Laboratorio di Nematologia Agraria del C.N.R.
70126 Bari - Italia

EFFECT OF *MELOIDOGYNE INCOGNITA* (KOFOID *ET* WHITE) CHITWOOD ON THE NICOTINE CONTENT OF TOBACCO (*NICOTIANA TABACUM* L.)

by
G. ZACHEO, F. LAMBERTI and R. D. DURBIN [1]

The nicotine content of the apical leaves of tobacco (*Nicotiana tabacum* L.) in plots treated with the nematicide 1,3 dichloropropene, was found to be lower than that of leaves from untreated plots (Lamberti *et al.*, 1971). Examination of the root systems of the plants revealed that those from the treated plots were only moderately attacked by root-knot nematodes, *Meloidogyne incognita* (Kofoid *et* White) Chitw, whereas roots from the untreated plots were severely galled. The question then arose as to whether the decreased nicotine content was the result of the nematicide treatment *per se* or whether nematode invasion might stimulate nicotine metabolism since the roots are the sole synthesizing organ (Dawson, 1942, 1945).

The results of experiments designed to discriminate between these two possibilities are reported herein.

Materials and methods

Aliquots of steam-sterilized sandy loam were mixed thoroughly in plastic bags with different quantities of 1,3 dichloropropene to obtain concentrations of 56.5, 113 and 169.5 ppm (v/v) which corresponded to about 100, 200 and 300 l/ha, respectively. Three days

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[1] Permanent address of the third author: USDA, Dept. Plant Pathology, Univ. Wisconsin, Madison. - The technical assistance of Mr. V. A. Melillo is acknowledged.

later each of the treated soils, plus a steam-sterilized control soil, was distributed to 20 1 l black plastic phytocells and ten days later planted with seedlings of tobacco « Xanthi-Yakà », 3 to 4 cm tall. Ten days after transplanting 2,000 second-stage larvae of *M. incognita* were placed in the root region of ten plants of each treatment. The other ten were left without nematodes to check the effect of the nematicide alone on the nicotine content.

In a second experiment, non-fumigated, steam-sterilized soil was used to test the effect of four densities of the nematode (10, 10^2, 10^3 and 10^4/plant) on nicotine content. The plants were grown in 2 l phytocells, ten replicates/density.

The phytocells were distributed at random on a bench in a green-house (25-26° C, 60-70% RH).

Twenty days after transplanting, leaves were harvested at intervals of about 20 days. Starting with the basal-most leaf of the plant, at each interval five leaves/plant were harvested.

Leaves from the first two intervals and second two intervals were bulked, then weighted, dried (50° C for 7 days) and powdered in a blender.

Nicotine was extracted by distillation of three aliquots (0.1-0.2 g dry wt) from each sample and determined spectrophotometrically (Dawson, 1945; Willits *et al.*, 1950). The same procedure was followed for determining the nicotine content of the roots. The number of second-stage larvae and eggs of *M. incognita* in the infested phitocells, and the weights of the roots systems were determined at the end of the experiment. The nematodes were extracted using a Baermann funnel, and the eggs were isolated using the sugar flotation method (Zacheo and Lamberti, 1974).

Statistical analysis was performed using Duncan's multiple range test (Duncan, 1955).

Results

In the first experiment, the nicotine content of the leaves from the first two harvests (lower leaves) was not affected by either the nematodes or the nematicide (Table I).

Conversely, at the later harvests (upper leaves), the nicotine content was higher in leaves from diseased plants. This effect persisted at the lowest rate of the nematicide, but at higher dosages,

Table 1 - *Nicotine content of tobacco plants and number of larvae and eggs of the nematode present in soil fumigated with different rates of 1,3 dichloropropene and inoculated with* M. incognita.

Rates of 1,3 dichloropropene application ppm	Nematode inoculum 2nd stage larvae	% content of Nicotine				Number of nematodes in 50 ml of soil			
		Lower leaves (the first 10 of each plant)		Higher leaves (the remaining 10 - 12)		2nd stage larvae		eggs	
0	2000	0.43	a	0.32	ab AB	1516	a	950	c A
	none	0.40	a	0.19	c C				
56.5	2000	0.45	a	0.33	a A	1250	a	1474	ab A
	none	0.43	a	0.24	abc ABC				
113	2000	0.35	a	0.26	abc ABC	1271	a	1501	a A
	none	0.43	a	0.20	a BC				
169.6	2000	0.41	a	0.26	abc ABC	1259	a	1217	abc A
	none	0.42	a	0.25	abc ABC				

Data flanked on the columns by the same letters are not statistically different; capital letters for P = 0.01, small letters for P = 0.05.

the nicotine content of the control and diseased plants were not significantly different.

At all rates of 1,3 dichloropropene, especially the highest (169.5 ppm), nematode invasion of the roots and reproduction was delayed by the toxic residue. On the roots only moderate galling was observed and there were less larvae and more eggs, as compared with the roots from non-fumigated soil.

The data from the second experiment were not statistically different at the 1% level because of the high degree of variability. However, at 5% probability the nicotine content was higher in the upper leaves and lower in the roots at inoculum densities of 10 and 100 nematodes/plant. At the higher inoculum densities, because of severe attacks of the nematode, the roots were severely galled and there was no more increase in nicotine content of the upper leaves but still the nicotine content of the roots decreased (Fig. 1 A).

Discussion

The nematicide had no direct effect on nicotine content but, indirectly as it reduced nematode activity, it caused a reduction in nicotine content.

Clearly though the nicotine content of the leaves was increased by nematode attack (Fig. 1 A). This increase was, in fact, positively correlated with inoculum potential (Fig. 1 B). On the other hand, there was a progressive decrease in the nicotine content of the roots as the inoculum was increased.

If one considers the nicotine content of the roots and upper leaves together at the end of the experiment, it can be noted that there is an increase in the diseased treatments with respect to the control (Fig. 1 C). Moreover, while the ratio of nicotine content of

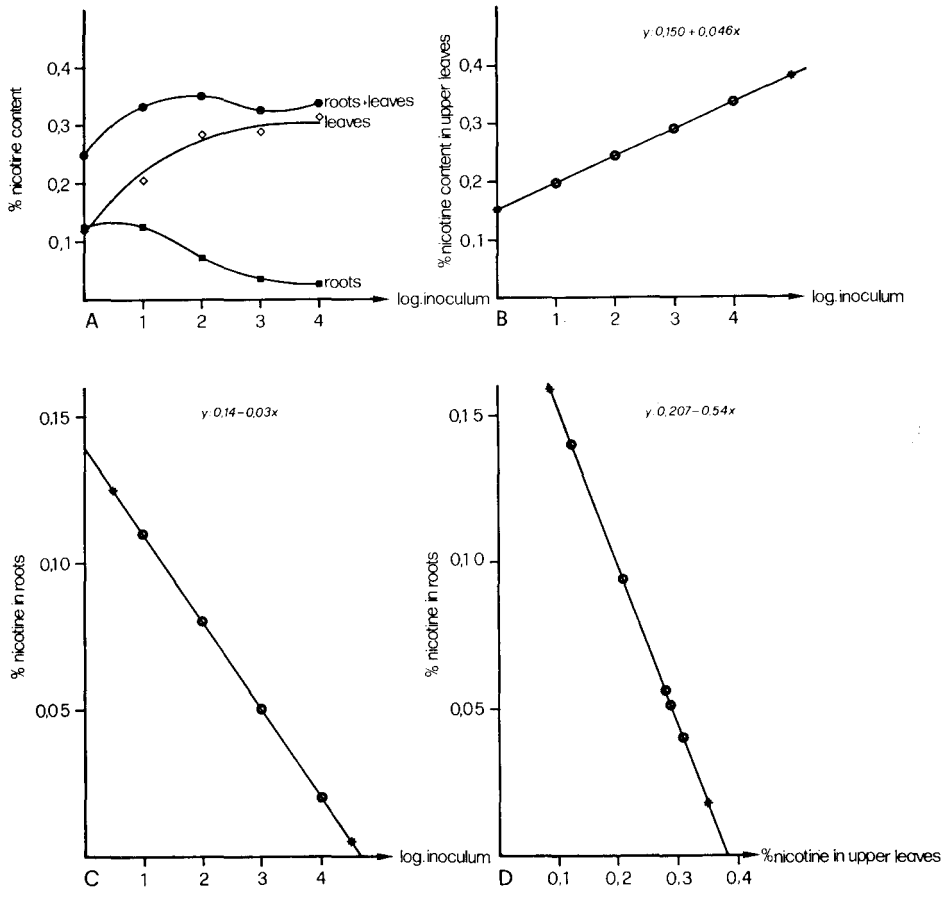

Fig. 1 - Effect of *Meloidogyne incognita* on the nicotine content of tobacco. A, relationships between inoculum density and nicotine content of roots and leaves. B and C, correlation between inoculum density and nicotine content of leaves and roots, respectively (r significant at 0.01 level). D, relationships between the nicotine content of leaves and roots (r significant at 0.01 level).

the upper leaves/nicotine content of the roots is one in the control, in the diseased plants the ratio is significantly higher. This indicates that the nematode not only induces a stimulus of synthesis, but also may stimulate nicotine translocation (Fig. 1 C, D).

We suggest that at least part of this increased nicotine content may be due to an augmentation of aspartic acid, which is a precursor of nicotinic acid and nicotine (Goodwin and Mercer, 1972), resulting from the action of proteolytic enzymes injected by the nematode into the host root cells (Veech and Burton, 1969; Viglierchio, 1971; Miller and Jenkins, 1964). That the free amino acid pool in root-knot infected roots is larger than in healthy roots has been amply demonstrated (Hanks and Feldman, 1963; Epstein and Cohn, 1971).

SUMMARY

The nicotine content of tobacco leaves is increased by root-knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitw. attack while its content in roots is decreased. There is also a positive correlation between overall nicotine content and amount of inoculum. These results indicate that the nematodes stimulate both synthesis and translocation of nicotine.

RIASSUNTO

Effetto di Meloidogyne incognita (*Kofoid* et *White*) *Chitwood sul contenuto nicotinico del tabacco* (Nicotiana tabacum *L.*).

Il contenuto in nicotina di foglie e radici di tabacco è rispettivamente aumentato e diminuito a seguito degli attacchi del nematode galligeno *Meloidogyne incognita* (Kofoid *et* White) Chitw. È stata osservata anche una correlazione positiva fra il contenuto di nicotina e la densità d'inoculo. Questi risultati indicano che i nematodi stimolano contemporaneamente la sintesi e il trasporto dell'alcaloide.

RÉSUMÉ

Effet de Meloidogyne incognita (*Kofoid* et *White*) *Chitwood, dans la teneur en nicotine du tabac* (Nicotiana tabacum *L.*).

Meloidogyne incognita (Kofoid *et* White) Chitw., provoque une augmentation de la nicotine dans les feuilles et une diminution dans les racines de tabac. Il y a aussi une corrélation positive entre la teneur en nicotine et l'intensité de l'inoculation. Ces résultats indiquent que les nématodes stimulent à la fois la synthèse et la migration de la nicotine.

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