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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

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Summary – Description is given of the methods used in the laboratory for *i)* culturing soil plant-parasitic nematodes, *ii)* determination of the effects of soil temperature, soil moisture, and host plants on their multiplication rate, *iii)* determination of their ability to survive soil desiccation and to enter anhydrobiosis during the dry season, *iv)* their life cycle and *v)* their potential pathogenicity. Culturing was tested for 54 species and successful with 45 of them. For the nine remaining species, the host plant cannot be determined for four species owing to the low numbers of available specimens; the other five species were probably mycetophagous.

Résumé – ***Méthodes de laboratoire utilisées pour l'étude de l'écologie et de la nocuité des Tylenchida, Longidoridae et Trichodoridae des zones humides et semi-arides de l'Afrique de l'Ouest.*** Description est donnée des techniques utilisées pour *i)* l'élevage de nématodes potentiellement phytoparasites, *ii)* la détermination des effets de la température, de l'humidité du sol et de la plante hôte sur les taux de multiplication, *iii)* la détermination de la capacité des nématodes à survivre à la dessication des sols et à entrer en anhydrobiose pendant la saison sèche, *iv)* l'étude du cycle biologique des espèces entrant en anhydrobiose, *v)* la nocuité des espèces vis-à-vis des cultures. L'élevage de 54 espèces a été tenté, avec succès pour 45 d'entre elles. Pour les neuf espèces restantes, la plante hôte n'a pu être déterminée pour quatre espèces à cause du faible nombre de spécimens disponibles; les cinq autres espèces étaient probablement mycétophages.

Key-words : Plant parasitic nematodes, laboratory technics, culture.

During studies on nematodes associated with crops in the rainy and semi-arid regions of West Africa, more than 50 potentially plant-parasitic species belonging to the Tylenchida, Longidoridae and Trichodoridae have been identified (Table 1). Nematological surveys allowed the determination of ecological trends regarding the influence of biotic and abiotic factors (Baujard & Martiny, 1994; in press *a, b*). Generally, as nothing is known about the ecology and pathogenicity of these species, laboratory studies are needed to determine their behaviour and their pathogenicity against the usual crops in these regions.

The aim of this paper is to describe the methods used for culturing soil nematodes, determination of the effects of soil temperature, soil moisture, and host plants on their multiplication rate, determination of their ability to survive to soil desiccation and to enter anhydrobiosis during the dry season, their life cycle and their potential pathogenicity against the main crops of this area.

Origin of nematodes

Nematodes were collected in different countries of West Africa (Mauritania, Mali, Niger, Senegal, Burkina Faso, Bissau Guinea) during both the rainy and dry seasons.

Nematode extraction from soil and roots

Nematodes were extracted from soil by elutriation (Seinhorst, 1962) and from roots in a mist chamber (Seinhorst, 1950). After elutriation, elutriates were put on Baerman trays and counting was done after 7 and 14 days on Baerman trays for the tylenchs and 1, 2, 3, 4, 7, and 21 days on Baermann trays for the Longidoridae and the Trichodoridae to *i)* ensure recovery of anhydrobiotic nematodes or juveniles hatched of eggs and *ii)* avoid the rotting of Longidoridae and Trichodoridae which were killed after two days in the water.

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Table 1. *List of species identified in West Africa and tentatively cultured in the laboratory (* = species unable to multiply in the laboratory)*

TYLENCHIDA Thorne, 1949

Tylenchidae Öerley, 1880

Coslenchus franklinae Siddiqi, 1981
Cephalenchus hexalineatus (Geraert, 1962) Geraert & Goodey, 1964
Cephalenchus lobus Dhanachand & Jairajpuri, 1980
* *Filenchus* cf. *facultativus* (Szczygiel, 1970) Raski & Geraert, 1987
* *Neothada cancellata* (Thorne, 1941) Khan, 1973

Anguinidae Nicoll, 1935 (1926)

* *Ditylenchus* sp. " Mali population "
Ditylenchus sp. " Niger population "

Belonolaimidae Whitehead, 1960

Tylenchorhynchus annulatus (Cassidy, 1930) Golden, 1971
Tylenchorhynchus germanii Fortuner & Luc, 1987
Tylenchorhynchus gladiolatus Fortuner & Amougou, 1974
Tylenchorhynchus indicus (Siddiqi, 1960) Fortuner & Luc, 1987
Tylenchorhynchus mashhoodi Siddiqi & Basir, 1959
Tylenchorhynchus phaseoli Sethi & Swarup, 1968
Tylenchorhynchus sulcatus de Guiran, 1967
Tylenchorhynchus ventralis (Loof, 1963) Fortuner & Luc, 1987
Tylenchorhynchus vulgaris Upadhyay, Swarup & Sethi, 1972
Trichotylenchus falciformis Whitehead, 1960
Trichotylenchus palustris (Merny & Germani, 1968) Seinhorst, 1963
Triversus annulatus (Merny, 1964) Sher, 1974

Pratylenchidae Thorne, 1949

Pratylenchus brachyurus (Godfrey, 1929) Filip'ev & Schuurmans Stekhoven, 1941
Pratylenchus loosi Loof, 1960
Pratylenchus sefaensis Fortuner, 1974
Pratylenchus zeae Graham, 1951
Radopholus similis (Cobb, 1893) Thorne, 1949

Hoplolaimidae Filip'ev, 1934

Aorolaimus macbethi (Sher, 1964) Fortuner, 1987
Aphasmatylenchus straturatus Germani, 1970
Aphasmatylenchus variabilis Germani & Luc, 1984
Helicotylenchus dihystera (Cobb, 1893) Sher, 1961
Helicotylenchus multicinctus (Cobb, 1893) Golden, 1954
Hoplolaimus pararobustus (Schuurmans Stekhoven & Teunissen, 1938) Sher, 1961
Hoplolaimus seinhorsti Luc, 1960
Scutellonema cavenessi Sher, 1964
Scutellonema clathricaudatum Whitehead, 1959
* *Rotylenchulus borealis* Loof & Oostenbrink, 1962
Rotylenchulus reniformis Lindford & Oliveira, 1940
Senegalonema sorghi Germani, Luc & Baldwin, 1984

Heteroderidae Filip'ev & Schuurmans Stekhoven, 1941

* Heteroderinae ind. only J2 recorded)
Meloidogyne sp.

Criconematidae Taylor, 1936

Criconemella curvata (Raski, 1952) Luc & Raski, 1981
Criconemella sphaerocephala (Taylor, 1936) Luc & Raski, 1981

Tylenchulidae Skarbilovitch, 1947

Paratylenchus pernoxius Siddiqi, Baujard & Mounport, 1993
* *Gracilacus* sp.

Genus incertae sedis

Paurodontus sp.

Aphelenchidae Bastian, 1865

* *Aphelenchus avenae* Bastian, 1865

DORYLAIMIDA Pearse, 1942

Longidoridae (Thorne, 1935) Meyl, 1961

Paralongidorus bullatus Sharma & Siddiqi, 1990
Paralongidorus duncani Siddiqi, Baujard & Mounport, 1993
Xiphinema elongatum Schuurmans Stekhoven & Teunissen, 1938
Xiphinema parasetariae Luc, 1958
Xiphinema savanicola Luc & Southey, 1980
* *Xiphinema* sp.

TRIPLONCHIDA Cobb, 1920

Trichodoridae (Thorne, 1935) Siddiqi, 1961

Paratrichodorus minor (Colbran, 1956) Siddiqi, 1974
Paratrichororus nanus (Allen, 1957) Siddiqi, 1974
Paratrichodorus rhodesiensis (Siddiqi & Brown, 1956) Siddiqi, 1974
Trichodorus eburneus De Waele & Carbonell, 1982

Culture methods

All experiments, except those on the life cycle, were done in pots made from PVC plastic tubes (4.5 cm diam, 17.5 cm high) with a small external PVC rim (6 mm high) on the top, and filled with sterilized sandy soil (Fig. 1 E). Studies on the life cycle were done in less high PVC tubes (9 cm high). Sandy soil used was collected near the laboratory (5.4 % clay, 0.6 % fine loam, 1.4 % coarse loam, 61.6 % fine sand, 31.2 % coarse sand; pH_{H20} 7.1, pH_{KCl} 6.2; 0.196 % total carbon; 0.027 % total nitrogen) for the cultures of species of Tylenchida or collected in a field in the centre of the peanut cropping area of Senegal (2.8 % clay, 1.0 % fine loam, 4.6 % coarse loam, 61.7 % fine sand, 30.4 % coarse sand; pH_{H20} 5.3, pH_{KCl} 4.9; 0.21 % total carbon;

0.019 % total nitrogen) for the species of Longidoridae and Trichodoridae which were unable to reproduce with the previous soil type. Tubes were put down as far as the rim (Fig. 1 D-E) into holes made on the top of closed and thermostated wooden boxes (Fig. 1 A). Constant soil temperature was achieved by air heating inside the boxes with two glow-lamps regulated by a thermostat. Constant soil moisture was achieved by adding twice a day an amount of water calculated by weighing to adjust tube weight to its theorical level (tube weight + label weight + dry soil weight + seed weight at seedling date + water amount needed to reach x % of soil moisture); this technique permited determination of water consumption in each tube during the experiments. Host plants were mostly cowpea (*Vigna unguiculata* (L.) Walp) cv. N 58 57, millet (*Pennisetum typhoides* Rich.) cv. Souna III, peanut (*Arachis hypogea* L.) cv. 55 437 and sorghum (*Sorghum vulgare* L.) cv. 51 69; when necessary (species unable to multiply on these plants or studies on the host range of some species), other plants have been tested. Nematodes were inoculated at 8 cm deep in the tube just before sowing of ten seeds per tube for millet, five seeds per tube for sorghum, and one two-day-old seedling per tube for peanut and cowpea. Nematodes were extracted from soil by elutriation.

Preliminary determination of host plant

When the host plant was unknown, the soil samples taken in the field were subdivided into several parts after homogenization. Subsamples (250 cm^3) were bioassayed by growing different plants at 30, 32, 34 and 36 °C constant soil temperature and 7 % constant soil moisture (one replication per temperature and plants) in a growth chamber. After 30 days, nematodes were extracted by elutriation and counted.

Stock cultures

Nematodes collected in the field were cultured in a growth chamber on the host plant at constant soil temperature and soil moisture. Cultures were renewed every 2 months afterwards by elutriation and inoculation of 100 hand-picked nematodes; these tubes constituted the stock cultures for all subsequent experiments.

Temperature experiments

Tubes were inoculated with determined numbers of nematodes, planted with the host plant, and maintained at four constant soil temperature levels (30, 32, 34, 36 °C) with seven replications for each temperature level, at constant soil moisture, for 60 days in a growth chamber with artificial lighting (16 h photoperiod).

Soil moisture experiments

Tubes were inoculated with a determined number of nematodes, planted with the host plant, and maintained at four constant soil moisture levels (5, 7, 9, 11 %) at constant soil temperature for 60 days in a greenhouse with natural lighting; the four treatments were replicated ten times in a completely randomized design.

Host-plant experiments and preliminary test for anhydrobiotic survival

Tubes were inoculated with determined numbers of nematodes, generally planted with one of the four host plants (cowpea, millet, peanut, sorghum), and maintained at constant soil temperature and soil moisture in the 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 constant soil temperature and weighed daily to follow the evolution of soil desiccation. Sixty days later, nematodes were extracted by elutriation.

Second experiment on anhydrobiosis

Seven tubes, each with a host plant, were inoculated with a determined number of nematodes and kept at constant soil temperature and constant soil moisture in the growth chamber. Nematodes were extracted from one tube by elutriation 60 days later; the six other tubes were allowed to dry another 60 days. Then the soil in each tube was thoroughly mixed. Nematodes were extracted from 50 g of soil from each tube and the rest of the soil was composited and equally distributed into six new PVC tubes topped with sterilized soil up to a volume of 250 cm^3. These tubes were planted with the host plant and kept at constant soil temperature and soil moisture. Nematodes were extracted by elutriation 60 days after planting.

Life-cycle determination for the species able to enter anhydrobiosis

Several stock cultures were allowed to desiccate in the growth chamber. One year after cessation of watering, the soil was thoroughly mixed. Nematodes were extracted from 50 g of soil and the rest of the soil was composited and equally distributed into several small (6 cm high) PVC tubes. These tubes were planted with the host plant at seed stage and rehumidified in the mist chamber in order to reproduce the effects of the first rainfall on the rainy season. Tubes were kept at constant soil temperature and soil moisture. Nematodes were extracted by elutriation for six replicates at regular times.

Pathogenicity

A separate experiment was conducted with each crop species at constant soil temperature and soil moisture in the growth chamber or in the greenhouse. Nematodes were inoculated on to each host at two inoculum levels. 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 and dry weights of roots and shoots were measured.

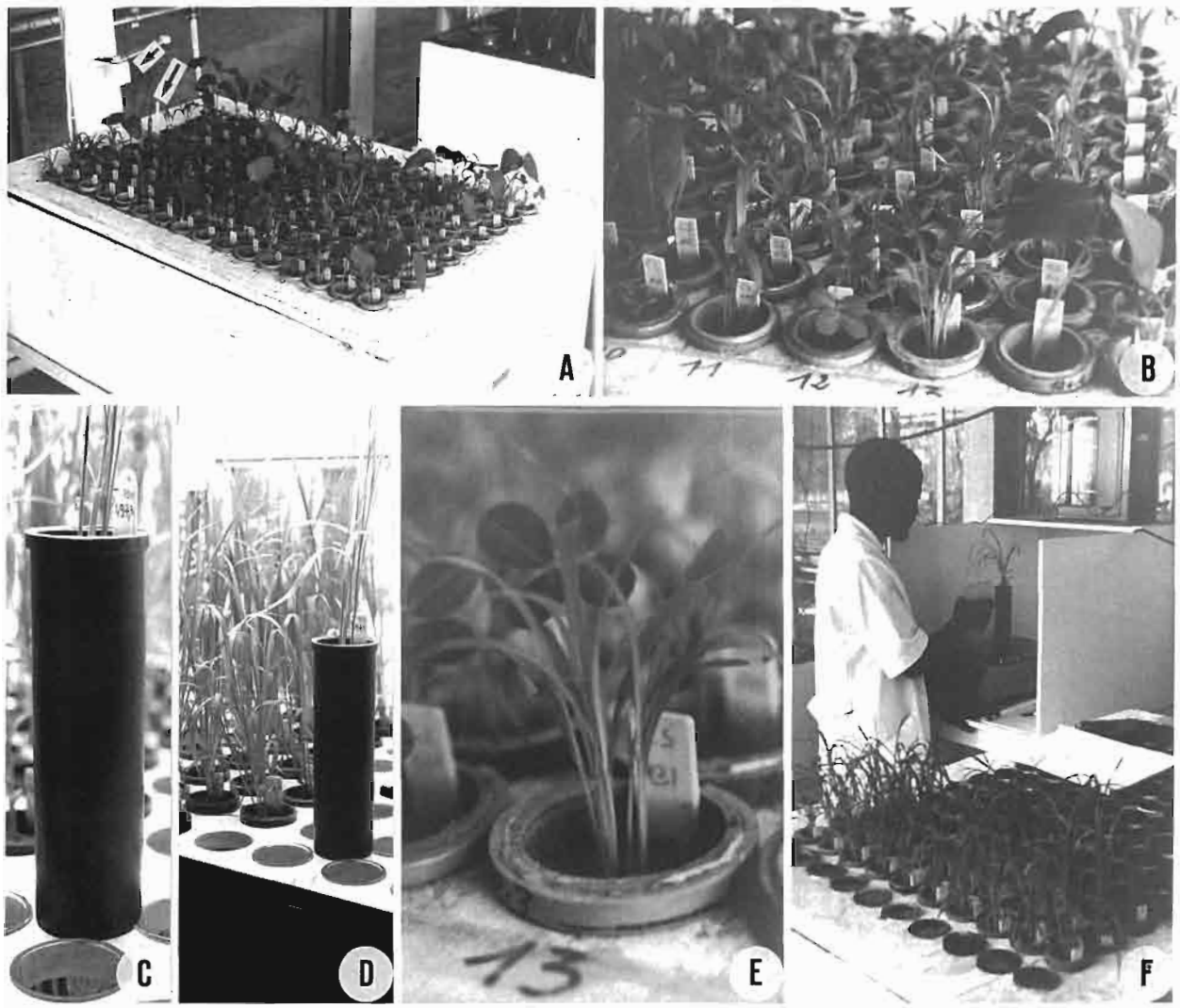

Fig. 1. *Culture of plant-parasitic nematodes in the laboratory. A : Thermostated wooden box (short arrow : probe of the thermostat; long arrow : control thermometer); B-E : PVC tubes used for rearing plant parasitic nematodes in the laboratory; F : Control of soil moisture by weighing.*

Conclusion

These techniques allowed the culture of 45 species out of 54 tested. Our success in rearing plant parasitic nematodes species from West Africa in the laboratory is probably due to our replication, as far as possible, of the ecological conditions of the sampling site (soil type, temperature, moisture, host). Only nine species could not be reared in the laboratory; five of them (*Filenchus* cf. *facultativus, Neothada cancellata, Ditylenchus* sp. " Mali population ", *Paurodontus* sp., *Aphelenchus avenae*) have probably mycetophagous habits; because the others four (*Rotylenchulus parvus,* Heteroderinae ind., *Gracilacus* sp., *Xiphinema* sp.) had been recorded in very low numbers during the nematological surveys (less than ten specimens at most), the determination of their host plant was unsuccessful.

References

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

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

BAUJARD, P. & MARTINY, B. (in press *b*). Characteristics of the soil nematode populations from the peanut cropping area of Senegal, West Africa. *J. Afr. Zool.*

SEINHORST, J. W. (1950). De betekenis van de grond voor het optreden van aanstasting door het stengelaatje (*Ditylenchus dipsaci* [Kühn] Filipjev). *Tijdschr. PlZiekt.*, 56 : 291-349.

SEINHORST, J. W. (1962). Modifications of the elutriation method for extracting nematodes from soil. *Nematologica,* 8 : 117-128.