Effect of Oryzalin and 1,1-Dimethylpiperidinium Chloride on Cotton and Tomato Roots Infected with the Root-knot Nematode, Meloidogyne incognita[1]

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Abstract: Oryzalin (3,5-dinitro-N^4,N^4-dipropyl-sulfanilamide) and BAS 083 (1,1-dimethylpiperdinium chloride) reduced root-knot infection in tomato roots when respectively applied as a soil drench at 20 ppm and 10,000 ppm. Oryzalin reduced knot counts with various intervals between treatment and inoculation. BAS 083 reduced knot counts only when applied before inoculation. Oryzalin was shown not to be a contact nematicide, and BAS 083 was only a weak one. Neither compound reduced penetration by infective larvae. Postinfection reduction in knot counts by Oryzalin and BAS 083 resulted, in part, from activation of natural defense mechanisms of the host. Giant-cell development in cotton roots inoculated with nematodes was inhibited by Oryzalin. Lateral root development was inhibited by BAS 083. *Key Words:* herbicides, growth regulators, physiology, resistance.

Herbicides and plant-growth regulators are chosen for use in agriculture on the basis of their selective phytotoxicity and activity in promoting or retarding growth. However, they sometimes affect crop plants in ways not intended. They may cause a plant to become either more susceptible or resistant to a disease organism (7). That may reflect either a direct effect on the disease organism or changes in the physiology and biochemistry of the crop plant that influence relations with the pest.

Several herbicides and plant-growth regulators have been shown to affect the development, growth, and reproduction of nematodes in various plant species (8, 13, 16). Romney et al. (14) reported that onion and bean plants grown in soils treated with the herbicide dimethyl tetrachloroterephthalate (DCPA) showed reduced susceptibility to root-knot nematode infection (evidenced by fewer root-knot galls). They suggested that the induced resistance could be correlated with an altered cell structure of the epidermis to resist the nematode *Meloidogyne hapla,* which, having a very delicate stylet, parasitizes only young thin-wall roots. In contrast, they observed that inhibition of the root and top growth of both alfalfa and tomato by root-knot nematodes was increased in the presence of trifluralin.

Davide and Triantaphyllou (5) studied the effect of foliar application of maleic hydrazide (MH) on sex differentiation of *Meloidogyne javanica* on tobacco and tomato plants. They found that plants treated with MH after inoculation had a higher incidence of males than controls, and suggested that sex reversal was due to suppression of giant-cell formation. Mjuge and Viglierchio (11) reported a reduction in the number of knots on tomato plants treated with MH. In contrast, Dropkin et al. (6) reported a loss of resistance to nematode infection in tomatoes when kinetin was applied to plants, an effect similar to that observed by Kochba and Samish (8) from studies of nematode resistance in several peach varieties.

We report here that 3,5-dinitro-N^4,N^4-dipropyl-sulfanilamide (Oryzalin; Elanco Products Company, Division of Eli Lilly and Company, Agricultural Research, Box 708, Greenfield, Indiana 46140) and 1,1-dimethylpiperidinium chloride (BAS 083; BASF Wyandotte Corp., Parsippany, New Jersey) reduced root-knot infection in tomato roots by activating the host-plant defenses, not through nematicidal properties. A study was done to determine the mechanism(s) by which Oryzalin and BAS 083 reduce root-knot-nematode infection in tomato plants and cotton.

MATERIALS AND METHODS

'Bonnie Best' and 'Floradel' tomato plants (*Lycopersicon esculentum*) and cotton (*Gossypium hirsutum,* 'M-8') were selected as test species because of their susceptibility to the root-knot nematode *Meloidogyne incognita.*

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Tomato plants were germinated and grown in the greenhouse in 10-cm-diam plastic pots containing a 4:1 mixture of sand and local topsoil. Oryzalin at 200 μg/pot in 4% acetone or BAS 083 at 130 mg/pot was applied as a soil drench to four sets of five plants—at 2 weeks before inoculation, 1 week before inoculation, inoculation, and 1 week after inoculation. Controls were a set of five plants treated with 4% acetone. All plants were 6 weeks old when inoculated with 2,000 infective second-stage nematode larvae/pot. Nematode infections or gall numbers were evaluated by knot counts. Roots were washed free of soil, placed in petri dishes, and spread apart, and knots were counted under a 2× magnifying lamp. The roots were then dried at 60 C and weighed. All experiments used a completely randomized block design and square-root transformations of the data were analyzed by analysis of variance with Dunnett's comparison of means or Duncan's multiple-range test.

Nematicidal effects of Oryzalin and BAS 083: Nematode larvae were incubated for 24 h in 20 ppm solution of Oryzalin and in 10,000 ppm BAS 083. The larvae were washed free of solutions in a 400-mesh sieve, suspended in water, and observed with a microscope. Tomato plants 4, 6, and 8 weeks old (small, medium, and large) were inoculated with treated and untreated larvae, or treated with BAS 083 at 130 mg/pot and inoculated with untreated larvae.

Treated tomatoes in untreated soil: Fifteen tomato plants were grown in a 15-cm pot for 6 weeks and then treated with BAS at 700 mg or Oryzalin at 1,250 μg in 10% acetone. Two days after treatment, five plants of each treatment and untreated controls were transplanted into new pots containing untreated soil. Five days after transplanting, these plants were inoculated with 2,000 nematode larvae per pot.

Larval penetration of cotton roots: Blotter rolls of cotton seedlings were prepared by methods of McClure and Robertson (10). Twenty-four hours after sowing, the germinating seeds were treated with 10 ppm Oryzalin. Twenty-four h later, about 100 larvae per root tip were applied. Forty-eight h after inoculation, roots were harvested, stained with acid fuchsin in ethanol and acetic acid, cleared in chloral hydrate, and observed under the microscope in lactophenol.

Giant-cell development in cotton roots: Forty-eight h after seeds were sown in blotter rolls, the germinating seeds were infected with about 200 larvae per root tip. Twenty-four h after inoculation, the cotton roots were treated with 10 ppm Oryzalin. Roots were harvested 72 and 96 h after inoculation, fixed in Navashin's solution under vacuum, embedded in paraffin, and sectioned. Sections were stained with basic fuchsin-indigo carmine, and photographed.

RESULTS

Oryzalin at 250 μg/pot reduced knot counts without affecting root dry weight (Fig. 1). Knot reduction occurred even when the interval between treatment and inoculation was varied. For example, Oryzalin reduced knot counts when applied one week after inoculation (Fig. 1).

Incubation of larvae for 24 h in 20 ppm Oryzalin did not reduce their ability to infect roots (Fig. 2). The only differences in galling in the experiment were correlated with age of plant at inoculation. The smaller plants (4 weeks old at inoculation) had fewer galls than larger plants (6 and 8 weeks old at inoculation). Examination under the microscope revealed no differences in activity between larvae incubated in Oryzalin solution and larvae incubated in dilute acetone. Knotting was reduced also in Oryzalin-treated plants transplanted

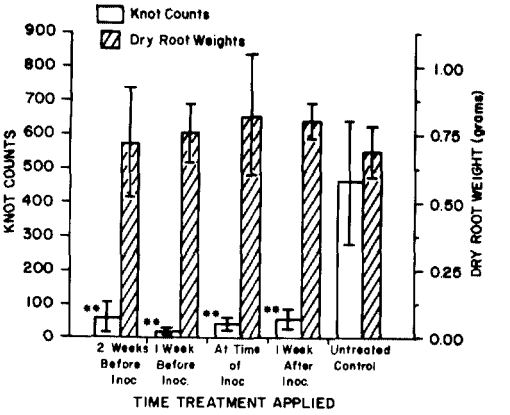

FIG. 1. Knot count and root weight in relation to the interval between application of Oryzalin and inoculation with *M. incognita*. Vertical lines indicate standard deviation, and ** indicates a difference from the control significant at the 1% level.

to untreated soil 5 days before inoculation (Fig. 3). Oryzalin affected both the penetration of the nematode larvae and linear growth of the cotton seedling roots. Larvae were confined to an area directly behind the root tip in treated seedlings, whereas in control seedlings larvae were found all along the root.

BAS 083 also reduced the knot counts of infected plants, although only in plants treated 1 or 2 weeks before inoculation. Knot counts were not affected in plants treated 1 week after inoculation (Fig. 4).

Incubating larvae in 10,000 ppm BAS 083 for 24 h before inoculation reduced their ability to infect the tomato roots. As with Oryzalin, smaller plants (4 weeks old at inoculation) had fewer galls (Fig. 5) than did larger plants (6 and 8 weeks old at inoculation). However, plants treated with 15 ml of 10,000 ppm BAS 083 1 week before inoculation had significantly fewer knots than plants inoculated with larvae that had been bathed in the same concentration of BAS 083 (Fig. 5). BAS-treated plants transplanted to untreated soil before inoculation were infected at the same level as control plants (Fig. 3).

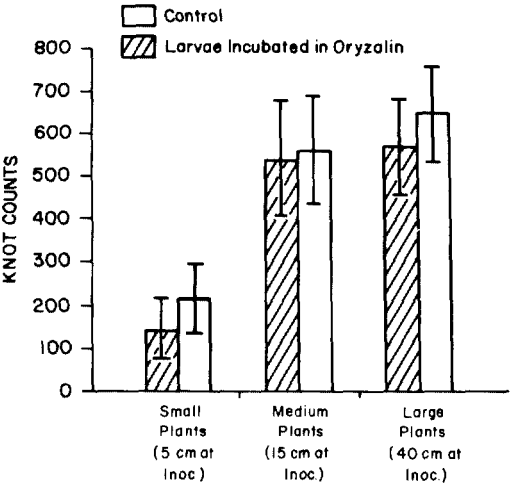

FIG. 2. Influence of larval incubation in 20 ppm Oryzalin for 24 h before inoculation with *M. incognita*. Vertical lines indicate standard deviation. Differences between means were not significant.

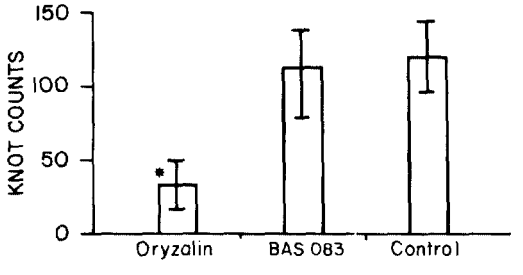

FIG. 3. Root-knot counts on treated plants transplanted to untreated soil before inoculation with *M. incognita*. Vertical lines indicate standard deviation, and * indicates a difference from the control significant at the 5% level.

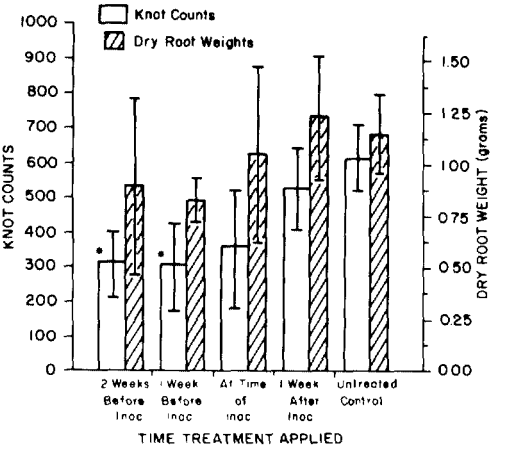

FIG. 4. Knot count and root weight in relation to the interval between application of BAS 083 and inoculation with *M. incognita*. Vertical lines indicate standard deviation, and * indicates a difference from the control significant at the 5% level.

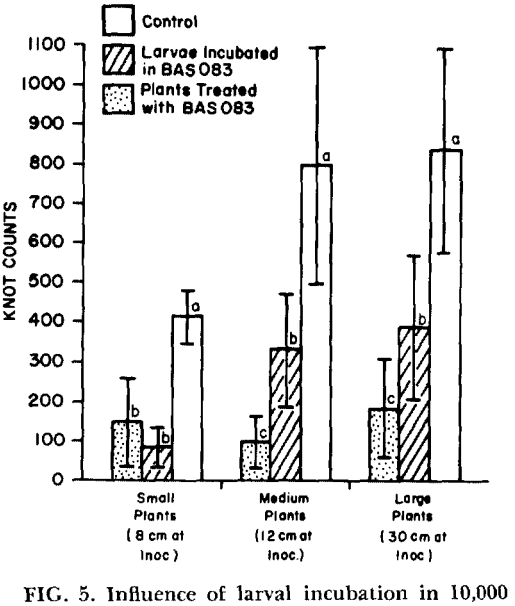

FIG. 5. Influence of larval incubation in 10,000 ppm BAS 083 for 24 h before inoculation with *M. incognita*. Vertical lines indicate standard deviation. Bars labeled with the same letter do not differ significantly at the 5% level.

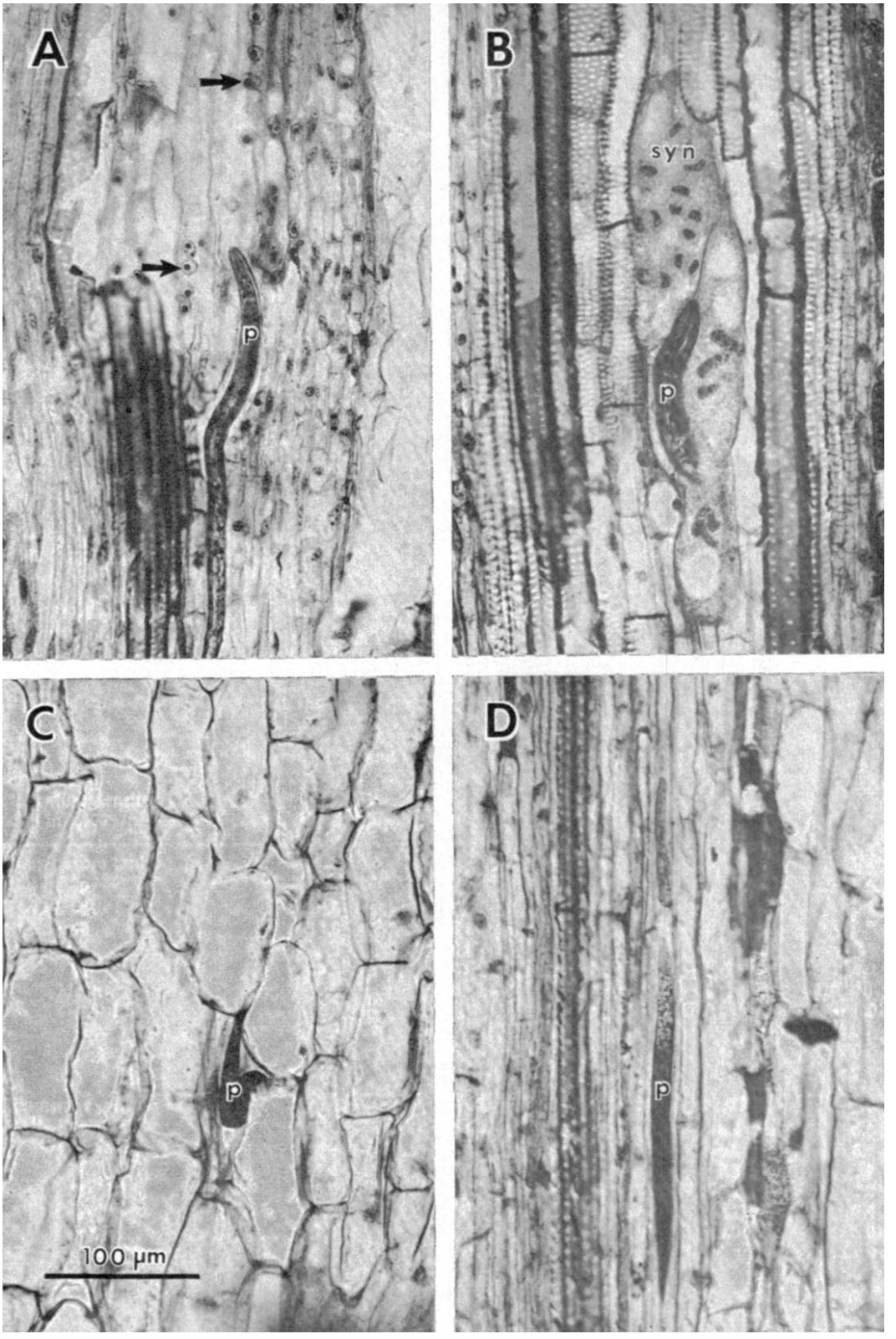

FIG. 6. Influence of Oryzalin on giant-cell formation by *Meloidogyne incognita* (p). A-B, untreated, 72 and 96 h after inoculation. Arrows indicate enlarged nuclei of developing syncytium (syn).

Giant-cell development in cotton roots inoculated with nematodes was inhibited by Oryzalin (Fig. 6). In untreated roots, giant cells were evident near the heads of nematodes at 72 h (Fig. 6a) and were large multinucleate structures by 96 h (Fig. 6b). No larvae were found in the cortex. Oryzalin-treated roots had no large multinucleate giant cells. Larvae were in the cortex 96 h after inoculation and were frequently coiled within or between cortical cells. Premolt growth was inhibited (Fig. 6c). Nematodes found in the stele failed to initiate giant cells, and vascular tissue had differentiated around their heads (Fig. 6d).

DISCUSSION

Romney et al. (14) reported that the herbicide DCPA prevented larval penetration by producing a mechanical barrier to the larvae, i.e., by thickening the cell walls of the epidermal tissue. Our results indicated that Oryzalin did not affect penetration *per se,* but, acted indirectly on the infection process through inhibition of giant-cell development in roots and confined larval penetration to the area just behind the root tips. The root tip is attractive to nematode larvae (15), and larvae of *Meloidogyne* penetrate the roots at this point (10). As the root elongates, more infection sites become available. Since Oryzalin stopped root growth and elongation, no new infection sites became available for the larvae to penetrate, resulting in an accumulation of many larvae at the tips of the roots and fewer knots or galls on the roots. Bartels and Hilton (1) reported that Oryzalin inhibited mitosis and root development by interfering with spindle microtubular activity.

More than larval penetration was affected by Oryzalin, since Oryzalin applied one week after inoculation also reduced knot counts. Our study of infected root tissue showed that giant-cell development was inhibited in Oryzalin-treated roots, and that nematode growth and development was prevented. Davide and Triantaphyllou (5) reported a similar result with maleic hydrazide (MH). MH treatment before inoculation prevented penetration, whereas treatment after inoculation reduced giant-cell development, increased the number of males in the population, and reduced galling. Orion and Minz (12) emphasized that nematode development is dependent upon the giant cells. They provided evidence that morphactin, a plant-growth regulator affecting mitosis, interfered with giant-cell formation and, hence, nematode development. Giant cells were smaller and had fewer nuclei in morphactin-treated plants than in control plants.

BAS 083 reduced the infectivity of larvae (Fig. 5), whereas Oryzalin did not (Fig. 2). BAS 083 did not reduce galling when treated plants were transplanted to untreated soil; Oryzalin did (Fig. 3). Although BAS may act partially as a contact nematicide, it also acted through the plant in some manner. A possible explanation of BAS 083 action was suggested by the observation that treated plants had fewer lateral roots than did control plants, and hence fewer sites for penetration by the nematode. The fewer galls on small plants than on large ones (Fig. 2, 5) supports the concept that the number of root tips at time of inoculation may be a limiting factor. Crittenden also (4) reported that plants with few lateral roots appeared to be less susceptible to nematode infection. Byrne et al. (2) observed that nematode larvae are frequently associated with lateral root primordia and that the nematode's feeding site was often the vascular connection between the primary and lateral roots. Reduction in lateral roots may also mean that BAS 083 was active in the pericycle of the root, where lateral roots are initiated. Since pericycle and adjacent tissues are the sites of nematode feeding, BAS 083 could be expected to influence nematode growth and reproduction.

Katan and Eshel (7) suggested that the biological activity of any pesticide is usually not restricted to the target organism. Both potentially harmful and beneficial secondary effects are to be expected. Trifluralin, a compound closely related to Oryzalin, is reported to increase damping-off by *Rhizoctonia solani* (3). We conclude that Oryzalin and BAS 083 herbicides show promising nematicidal properties, but that field testing of these and other broad-spectrum compounds should be carefully controlled, with close monitoring of non-target organisms.

LITERATURE CITED

1. BARTELS, P. G., and J. L. HILTON. 1973. Comparison of Trifluralin, Oryzalin, pronamide, propham and colchicine treatments on microtubules. Pest. Biochem. Physiol. 3: 462-472.
2. BYRNE, J. M., T. C. PESCRETA, and J. A. FOX. 1977. Vascular pattern change caused by a nematode, Meloidogyne incognita, in lateral roots of Glycine max (L) Merr. Am. J. Bot. 64:960-965.
3. CHANDLER, J. M., and P. W. SANTELMANN. 1968. Interactions of four herbicides with Rhizoctonia solani on seedling cotton. Weed Sci. 16:453-456.
4. CRITTENDEN, H. W. 1954. Factors associated with root-knot nematode resistance in soybeans. Phytopathology 44:388 (Abstr.).
5. DAVIDE, R. G., and A. C. TRIANTAPHYLLOU. 1968. Influence of the environment on development and sex differentiation of root-knot nematode. III. Effect of foliar application of maleic hydrazide. Nematologica 14:37-46.
6. DROPKIN, V. H., J. P. HELGESON, and C. D. UPPER. 1969. The hypersensitivity reaction of tomato resistance to Meloidogyne incognita; reversal by cytokinins. J. Nematol. 1: 55-61.
7. KATAN, J., and Y. ESHEL. 1973. Interactions between herbicides and plant pathogens. Residue Rev. 45:145-177.
8. KOCHBA, J., and R. M. SAMISH. 1971. Effect of kinetin and 1-naphthylacetic acid on root knot nematodes in resistant and susceptible peach root stocks. J. Am. Soc. Hort. Sci. 96: 458-461.
9. KOCHBA, J. and R. M. SAMISH. 1972. Effect of growth inhibitors on root knot nematodes in peach roots. J. Am. Soc. Hort. Sci. 97:178-180.
10. McCLURE, M. A., and J. ROBERTSON. 1973. Infection of cotton seedlings by Meloidogyne incognita and a method of producing uniformly infected root segments. Nematologica 19:428-434.
11. MJUGE, S. G., and D. R. VIGLIERCHIO. 1975. Influence of growth promoters and inhibitors on tomato plants infected with Meloidogyne incognita and M. hapla. Nematologica 21: 476-477.
12. ORION, D., and G. MINZ. 1971. The influence of morphactin on the root-knot nematode Meloidogyne javanica and its galls. Nematologica 17:107-112.
13. PEACOCK, F. C. 1963. Systemic inhibition of root-knot eelworm (Meloidogyne incognita) on tomato. Nematologica 9:581-583.
14. ROMNEY, R. K., J. L. ANDERSON, and G. D. GRIFFIN. 1974. Effects of DCPA on seedling infection by root-knot nematode. Weed Sci. 22:51-54.
15. WALLACE, H. R. 1964. The biology of plant parasitic nematodes. St. Martin's Press, N.Y. 280 p.
16. WEBSTER, J. M. 1967. Some effects of 2,4-dichlorophenoxyacetic acid herbicides on nematode infested cereals. Plant Pathol. 16: 23-26.