

# Ecology and pathogenicity of the Hoplolaimidae (Nemata) from the sahelian zone of West Africa.

## 6. *Hoplolaimus pararobustus* (Schuurmans Stekhoven & Teunissen, 1938) Sher, 1963 and comparison with *Hoplolaimus seinhorsti* Luc, 1958

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**Summary** – The geographical distribution, population dynamics and vertical distribution in the soil were studied for the nematode *Hoplolaimus pararobustus*. The factors influencing the multiplication and survival rates of *H. pararobustus* and *H. seinhorsti* were studied in the laboratory. Both species appeared adapted to the ecological conditions of the semi-arid tropics of West Africa. The absence of *H. seinhorsti* from these ecological zones remains unexplained; the possibility of the loss of reproduction capacity after anhydrobiosis is suggested. A the inoculum levels tested, these two species did not have any pathogenic effects on the plants with the exception of *H. pararobustus* which induced a reduction of root fresh weight for cowpea.

**Résumé** – *Écologie et nocuité des Hoplolaimidae (Nemata) de la zone sahélienne de l'Afrique de l'Ouest. 6. Hoplolaimus pararobustus (Schuurmans Stekhoven & Teunissen, 1938) Sher, 1963 et comparaison avec Hoplolaimus seinhorsti Luc, 1958* – La répartition géographique, la dynamique et la répartition verticale des populations sont étudiées pour le nématode *Hoplolaimus pararobustus*. Les facteurs influençant les taux de multiplication et de survie sont étudiés au laboratoire pour *H. pararobustus* et *H. seinhorsti*. Ces travaux montrent que ces deux espèces sont parfaitement adaptées aux conditions climatiques régnant dans la zone sahélienne ouest africaine. L'absence d'*H. seinhorsti* de ces biotopes reste donc inexplicquée; l'éventualité d'une perte de capacité reproductrice après l'entrée en anhydrobiose est évoquée. Aux taux d'inoculum testés, ces deux espèces n'ont pas d'effets sur la croissance des plantes à l'exception d'*H. pararobustus* qui induit une diminution du poids frais des racines pour le niébé.

**Key-words** : *Hoplolaimus pararobustus*, *Hoplolaimus seinhorsti*, nematode, West Africa, geographical distribution, population dynamics, vertical distribution, soil temperature, soil moisture, host plant, multiplication rate, anhydrobiosis pathogenicity.

Species of the genus *Hoplolaimus* are suspected or known to be parasites of numerous tropical crops in the world (Luc *et al.*, 1990). In Africa, two species of this genus, *Hoplolaimus pararobustus* (Schuurmans Stekhoven & Teunissen, 1938) Sher, 1963 and *H. seinhorsti* Luc, 1958, are widely distributed (Siddiqi, 1974; Van den Berg, 1976). *H. pararobustus* is identified in the soils of semi-arid tropics of West Africa in Mauritania (Baujard & Martiny, 1995 *a*), Senegal (Baujard & Martiny, 1995 *b*), Mali (Baujard & Martiny, 1994), Burkina Faso (Sharma, 1989), Niger (Sharma *et al.*, 1988, 1990, 1992), where *H. seinhorsti* has never been recorded.

This sixth paper on the ecology and pathogenicity of the Hoplolaimidae (Baujard & Martiny, 1995 *c, d, e, f, g*), presents the results of field and laboratory studies on *H. pararobustus* and *H. seinhorsti*.

### Material and methods

Studies on geographical distribution, field population dynamics and vertical distribution have been conducted as previously described (Baujard & Martiny, 1995 *c*). Unless otherwise stated, nematode extractions, nematode cultures and techniques, host plants and cultivars (peanut [*Arachis hypogea* L. cv. 55 437], millet [*Pennisetum typhoides* Rich. cv. Souna III], sorghum [*Sorghum vulgare* L. cv. 51 69], cowpea [*Vigna unguiculata* (L.) Walp.] cv. N 58 57 used for laboratory studies are those described by Baujard (1995).

### ORIGIN OF NEMATODES AND LABORATORY STOCK CULTURE CONDITIONS

*H. pararobustus* : nematodes originated from samples taken at Dombe, km 5 Diourbel to Thies road, Senegal,

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in a field previously cropped with millet in June 1981 during the dry season. The nematodes were extracted in August 1983 and reared on millet in the laboratory. From August 1983 to May 1989, the nematodes were reared without control of soil temperature or moisture; from May 1989 until May 1992, the soil temperature and moisture were kept constant respectively at 34 °C and 10 %.

*H. seinhorsti* : nematodes originated from a yam tuber from Martinique, French West Indies, sent to us by Dr. P. Cadet in May 1989. Nematodes were extracted in a mist chamber and reinoculated on *Zea mays* cv. HVB1, kenaf (*Hibiscus cannabinus* L.), sorghum and millet, and maintained at 32 °C constant soil temperature and 10 % constant soil moisture.

#### SOIL TEMPERATURE

*H. pararobustus* : in a first experiment, tubes were inoculated with 25 hand-picked nematodes (mixture of all stages) originating from 40-day-old stock cultures, planted with millet, and maintained at four constant soil temperature levels (30, 32, 34 or 36 °C) with seven replications for each temperature level, at 7 % constant soil moisture, for 75 days in a growth chamber with artificial lighting (16-h photoperiod). In a second experiment, tubes were inoculated with 67 ± 5 nematodes (mixture of all stages) originating from 100-day-old stock cultures, planted with sorghum, and maintained at four constant soil temperature levels (30, 32, 34 or 36 °C) with seven replications for each temperature level, at 10 % constant soil moisture, for 60 days in a growth chamber.

*H. seinhorsti* : tubes were inoculated with 91 ± 7 nematodes (mixture of all stages) originating from stock cultures on kenaf, millet and sorghum, planted with sorghum, and maintained at four constant soil temperature levels (30, 32, 34 or 36 °C) with seven replications for each temperature level, at 10 % constant soil moisture, for 60 days in a growth chamber.

#### SOIL MOISTURE

*H. pararobustus* : in a first experiment, tubes were inoculated with 110 hand-picked nematodes (mixture of all stages) originating from the first soil temperature experiment, planted with millet, and maintained at four constant soil moisture levels (5, 7, 9 or 11 %) at 34 °C constant soil temperature for 75 days in a greenhouse with natural lighting; the four treatments were replicated ten times in a completely randomized design. In a second experiment, tubes were inoculated with 94 ± 10 nematodes (mixture of all stages) originating from a 75-day-old stock culture on millet, planted with sorghum, and maintained at four constant soil moisture levels (5, 7, 9 or 11 %) at 34 °C constant soil temperature for 60 days in a greenhouse; the four treatments were replicated ten times in a completely randomized design.

*H. seinhorsti* : tubes were inoculated with 152 ± 12 nematodes (mixture of all stages) originating from soil and sorghum roots from the previous experiment on soil temperature, planted with sorghum, and maintained at four constant soil moisture levels (5, 7, 9 or 11 %) at 32 °C constant soil temperature for 60 days in a greenhouse; the four treatments were replicated ten times in a completely randomized design.

#### HOST PLANTS AND TEST FOR ANHYDROBIOTIC SURVIVAL

*H. pararobustus* : tubes were inoculated with 143 ± 14 nematodes (mixture of all stages) originating from a 100-day-old stock culture on millet, planted with one of the four host plants (cowpea, millet, peanut or sorghum), and maintained at 34 °C constant soil temperature and 10 % constant soil moisture in a greenhouse. The four treatments were replicated 20 times in a completely randomized design. After 60 days, nematodes were extracted from ten replications to obtain final population counts. At this time, watering was stopped for the ten remaining replications of each treatment. These tubes were kept at 34 °C constant soil temperature and weighed daily to follow the evolution of soil desiccation. Sixty days later, nematodes were extracted by elutriation.

*H. seinhorsti* : tubes were inoculated with 103 ± 7 nematodes (mixture of all stages) originating from sorghum roots of the previous experiment on soil moisture, planted with one of the four host plants (cowpea, millet, peanut or sorghum), and maintained at 32 °C constant soil temperature and 10 % constant soil moisture in a greenhouse. The experiment was conducted as described for *H. pararobustus*.

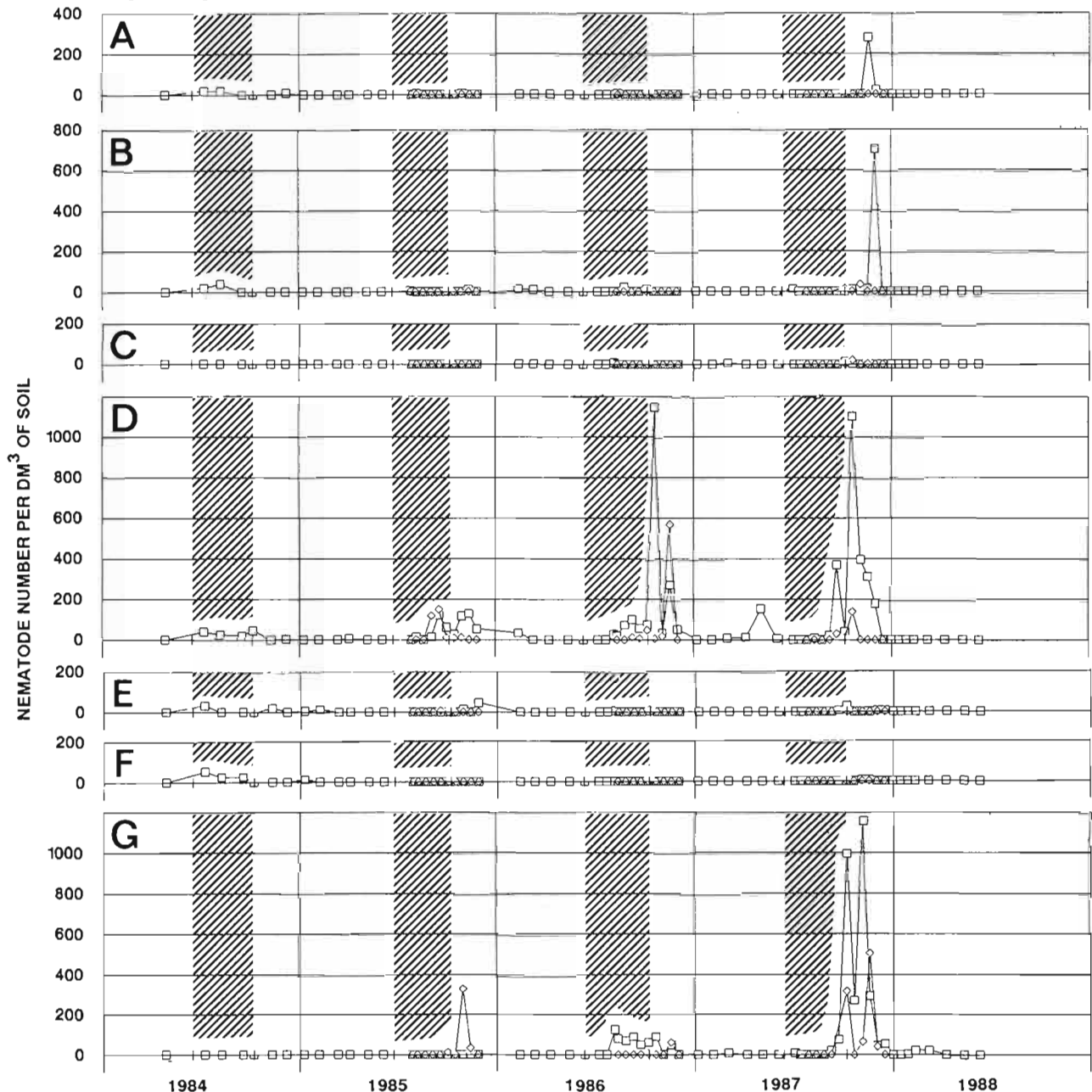
#### CULTURE DURATION

This experiment was conducted only with *H. pararobustus* : 100 hand-picked nematodes (mixture of all stages) originating from a 60-day-old stock culture on millet at constant soil temperature (34 °C) and soil moisture (10 %) were inoculated on millet at constant soil temperature (34 °C) and soil moisture (10 %) for three different durations (60, 75, 100 days) with seven replications per treatment in a growth chamber. At the end of each culture period, nematodes were extracted, counted and reinoculated in the same conditions (inoculum level, host-plant, soil temperature and moisture, durations) three times. In addition, nematodes originating from the « 75 days » treatment were reinoculated in the same experimental conditions for a culture duration of 60 days; those originating from the « 100 days » treatment were reinoculated in the same experimental conditions for a culture duration of 60 and 75 days in order to evaluate the effect of the previous culture duration on the multiplication rate.

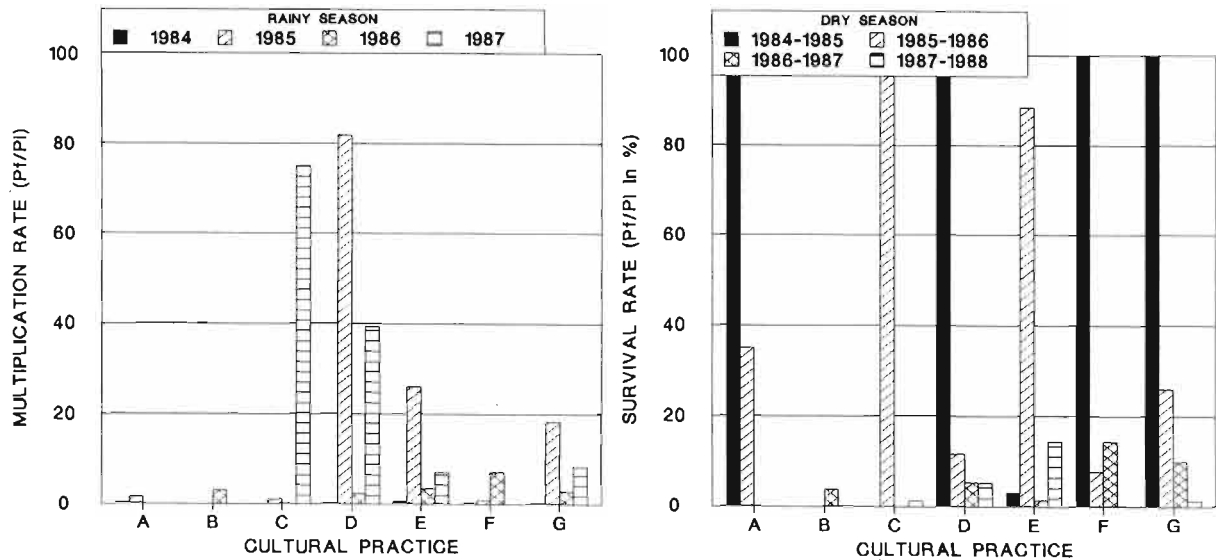
## PATHOGENICITY TO COWPEA, MILLET, PEANUT AND SORGHUM

*H. pararobustus* : separate experiments were conducted with each crop species at 34 °C soil temperature and 10 % soil moisture in the greenhouse. Nematodes (mixture of all stages) originating from 100-day-old stock

cultures on millet were inoculated onto each host at two inoculum levels : onto peanut at  $500 \pm 50$  or  $1000 \pm 100$ , onto millet at  $220 \pm 20$  or  $440 \pm 40$  in a first experiment and at  $500 \pm 20$  or  $1000 \pm 40$  in a second experiment, onto sorghum at  $500 \pm 40$  or  $1000 \pm 80$ , onto cowpea at  $350 \pm 40$  or  $700 \pm 80$  nematodes per tube. Nematode effects were compared to control plants without nema-



**Fig. 1.** Population dynamics of *Hoplolaimus pararobustus* according to the cultural practices. A : Peanut monoculture; B : Peanut-millet rotation without nematicidal treatment; C : Peanut-millet rotation with nematicidal treatment; D : Millet monoculture; E : Sorghum monoculture; F : Cowpea monoculture; G : Permanent fallow (Hatched areas : rainy seasons; squares : soil population; diamonds : root population).



**Fig. 2.** Multiplication and survival rates of *Hoplolaimus pararobustus* according to the cultural practices and year of observations (See Fig. 1 for the legend).

todes. The three treatments were replicated ten times in a completely randomized design. After 40 days, nematodes were extracted from soil and roots to determine the multiplication rate, and the fresh weight of roots and fresh and dry weights of shoots were measured.

*H. seinhorsti*: a separate experiment was conducted only with sorghum and cowpea at 32 °C soil temperature and 10 % soil moisture in the greenhouse. Nematodes (mixture of all stages) originating from roots of the soil moisture experiment were inoculated at two inoculum levels ( $325 \pm 23$  or  $650 \pm 46$  nematodes per tube) and other nematodes (mixture of all stages) originating from soil and roots of a 49-day-old stock culture on sorghum were inoculated at two inoculum levels onto cowpea ( $500 \pm 35$  or  $1\ 000 \pm 70$  nematodes per tube). Nematode effects were compared to control plants without nematodes. The three treatments were replicated ten times in a completely randomized design. After 40 days, nematodes were extracted from soil to determine the multiplication rate, and the fresh weight of roots and the fresh and dry weights of shoots were measured.

## Results

### GEOGRAPHICAL DISTRIBUTION AND HOSTS OF *H. PARAROBUSTUS* IN SENEGAL

*H. pararobustus* is a ubiquitous species in the soils of Senegal where it occurred during the dry and the rainy seasons in low numbers (0-2 000 nematodes per  $\text{dm}^3$ ) associated with pluvial crops [*Arachis hypogea* L., *Pennisetum typhoides* Rich., *Sorghum vulgare* L., *Vigna unguiculata* (L.) Walp., *Hibiscus sabdariffa* L.] and several annual and wild plants [*Cenchrus biflorus* Roxb., *Ses-*

*bania pachycarpa* DC., *Andropogon guayanus* Hack., *Icacina senegalensis* A. Juss., *Piliostigma reticulatum* (DC.) Hochst., *Acacia albida* Del., *Acacia Senegal* (L.) Willd., *Acacia nilotica* (L.) Willd., *Acacia tortilis* ssp. *raddiana* Savi., *Prosopis juliflora* DC.]. It has never been found in the rhizosphere of *Euphorbia balsamifera* Ait., a plant commonly used as a green fence in this area.

### FIELD STUDIES ON *H. PARAROBUSTUS*

#### Population dynamics

The soil population increased from the beginning to the end of the rainy season slowly in the rhizosphere of peanut, sorghum, cowpea and fallow plants and strongly in the rhizosphere of millet. Nematodes invaded the root system only on millet where root populations never exceeded 200 nematodes per root system. At the end or just after the end of the rainy season, the population density decreased rapidly and remained constant throughout the dry season (Fig. 1). Multiplication rates during the rainy season varied from 0 to 80 and survival rates during the dry season from 0 to 100 % according to the crop and to the year. For millet, sorghum, cowpea, and fallow, an alternation of rainy seasons with high and low multiplication rates occurred (Fig. 2).

Although millet appeared to be a good host for the nematode, no multiplication was recorded with this plant in 1985 and 1987 on the plots under the peanut-millet rotation (Fig. 1).

#### Vertical distribution in the soil

The nematode was erratically distributed in the soil, from the surface down to a depth of 80 cm; it was mostly predominant in the upper layers of the soil (Fig. 3).

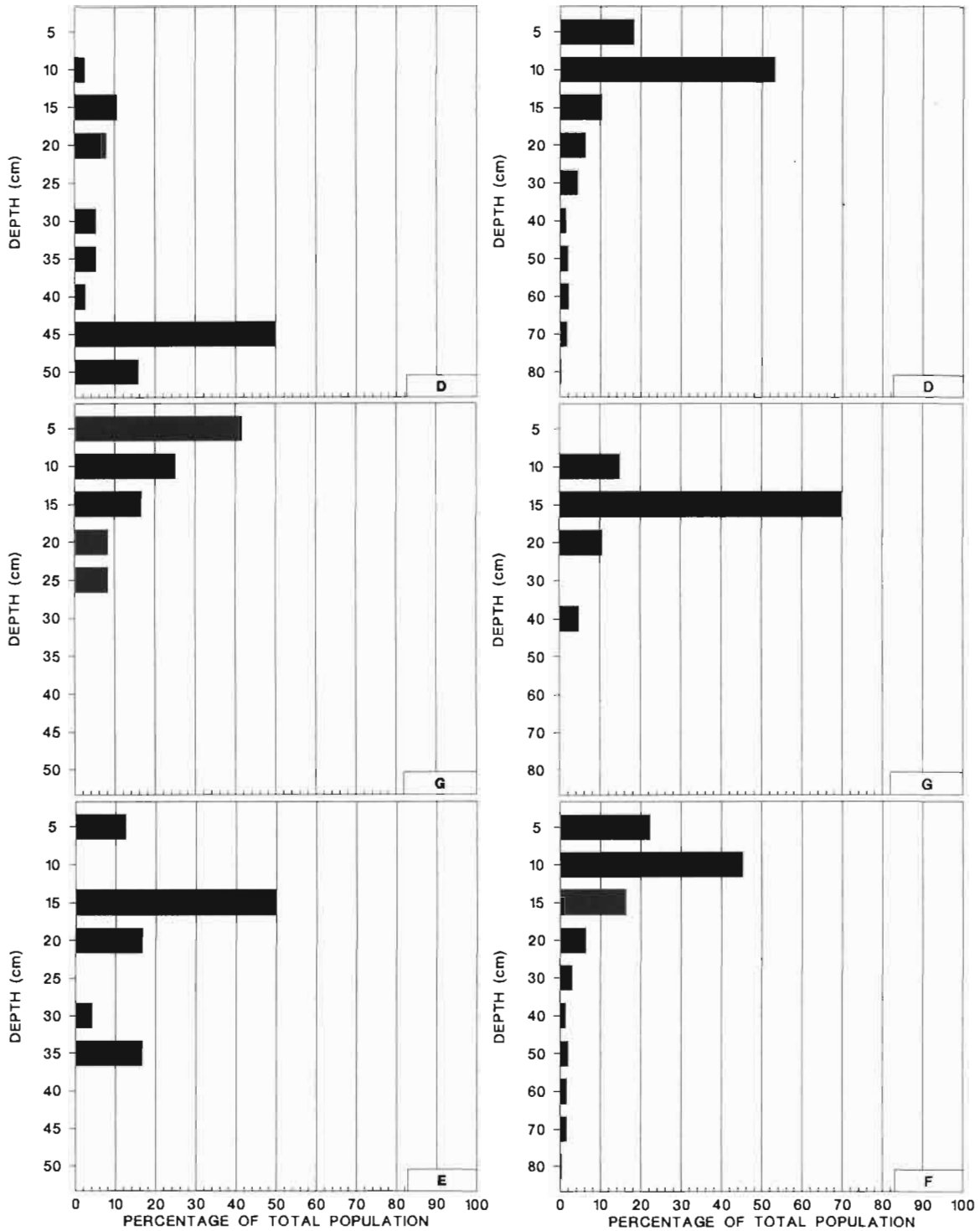


Fig. 3. Vertical distribution of *Hoplolaimus pararobustus* according to the cultural practices and the season of observation. (See Fig. 1 for the legend; left column : dry season; right column : rainy season).

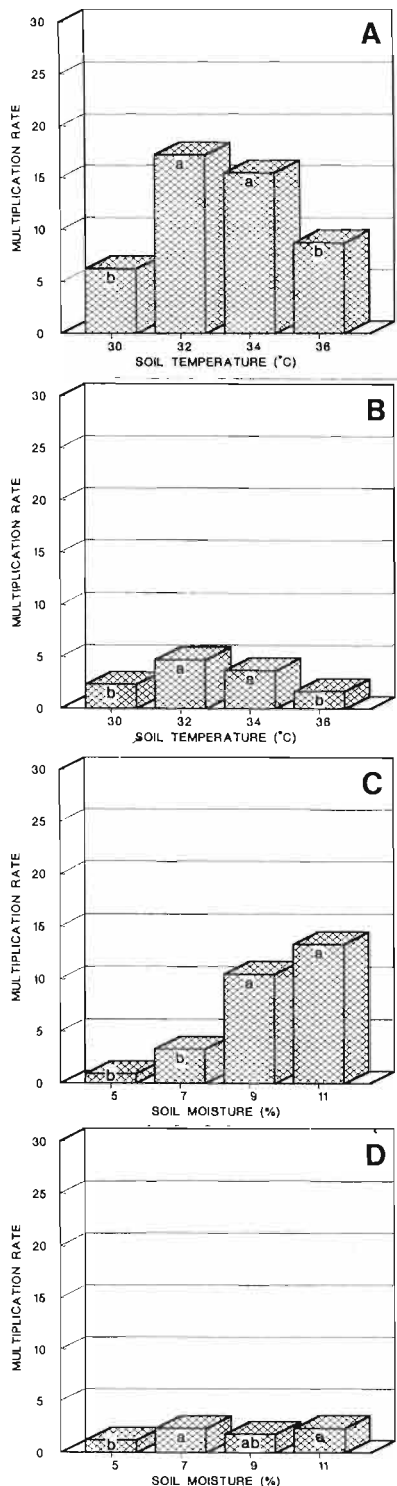


Fig. 4. Effects of soil temperature (A, B), and soil moisture (C, D) on the multiplication rate of *Hoplolaimus pararobustus* on millet (A-C) and sorghum (B, D). (In each experiment, data followed by the same letter are not significantly different at  $P \leq 0.05$ ).

LABORATORY STUDIES

Observations on laboratory stock cultures

*H. pararobustus* : during the first period of stock culture, multiplication rates varied from 0.12 to 2.77 ( $\bar{x} = 2.22 \pm 1.76$ ,  $n = 29$ ) and during the second period, from 1.01 to 41.6 ( $\bar{x} = 9.61 \pm 10.09$ ,  $n = 36$ ). Sex ratio varied from 36 to 150 % ( $\bar{x} = 87 \pm 28$ ,  $n = 12$ ).

*H. seinhorsti* : the nematode reproduced on maize, millet, sorghum and kenaf with multiplication rates of 3-15, 20-31.9, 9.84-74.22, and 46.65 respectively. No males occurred in the stock cultures.

Factors affecting the multiplication rate

Soil temperature affected significantly the multiplication rate of both the species in the same way, higher multiplication occurring at 32-34 °C (Figs 4, 7). Soil moisture affected significantly the multiplication rate only of *H. pararobustus* on millet and sorghum (Figs 4, 7). The use of millet or sorghum as host plant for *H. pararobustus* in these experiments did not modify these trends. The lower multiplication rates observed for *H. pararobustus* under sorghum in comparison with millet can be related to the effect of host plant and/or of the effect of the duration of the experiment (75 days with millet vs 60 days with sorghum). Host plants had a significant effect on the multiplication rate of both species. All the plants allowed the reproduction of *H. seinhorsti*, whereas only millet and sorghum allowed it for *H. pararobustus*. Millet appeared to be the best host for *H. pararobustus* and cowpea for *H. seinhorsti* (Figs 5, 6). Mul-

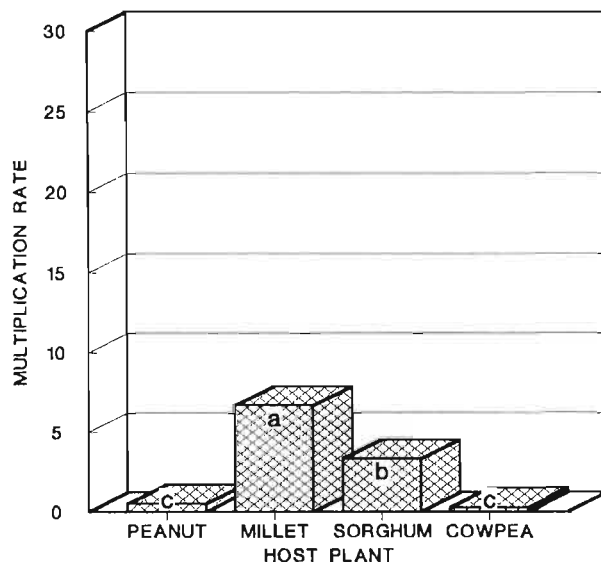


Fig. 5. Effects of host plants on the multiplication rate of *Hoplolaimus pararobustus*. (In each experiment, data followed by the same letter are not significantly different at  $P \leq 0.05$ ).

tiplication rates recorded for *H. seinhorsti* under sorghum in this experiment were four to five times higher than in the experiments on soil temperature and soil moisture (Fig. 6). This difference could really be related only to the origin of the inoculum : soil and root populations in the experiments on soil temperature and moisture *vs* only root populations in the experiment on host plant.

The multiplication rate of *H. pararobustus* increased with an increase in the duration of the culture; the duration of the previous culture did not affect the multiplication rate of the nematode (Fig. 6).

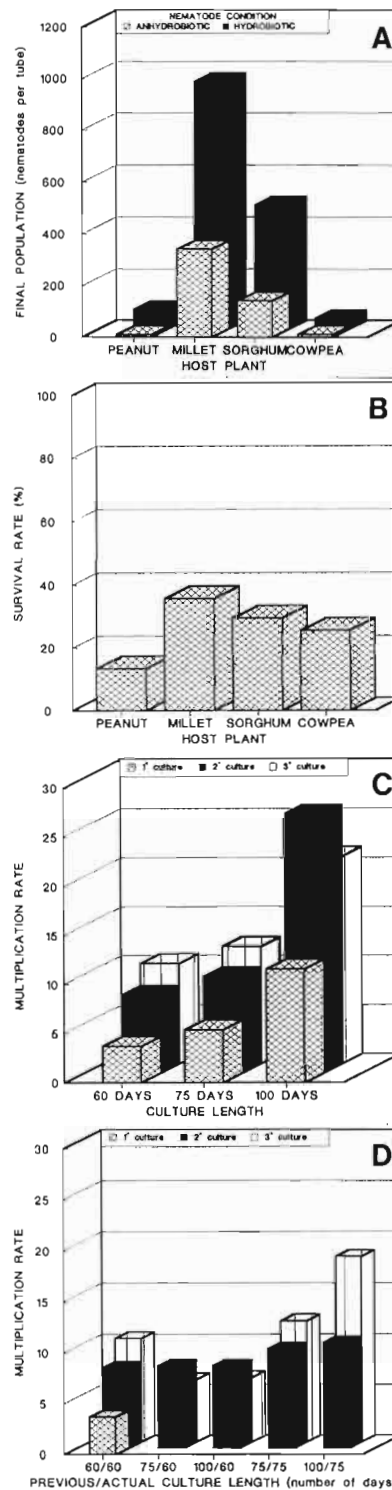
Root population levels with millet and sorghum were constant in these experiments; they were not affected by environmental factors and differed according to the species : 20-68.5 % for *H. pararobustus* *vs* 66.1-85.8 % for *H. seinhorsti*; with poor host plants, these levels were below 10 % (Table 1).

*Ability to enter anhydrobiosis*

Cessation of watering induced a decrease of soil moisture down to 0.2 % in 5 days for *H. pararobustus* and down to 0.5 % in 15 days for *H. seinhorsti*. Nematodes survived soil desiccation over a 60-day period according to the size of the population before watering was stopped for the four host plants for *H. pararobustus* (Fig. 6);

**Table 1.** Percentages of the population of *Hoplolaimus pararobustus* and *Hoplolaimus seinhorsti* in the roots at the end of the different experiments on multiplication rate. (ND = not determined).

Experiment	Treatment	Root population as a % of total tube population		
		<i>H. pararobustus</i>		<i>H. seinhorsti</i>
		Millet	Sorghum	
Soil temperature	30 °C	68.5	31.8	82.2
	32 °C	63.3	52.2	74.4
	34 °C	68.4	31.3	78.3
	36 °C	54.9	45.4	66.1
Soil moisture	5 %	28.0	20.0	78.1
	7 %	41.4	34.5	73.2
	9 %	59.3	34.1	73.0
	11 %	45.7	39.3	78.6
Host plants	peanut	1.5		9.1
	millet	38.5		69.1
	sorghum	44.2		80.1
	cowpea	2.8		85.8
Culture length	60/60	42.1		ND
	75/75	41.4		ND
	100/100	35.6		ND



**Fig. 6.** Effects of soil desiccation on soil population (A) and survival rate (B) and effect of culture length on multiplication rate (C, D) of *Hoplolaimus pararobustus*.



**Table 2.** Percentages of the population of *Hoplolaimus pararobustus* and *Hoplolaimus seinhorsti* in the roots at the end of the different experiments on pathogenicity. (ND = not determined).

Experiment	Treatment	Root population as a % of total tube population	
		<i>H. pararobustus</i>	<i>H. seinhorsti</i>
Peanut	* 500	2.9	ND
	* 1 000	2.3	ND
Millet	* 220	36.0	ND
	* 440	29.1	ND
	* 500	61.3	ND
	* 1 000	58.5	ND
Sorghum	* 500	32.3	ND
	* 1 000	30.8	ND
	* 325	ND	81.4
	* 650	ND	85.6
Cowpea	* 350	56.4	ND
	* 700	61.4	ND
	* 500	ND	91.3
	* 1 000	ND	90.4

\* Inoculum per tube.

for *H. seinhorsti*, the number of nematodes surviving soil desiccation was constant for millet, sorghum and cowpea (Fig. 7). Survival rates varied according to the host plant and to the nematode species : 13-36 % for *H. pararobustus* vs 9-22 % for *H. seinhorsti* (Figs 6, 8).

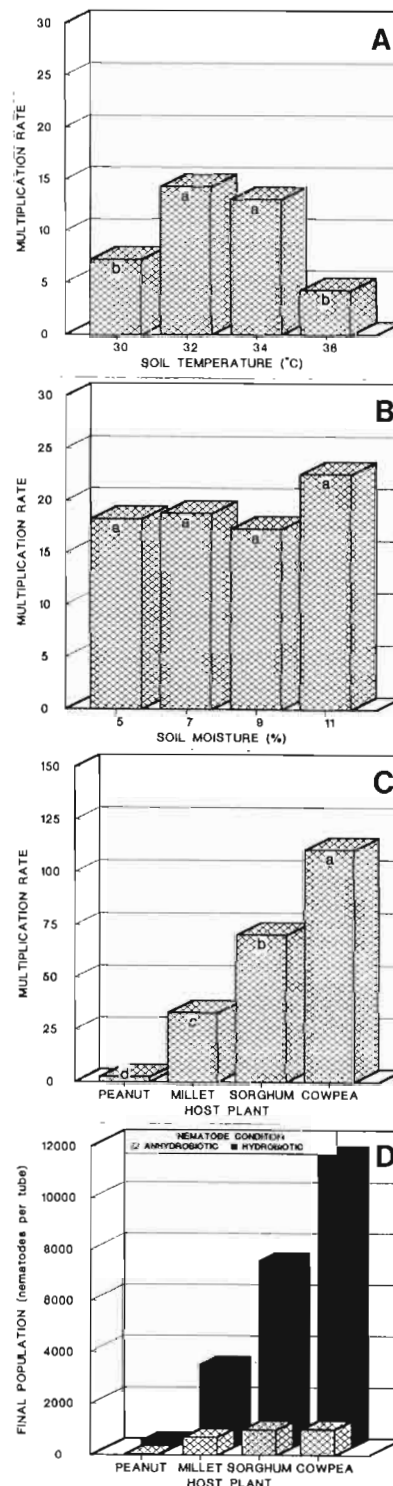
*Pathogenicity*

Neither species had any significant effect on growth of plants at the inoculum levels tested on peanut, millet, sorghum or cowpea (Tables 3, 4). Only *H. pararobustus* induced a reduction of cowpea fresh root weight at the rate of 1000 nematodes per plant. Root population levels varied as in the previous experiments (Table 2).

**Discussion**

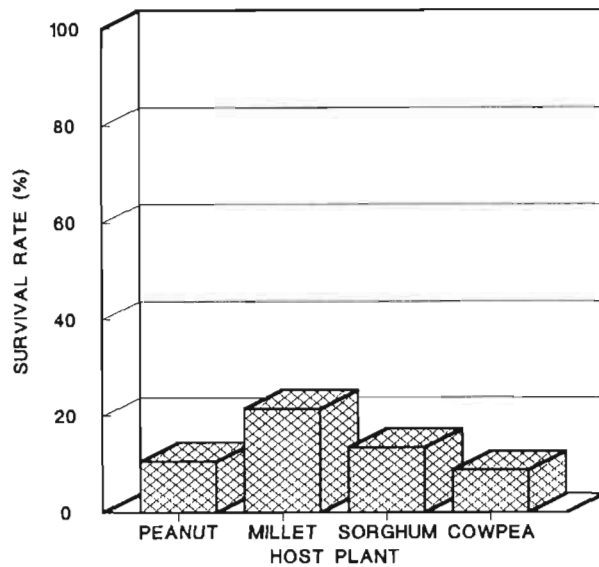
Laboratory experiments conducted with *H. pararobustus* confirmed the field observations on population dynamics and supported the currently known distribution of this species from wet to semi-arid tropics of West Africa. Cereals are good hosts for this species which is able to support high soil temperature levels and to enter anhydrobiosis. Reproduction is dependent directly and indirectly (duration of activity in the soil) on the soil moisture.

These experiments showed that *H. seinhorsti* exhibited the same behaviour as *H. pararobustus* in relation to temperature and soil moisture. It appeared better adapted to the ecological conditions of the sahelian zone of West Africa since it is more polyphagous and its multi-



**Fig. 7.** Effects of soil temperature (A), soil moisture (B) and host plants (C) on the multiplication rate and effects of soil desiccation on soil population (D) of *Hoplolaimus seinhorsti*. (In each experiment, data followed by the same letter are not significantly different at  $P \leq 0.05$ ).





**Fig. 8.** Effects of host plants and soil desiccation on survival rate of *Hoplolaimus seinhorsti*.

**Table 3.** Multiplication rate and effects of *Hoplolaimus pararobustus* on peanut and millet. (Numbers followed by the same letter are not significantly different at  $P \leq 0.05$ ).

Plant	Inoculum	Multiplication rate	Fresh weight (g)		Dry weight (g)
			Roots	Shoots	Shoots
Peanut	0	-	2.34 a	7.95 a	1.37 a
	500	0.67	2.03 a	6.96 a	1.15 a
	1000	0.48	2.03 a	7.71 a	1.32 a
Millet	0	-	4.19 a	4.10 a	0.64 a
	220	0.70	2.99 a	4.41 a	0.72 a
	440	0.29	2.98 a	4.95 a	0.85 a
Sorghum	0	-	3.08 a	5.63 a	0.78 a
	500	4.68	2.53 a	5.82 a	0.78 a
	1000	3.96	2.52 a	5.76 a	0.85 a
Cowpea	0	-	4.91 a	5.75 a	1.00 a
	500	1.19	4.71 a	6.06 a	1.02 a
	1000	1.27	4.87 a	6.12 a	1.10 a
Cowpea	0	-	4.98 a	5.07 a	1.02 a
	350	1.14	4.79 a	5.98 a	1.18 a
	700	1.33	4.01 b	6.28 a	1.28 a

plication rate was not affected by soil moisture. Therefore, the absence of this species from the semi-arid tropics of West Africa remains unexplained. The experiment on anhydrobiotic survival showed that the

**Table 4.** Multiplication rate and effects of *Hoplolaimus seinhorsti* on peanut and millet. (Numbers followed by the same letter are not significantly different at  $P < 0.05$ ).

Plant	Inoculum	Multiplication rate	Fresh weight (g)		Dry weight (g)
			Roots	Shoots	Shoots
Peanut	0	-	2.71 a	5.07 a	0.80 a
	325	15.12	2.37 a	5.20 a	0.77 a
	650	13.99	2.21 a	4.89 a	0.76 a
Cowpea	0	-	3.76 a	6.32 a	1.28 a
	500	2.29	4.29 a	6.54 a	1.21 a
	1000	1.88	4.00 a	6.82 a	1.20 a

survival rate of *H. seinhorsti* is 50 % that of *H. pararobustus* and independent of the size of the population before-soil drying. Survival ability to the long dry season (up to 9 months) occurring in semi-arid tropics of West Africa remains unknown since the experiment was conducted only for 60 days and without any evaluation of the reproduction capacity of the nematodes after anhydrobiosis.

*H. pararobustus* appeared pathogenic only on cowpea, inducing a reduction of fresh weight of roots. Little is known about pathogenicity of these two species. Goodey (1957) and Whitehead (1959) respectively reported the presence of *H. pararobustus* under the names *H. proporicus* and *H. augustulatus* in the roots of oil palm and banana exhibiting rotting symptoms. No laboratory data are available on the pathogenicity of this species. Bridge (1973) showed that *H. seinhorsti* parasitized cowpea in Nigeria, whose roots exhibited necrosis in the field. The nematode was also found associated with poor growth of pigeon pea in India (Sikora & Greco, 1990).

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