenchus* species (Goodey, Franklin & Hooper, 1965). Recent studies of rotational cropping in banana plantations in the French West Indies (Ternisien & Melin, 1989) showed that the legumes *Canavalia ensiformis, Crotalaria juncea, Desmodium distortum* and *Mucuna pruriens* maintained *R. similis* in the soils, while the grass species *Sorghum* spp. and *Brachiara decubens* were not hosts of this nematode. As these grasses (especially *B. decubens*) provide bio-fertilising elements in soils, they were considered the most worthwhile crops in rotation with banana (Ternisien, 1989) but sorghum was infested by *Helicotylenchus* and *Meloidogyne* spp., and *B. decubens* by *Meloidogyne* spp.

Flooding has a similar effect on the selection of nematode species: *R. similis* is eradicated while the other species will survive in the soil.

Thus, both fallowing and flooding reduce *R. similis* population densities from highly damaging to less damaging levels. The development of useful cultural control methods will

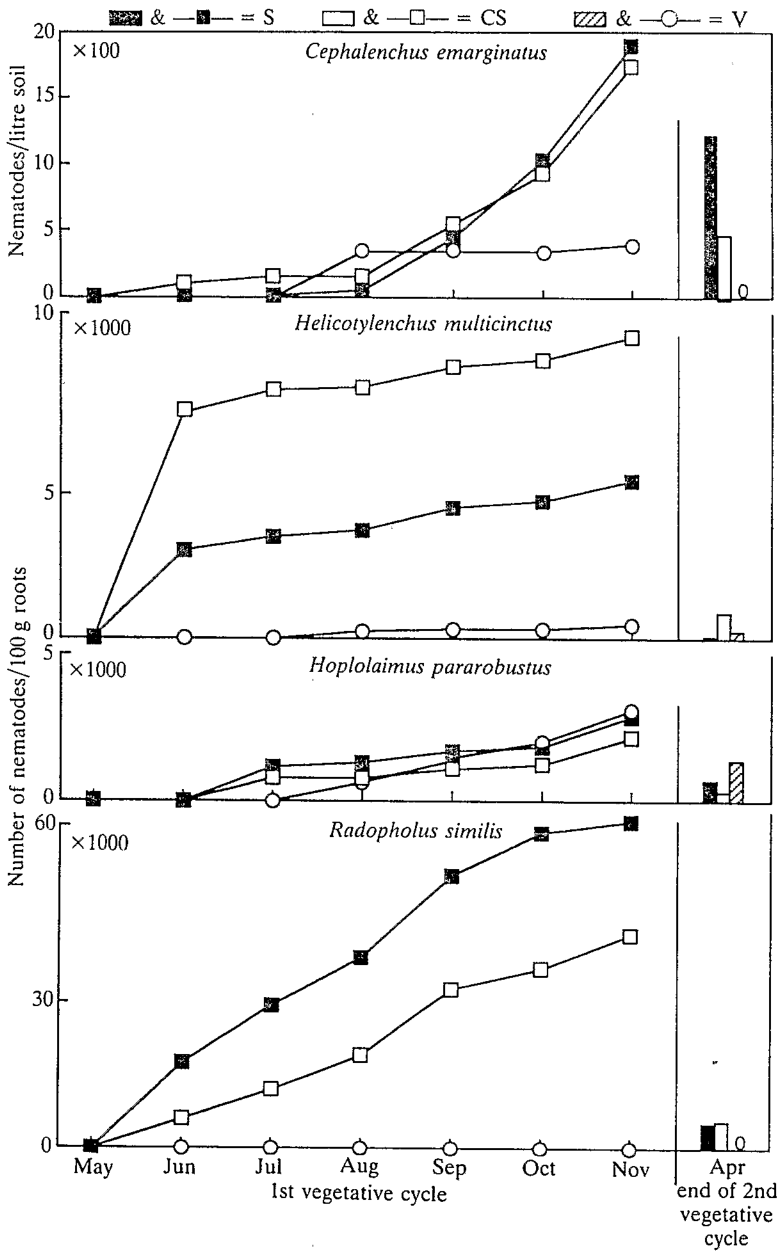

Fig. 4. Nematode populations in roots and soil under bananas cultivated on a clay-peat soil in the Nieky region after a 10-week flooding period. Treatments: i) planting with suckers (S); ii) planting with suckers coated with 1.2 g a.i. of liquid fenamiphos (CS); iii) planting with *vitro*-plants (V).

depend greatly on the choice of the planting material and, in these conditions, it is evident that *vitro*-plants will provide the best option.

Influence of the planting material

Quite apart from their distinct agro-physiological features, bullheads and suckers are also very different from *vitro*-plants in their host potential: the first two are always infested by

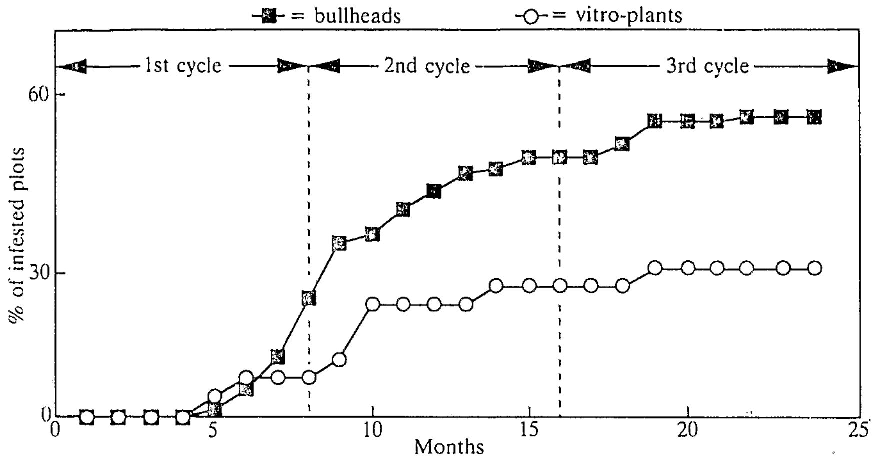

Fig. 5. Frequency of the plots in which infestation levels exceeded 4000 *Radopholus similis* per gram of roots, replanted with banana bullheads or vitro-plants after fallowing (Mateille, Adjovi & Hugon, 1992).

R. similis and sometimes also harbour other species (Quénéhervé & Cadet, 1985), while micropropagated banana plants are nematode-free.

In the field, different soil characteristics (texture, organic matter content, pH, soil biota) and the type of cultivated *Musa* result in the development of unique nematode communities (Quénéhervé, 1988) depending on: i) the endemic nematode species; and ii) the 'imported' nematode species (e.g. *R. similis*) within the banana-plant material. In these experiments four situations were described and it was clear that the use of *vitro*-plants in soil containing fewer pathogenic nematodes gave the best results. In contrast to the use of bullheads and suckers, planting *vitro*-plants after fallowing or flooding did not introduce any new species into the nematode community.

In banana farming in Côte d'Ivoire, when plots are properly treated (fallow or flooding), farmers can grow at least three vegetative cycles without any need for nematicide application. Agronomic studies (Mateille *et al.*, 1992) showed that the use of *vitro*-plants after fallow or flooding gave the best yields (rapid growth, decreased incidence of toppling, and greater bunch weights).

Fallowing and flooding (when flooding is feasible, e.g. on "polders") are two interesting nematode control measures in bananas. They both significantly decreased nematode populations (actually eradicating *R. similis* in certain circumstances) and consequently resulted in better yields. Both control techniques are improved by the use of *vitro*-plants free of nematodes so that, after weed fallow or flooding, further nematicide application was not required until at least the third vegetative cycle in our study.

It is also important to know for how long nematicide treatments for other pathogenic nematode species can be delayed. These include the known banana pathogen *H. multicinctus* (Minz *et al.*, 1960), but information on the pathogenicity to banana of *H. pararobustus* and *C. emarginatus* is scarce. During the past 20 years, *H. pararobustus* has spread all over the banana growing area of Côte d'Ivoire (Fargette & Quénéhervé, 1988; Luc & Vilardebo, 1961) and recent research has demonstrated its pathogenicity on banana cv. Poyo (Mateille, 1992, 1993). Similar threats could be posed by other species formerly considered to be only minor parasites (e.g. *Meloidogyne* spp., *Rotylenchulus reniformis, C. emarginatus*); thus, their pathogenicity should be studied.

Besides allowing genetic improvement of bananas for resistance to nematodes (not only to *R. similis* but to other parasitic banana species too), the new banana micropropagation techniques have resolved the problem of transmitting plant-parasitic nematodes to nematode-free sites on planting material. As weed fallows do not eradicate all pathogenic banana nematodes, further research should be conducted to seek alternative non-host plants for use as cover crops.

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