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Ecology and pathogenicity of the Hoplolaimidae (Nemata) from the sahelian zone of West Africa. 7. *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961 and comparison with *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956

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Summary – The geographical distribution and field host plants, population dynamics and vertical distribution were studied for the nematode *Helicotylenchus dihystera*. The factors influencing the multiplication rate and the effects of anhydrobiosis were studied for *H. dihystera* and *H. multicinctus* in the laboratory and showed that absence of *H. multicinctus* from semi-arid tropics of West Africa might be explained by the effects of high soil temperature on multiplication rate and low survival rate after soil desiccation during the dry season. The field and laboratory observations showed that anhydrobiosis might induce a strong effect on the physiology of *H. dihystera,* nematode numbers being higher after soil desiccation during the dry season. *H. dihystera* appeared pathogenic to peanut and millet.

Résumé – ***Écologie et nocuité des Hoplolaimidae (Nemata) de la zone sahélienne de l'Afrique de l'Ouest.* 7. Helicotylenchus dihystera *(Cobb, 1893) Sher, 1961 et comparaison avec* Helicotylenchus multicinctus *(Cobb, 1893) Golden, 1956*** – La répartition géographique et les plantes hôtes, la dynamique des populations et la répartition verticale ont été étudiées pour le nématode *Helicotylenchus dihystera*. Les facteurs influençant le taux de multiplication et les effets de l'anhydrobiose ont été étudiés au laboratoire pour *H. dihystera* et *H. multicinctus,* montrant que l'absence d'*H. multicinctus* de la zone sahélienne d'Afrique de l'Ouest pourrait être expliquée par les effets des fortes temperatures du sol et le faible taux de survie après le dessèchement du sol. Les observations conduites au champ et au laboratoire montrent que l'anhydrobiose induirait de profondes modifications physiologiques chez *H. dihystera,* les taux de population dénombrés étant plus élevés après qu'avant le dessèchement du sol. La nocuité d'*H. dihystera* vis-à-vis de l'arachide et du mil est démontrée.

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

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

Helicotylenchus dihystera (Cobb, 1893) Sher, 1961 and *H. multicinctus* (Cobb, 1893) Golden, 1956 are two widely distributed, tropical and polyphagous nematode species (Siddiqi, 1972, 1973). *H. dihystera* was detected in most of the countries of West Africa in both semi-arid and rainy tropics : Ivory Coast, Liberia, Nigeria, Senegal (Sher, 1966), Cameroun (Ali & Geraert, 1975), Benin (Sharma, 1989), Burkina Faso (Cadet, 1986), Mali (Baujard & Martiny, 1994), Mauritania (Baujard & Martiny, 1995 *a*). *H. multicinctus* is widely distributed in banana growing regions worldwide (Sher, 1966), but has only been recorded one time in the semi-arid tropics of West Africa in fruit and vegetable gardens of Gambia (Merny *et al.,* 1974).

Material and methods

Studies on geographical distribution, field population dynamics, and vertical distribution have been conducted as previously described (Baujard & Martiny, 1995 *b*). Unless otherwise stated, nematode extraction, nematode cultures, technics, 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).

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Origin of nematode and laboratory stock culture conditions

H. dihystera originated from soil samples taken at N'Dindy, km 25, Diourbel to Darou Mousty road, Senegal, in 1982 during the dry season. The soil was kept in the laboratory and cropped with millet in the greenhouse two years later. The nematodes were extracted after 2 months and reared on millet at 34 °C constant soil temperature and 10 % constant soil moisture in the laboratory until May 1992.

H. multicinctus originated from roots of banana collected at Bula, Guinea-Bissau in April 1990. Nematodes were extracted in a mist chamber and reared on cowpea at 30 °C constant soil temperature and 10 % constant soil moisture in the laboratory until May 1992.

Soil temperature

H. dihystera : in a first experiment, tubes were inoculated with ten 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 10 % constant soil moisture, for 75 days in a growth chamber with artificial lighting (16-h photoperiod). In a second experiment, tubes were inoculated with 97 ± 11 nematodes (mixture of all stages) originating from 75-day-old stock cultures, planted with millet, and maintained at 9 % constant soil moisture, for 60 days in a growth chamber. In a third experiment, tubes were inoculated with 96 ± 6 nematodes (mixture of all stages) originating from 60-day-old stock cultures, planted with sorghum, and maintained at 10 % constant soil moisture, for 60 days in a growth chamber.

H. multicinctus : tubes were inoculated with 46 ± 2 nematodes (mixture of all stages) originating from roots of stock cultures on cowpea, planted with cowpea, and maintained at 10 % constant soil moisture for 60 days as mentioned above.

Soil moisture

H. dihystera : in a first experiment, tubes were inoculated with 557 ± 39 nematodes (mixture of all stages) originating from 90-day-old stock cultures, 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 89 ± 10 nematodes (mixture of all stages) originating from a 60-day-old stock culture on millet, planted with sorghum, and maintained for 60 days as mentioned above.

H. multicinctus : tubes were inoculated with 87 ± 8 nematodes (mixture of all stages) originating from soil and roots from 60-day-old stock cultures, planted with cowpea and maintained at 30 °C constant soil temperature for 60 days as mentioned above.

Host plants and anhydrobiotic survival

H. dihystera : in a first experiment, tubes were inoculated with 991 ± 134 nematodes (mixture of all stages) originating from the first experiment on soil moisture, planted with one of the four host plants (cowpea, millet, peanut or sorghum), and maintained at 34 °C constant soil temperature and 11 % constant soil moisture in a greenhouse. The four treatments were replicated ten times in a completely randomized design. After 75 days, nematodes were extracted. In a second experiment, tubes were inoculated with 89 ± 10 nematodes (mixture of all stages) originating from a 60-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 twenty 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 10 remaining replications of each treatment. These tubes were kept at 34 °C constant soil temperature and weighed daily to follow the progress of soil desiccation. Sixty days later, nematodes were extracted by elutriation.

H. multicinctus : tubes were inoculated with 87 ± 8 nematodes (mixture of all stages) originating from soil and roots of 60-day-old stock cultures, planted with one of the four host plants (cowpea, millet, peanut or sorghum), and maintained at 30 °C constant soil temperature and 10 % constant soil moisture in a greenhouse. Experiment design was the same as mentioned above for the second experiment with *H. dihystera;* during soil desiccation, tubes were kept at 30 °C constant soil temperature.

Pathogenicity of *H. dihystera* to millet and peanut

Two separate experiments were conducted with each crop species at 34 °C soil temperature and 10 % soil moisture in a greenhouse. Nematodes (mixture of all stages) originating from 60-day-old stock cultures on millet were inoculated onto each host at two inoculum levels : 500 ± 30 or 1000 ± 60. 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 and roots to determine the multiplication rate, and the fresh weight of roots and fresh and dry weights of shoots were measured.

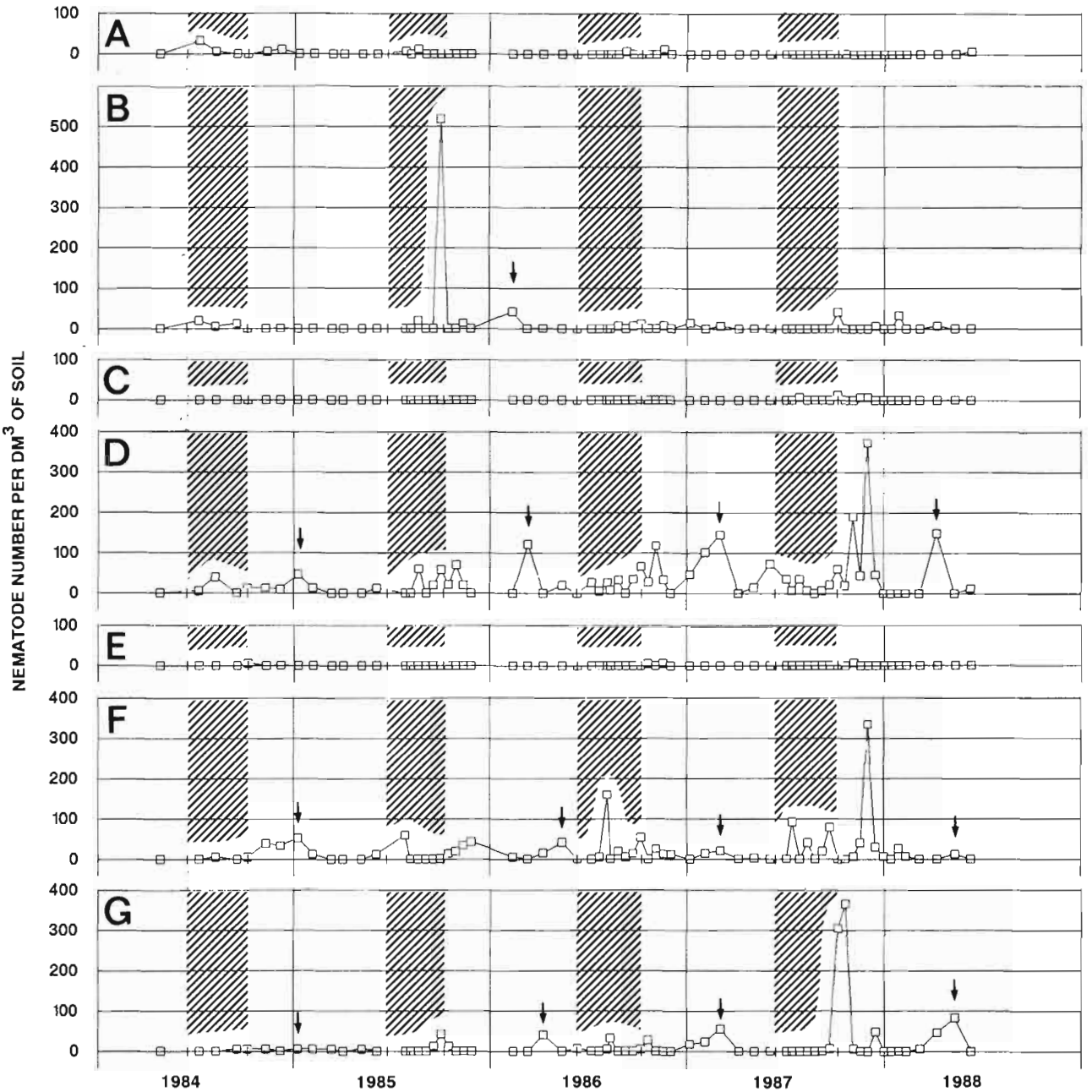

Fig. 1. *Population dynamics of* Helicotylenchus dihystera *according to the cultural practices. A : Peanut monoculture; B : Peanut-millet rotation without nematicidal treatment; C : Peanut-millet rotation with nematicidal treatment; D : Millet monoculture; E : Sorghum monoculture; F : Cowpea monculture; G : Permanent fallow.* (Hatched areas = rainy seasons; the arrows indicate " abnormal " population increases during the dry season).

Results

Geographical distribution and hosts of *H. dihystera* in Senegal

H. dihystera is a ubiquitous species in the soils of Senegal where it occurred during the dry and the rainy seasons in low numbers (0-2000 nematodes per dm^3) associated with several plants : *Arachis hypogea* L., *Pennisetum typhoides* Rich., *Sorghum vulgare* L., *Vigna unguiculata* (L.) Walp., *Hibiscus sabdariffa* L., *Cenchrus biflorus* Roxb., *Sesbania 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 green fence in this area.

Field studies on *H. dihystera*

Population dynamics

In the field, multiplication of *H. dihystera* occurred only on millet, cowpea and fallow plants. Multiplication occurred on peanut only during the first rainy season (Fig. 1). The soil population increased from the middle of the rainy season up to the beginning of the following dry season. No nematodes were recovered from plant roots. During all the dry seasons, increases in population densities were detected (see arrows on Fig. 1) although nematodes were in anhydrobiotic condition (soil moisture below 0.2 %). Multiplication rates during the rainy season varied from 0 to 100 and survival rates during the dry season from 0 to more than 100 % according to the crop and to the year (Fig. 2).

Vertical distribution in the soil

Vertical distribution pattern differed according to the cultural practice and time of observation. During the dry season, *H. dihystera* was erratically distributed in the soil for millet and fallow, from the top down to 50 cm deep. For cowpea, it appeared more abundant in the upper layers of the soil. During the dry season, the nematodes were distributed more homogeneously under millet and fallow; they were not recovered from cowpea (Fig. 3).

Laboratory studies

Factors affecting the multiplication rate

Soil temperature affected significantly the multiplication rate of *H. dihystera* with millet; higher multiplication rates occurred at 34 °C and were not affected by either the inoculum level or the duration of the experiment (Fig. 4 A, B). With sorghum, the soil temperature did not affect significantly the multiplication rate, which was lower than with millet (Fig. 4 C). Soil temperature affected significantly the multiplication rate of *H. multicinctus,* higher multiplication occurring at the lowest temperature tested (Fig. 5).

Soil moisture affected significantly the multiplication rate of *H. dihystera* but not *H. multicinctus* (Figs 4 D, E, 5). The nematode response to soil moisture level differed slightly according to the host plant (Fig. 4 D, E); comparison of the first two experiments on soil temperature and of the first experiment on soil moisture showed that, for *H. dihystera* on millet, the increase of

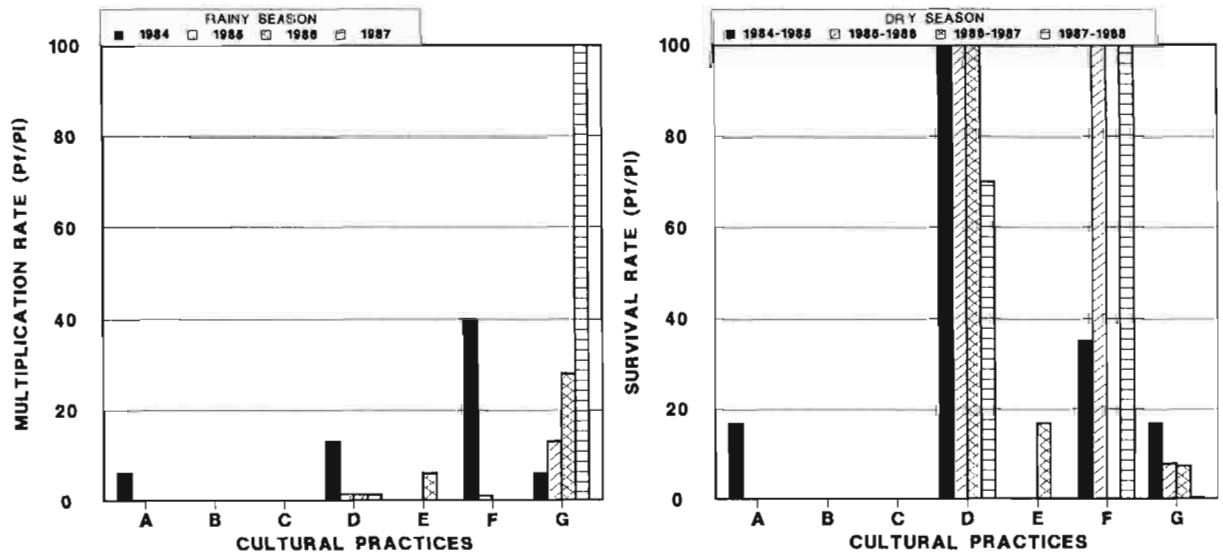

Fig. 2. *Multiplication and survival rates of* Helicotylenchus dihystera *according to the cultural practices and year of observations* (See Fig. 1 for the legend).

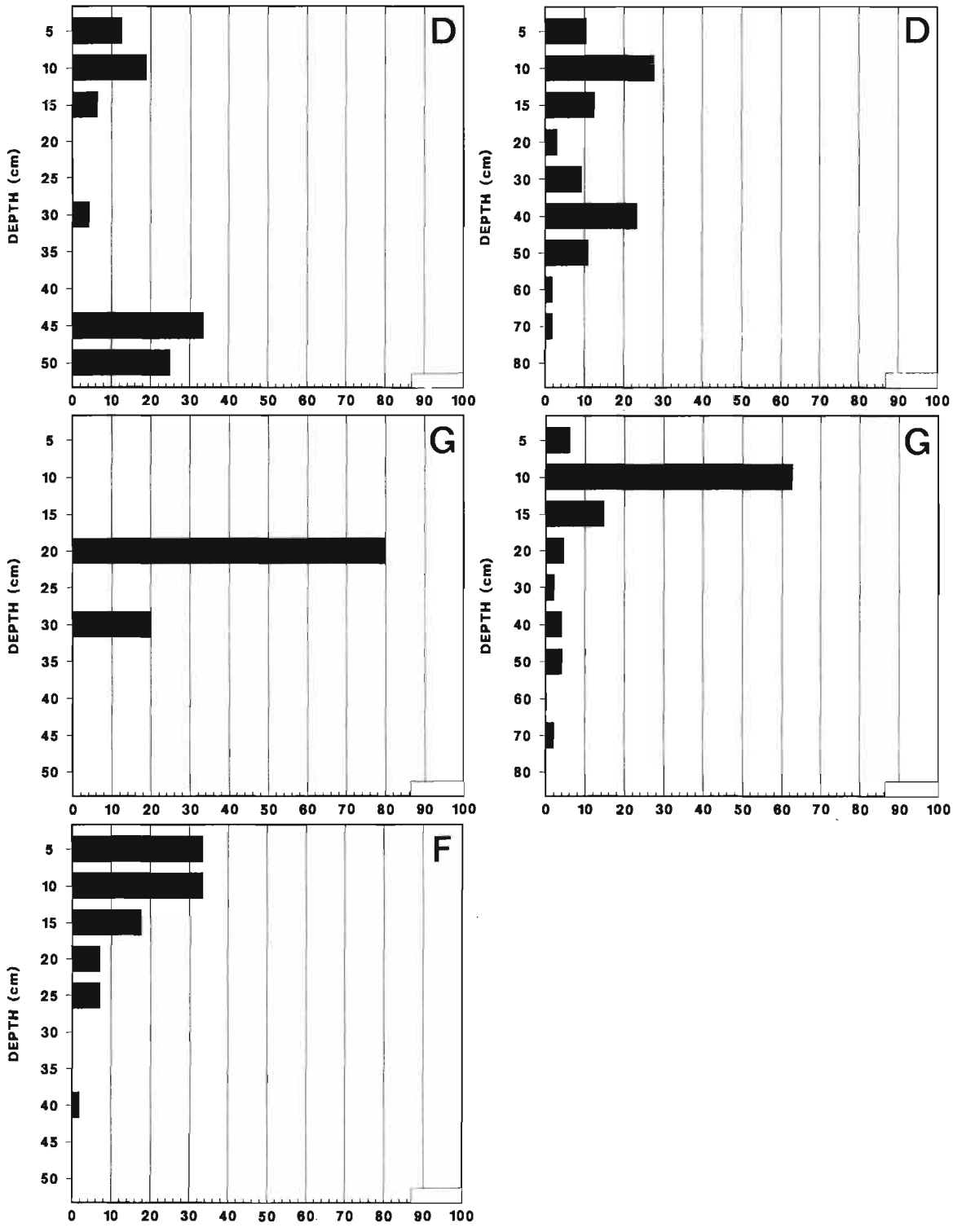

Fig. 3. *Vertical distribution of* Helicotylenchus dihystera *according to the cultural practices and the season of observation* (See Fig. 1 for the legend; left column : dry season; right column : rainy season).

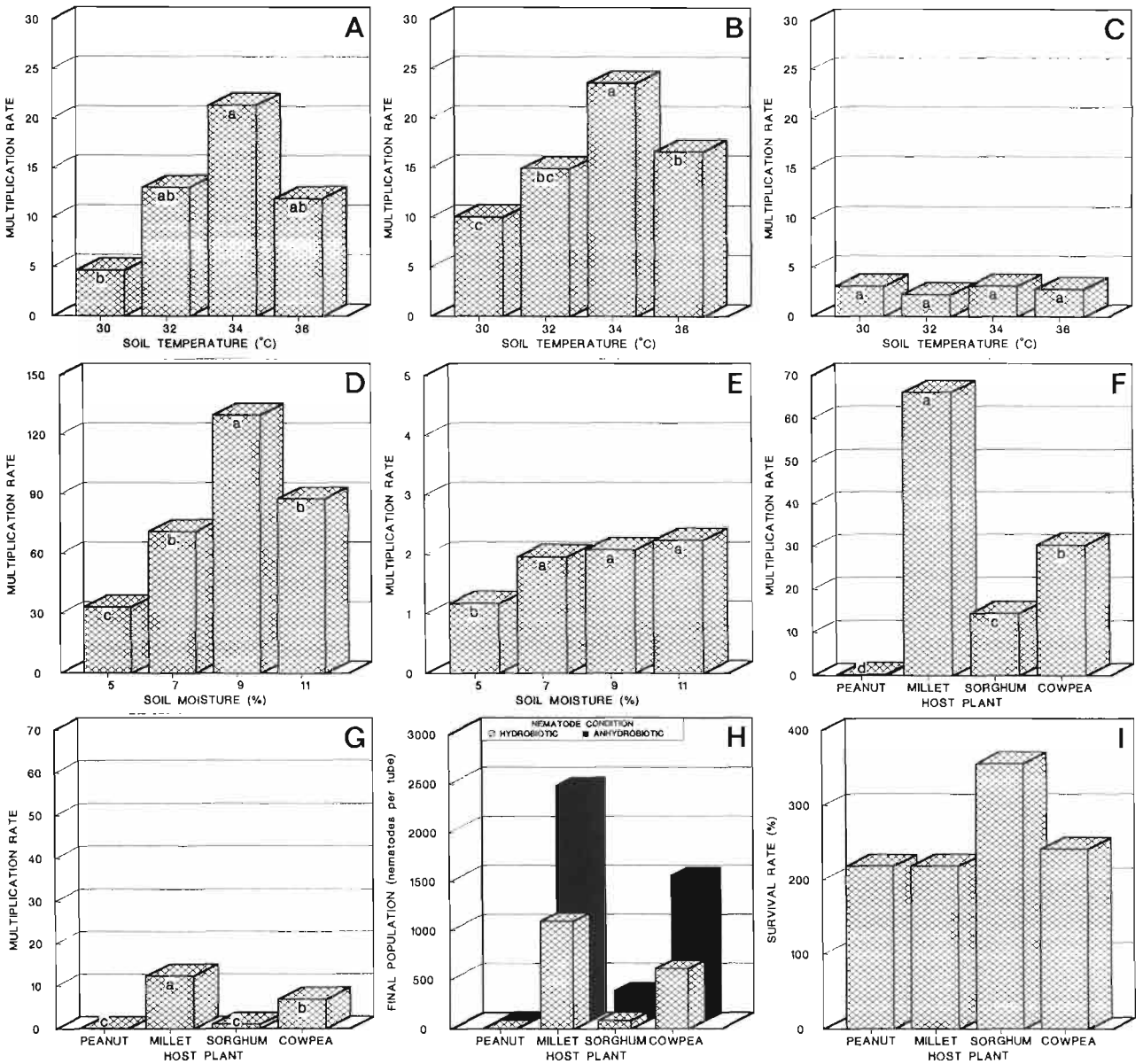

Fig. 4. *Effects of soil temperature (A, B : experiments with millet; C : experiment with sorghum), soil moisture (D, E : experiments with millet and sorghum respectively) and host plants (F : first experiment with an inoculum of 991 nematodes per tube; G : second experiment with an inoculum of 89 nematodes per tube) on the multiplication rate and survival (H, I) of* Helicotylenchus dihystera (In each experiment, data followed by the same letter are not significantly different at $P < 0.05$).

the inoculum level (557 *vs* < 97) induced an increase of the multiplication rate (Fig. 4 A, B, D).

Host plants had a significant effect on the multiplication rate of both the species; all the plants except peanut allowed the reproduction of *H. dihystera* whereas only cowpea allowed it for *H. multicinctus;* millet appeared to be the best host for *H. dihystera* (Figs 4 F, G, 5). Multiplication rates for *H. dihystera* in the two experiments on host plants confirmed that the increase of the inoculum levels (991 *vs* 89) induced an increase of the multiplication rates on millet, sorghum and cowpea (Fig. 4, F, G).

Root population levels were not affected by environmental factors and differed according to the species :

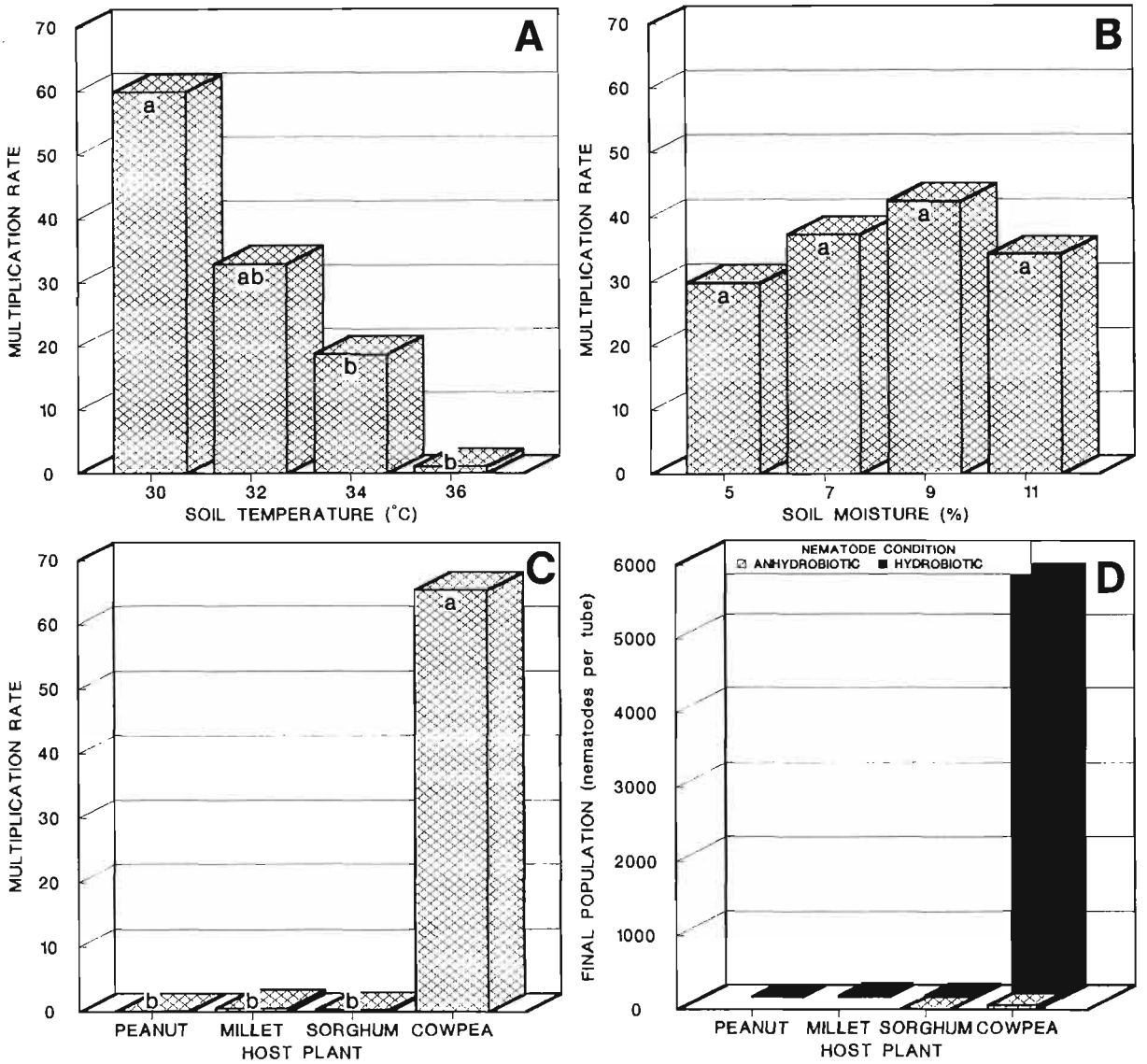

Fig. 5. *Effects of soil temperature (A), soil moisture (B) and host plants (C-D) on the multiplication rate and survival of* Helicotylenchus multicinctus (In each experiment, data followed by the same letter are not significantly different at $P < 0.05$).

less than 20 % for *H. dihystera vs* 36-67 % for *H. multicinctus;* with non-host plants of *H. multicinctus,* these levels were below 20 % (Table 1).

Ability to enter anhydrobiosis

Cessation of watering induced a decrease of soil moisture down to 0.2 % in 5 days for *H. dihystera* and 15 days for *H. multicinctus*. Nematode numbers recovered after soil desiccation for 60 days for *H. dihystera* were higher than before watering was ceased, although soil moisture level did not allow reproduction of the nematode. These numbers varied according to the size of the population before the halt to watering for the four host plants for *H. dihystera* (Fig. 4 H); for *H. multicinctus,* the number of nematodes surviving soil desiccation was low and constant for sorghum and cowpea (Fig. 5). Survival rates varied according to the host plant and to the nematode species : 200-350 % for *H. dihystera vs* 0.9-14 % for *H. multicinctus* (Figs 4 I, 6).

Pathogenicity

H. dihystera induced a significant ($P < 0.05$) reduction of root fresh weight for peanut and millet, and a significant reduction of fresh- and dry- shoot weights of peanut (Table 2).

Table 1. *Percentages of the population of* Helicotylenchus dihystera *and* Helicotylenchus multicinctus *in the roots at the end of the different experiments on multiplication rate and pathogenicity (ND = not determined).*

Experiment	Treatment	Root population as a % of total tube population		
		H. dihystera		*H. multicinctus*
Soil temperature		On millet	On sorghum	On cowpea
	30 °C	3.2-0.8	3.5	54.6
	32 °C	10.8-2.5	6.7	42.9
	34 °C	3.0-1.9	5.4	36.0
	36 °C	0 -4.0	2.1	0
Soil moisture		On millet	On sorghum	On cowpea
	5 %	0.5	3.2	66.9
	7 %	0.4	3.0	58.0
	9 %	0.6	2.3	57.0
	11 %	0.9	4.4	55.4
Host plants		* 991 nematodes	* 89 nematodes	* 89 nematodes
	peanut	8.4	0	0
	millet	12.3	2.1	15.9
	sorghum	3.1	3.4	7.9
	cowpea	17.4	19.8	57.7
Pathogenicity				
peanut	*500 nematodes	2.5		ND
	*1000 nematodes	3		ND
millet	*500 nematodes	6.6		ND
	*1000 nematodes	5.7		ND

* inoculum per tube.

Table 2. *Multiplication rate and effects of* Helicotylenchus dihystera *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	-	2.23 *a*	7.58 *a*	1.20 *a*
	500	0.24	1.84 *b*	6.63 *b*	0.99 *b*
	1000	0.25	1.69 *b*	6.75 *b*	1.02 *b*
Millet	0	-	2.81 *a*	3.76 *a*	0.52 *a*
	500	7.60	2.16 *ab*	3.54 *a*	0.49 *a*
	1000	6.55	1.84 *b*	3.19 *a*	0.40 *a*

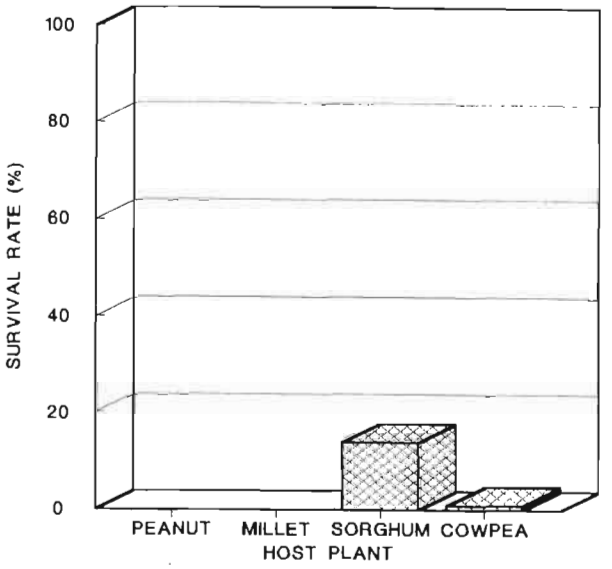

Fig. 6. *Effects of host plants on the survival rate of* Helicotylenchus multicinctus.

Discussion

Laboratory experiments conducted with *H. dihystera* and *H. multicinctus* showed differences in nematode responses to the environment. *H. dihystera* is a polyphagous species with an ectoparasitic behaviour, able to reproduce at high soil temperature and to enter anhydrobiosis. As previously described with *Hoplolaimus pararobustus* (Baujard & Martiny, 1995 *g*), the host plant affected the response of the nematode to soil temperature and soil moisture. The multiplication rate of the nematode is also affected by *i)* the level of inoculum and *ii)* the duration of the experiment. The recovery of at least twice as many *H. dihystera* after soil desication cannot be attributed to the multiplication of the nematode during short (5 days) phases of soil desiccation but more probably indicates a change in the behaviour of the nematode, possibly induced by anhydrobiosis. This change could consist in : *i)* hatching of eggs previously in diapause in moist soil, hatching induced by alternation of anhydrobiotic and hydrobiotic periods and/or *ii)* breaking by anhydrobiosis of quiescence or diapause of juvenile and adult stages in moist soils unable to go through the Baermann trays after elutriation. Such behaviour has never been previously reported in plant parasitic nematodes (see review by Antoniou, 1989) and might explain the increases registered in soil population densities during the dry season (see arrows on Fig. 1) and also the high survival rates recorded at Nebe (Fig. 2).

The biology of *H. multicinctus* differs from that of *H. dihystera* in the following : *i)* it exhibited an endoparasitic behaviour; *ii)* its multiplication rate appeared limited by high soil temperature levels; *iii)* cereals are poor or non-hosts for the nematode as previously reported by

several authors (Sher, 1966; Caveness, 1967; Stoyanov, 1967; Siddiqi, 1973) and *iv)* survival rates after soil desiccation are low. These characteristics might explain the absence of *H. multicinctus* from the semi-arid tropics of West Africa.

The negative effects of *H. dihystera* on peanut root and shoot growth increase the number of species in Hoplolaimidae producing pathogenic effects in this area (Germani, 1981; Baujard & Martiny, 1995 *e*). Experiments on the pathogenicity of *Scutellonema cavenessi* on peanut were conducted by Germani (1981) with a mixture of hoplolaimid species with among others *Helicotylenchus* sp. (more probably *H. dihystera* since the soil used originated from the peanut cropping area of Senegal). Thus, since the pathogenicy of *S. cavenessi* had not been reproduced in the laboratory (Baujard & Martiny, 1995 *c*), the pathogenicity recorded previously remains doubtful.

References

ALI, S. S. & GERAERT, E. (1975). *Helicotylenchus* species from Cameroon. *Meded. Fac. Landbouww. Rikjsuniv. Gent,* 40 : 517-520.

ANTONIOU, M. (1989). Arrested development in plant parasitic nematodes. *Helminth. Abstr., Ser. B :* 58 : 1-19.

BAUJARD, P. (1995). Laboratory methods used for the study of the ecology and pathogenicity of Tylenchida, Longidoridae and Trichodoridae from rainy and semi-arid tropics of West Africa. *Fundam. appl. Nematol.,* 18 : 63-66.

BAUJARD, P. & MARTINY, B. (1994). Études nématologiques au Mali, Afrique de l'Ouest. I. Prospections de deux zones arachidières sur l'arachide et le mil. *J. afr. Zool.,* 108 : 217-226.

BAUJARD, P. & MARTINY, B. (1995 *a*). Études nématologiques en Mauritanie et au Niger, Afrique de l'Ouest : nématodes associés au mil sauvage *(Pennisetum violaceum). J. afr. Zool.,* 109 (in press).

BAUJARD, P. & MARTINY, B. (1995 *b*). Ecology and pathogenicity of the Hoplolaimidae (Nemata) from the sahelian zone of West Africa. 1. Field Studies on *Scutellonema cavenessi* Sher, 1964. *Fundam. appl. Nematol.,* 18 : 261-269.

BAUJARD, P. & MARTINY, B. (1995 *c*). Ecology and pathogenicity of the Hoplolaimidae (Nemata) from the sahelian zone of West Africa. 2. Laboratory studies on *Scutellonema cavenessi* Sher, 1964. *Fundam. appl. Nematol.,* 18 : 335-345.

BAUJARD, P. & MARTINY, B. (1995 *d*). Ecology and pathogenicity of the Hoplolaimidae (Nemata) from the sahelian zone of West Africa. 3. *Scutellonema clathricaudatum* Whitehead, 1959. *Fundam. appl. Nematol.,* 18 : 347-353.

BAUJARD, P. & MARTINY, B. (1995 *e*). Ecology and pathogenicity of the Hoplolaimidae (Nemata) from the sahelian zone of West Africa. 4. The genus *Aphasmatylenchus* Sher, 1965. *Fundam. appl. Nematol.,* 18 : 355-360.

BAUJARD, P. & MARTINY, B. (1995 *f*). Ecology and pathogenicity of the Hoplolaimidae (Nemata) from the sahelian zone of West Africa. 5. *Aorolaimus macbethi* (Sher, 1964) Fortuner, 1987. *Fundam. appl. Nematol.,* 18 : 427-433.

BAUJARD, P. & MARTINY, B. (1995 *g*). Ecology and pathogenicity of the Hoplolaimidae (Nemata) from the sahelian zone of West Africa. 6. *Hoplolaimus pararobustus* (Schuurmans Stekhoven & Teunissen, 1938) Sher, 1963 and a comparison with *Hoplolaimus seinhorsti* Luc, 1958. *Fundam. appl. Nematol.,* 18 : 435-444.

CADET, P. (1986). Évolution des nématodes ectoparasites dans la rhizosphère de la canne à sucre au Burkina Faso. *Revue Écol. Biol. Sol,* 23 : 205-213.

CAVENESS, F. E. (1967). Shadehouse host ranges of some Nigerian nematodes. *Pl. Dis. Reptr,* 51 : 33-37.

GERMANI, G. (1981). Pathogenicity of the nematode *Scutellonema cavenessi* on peanut and soybean. *Revue Nématol.,* 4 : 203-208.

MERNY, G., FORTUNER, R. & LUC, M. (1974). Nématodes associés aux cultures maraîchères en Gambie. *Agron. trop., Nogent,* 29 : 702-707.

SHARMA, S. B. (1989). Further infestigations on the role of plant-parasitic nematodes in crop growth variability of groundnut in Niger. *Legumes Pathol. Progr. Rep., ICRISAT, India,* 8 : 61 p.

SHER, S. A. (1966). Revision of the Hoplolaiminae (Nematoda). VI. *Helicotylenchus* Steiner, 1945. *Nematologica,* 12 : 1-56.

SIDDIQI, M. R. (1972). *Helicotylenchus dihystera. C.I.H. Descript. Pl. parasit. Nematodes,* Set 2, N° 23 : 3 p.

SIDDIQI, M. R. (1973). *Helicotylenchus multicinctus. C.I.H. Descript. Pl. parasit. Nematodes,* Set 2, N° 23 : 3 p.

STOYANOV, D. (1967). Additions to host records of *Meloidogyne* sp., *Helicotylenchus multicinctus* and *Rotylenchulus reniformis. Nematologica,* 13 : 173.