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Istituto di Nematologia Agraria, C.N.R. - 70126 Bari, Italy
Plant Protection Department, CARI - Suakoko, Liberia

RELATIONSHIPS BETWEEN *XIPHINEMA IFACOLUM* AND RICE IN LIBERIA

by
F. LAMBERTI, T. BLEVE-ZACHEO, D.K. TUOPAY, A. CIANCIO and J.B. BOIBOI

Xiphinema ifacolum Luc, 1961 is widespread in Liberia and often occurs in rice fields within patches of stunted plants. In particular, large numbers of the nematode were found in the rhizosphere of declining plants in a rice field at Suakoko (Fig. 1). It was therefore thought interesting to study the relationships between this longidorid nematode species and rice plants, *Oryza sativa* L.

Materials and Methods

The heavily infested above mentioned field at Suakoko, consisting of a sandy loam, was ploughed 30-40 cm deep, rotavated and divided into 24, 5×3 m plots separated from each other by an interspace of 1 m and distributed at random in four blocks. On 14 July 1986 either the wettable powder of methomyl at the rate of 8 kg a.i./ha or the granular formulation of carbofuran at the rates 4, 8, 10 and 12 kg a.i./ha were broadcast on the plot surface and incorporated to a depth of 10-15 cm; 4 plots were left untreated as control. The next day the plots were sown with three rice seeds spaced at 20 cm in 16 rows per plot 25 cm apart; the crop was grown as rainfed upland. On September 18 it was fertilized with 20 kg/ha of ammonium sulphate and weeded by hand when necessary.

A month after sowing (20 August) the number of plants that had emerged were counted in a 0.5 diameter circle at the center of each plot. On 3 and 23 September and 15 October five plants were taken at random

Fig. 1 - Patchy appearance of a rice field infested by *Xiphinema ifacolum* at Suakoko.

Fig. 2 - Plant emergence and growth in a plot treated with 10 kg a.i./ha of carbofuran.

Fig. 3 - Plant emergence and growth in a control plot.

from each circle and their height measured; the number of tillers per plant was also recorded on the last date. Population densities of *X. ifacolum* were assessed by extracting by Cobb's sieving technique from a 200 ml aliquot of a composite soil sample collected on 25 September from the rhizosphere at three different places in each plot. The experiment was discontinued on 2 December, 1986 when straw weight and seed weight were recorded after the crop from each plot had been dried and winnowed.

Data were statistically analyzed by Duncan's multiple range test and correlation coefficients were calculated.

Pathogenicity tests were conducted during the period October-December 1986 in a screenhouse at Suakoko. Three lots of 20, 100 ml plastic pots were filled with soil collected from the experimental field, sterilized by dry heat and air dried for one year and two lots with soil collected from the same experimental field on 10 October either from a control plot (soil samples collected on 25 September contained about 40 *X. ifacolum* per 200 ml of soil) or from a plot treated with 12 kg/ha of carbofuran (no *X. ifacolum* were detected in samples collected on 25

September). On 17 October a one week old seedling of rice cv. Lac 23 white was planted in each pot. A suspension of 10-12 *X. ifacolum* was poured onto the roots of the plantlets in 20 pots containing sterile soil, and a suspension of about 50 *Helicotylenchus pseudorobustus* (Steiner) Golden and 10 *Criconemella curvata* (Raski) Luc *et* Raski was added to each of another 20 pots; 20 pots were left uninoculated as control.

Plant heights were recorded weekly starting from October 27 until December 18. Plant and root weights were recorded at the end of the experiment on December 19.

Data were analyzed by means of the Student's «t» test.

Histopathological and ultrastructural studies were carried out at Bari. Groups of ten *X. ifacolum* were put in 5 cm diameter clay pots containing sterilized sand with a germinated seed of rice cv. Lac 23 white and kept in a growth chamber at 24°C. One and two weeks later swollen root tips and galls were fixed in 3% glutaraldehyde in 0.05 M uranyl acetate, dehydrated in a graded ethanol series and embedded in Spurr's medium. Sections 2 μm thick were stained with toluidine blue and observed with a light microscope. Ultrathin sections were cut with a LKB ultratome III, stained in uranyl acetate and lead citrate and examined in a Philips 400 T electron microscope.

Results

Plant emergence was significantly increased by treatments with carbofuran at the rates of 8, 10 and 12 kg a.i./ha (Figs 2 and 3) and all treatments showed a beneficial effect on the plant growth during the period of the experiment (Table I). The number of tillers was not affected. Straw and grain weights were significantly higher in the plots treated with all doses of carbofuran with respect to the control, while only the plots treated with 10 kg a.i./ha of carbofuran yielded significantly more straw than those treated with methomyl. All treatments caused statistically significant reduction in the population densities of *X. ifacolum* in the soil but there were no significant differences between nematicide treatments.

The effect of carbofuran treatments on *X. ifacolum* numbers in the soil and the relation with plant growth and yield is clearly evident in Table II where the coefficients of correlation between the variables examined are given. The correlation between plant height one and a half months after sowing and the number of *X. ifacolum* in the soil is shown in Fig. 4.

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. plants in the test area (0.5 m diam)	Average plant height (cm) 3 Sept.	23 Sept.	15 Oct.	No. tillers per plant*	Straw weight (kg)	Grain weight (kg)	No. *X. ifacolum* per 200 ml soil
Methomyl	8	26.2ab	46.7 Bb	57.5ab	79.7a	4.8	8.1A Bb	0.8bc	12Aa
Carbofuran	4	28.2ab	50.5A Bb	62.5a	87.5a	5.0	10.3Aa b	1.5ab	5Aa
Carbofuran	8	35.5a	51.5Aa Bb	63.7a	81.5a	5.6	10.4Aa b	1.1ab	1Aa
Carbofuran	10	39.7a	51.2Aa Bb	64.5a	87.7a	4.9	11.6Aa	1.8a	2Aa
Carbofuran	12	34.7a	52.5Aa	65.5a	81.7a	5.0	10.5Aa b	1.6ab	1Aa
Control	—	13.5 b	32.5	52.5 b	66.7	5.3	4.4 B	0.2 c	32

N.B. - Data flanked in any column by the same letter are not statistically different (capital letters for P=0.01; small letters for P=0.05); * F-test not-significant.

Table II - *Coefficients of correlation between the variables examined.*

Carbofuran dose	Plant height 3 Sept.	Plant height 15 Oct.	Straw production	Grain yield	Plant density	Nematode population
Carbofuran dose 1	.767***	.454	.680***	.578	.329	—.804***
Plant height 3 Sept.	1	.765***	.925***	.782***	.508	—.935***
Plant height 15 Oct.		1	.791***	.736***	.407	—.657***
Straw production			1	.843***	.402	—.834***
Grain yield				1	.211	—.641*
Plant density					1	—.456
Nematode population						1

*** r significant for P=0.001; * r significant for P=0.05

In the pathogenicity test in the screenhouse there was a significant suppression in growth of the plants in the pots containing field soil from either the treated or untreated plots (Table III). Inoculation with *C. curvata* and *H. pseudorobustus* or *X. ifacolum* did not affect plant growth although the root systems of the plants grown in pots inoculated with *X. ifacolum* were less developed compared with the control.

The histopathological studies indicated that *X. ifacolum* fed exclusively on the tips of the rice roots. As a response to the feeding activity of the nematode the tips started to swell and ceased growth (Fig. 5a). Swollen tips were distorted and produced new lateral roots (Fig. 5b). Sections through galled tips, one week after nematode inoculation, showed groups of collapsed or necrotic cells. These resulted from the direct injury caused by the nematode's odontostyle and the removal of cell contents. Near the damaged area the cells were hypertrophied and multinucleate (Fig. 5d and e), while in the corresponding region of the unattacked roots only rows of small uninucleate cells were present (Fig. 5c). Modified cells in two week old galls were almost devoid of cytoplasm, contained several well preserved nucleoli and showed processes of cell wall dissolution (Fig. 5e).

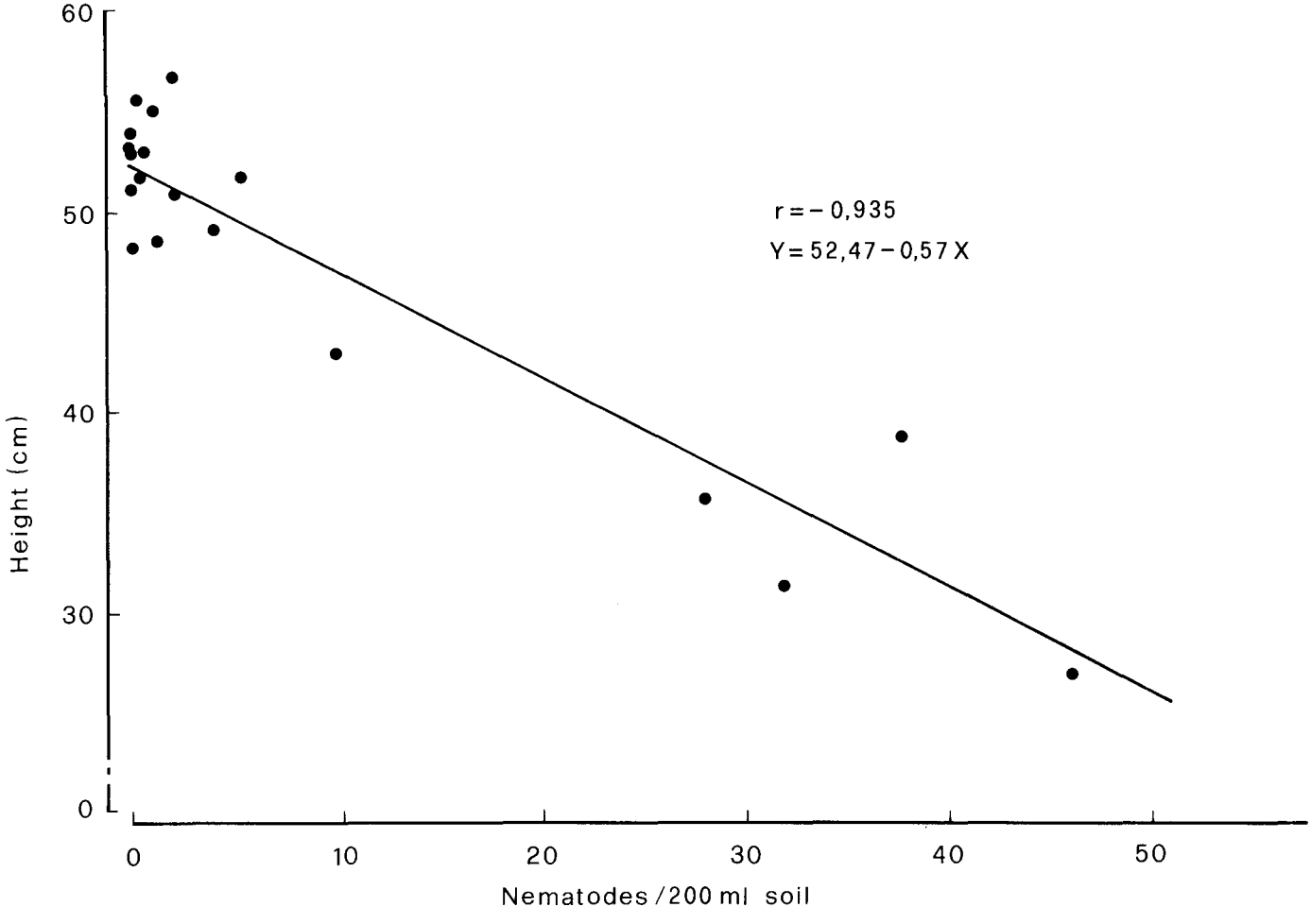

Fig. 4 - Correlation between height of plants $1^1/_2$ months after sowing and number of *X. ifacolum* per 200 ml of soil.

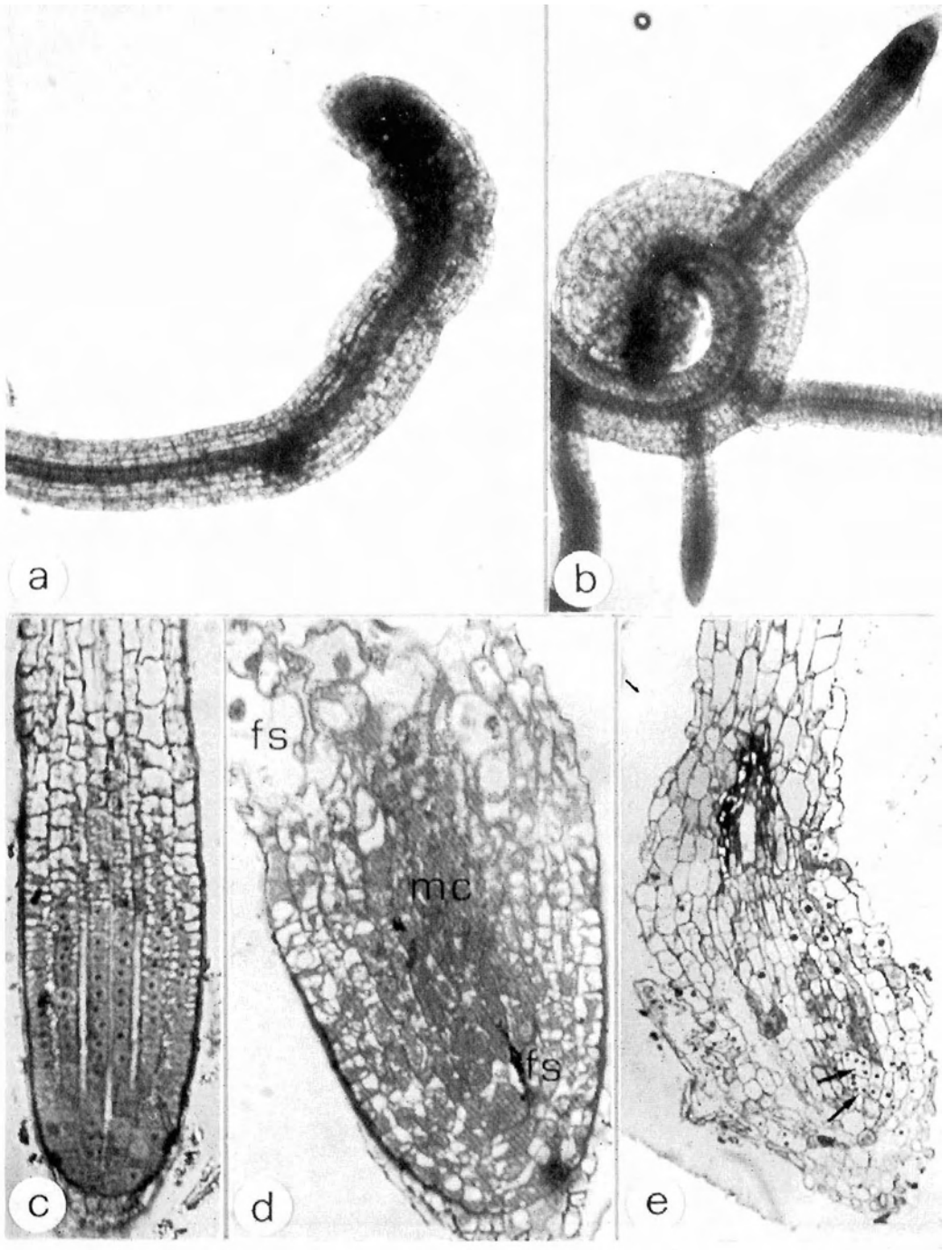

Fig. 5 - Damage caused by *X. ifacolum* feeding on rice roots. a) Attacked root tip swollen and highly deformed; b) galled tip stopped growing and lateral roots emerged at various sites behind the terminal swelling; c) longitudinal section through an unattacked root tip; d) longitudinal section through swollen root tip, one week after nematode inoculation, showing hypertrophied meristematic cells (mc) beneath the feeding site (fs); e) longitudinal section through a galled root tip, 15 days after nematode inoculation; the feeding site is represented by rows of necrotic cells; the meristematic tissue appears devoid of cytoplasm with partially dissolved cell walls. In the hypertrophied multinucleate cells some cytoplasm and nucleoli (arrow) are still visible.

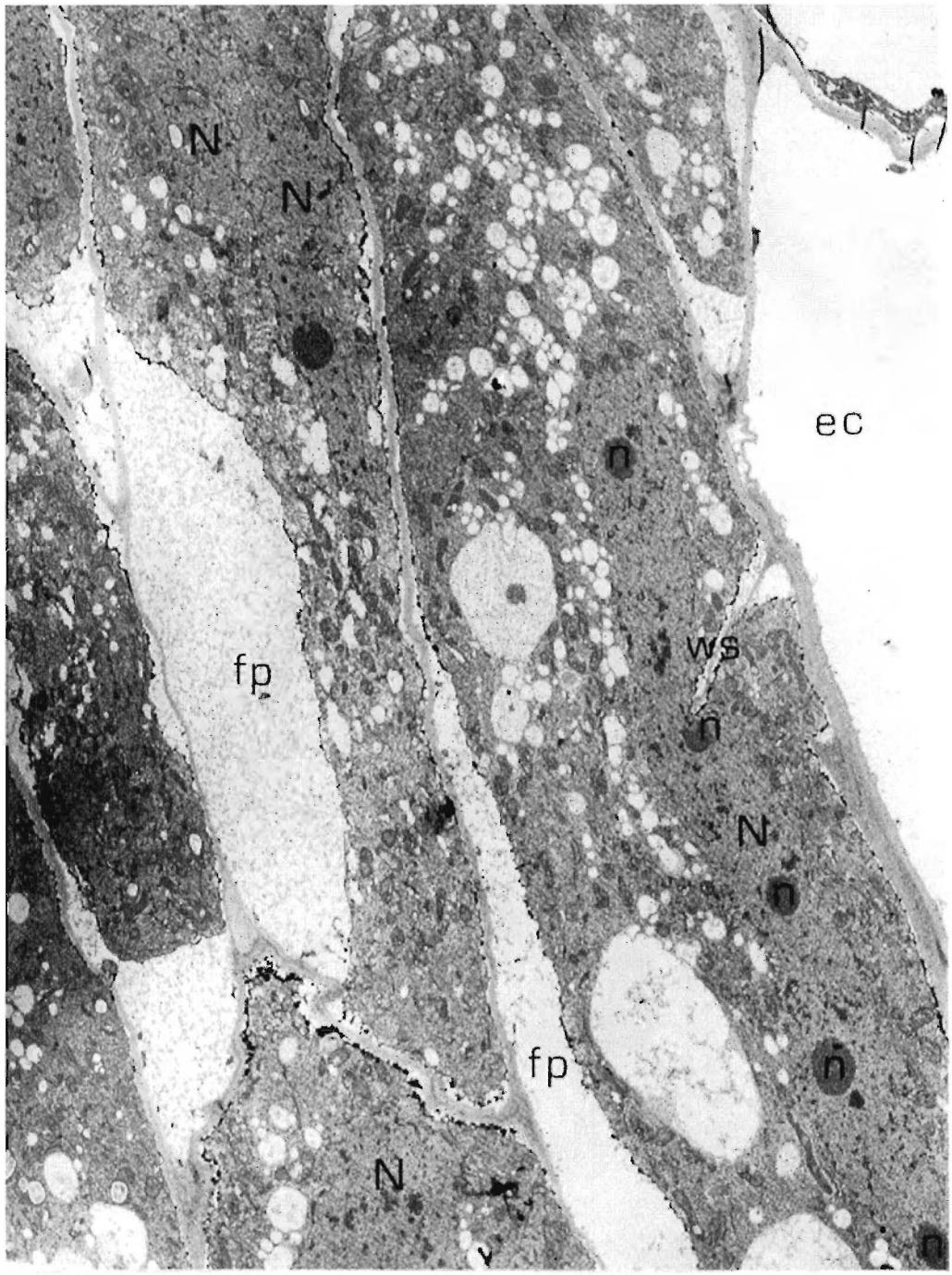

Fig. 6 - Micrograph of a longitudinal section through a parasitized swollen root tip one week after nematode inoculation. Almost empty (ec), and hypertrophied meristematic cells are present close to the feeding site. Note the abnormal nuclei (N), one of which has five nucleoli (n), cell wall stubs (ws) in the cytoplasm rich in organelles. All the tissue shows the process of false plasmolysis (fp).

Table III - *Growth development of rice cv. Lac 23 white in differently treated soil*

Treatment	Average plant height at different dates (cm)						Plant weight g	Root weight g
	23 Oct.	3 Nov.	10 Nov.	24 Nov.	11 Dec.	18 Dec.		
From the field								
Carbofuran treated	11.5**	19.8**	21.2***	25.1***	26.3***	28.5***	1.2***	0.8***
Control	12.3*	16.4***	21.9***	24.4***	25.6***	27.2**	0.7***	0.4***
Sterilized								
with *X. ifacolum*	16.2	26.0	39.4	45.7	53.1	57.4	5.7	3.4**
with *Helic* + *Cricon*	19.2	29.9	41.4	48.5	54.9	59.7	5.8	4.3
Control	16.3	27.2	38.5	47.8	55.6	58.1	6.6	4.9

N.B. - Significantly different from the control * for P=0.05; ** for P=0.01; *** for P=0.001.

Almost empty cells, delimiting the feeding site, were observed in ultrathin sections of one week old swollen root tips. They were surrounded by abnormally large multinucleate cells, many times the size of the normal ones (Fig. 6). Nuclei were slightly enlarged, considerably lobed and sometimes with numerous nucleoli. No cell plates were observed; wall stubs, however, were present along the cell walls. Such stubs probably resulted from an incomplete fusion of cell-plate vesicles. Multinucleate cells showed the typical activity of meristematic tissue, with dense cytoplasm rich in organelles, and without intercellular spaces. The disconnection of the tonoplast from the cell wall was indicative of false plasmolysis (Fig. 6).

Discussion and Conclusions

Xiphinema ifacolum must be considered a serious pest of rice in sandy soils in Liberia. The field experiment has shown significant negative correlations between nematode population densities and plant growth and production. Nematode control and consequent increases in yield can be obtained by soil application of the granular formulation of carbofuran. Broadcast applications at the rates of 8-10 kg a.i./ha at planting gave technically satisfactory results although the economics of such treatments under the local conditions have yet to be evaluated.

The pathogenicity test indicates that probably *X. ifacolum* interacts in the field synergistically with other root pathogens. Plant growth was greatly suppressed in non sterilized field soil whether it came from plots with either high or undetectable (certainly there were eggs) nematode population densities. However, in sterile soil with *X. ifacolum* alone, even at low population densities, the root systems were reduced by approximately 30% after two months, and it could be assumed that root reduction would continue to increase if the plants had been allowed to grow further, eventually affecting the overall plant development, as nematode populations increased. Field observations have shown that *X. ifacolum* multiplies actively on the variety of rice used in the experiment.

Conversely, *H. pseudorobustus* and *C. curvata* at the inoculum levels used had no obvious effect on plant growth.

The response of roots and root cells to feeding by *X. ifacolum* resembles those observed for other longidorid nematodes (Cohn, 1970; Weischer and Wyss, 1976; Bleve-Zacheo *et al.*, 1979). The most relevant changes in the histology of swelling root tips are groups of hypertrophied multinucleate

cells beneath the necrotic cortical layers, representing the feeding site. These cells show features of high metabolic activity, with dense cytoplasm and nuclear hypertrophy. Cell wall stubs and the presence of numerous nucleoli indicate the failed cytodieresis. The plasma membrane of the multinucleate cells is disconnected from the cell wall on the opposite side of the necrotic cells because of the demand of nutrients from the parasite: a flux of metabolism moves towards the feeding site.

SUMMARY

A field experiment undertaken at Suakoko in Liberia demonstrated that *Xiphinema ifacolum* Luc suppresses growth and yield of rice, *Oryza sativa* L. Nematode population reduction and yield increase were obtained with the soil application of carbofuran at the rates of 4 to 12 kg a.i./ha. Pathogenicity tests indicated that the nematode may interact with other root pathogens although a density of 10 *X. ifacolum*/100 ml soil alone caused about 30% reduction in the root system with respect to the control over a period of two months. The response of roots and root cells to the feeding of *X. ifacolum* resembles that observed for other longidorid nematodes.

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