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PATHOGENICITY AND REPRODUCTION OF TWO SPECIES OF *XIPHINEMA* ON SELECTED VEGETABLE CROPS IN LIBERIA

by
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Summary. Population densities of *Xiphinema ifacolum* increased fourfold on cowpea, eggplant and tomato and six to ten fold on okra, over a period of four months. Growth of cowpea, okra cv. Better Five, at higher population densities, but not cv. Clemson Spineless and tomato were suppressed by the attacks of the nematode; it also affected growth and yields of pepper in the field, where it could be controlled by applications of carbofuran and prophos. Cowpea, okra and eggplant did not appear to be good hosts for *X. longicaudatum* which, however, severely depressed the growth of eggplant.

Xiphinema ifacolum Luc is one of the most common plant parasitic nematodes in Liberia where it is the causal agent of a decline of rice (Lamberti *et al.*, 1987 and 1991). It also occurs in the rhizosphere of various trees and vegetable crops and often patches of stunted plants are associated with large numbers of the nematode in the soil.

The effect of *X. ifacolum* on growth and yield of selected vegetable crops and their host suitability to the nematode was investigated. Experiments were carried out in the field with cowpea [*Vigna unguiculata* (L.) Walp.] and okra [*Abelmoschus esculentus* (L.) Moench.] and pathogenicity tests were undertaken in the screenhouse with cowpea, okra, eggplant (*Solanum melongena* L.) and tomato (*Lycopersicon esculentum* Mil.).

The main ultrastructural changes induced in cowpea roots by feeding of the nematode were investigated by electron microscopy.

Xiphinema longicaudatum Luc occasionally occurs in the rhizosphere of declining plants in mixed populations with *X. ifacolum*. To investigate its effect on plant growth, pathogenicity tests were undertaken in the screenhouse with cowpea, okra and eggplant.

Materials and methods

All the experiments were conducted in fields or in screenhouses at C.A.R.I., Suakoko. Fields were ploughed 30-40 cm deep, rotovated and divided into 30, 4 x 2 m plots separated from each other by an interspace of 0.5 m and distributed at random in five blocks.

On 12 August, 1987 methomyl wettable powder at 8 kg a.i./ha, or carbofuran or prophos granules at 8 and 10 kg. a. i./ha, were broadcast on the plot surface and incorporated to a depth of 10 cm. Five plots were left untreated as control. The next day the plots were sown with three seeds per hole, spaced at 20 cm (between plants in a row) x 50 cm (between rows) for cowpea cv. Botota Local and at 75 x 80 cm for okra cv. Better Five. A composite soil sample was collected on 21 August, nine days after treatment, from the rhizosphere of emerging plantlets at three different sites in each plot. *X. ifacolum* were extracted from 200 ml aliquot by Cobb's sieving technique to ascertain initial population densities.

On 21 September all plots were sprayed with carbaryl to control insects. Soon after the okra field was abandoned because of the erratic germination of seeds and erratic emergence of plantlets. The cowpea plots were weeded by hand, when necessary. The height of the plants in the two central rows of each plot were recorded on 15 and 30 September and on 15 October. Fresh pods were harvested on 21 and 30 October, and 22 November.

On 23 November the experiment was discontinued and the final population densities of *X. ifacolum* were ascertained as indicated earlier.

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

The pathogenicity tests consisted of two series. For the first one, carried out between May and August 1987, one week old seedlings of cowpea (cv. Botota Local), okra (cv. Clemson Spineless), eggplant (cv. Black Beauty) and tomato (cv. Cl 1131 - 0 - 0 - 43 - 8 - 1 of the Asian Vegetable Research and Development Center) were planted (between 5 and 10 May) in 0.5 l (10 cm diam) plastic pots (for okra 1 l 12 cm diam pots were used) filled with air dried river sand free from nematodes. At planting, hand picked nematode suspensions (20 females or preadults in about 25 ml of tap water) were poured directly onto the roots of each of the following seedlings: cowpea, 15 pots with *X. ifacolum* and 15 with *X. lon-*

Fig. 1 - Root systems of cowpea plants grown in pots infested by *Xiphinema ifacolum* (control at left).

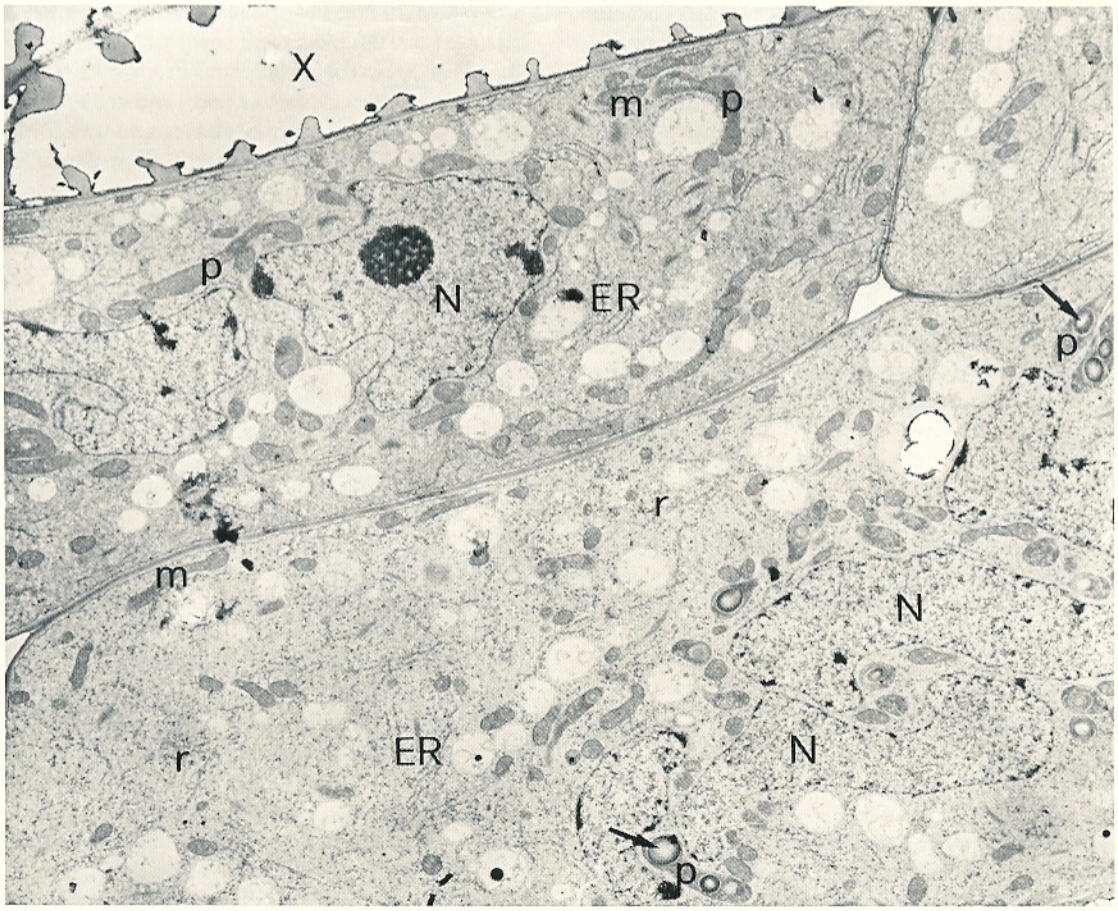

Fig. 2 - Micrograph of longitudinal section through a cowpea root tip exposed to *X. ifacolum*: cells in the area of elongation, adjacent to xylem vessels (X), show a coenocytic process; nuclei (N) are amoeboid with reticulate nucleoli; numerous organelles such as endoplasmic reticulum (ER), ribosomes (r), mitochondria (m) and plastids (p) with starch granules (arrow) are detectable in the cytoplasm (x 5500).

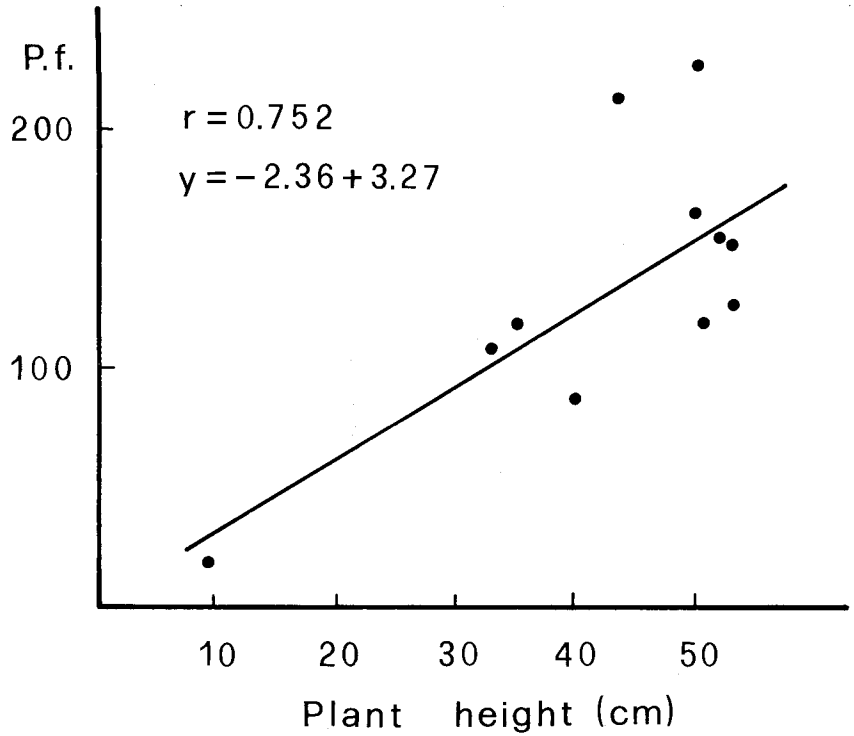

Fig. 3 - Correlation between plant height on 30 July and final population (Pf) of *X. ifacolum* in pots planted with okra cv. Clemson Spineless (significant for P = 0.01).

gicaudatum; okra, 12 pots with *X. ifacolum* and 12 with *X. longicaudatum*; eggplant, 12 pots with *X. ifacolum* and 6 with *X. longicaudatum*; tomato, 12 pots with *X. ifacolum*. There was always an equal number of uninoculated control plants (they were inoculated with plain tap water only).

Plant heights were recorded on 1 June for cowpea and 1, 15 and 30 June and 15 and 30 July for okra, eggplant and tomato. Plant weights and numbers of nematodes per pot were determined at the end of the experiments, all of which were discontinued between 17 and 19 August.

The second series of pathogenicity tests was undertaken with *X. ifacolum* only: from 18 August to 23 November, 1987, for cowpea cv. Botota Local (15 pots), from 19 August to 25 November for okra cv. Better Five (12 pots) and from 20 August to 2 December for eggplant cv. Black Beauty (22 pots). An equal number of uninoculated pots per each plant species served as control. In these tests the number of nematodes inoculated per pot varied from 18 to 144 for cowpea, from 19 to 229 for okra and from 25 to 162 for eggplant. They were all mobile stages, except males, with 10 - 15% gravid females. The final population of each pot of the previous experiment served as an initial population for the next. The inoculum for eggplant consisted of the nematodes of the previous eggplant experiment for 12 pots and

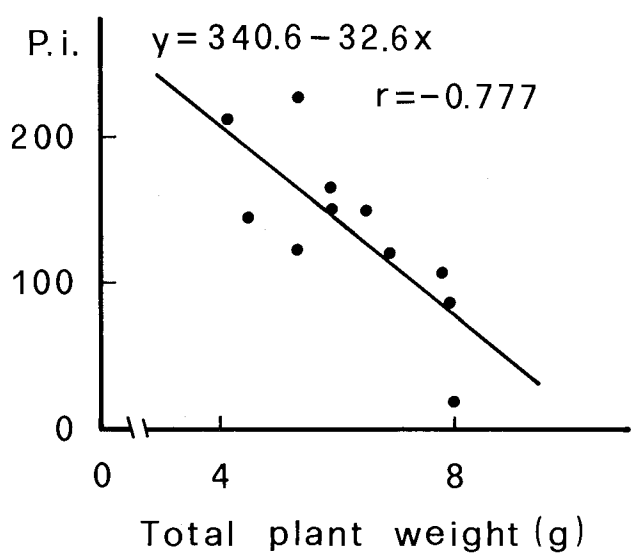

Fig. 4 - Correlation between plant weight and initial population (Pi) of *X. ifacolum* in pots planted with okra cv. Better Five (significant for P = 0.01).

of the nematodes of the previous tomato experiment for the remaining 10. Plant heights were recorded on 15 and 30 Septemebr and on 15 and 30 October for all plants; for egg-plant an additional measurement was made on 16 November. At the conclusion of the tests, plant weights and the number of nematodes per pot were determined.

The data of the pathogenicity experiments were compared statisticaly by means of Student's "t" test; correlation coefficients were also calculated.

For electron microscopy studies cowpea cv. Botola Local seedlings were transplanted in the 5 cm diam pots containing 10 ml sterilized sand. Each pot was inoculated with 5 adult females of *X. ifacolum*. The pots were kept in a growth chamber at 24 °C. Five days after inoculation swollen root tips were fixed in 3% gluteraldehyde in 0.05 M sodium cacodylate buffer at pH 7.2 for four hours, then rinsed in the same buffer and post-fixed in 2% osmium tetroxide for four hours at 4 °C, followed by staining in 0.5% aqueous uranyl acetate, dehydration in an ascending series to absolute ethanol and embedding in Spurr's medium. Ultrathin sections were cut with a LKB ultratome III, stained with uranyl acetate and lead citrate and examined with a Philips 400T electron microscope.

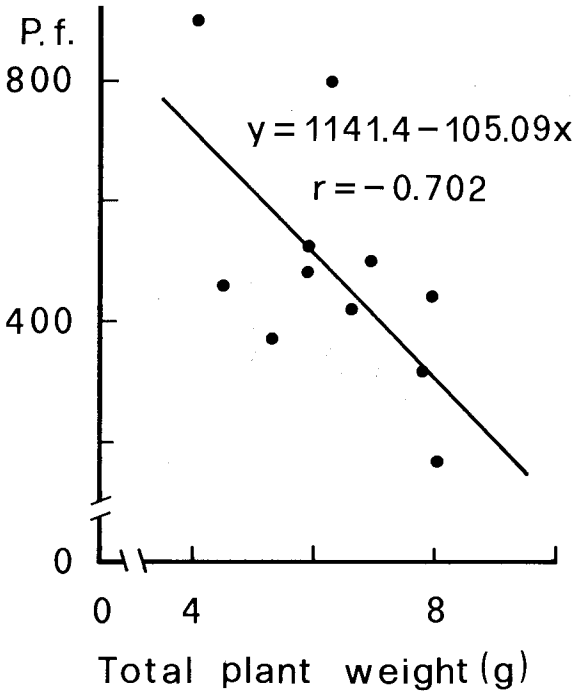

Fig. 5 - Correlation between plant weight and final population (Pf) of *X. ifacolum* in pots planted with okra cv. Better Five (significant for P = 0.01).

Results and discussion

Cowpea. Initial population densities of *X. ifacolum* in the field were too low and erratic to significantly affect plant growth and yields (Table I). However, it is interesting that nematicidal treatment with carbofuran and prophos suppressed reproduction of the nematode.

The pathogenicity tests undertaken from May to August indicated that *X. ifacolum* can retard plant growth and root development (Fig. 1) while there was no significant effect with *X. longicaudatum* (Table II). The final nematode population densities (average 81 nematodes per pot, a range 18 to 144) showed that *X. ifacolum* reproduced well; in fact many gravid females and first and second stage juveniles were present in the pots.

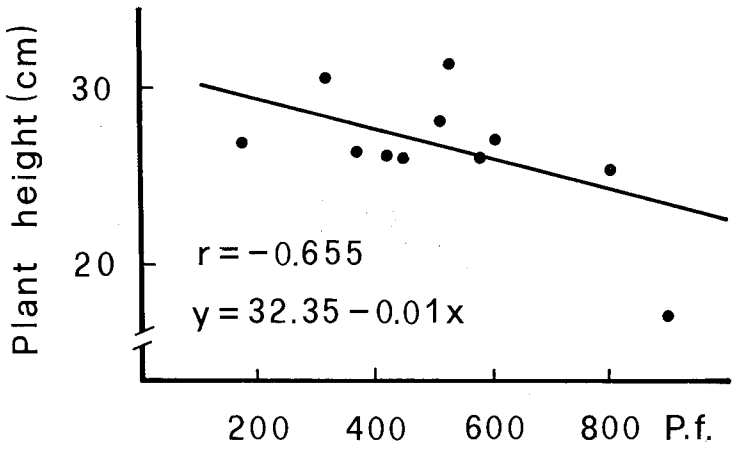

Fig. 6 - Correlation between final population (Pf) of *X. ifacolum* and plant height on 30 October in pots planted with okra cv Better Five (significant for P = 0.05).

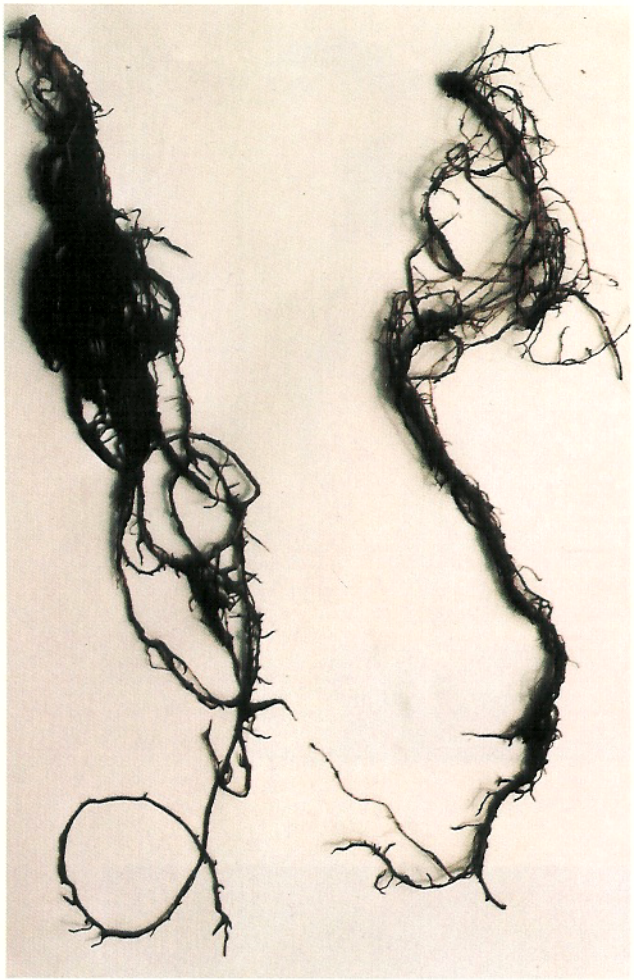

Fig. 7 - Root system of okra cv Better Five, grown in pots infested by *X. ifacolum* (control at left).

Populations of *X. longicaudatum* decreased from 20 to 3 individuals per pot; however, of a total of 50 nematodes, 11 were gravid females and the juveniles were mostly preadult stages. This suggests that cowpea is a poor host for this species but a low rate of reproduction enables it to survive with an extended life cycle.

The second series of tests carried out from August to December, did not confirm the effect of *X. ifacolum* on the growth of cowpea. Nematode numbers decreased from an average of 81 to 18 (range 6 to 37) per pot. There was a prevalence of females that appeared to be in good nutritional state (dark intestine) but they were mostly without eggs in the uteri.

As reported for other species of longidorids (Bleve Zacheo *et al.*, 1987), the main cellular change associated with the feeding of *X. ifacolum* on cowpea roots was the production of a coenocyte at the feeding site; this had two or more enlarged nuclei with markedly lobed contours (Fig. 2). These nuclei were packed together and contained nucleoli with intranuclear vacuoles. The cytoplasm of modified cells showed proliferation of rough endoplasmic reticulum arranged in parallel strands, scattered ribosomes and numerous mitochondria and plastids, all indicating active synthesis.

Coenocytes are the nutrient source of the nematode and continue to synthetize under the effect of the stimulus of nematode feeding.

Okra - Neither *X. ifacolum* nor *X. longicaudatum* affected growth of cv. Clemson Spineless. However, nematode numbers increased from 20 to 127 ± 19.5 within a range from 19 to 229 per pot for the former species, and decreased from 20 to 12 ± 1.7 within a range from 4 to 22 per pot for the latter species. In both cases populations were composed of all the motile stages including gravid females and first stage juveniles, indicating that reproduction was in

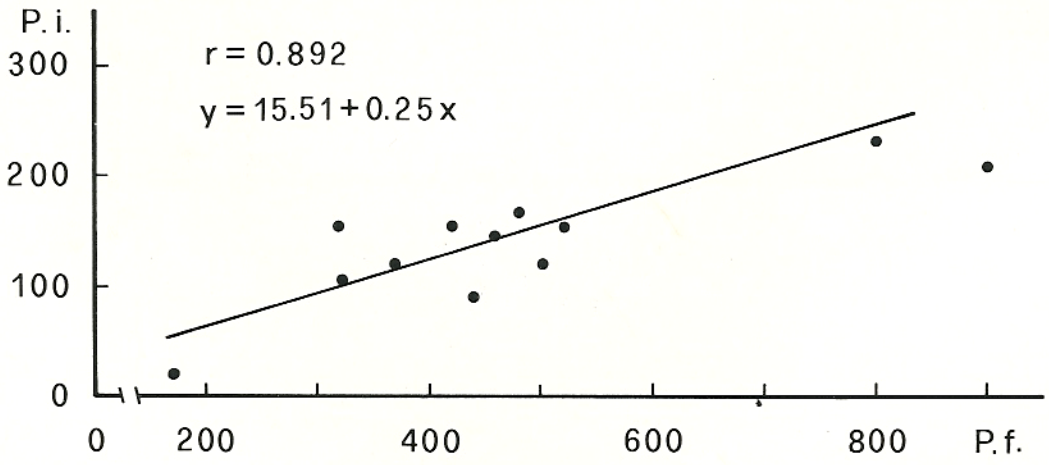

Fig. 8 - Correlation between final (Pf) and initial (Pi) population of *X. ifacolum* in pots planted with okra cv Better Five (significant for P = 0.01).

Fig. 9 - Growth of eggplant in pots infested by *X. longicaudatum* (control at right).

Fig. 10 - Root systems of eggplant grown in pots infested by *X. longicaudatum* (control at left).

Fig. 11 - Root systems of eggplant grown in pots infested by *X. ifacolum* (control at left).

Fig. 12 - Growth of tomato plants in pots infested by *X. ifacolum* (two control plants at left).

Fig. 13 - Root systems of tomato plants grown in pots infested by *X. ifacolum* (control at left).

progress; it was most active on the best growing plants as demonstrated by the positive correlation between final population densities of *X. ifacolum* and heights of the plants on 30 July (Fig. 3).

In the second pathogenicity experiment with the cv. Better Five there was a negative significant correlation between both the initial or final population densities of *X. ifacolum* and total weight of plants (Figs. 4 and 5) and between final population densities and plant height on 30 October (Fig. 6). The root systems of plants exposed to the nematodes were reduced in size compared with the control (Fig. 7) and population densities increased from an average of 127 to 489 nematodes per pot (range 168 to 900). When the test was discontinued the plants were still in an actively growing vegetative stage and consequently nematode reproduction was still active, with a higher reproductive rate at the higher initial population densities, as shown by the positive correlation between final and initial numbers of nematodes (Fig. 8). This was confirmed by the fact that populations contained many gravid females and first and second juvenile stages.

Egglant - In the first pathogenicity test *X. ifacolum* did not significantly affect growth but *X. longicaudatum* did (Table III). Plants inoculated with *X. longicaudatum* were stunted and had a reduced root system (Figs. 9 and 10). *X. ifacolum* increased its initial population densities fourfold (79 ± 15 nematode per pot; range 63 to 162); the numbers of *X. longicaudatum* declined (Table III).

On the August-December test there was an initial decline of plants in the pots infested by *X. ifacolum*; but later they recovered and when the experiment was discontinued they were not statistically different from the control plants (Table IV). The nematode populations remained stationary with a final mean density of 77 (range 16 to 348) nematodes per pot and with a prevalence of juvenile stages but very few gravid females, indicating perhaps that after an intensive initial egg laying reproduction had stopped or slowed. However, in the pots with lower final population densities the roots systems of the plants were stubby (Fig. 11) and the intestines of the nematodes were transparent and devoid of granules, indicating that they had not fed for a long time.

Tomato - X. ifacolum significantly suppressed growth (Table V; Figs. 12 and 13). Nematode densities increased fourfold within a range of 28 to 158 nematodes per pot.

The plant response to the nematode infestation was

TAB. I - *Yields of cowpea and population densities of* Xiphinema ifacolum *in soil treated with different nematicides*

Treatment	Rate (Kg. ai/ha)	Yield (g of fresh pods/plot)	Nematode populations in 200 ml soil	
			initial	final
Methomyl	8	290 a	5.2 a	15.6 A
Prophos	8	408 a	3.8 a	4.2 BC
Prophos	10	360 a	1.2 a	2.4 C
Carbofuran	8	294 a	36 a	2.4 C
Carbofuran	10	438 a	2.8 a	3.2 C
Control	–	293 a	3 a	12.4 AB

N. b.: Data flanked in any calumn by the same letter are not statistically different; small letters for P = 0.05, capital letters for P = 0.01.

TAB. II - *Effect of* X. ifacolum *and* X. longicaudatum *on growth of cowpea*

Nematodes species	Total plant weight (g)	Root weight (g)	Plant height on 1 July (cm)	Final nematode population/pot ± SE
X. ifacolum	3.4*	1.6*	17.5	81 ± 10.5
X. longicaudatum	4.7	2.1	16.3	3.2 ± 0.4
Control	5.4	2.9	18.6	

* Statistically different with respect to the control for P = 0.05.

TAB. III - *Effect of* X. ifacolum *and* X. longicaudatum *on growth of eggplants during the period May - August*

Nematode species	Plant height (cm) 1 June	15 June	30 June	15 July	30 July	Total plant weight (g)	Root weight g	Final nematode population/pot ± SE
X. ifacolum	5.7	7.9	14.3	19.6	22.0	21.8	12.6	79 ± 15
X. longicaudatum	5.4	8.5	15.0	19.7	19.7*	5.8**	2.4**	2.4 ± 1
Control	6.3	9.7	16.3	21.2	24.0	21.2	11.4	

Statistically different with respect to the control: * for P = 0.05 and ** for P = 0.01.

TAB. IV - *Effect of* X. ifacolum *on growth of eggplant during the period August - December*

	Plant height (cm) 15 Sept.	30 Sept.	15 Oct.	30 Oct.	16 Nov.	Total plant weight (g)	Root weight g	Final nematode population/pot
Nematodes	3.8	5.7*	9.6*	12.8	17.4	7.6	3.1	77
Control	4.0	8.0	12.1	15.1	19.3	8.5	3.5	

Statistically different with respect to the control: * for P = 0.05 and ** for P = 0.01.

TAB. V - *Effect of* X. ifacolum *on growth of tomato*

	Plant height (cm) 1 June	15 June	30 June	15 July	30 July	Total plant weight (g)	Root weight g	Final nematode population/pot ± SE
Nematodes	7.4	11.6	20.4*	28.4*	36.1*	5.5*	1.4**	81 ± 14
Control	8.0	14.7	28.2	38.8	50.5	9.1	2.8	

Statistically different with respect to the control: * for P = 0.05 and ** for P = 0.01.

Fig. 15 - Patchy appearance of a hot pepper field infested by *X. ifacolum*.

Fig. 16 - Stunted and yellowish plants of hot pepper in a field infested by *X. ifacolum*.

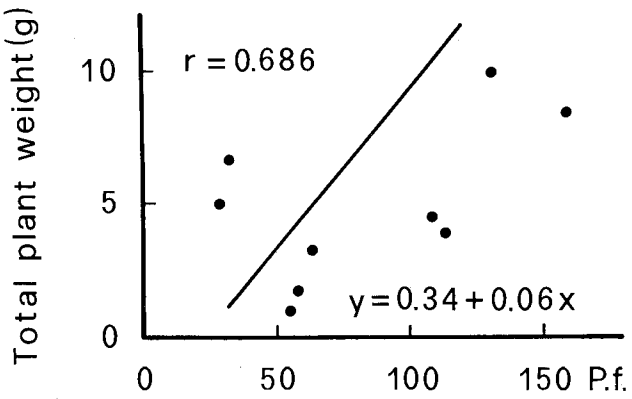

Fig. 14 - Correlation between final population (Pf) of *X. ifacolum* and weitght of tomato plants grown in pots infested by *X. ifacolum* (significant for P = 0.05).

variable and the highest final population densities were again associated with the better growing plants (Fig. 14).

Pepper - *X. ifacolum* was found in high numbers (over 500 nematodes per 300 ml of soil) in the rhizosphere of declining plants of hot pepper (*Capsicum annuum* L.). The field was patchy and plants were small and yellowish (Figs. 15 and 16). Root tips were distorted and swollen.

Conclusions

In Liberia, *X. ifacolum* is a pest of cowpea, okra, eggplant and tomato, that all must be considered as good hosts for the nematode.

Cowpea suffered growth suppression of 37% and root weight reduction of 45% with respect to the control in one experiment, but was not affected in a second pathogenicity test. However, for some unknown reason, reproduction of the nematode did not occur during this second test and population densities had much decreased compared to the inoculum levels.

Different cultivars of okra showed a varying reaction to *X. ifacolum*. There was no significant effect on cv. Clemson spineless but the growth of cv. Better Five was suppressed, however at initial population densities as high as about 500 *X. ifacolum* per pot. Since plants were still growing and nematode reproduction still continuing, when the test was terminated, it could be assumed that growth suppression would have been greater at a more advanced stage.

The growth of eggplant was significantly suppressed by *X. ifacolum* only at the beginning of the experiment at higher initial inocolum levels; later the plants recovered and grew well.

X. ifacolum clearly suppressed plant growth (by 40% compared to the control) and root development (by 50% with respect to the control) in tomatoes.

Field observations indicate that the growth and yield of pepper can also be suppressed by *X. ifacolum*.

The field experiment indicates that this species can be controlled by the application of nematicides such as carbofuran and prophos.

None of the three plant species, cowpea, okra and eggplant can be considered good host for *X. longicaudatum*. However, okra supported higher population densities than the other two.

X. longicaudatum suppressed total plant and root weights of eggplant cv. Black Beauty by 73 and 79% respectively, compared to the control, indicating that it might be a serious pest of this crop.

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