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NEMATODE THREATS TO RICE IN LIBERIA

by

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Summary. The relationship between rice and seven plant parasitic nematodes in Liberia are discussed. *Xiphinema ifacolum* is a widespread species and must be regarded as a major pest. *Mesocriconema curvatum* and *M. onoense* cause damage at high population densities and in wet and clay soils. *Hemicycliophora typica* is responsible for the destruction of feeder roots. *Helicotylenchus dihystera* is cosmopolitan and when numerous may affect yields to an economic extent. *Heterodera sacchari* could be a serious pathogen but has a very restricted distribution. Finally, *Meloidogyne incognita* is widespread but it was rarely found in association with rice.

During a survey of plant parasitic nematodes undertaken in 1985-1987 in Liberia, several upland and lowland rice fields were sampled. In some instances poor growth of plants was associated with high populations of plant parasitic nematodes or the presence of major plant parasitic nematodes known to affect rice in other areas of the world. The pathogenicity of several selected species in Liberia was investigated by field observations, replicated experiment tests and histopathological studies.

This paper reports on the relationships between rice (*Oryza sativa* L.) and seven nematode species: *Helicotylenchus dihystera* (Cobb) Sher; *Hemicycliophora typica* de Man; *Heterodera sacchari* Luc et Merny; *Meloidogyne incognita* (Kofoid et White) Chitw.; *Mesocriconema curvatum* (Raski) Loof et De Grisse; *M. onoense* (Luc) Loof et De Grisse and *Xiphinema ifacolum* Luc.

Materials and methods

A field experiment was undertaken during the period August-December 1987 at Suakoko in a sandy loam infested by *X. ifacolum* and *M. curvatum*. The soil was ploughed 30-40 cm deep, rotavated and divided into 24, 5 × 3 m plots, separated by an interspace of 1 m from each other, and distributed at random in four blocks. On August 5 either the methomyl wettable powder, at the rate of 8 kg a.i./Ha, or the granular formulation of carbofuran, at the rates of 4, 8, 10 and 12 kg a.i./ha were broadcast on the plot surface and incorporated to a depth of 10 cm; four plots were left untreated as a control. The next day the plots were sown with lots of three rice seeds, cv. Lac 23 white, spaced at 20 cm in 15 rows per plot 20 cm apart; the

crop was grown as rainfed upland. All plots were fertilized with 60 kg/ha of NPK on 28 September, sprayed with carbaryl at tillering to control insects and weeded by hand when necessary.

A month after sowing (4 September) the plants that had emerged in a 0.5 m diameter circle at the centre of each plot were counted. On 28 September, 19 October and 9 November, five plants were taken at random from each circle and their height measured; the number of tillers per plant was also recorded on 9 October. Population densities of *X. ifacolum* and *M. curvatum* were assessed through extraction by the Cobb's sieving decanting technique, from a 200 ml aliquot of a composite soil sample collected on 20 August, two weeks after sowing, and on 26 November, three weeks before harvest, from the rhizosphere at three different sites from each plot. The experiment was discontinued on 17 December when straw and seed weights were recorded, after the crop from each plot had been dried and winnowed.

Two pathogenicity tests were conducted during the periods 6 May-18 August and 21 August-30 November, respectively, in a screenhouse at Suakoko. For the first experiment, two lots of 12, 0.5 l plastic pots were filled with dried river sand free of any nematodes. On 6 May groups of hand picked *X. ifacolum*, with prevalence of adult females and preadult juveniles, were put in 12 pots concomitantly with a one week old seedling of rice cv. Lac 23 White; 12 pots were left uninoculated as a control. Plant heights were recorded on 1 and 29 June and on 15 and 30 July. Total plant and root weights and the number of nematodes per pot were recorded at the end of the experiment on 18 August.

For the second experiment the same procedure was adopted, except that the inoculation density of *X. ifacolum* varied from 51 to 214 nematodes in the 12 inoculated pots. In fact the nematode populations for each of the replicates in the previous experiment containing all stages (except males and eggs) with 10-15% gravid females, were used for the equivalent replicate in this second experiment. Plant heights were recorded on 15 and 30 September, 15 and 30 October and 16 November. Total plant and root weights and number of nematodes were determined on 30 November.

Data were analyzed by Student's «t» test and Duncan's multiple range test, and correlation coefficients were calculated.

Histopathological and ultrastructural studies were carried out at Bari with *H. typica*. A suspension of 30 hand-picked nematodes from a Ganda, Liberian population was pipetted onto the rhizosphere of each five day old rice seedling cv. Lac 23 White grown in sterilized sand in 5 cm diameter clay pots. The plants were maintained in growth chambers at 22 °C and at 2 and 5 days after inoculation samples of roots were removed and washed. Specimens were prepared for transmission electron microscope obser-

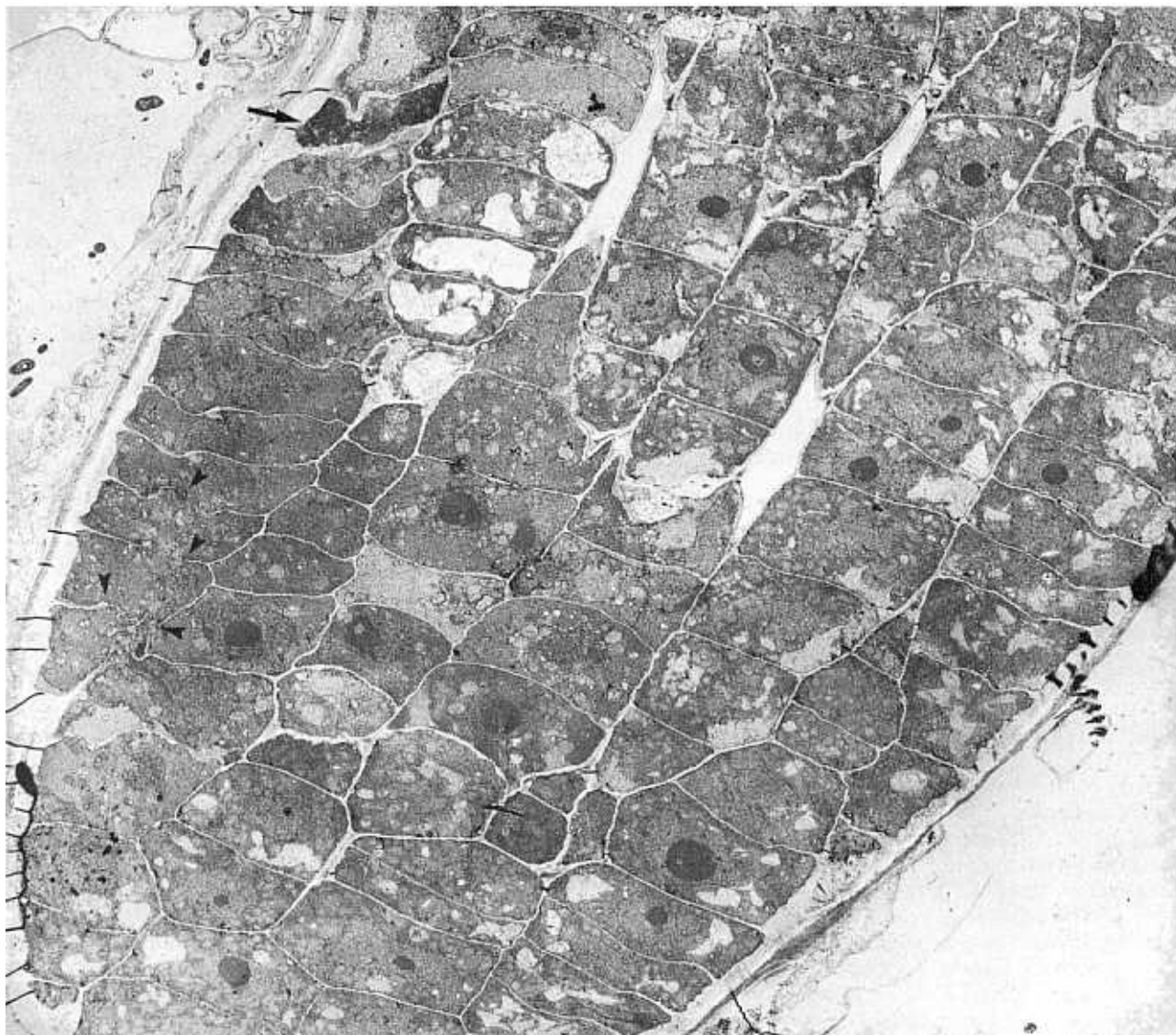


Fig. 1 - Micrograph of a longitudinal section through a rice rootlet attacked by *Hemicycliophora typica*. The cells of the rhizodermis are affected two days after inoculation. Note a necrotic cell (arrow) due to the stylet insertion. The remaining row of the rhizodermal cells show cell wall breakdown (arrowheads). All the meristematic tissue is extensively injured (X 2400).

vations according to the procedure of Bleve-Zacheo *et al.* (1987).

Results and discussion

Helicotylenchus dibystrera is widespread in Liberia and is often found in upland rice fields alone or associated with *Mesocriconema* spp. or other plant parasitic nematodes; it is considered to be the cause of a rice decline in Mauritius (Chinappen *et al.*, 1988).

Hemicycliophora typica populations from Liberia showed feeding behaviour on rice seedlings cv. LAC 23 White similar to that of a *H. typica* population from Mau-

ritius on cv. LAC 23 Red (Bleve-Zacheo *et al.*, 1987). Lateral roots were retarded and eventually as growth stopped the tips became swollen. Cytological changes observed were progressive cell wall dissolution, starting from the first layer of the cortical cells below the rhizodermis (Fig. 1). Extensive condensation of the cell content resulted in the disappearance of cell organization with the membrane system completely deranged. The nuclei with amoeboid outline were scattered in the amorphous syncytium and contained dense nucleoli (Fig. 2). The nematode inserted its stylet into the cells and ingested the contents, but its main food source was the rows of meristematic cells adjacent to the feeding site. Lysis of the cells due to the action

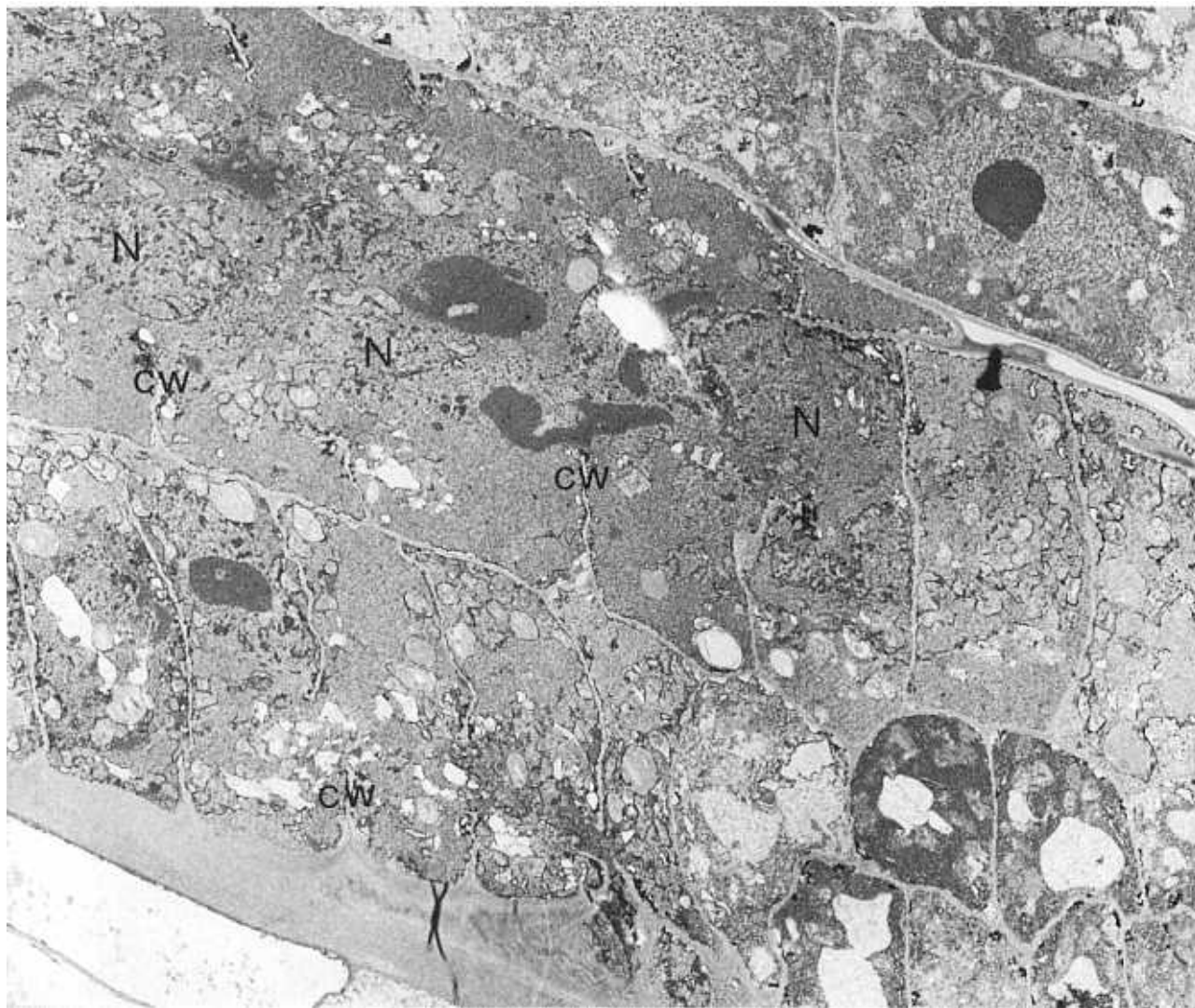


Fig. 2 - Longitudinal section through a rice root tip five days after inoculation of *H. typica*. The process of dissolution of the cell walls (cw) also involves the cortical cells. A large syncytium with amoeboid nuclei (N) is evident. The cytoplasm in both rhizodermal and cortical cells has become amorphous, devoid of organelles (X 5300).



Fig. 3 - Rice field with patches of stunted growth caused by *Meloidogyne incognita*.



Fig. 4 - Retarded growth of rice plants infested by *M. incognita*.

of nematode enzymes, was very intense and spread so rapidly that the root tip was completely destroyed within one week (Bleve-Zacheo *et al.*, 1987).

Heterodera sacchari was found only in one location at Ganta. The feeding activity of the nematode on the roots caused cell wall disintegration leading to the formation of large, intercommunicating syncytia (Vovlas *et al.*, 1986). Root vascular structures were completely disorganized.

Meloidogyne incognita was also rare. It was found at Nagbein in the roots of young rice plants (Lamberti *et al.*, 1988) showing only slightly retarded growth. Soil samples collected from the rhizosphere of the plants, however, also contained specimens of *M. curvatum* and *H. dibytera*. There was a more dramatic situation in a field at Malekie, near Gbanga, where patches (Fig. 3) of stunted and yellowish plants (Fig. 4) were widespread.

Mesocriconema curvatum (Fig. 5, 6) was found in very

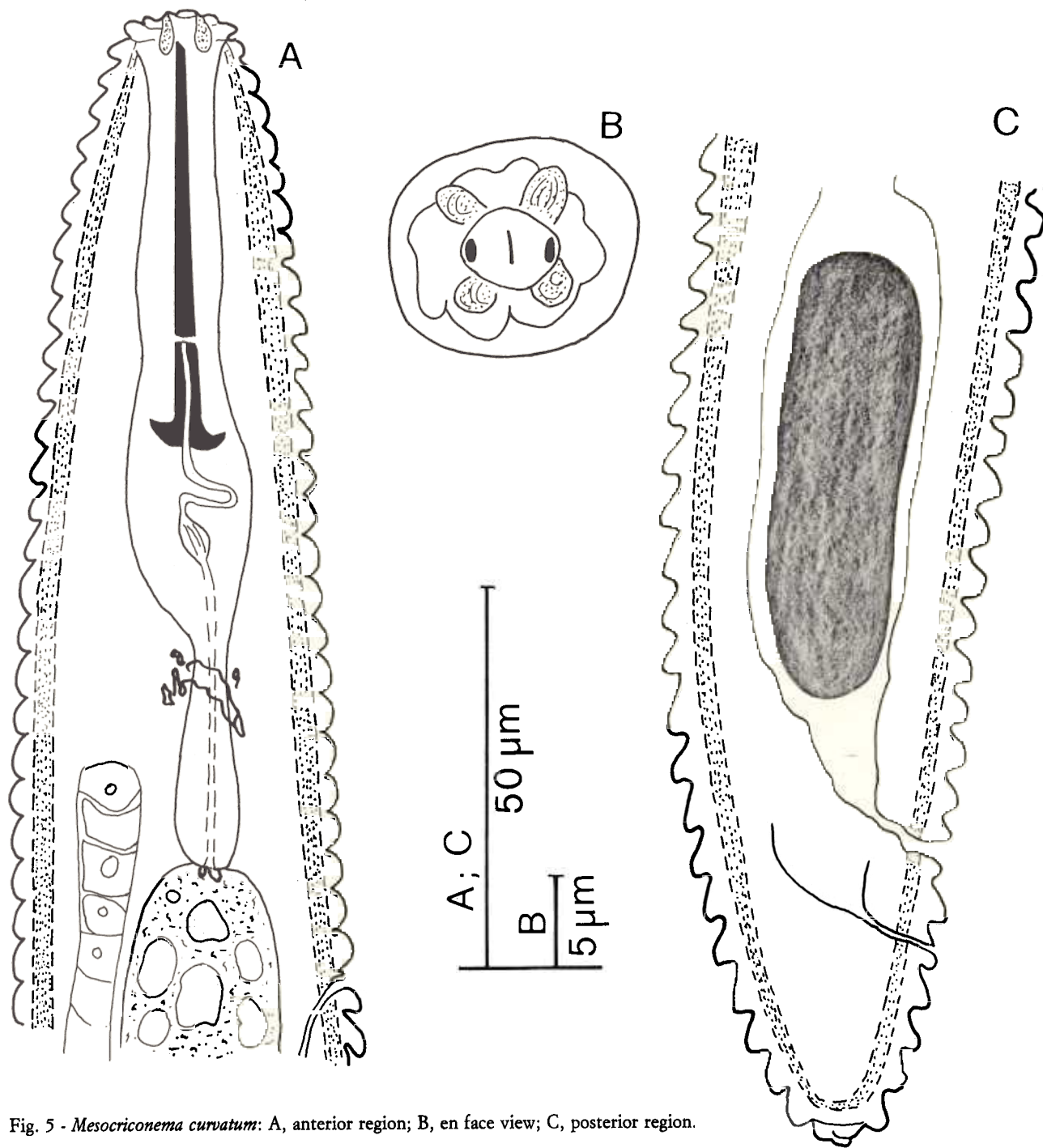


Fig. 5 - *Mesocriconema curvatum*: A, anterior region; B, en face view; C, posterior region.

low numbers (from 22-24 to 50-55 specimens per 200 ml soil) in plots of the field trial at Suakoko and had almost disappeared by the end of the experiment, which suffered severe drought in the last three months. However, in a cultivar trial of swamp rice in May 1987 at Suakoko some plots had patches of stunted yellowish plants (Fig. 7). Very high numbers (several thousands per 0.5 l soil) of the nematode were found in soil samples collected from the rhizosphere of those plants. Plant decline had further progressed (Fig. 8) at harvest (August 1987) although at this time the nematode population had decreased to very low levels.

Mesocriconema onoense (Fig. 9, 10) was found in various upland rice fields with yellowish and declining plants, including one at Tubmanburg (Fig. 11). Soil populations varied from low (200 to 500 specimens per 0.5 l of soil) to medium-high (2000-3000 spec. per 0.5 l soil). This species is reported to affect rice in the U.S.A. (Hollis, 1977) and in Mauritius (Chinappen *et al.*, 1988).

Females of *M. onoense* are distinguishable from *M. curvatum* by the larger number of body annuli (111 vs 78-108), the shorter stylet (40-51 μ m vs 47-68), the number of body annules from vulva to posterior end (8-14 vs 5-10) and the ratio length of the stylet/total body length (7-10 vs 11-17) (De Grisse, 1965).

Xiphinema ifacolum is common in rice fields in Liberia and often is associated with declining plants. Its relationship to rice was studied by Lamberti *et al.* (1987), who reported that it suppressed plant growth and yield in the field. These authors, however, postulated that severe damage could be attributed to a synergistic interaction with

other soilborne pathogens, although it was observed that nematode feeding caused substantial disorder in the rootlets.

In infested fields the emergence of the plantlets was patchy (Fig. 12). Young plants were stunted and bushy compared to healthy ones (Fig. 13); roots were stubby and distorted (Fig. 14).

The field trial carried out from August to December 1987 at Suakoko showed that nematicidal treatments improved plant growth during the first 70 days after sowing, but they failed to provide longer protection nor did treatments significantly increase plant emergence, tillering and straw weight, although grain yields were significantly increased with respect to the control. A negative correlation was found between initial population densities of *X. ifacolum* and straw and grain yields; however this was not statistically significant probably because of the low nematode densities involved, the erratic distribution of the nematodes and a severe drought which adversely effected yields throughout the whole region.

At the end of the first pathogenicity test carried out in a screenhouse from May to August, total and root weights of the plants grown in pots infested with 20 *X. ifacolum* averaged 17.4 and 12.4 g per plant, respectively, compared to the control plants with mean weights of 22.7 and 17.5 g, respectively. These differences were not statistically significant, nor were the negative correlations of final nematode population densities and total plant and root weights. However, the data clearly indicated that *X. ifacolum* reproduced well on rice. In fact, from an initial population, of 20 nematodes per pot, final densities averaged 98 ± 15 (mean \pm S.E.) nematodes per pot after four months, with a range between 53 and 214. All stages of the

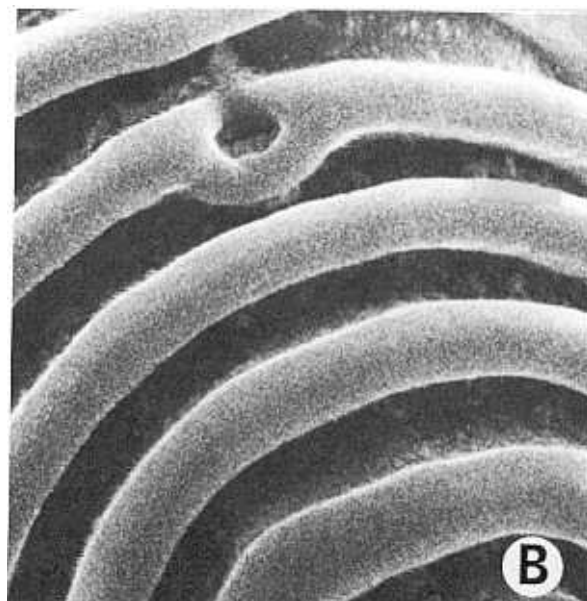
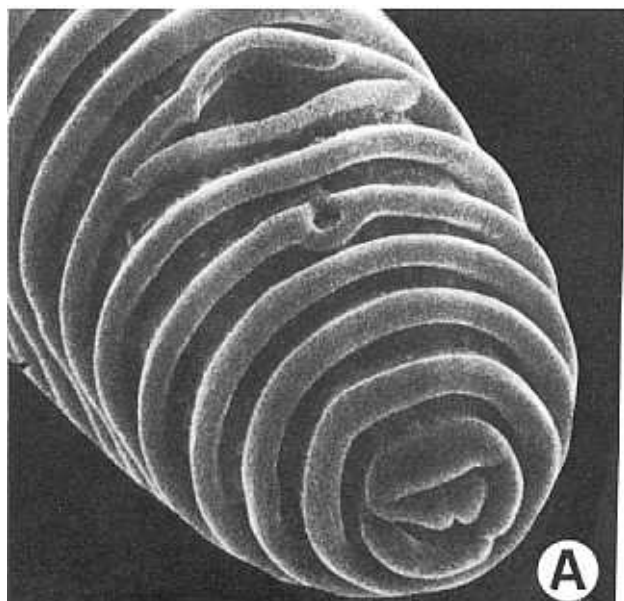


Fig. 6 - *M. curvatum*: A, posterior region; B, anal area.



Fig. 7 - Rice field with patchy growth in soil infested by *M. curvatum*.



Fig. 8 - The same rice fields as Fig. 7 at harvest.

nematode were present at the end of the experiment with about 10-15% of mature and/or gravid females and numerous first and second stage juveniles, indicating active reproduction in progress.

The results of the second pathogenicity test, undertaken in the screenhouse during the period August to November, demonstrated statistically significant negative correlations between either initial or final population densities of *X. ifacolum* and total plant and root weights and plant heights measured on 16 November (Table II). More-

over, initial and final population densities of the nematode were positively correlated (Fig. 15), indicating that *X. ifacolum* was still actively reproducing. In fact, population increased from an average number of 98 to 132 nematodes per pot, within a range between 19 and 442 specimens per pot, mainly first and second stage juveniles. Higher population densities occurred in the pots in which the plants had smaller root systems (Fig. 16), confirming that *X. ifacolum* affects rice growth and suggesting that heavy infestations might be destructive to this crop.

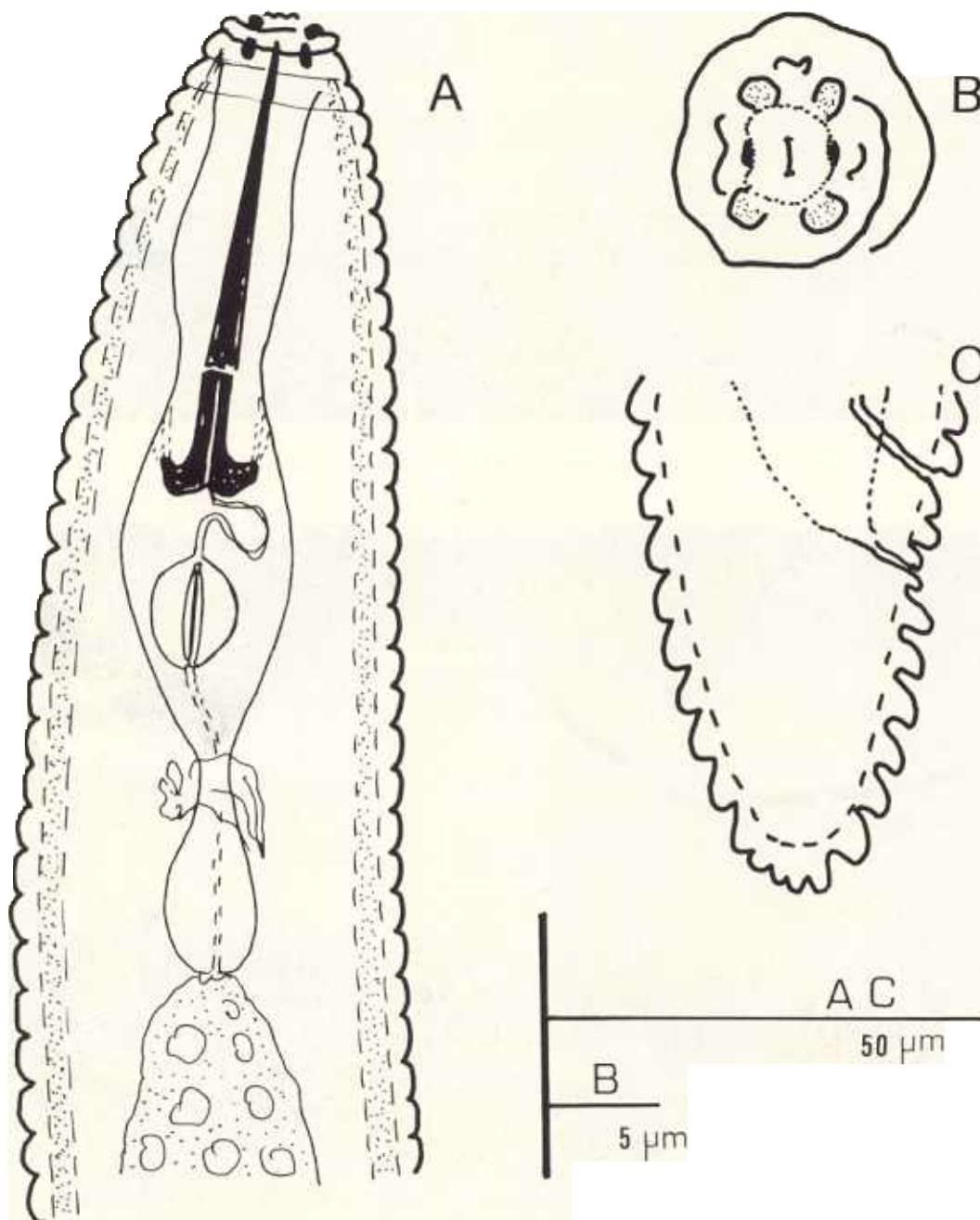


Fig. 9 - *M. onoense*: A, anterior region; B, en face view; C, posterior region

TABLE I - Effect of nematicidal treatments on growth and yield of rice cv. LAC 23 White in soil infested by *Xiphinema ifacolum*.

Treatment	Rate kg a.i./ha	No. of plants in the test area (0.5 m diam)	No. of tillers in the test area (0.5 m diam)	Average plants heights (cm)			Weights (kg)		No. of <i>X. ifacolum</i> per 200 ml soil	
				28 Sept.	19 Oct.	9 Nov.	Straw	Grain	20 Aug. (initial)	29 Nov. (final)
Methomyl	8	18 a	39 a	56.4 a	97.2 bc	96.0 :	10.1 a	0.17 a	23	16
Carbofuran	4	20 a	53 a	54.6 a	98.0 ab	101.4 :	13.4 a	0.27 a*	11	28
Carbofuran	8	20 a	48 a	54.0 a	95.6 ab	99.2 :	13.3 a	0.45 a**	10	12
Carbofuran	10	21 a	50 a	54.1 a	98.9 ab	99.7 :	13.6 a	0.27 a*	13	7
Carbofuran	12	22 a	61 a	58.7 a	99.9 a	99.6 :	11.0 a	0.37 a*	8	12
Control	—	18 a	48 a	43.0 b	88.6 c	94.3 :	11.2 a	0.09 a	12	27

N.B. Data flanked in any column by the same letter do not differ statistically for $P = 0.05$; * for $P = 0.05$; ** for $P = 0.01$ statistically different from the control according to Student's 't' test.

TABLE II - Coefficient of correlation between the variables examined in the second pathogenicity test in the screenhouse.

	Plant height on 16 Nov.	Total plant weight	Root weight	Final pop. density of <i>X. ifacolum</i>	Initial pop. density of <i>X. ifacolum</i>
Plant height 16 Nov.	—	0.637 **	0.542 **	-0.463 *	-0.547 **
Total plant weight		—	0.971 ***	-0.444 *	-0.480 *
Root weight			—	-0.475 *	-0.488 *
Final pop. density <i>X. ifacolum</i>				—	0.897 **
Initial pop. density <i>X. ifacolum</i>					—

***: significant for $P = 0.001$; **: significant for $P = 0.01$; *: significant for $P = 0.05$.

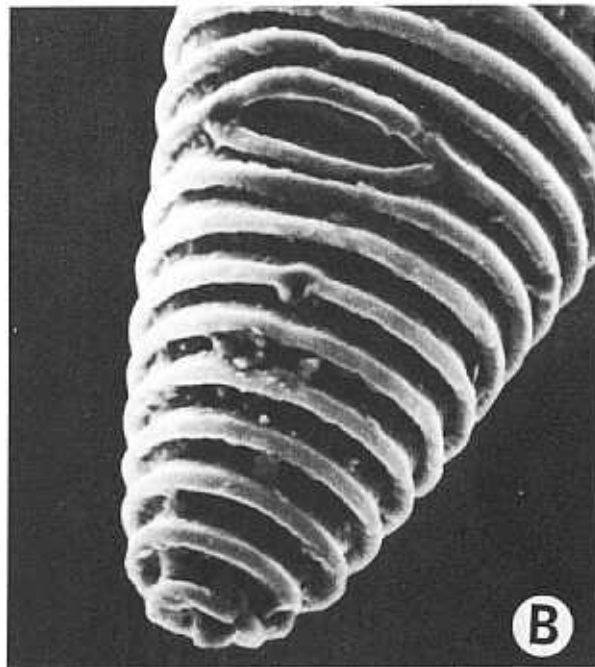
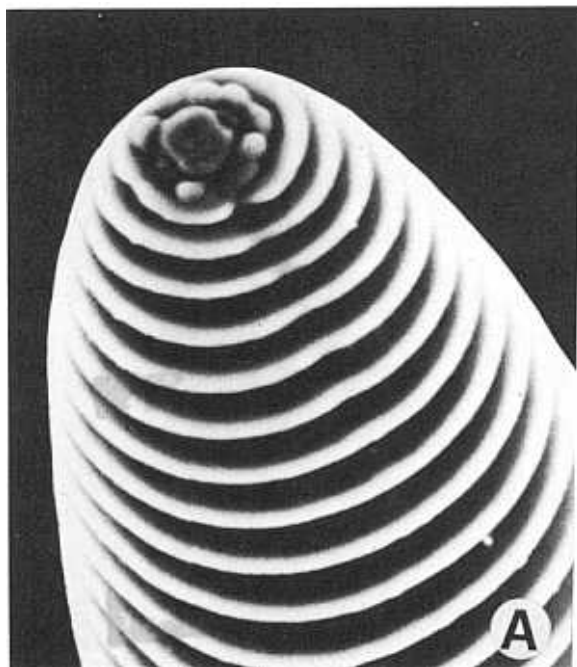
Fig. 10 - *M. onoense*: A, anterior region; B, posterior region.



Fig. 11 - Yellowish and declining rice plants in soil infested by *M. onoense*.



Fig. 12 - Rice field with patchy growth in soil infested by *Xiphinema ifacolum*.



Fig. 13 - Stunted rice plants in soil infested by *X. ifacolum*.

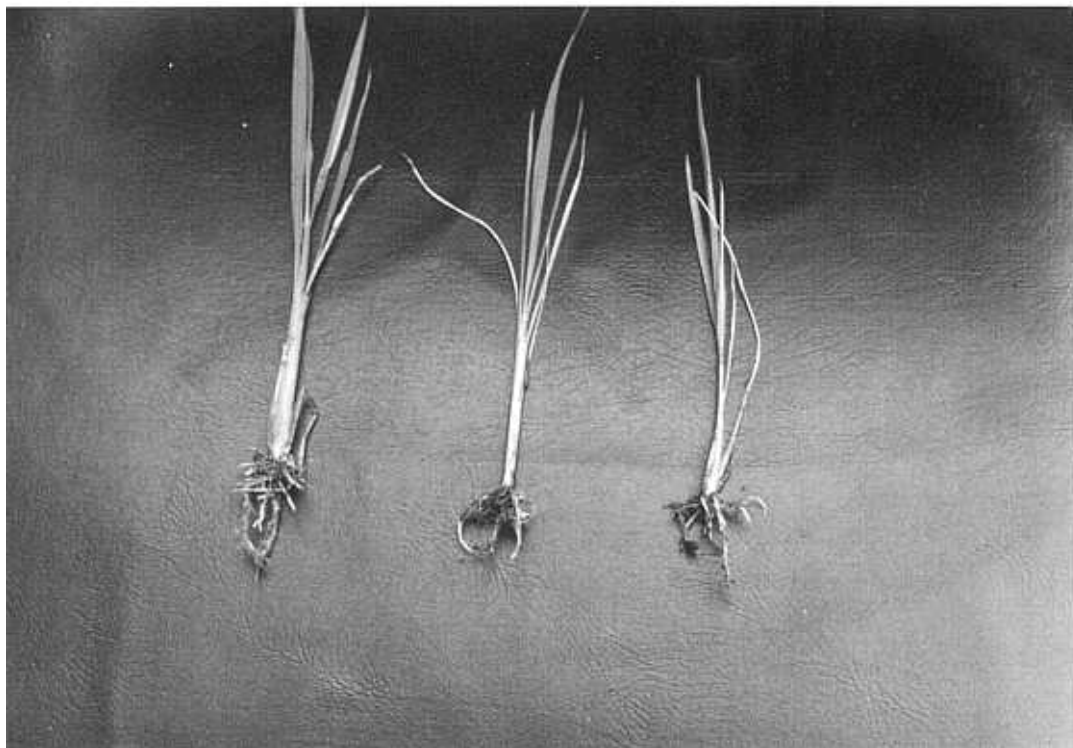


Fig. 14 - Root systems of rice plants grown in soil naturally infested by *X. ifacolum*.

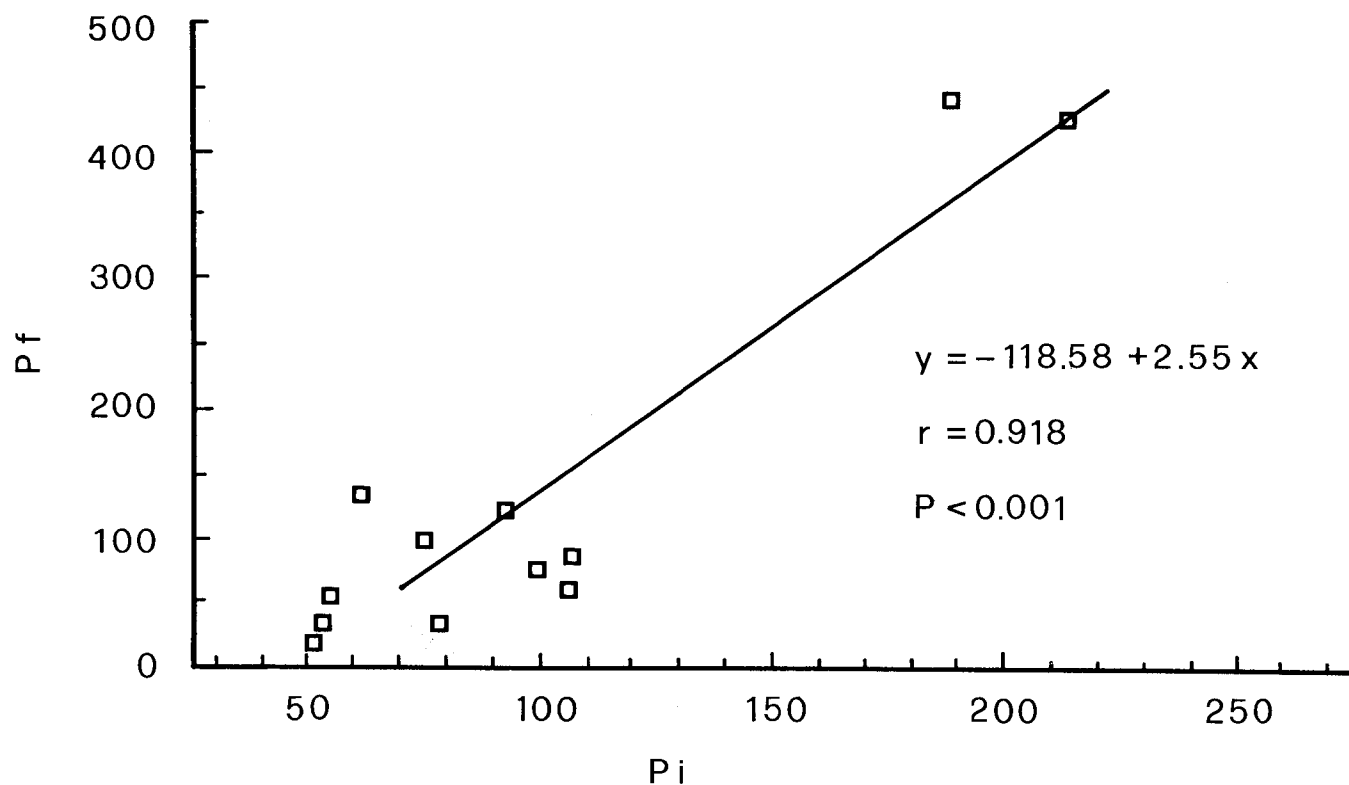


Fig. 15 - Linear correlation between initial (P_i) and final (P_f) population densities of *X. ifacolum* in pots planted with rice.

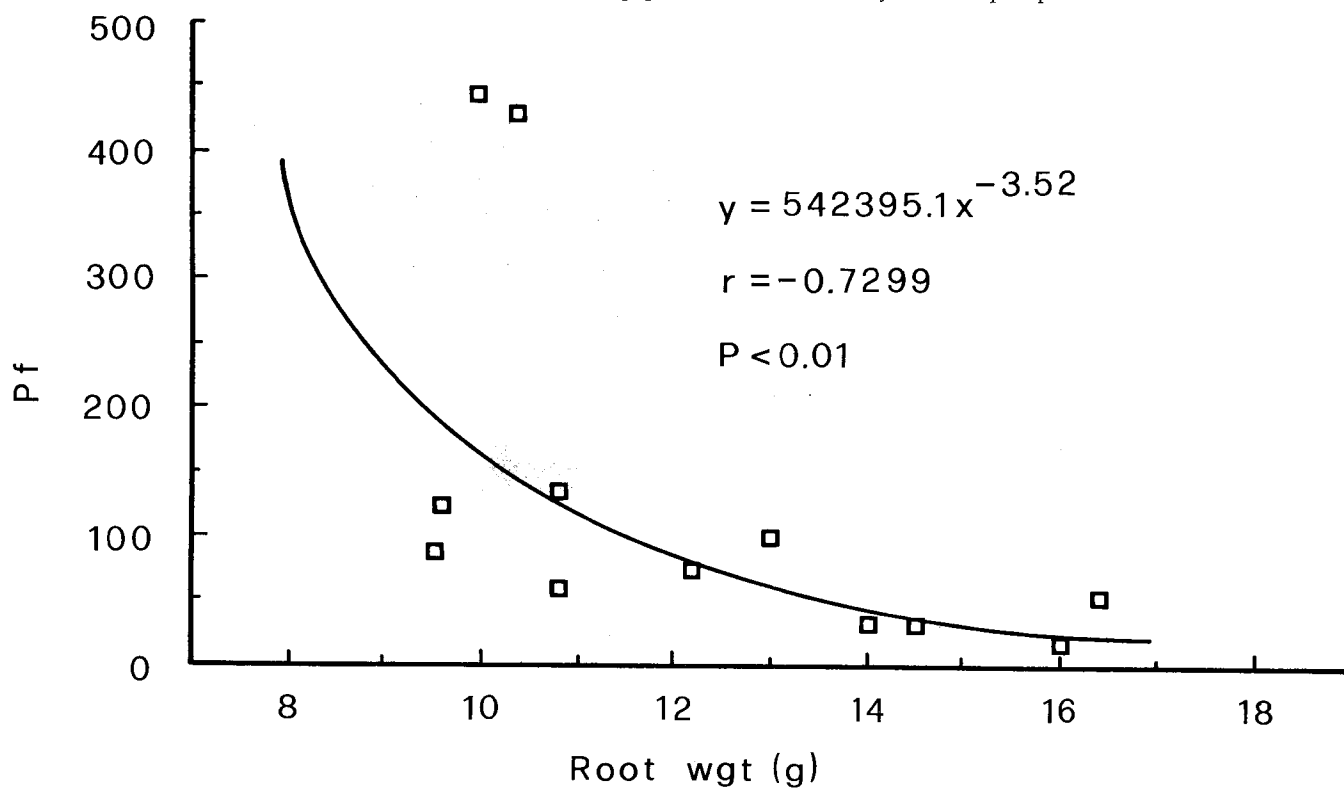


Fig. 16 - Exponential correlation between root weight of rice plants grown in pots and final population density (P_f) of *X. ifacolum*.

Conclusions

The results of this work clearly indicate that *X. ifacolum* must be regarded as a major nematode pest of rice. The extent of the damage it can cause obviously depends on population densities and environmental, edaphic and pedoclimatic factors influencing plant growth and nematode survival in soil. However, growth suppression and crop losses are likely to be aggravated by concomitant infection by other soilborne pathogens.

Mesocriconema curvatum and *M. onoense* may be regarded as minor parasites which can cause damage at large population densities and in moist soils with high clay content.

Conspicuous root damage is produced by *H. typica* but the extent of crop losses induced by its feeding is still unknown. It is certainly related to the severity of the attack which, if massive, can destroy all the feeder roots.

Helicotylenchus dihystera is a cosmopolitan species, invariably associated with many crops in mixed populations with other plant parasitic nematodes. Its occurrence in high population densities in rice fields may cause damages of economic importance.

A serious threat is undoubtedly constituted by *H. sacchari*, as is evident with most of the cyst nematode species. Fortunately it was detected only in a single field in Liberia and due to the large extent of the survey undertaken in the whole country it can be assumed that its distribution is restricted.

Meloidogyne incognita is widespread in the country but seems to be rarely associated with rice. Nevertheless its occurrence in rice fields should not be overlooked especially

in upland fields where it can become an important pest (Bridge *et al.*, 1990).

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