

Effect of Phenamiphos on *Heterodera schachtii* and *Meloidogyne javanica*¹

N. Greco and I. J. Thomason²

Abstract: Aqueous solutions of technical-grade phenamiphos [ethyl 3-methyl-4-(methylthio)phenyl (1-methylethyl) phosphoramidate] were used in hatching chambers to test, under laboratory conditions, the effect of phenamiphos on the hatching and movement of *Meloidogyne javanica* and *Heterodera schachtii*. Hatch of *M. javanica* and *H. schachtii* eggs was depressed 70 and 88% by nematicide at 0.48 and 4.80 µg/ml, respectively. The infectivity of second-stage larvae of both species was affected by concentrations as low as 0.01 µg/ml. At least 0.5 µg/ml was required to decrease the movement of larvae of *M. javanica* and *H. schachtii*. To decrease the movement of *H. schachtii* males toward females, 10 µg/ml was required. In a field experiment using a 15% granular formulation, 5 kg/ha a.i. significantly reduced infection of sugarbeet roots by *H. schachtii*. **Key Words:** nematicide, cyst nematode, root-knot nematode, mode of action.

Nonfumigant granular nematicides such as oxime carbamates and organophosphates offer promise in control of plant-parasitic nematodes because they are not highly phytotoxic (3). This promise has stimulated investigations of the effectiveness of these nematicides in the field (1,2,5,9,10,11) and laboratory (4,8,12,13). Phenamiphos [ethyl 3-methyl-4-(methylthio)phenyl (1-methylethyl) phosphoramidate] has been studied extensively for controlling plant-parasitic nematodes in the field (1,2,10,11) but there is less information on its effect on nematode behavior. Available knowledge is concerned mostly with the hatching of *Meloidogyne* spp. eggs (8) and of *Heterodera schachtii* Schmidt cysts (13). Hough and Thomason (4) obtained information on the effects of aldicarb on the behavior of *H. schachtii* and *Meloidogyne javanica* (Treub) Chitwood. The laboratory and field experiments reported in this paper were designed to test the effects of phenamiphos on: 1) hatching of eggs of *M. javanica* and *H. schachtii*; 2) movement and infectivity of second-stage larvae of both species; and 3) movement of *H. schachtii* males toward females.

MATERIALS AND METHODS

Technical phenamiphos was used for all experiments except for the field test in which a 15% granular material was used. Each treatment was replicated four times, and the data were subjected to an analysis of variance and where appropriate to Duncan's Multiple Range test.

Hatching: The chamber used for hatching studies was similar to that of Hough and Thomason (4). Each chamber consisted of three blocks of plexiglass in which four holes were drilled. On the bottom of the upper and middle blocks, a nylon screen of about 40-mesh was placed to hold 4.5 ml of a 150–250-µm quartz sand in between. The holes in the lower block were connected with a small tube which served as a drain when the chemical was to be washed from the chamber in order to collect larvae. These holes were stoppered during the experiment, which lasted 40 days for *M. javanica* and 50 days for *H. schachtii*. *M. javanica* egg masses were picked from infected roots of lima bean, *Phaseolus lunatus* L., Line L-136. *H. schachtii* cysts were extracted by elutriation from soil collected near Salinas, California. Two experiments, one with 30 egg masses of *M. javanica* averaging 550 eggs each, and the other with 60 cysts with an average of 138 eggs each, were established by placing the egg masses or cysts in sand in test chambers. A hatch-

Received for publication 2 February 1978.

¹Work reported herein done while the senior author was on a study leave at the University of California, Riverside.

²Department of Nematology, University of California, Riverside 92521. Present address of senior author: C.N.R.-Laboratorio di Nematologia Agraria applicata ai vegetali, Via Amendola, 165/A, 70126 Bari, Italy.

ing solution (3 mM solution of $ZnCl_2$ for *H. schachtii* and Sorensen's phosphate buffer at pH 7 for *M. javanica*) with the desired nematicide concentration was added to the sand, and the chambers were stoppered at both ends. The solutions containing 0.0, 0.48, 4.80, and 48.00 $\mu\text{g}/\text{ml}$ of phenamiphos used in this test were prepared fresh every 5 days. Each treatment was replicated 4 times. The chambers containing *M. javanica* egg masses were incubated at 27 C, whereas the incubation temperature for *H. schachtii* alternated: 2 days at 15 C and 3 days at 24 C. The hatched larvae were collected every 5 days by washing each chamber with 20 ml of fresh chemical solution. The elutriate was filtered on a 3- μm Millipore filter, washed to remove the chemical, collected in a beaker, and counted. The larvae of *M. javanica* were inoculated onto tomato, *Lycopersicon esculentum* L., cv. Pearson, and those of *H. schachtii* onto sugarbeet, *Beta vulgaris* L., cv. U.S. H 10. Seedlings with two true leaves were transplanted into 200-ml polystyrene cups (filled with river sand for tomato, and with sandy loam for sugarbeet) and fertilized with Hoagland's nutrient solution 5 days before the inoculation. All cups were then placed in larger plastic pots filled with soil and arranged in a constant-temperature tank at 27 C. Five days were allowed for the nematode to enter the roots before the seedlings were removed, rinsed gently in tap water, stained with lactophenol and acid fuchsin, and macerated. Larvae were counted and expressed as the percent of the initial inoculum. To determine the status of unhatched eggs, these were inoculated onto seedlings of their host plants. At 21 days the roots were stained and macerated, and the nematodes were counted. Numbers are expressed as percentages of the unhatched eggs.

Because eggs of *H. schachtii* did not hatch until 30 days, it was difficult to evaluate the time required for the chemical to affect the emergence of the larvae, and a second hatching experiment was conducted with *H. schachtii*. This time, a population of *H. schachtii* from near Oxnard, California, was grown in the greenhouse on sugarbeets, and 40 newly formed cysts with an average of 217 eggs per cyst were used in each chamber. Only 1.7% of the eggs were

found to be infected by fungus as determined by the method of Kerry and Crump (6). In this second experiment, hatching solution without phenamiphos was used for the first 15 days. At that time, emergence of the larvae was judged satisfactory and phenamiphos was added.

Infectivity: To determine the infectivity of the second-stage larvae of *M. javanica* and *H. schachtii* in the presence of the phenamiphos, columns were used according to Hough and Thomason (4) and left in a growth chamber at 27 C with 14 h of light per day. Each column, made from a plexiglass tube, was 3.9 cm in diameter and 12.5 cm high, and a porous ceramic disk having a 1/2-bar air-entry value was placed on the bottom. Columns were filled with a 150–250- μm quartz sand, which was wetted with Hoagland's nutrient solution and the tension adjusted to 22 mbar. Tomato and sugarbeet seedlings (fourth-leaf stage) were transplanted into columns 3 days before being inoculated. Then the nutrient solution was flushed and replaced with solutions containing phenamiphos at 0.0, 0.01, 0.10, and 1.00 $\mu\text{g}/\text{ml}$. Larvae were pretreated for 8 h in these solutions, and then 3000 *H. schachtii* and 4000 *M. javanica* were inoculated at the top of each column, and columns were covered with Parafilm (American Can Co., Neenah, Wisconsin) to avoid evaporation. To obtain larvae of *H. schachtii* for this experiment, cysts grown on sugarbeet in a greenhouse were incubated in 5-cm-diameter plastic petri dishes containing 5 ml of 3 mM $ZnCl_2$ solution, and larvae were collected every 2 days. *M. javanica* larvae were obtained from lima bean roots incubated in aerated water. To separate larvae from eggs, the suspension was concentrated on a 500-mesh sieve and poured on a Baermann funnel, where it was left for 18 h before larvae were collected. Three days after the larvae were introduced, the seedling roots were rinsed in tap water, stained and macerated, and the larvae inside the roots were counted and reported as a percentage of those added.

Movement of second-stage larvae: The effect of phenamiphos on larval movement was studied with columns similar to those in the infectivity test, pre-cut in five 2.5-cm-high sections, having at the bottom a porous ceramic disk of 1/4-mbar air-entry value.

The sand size and solution tension were similar to those in the larval infectivity test, and phenamiphos concentrations were 0.0, 0.1, 0.5, 1.0, and 5.0 $\mu\text{g}/\text{ml}$. Each treatment was replicated 4 times. Two-thousand second-stage larvae per column, obtained as described above, were preexposed to phenamiphos concentrations for 12 h at 15 C before addition on the top of each column. The columns were kept at 27 C. Three days later, the column was disassembled and the sand from each section was suspended in water. The suspension was then decanted and concentrated on a 500-mesh sieve, and the larvae were counted. The results are expressed as a percentage of the total number recovered from the column.

Movement of H. schachtii males: *H. schachtii* male movement toward females was studied in columns similar to those used for larvae movement. However, 250–500 μm sand and a 1/2-bar ceramic porous disk were used with the tension adjusted to 15 mbar. Males were collected from field soil. Females were picked from sugarbeet roots grown in the greenhouse. One hundred and fifty males were inoculated in the middle section of the column, and 30 females were placed on the top (except in a control series without females). Phenamiphos treatments were made in concentrations of 0.0, 0.001, 0.010, 0.100, 1.000, and 10.000 $\mu\text{g}/\text{ml}$. Each treatment was replicated 4 times. Three days were allowed for the males to move through the column, which was kept at 26 C. Males were recovered and expressed as above.

Field experiments: Field experiments were designed to determine the effectiveness of several rates of phenamiphos for suppressing the infection of sugarbeet roots by *H. schachtii*. A 15% commercial granular formulation was tested at 0.0, 1.25, 2.50, 5.00, 7.50, 10.00, and 12.50 kg active ingredient/ha. Planting beds were 120 cm long and 32.5 cm wide, and centers of beds were 75 cm apart. Because soil inoculum was only two eggs/ml, 3 liters of soil containing six eggs/ml were broadcast on 60-cm-long bands in the middle of each plot. Phenamiphos doses were distributed over each plot and rototilled into the soil to a 15-cm depth. The experimental design was

a randomized complete block, with 4 replicates of each treatment.

Sugarbeet seeds were then machine-sown in 1 row in each plot. Fifty-one days after the plants had emerged, four plants in the middle of each plot were removed, the feeder roots were cut off and macerated, and *H. schachtii* were counted and expressed as nematodes/3 g roots.

RESULTS

Hatching: All phenamiphos concentrations reduced ($P = 0.05$) the hatch of *M. javanica* compared with the control (Fig. 1A), and 4.8 $\mu\text{g}/\text{ml}$ stopped the emergence of larvae in 5 days. *H. schachtii* (Fig. 1B,C) was less sensitive than *M. javanica* to phenamiphos. At 0.48 $\mu\text{g}/\text{ml}$, *M. javanica* egg hatch was reduced but *H. schachtii* eggs hatched as readily as in the untreated control. At 4.8 $\mu\text{g}/\text{ml}$ phenamiphos the hatch of *H. schachtii* eggs was stopped in 5 days. The effect of phenamiphos on larvae recovered from the above tests was similar in that *M. javanica* larvae were significantly less infective than the check at all concentrations, and *H. schachtii* larvae were less infective only at the two higher concentrations (Table 1). When roots were inoculated with egg masses remaining at the end of the hatching tests, a few eggs of *M. javanica* unhatched in 0.48 $\mu\text{g}/\text{ml}$ of phenamiphos produced larvae which infected roots (Table 2). Phenamiphos at 0.48 $\mu\text{g}/\text{ml}$ was sufficient to significantly inhibit hatch but the effect was reversible and a number of larvae hatched from eggs and entered hosts roots. At the two higher concentra-

Table 1. Numbers of larvae found in roots of host plants, expressed as percentages of initial larval inoculum.* Larvae from eggs exposed to four concentrations of phenamiphos in the hatching solution.

Phenamiphos concentration	<i>Heterodera schachtii</i>		<i>Meloidogyne javanica</i>
	Experiment I	Experiment II	
0.00 $\mu\text{g}/\text{ml}$	12.8 a	6.1 a	7.5 a
0.48 $\mu\text{g}/\text{ml}$	6.6 ab	5.5 a	2.1 b
4.80 $\mu\text{g}/\text{ml}$	3.4 b	1.1 b	0.6 bc
48.00 $\mu\text{g}/\text{ml}$	0.0 c	0.0 c	0.1 c

*Each figure is the mean of four replicates. Means followed by different letters differ ($P = 0.05$) using Duncan's multiple-range test.

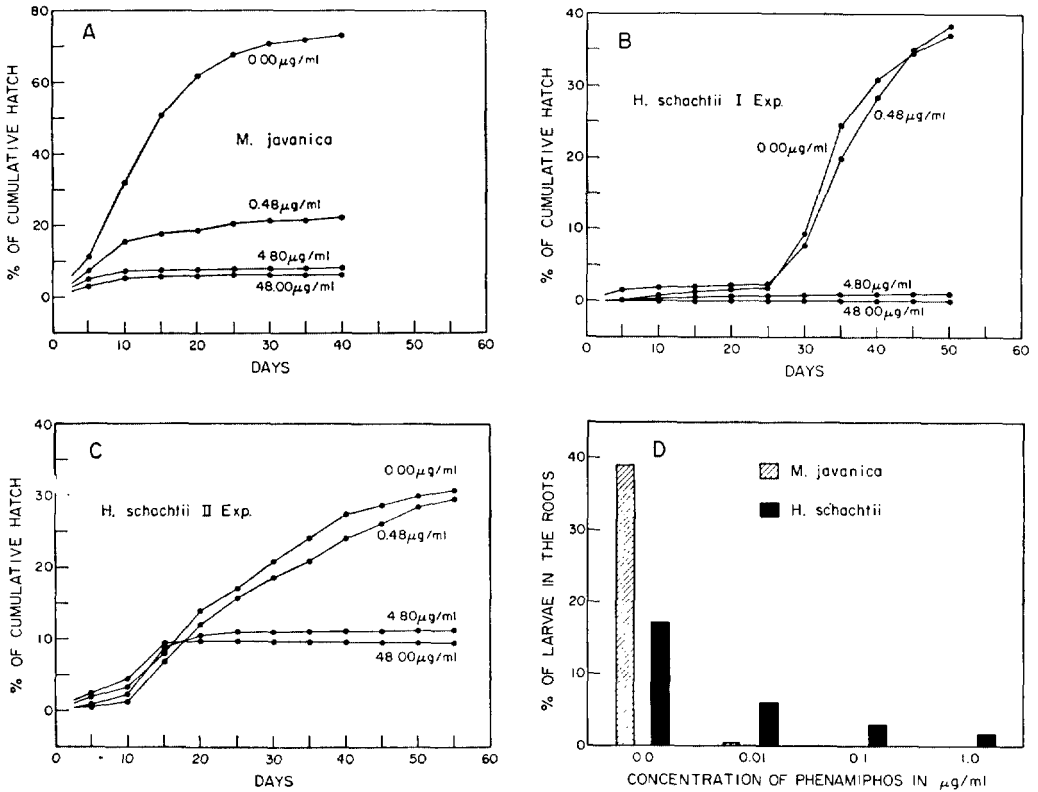


Fig. 1. Effect of phenamiphos on the hatching of: A) *Meloidogyne javanica*; B) *Heterodera schachtii* experiment I; and C) *Heterodera schachtii* experiment II. D) Effect of different concentrations of phenamiphos on the infectivity of second-stage larvae of *M. javanica* and *H. schachtii*.

Table 2. Nematodes recovered from host roots inoculated with cysts or egg masses containing eggs unhatched at the end of the hatching test. Numbers are percentages of eggs unhatched when test hosts were planted.^a

Phenamiphos concentration	<i>Heterodera schachtii</i>		<i>Meloidogyne javanica</i>
	Experiment I	Experiment II	
0.00 µg/ml	0.8 b	0.2 b	0.4 ab
0.48 µg/ml	2.0 ab	0.5 ab	1.4 a
4.80 µg/ml	4.2 a	1.4 a	0.1 bc
48.00 µg/ml	0.4 c	0.0 b	0.0 c

^aEach figure is the average of four replicates. Averages followed by different letters differ ($P = 0.05$) using Duncan's multiple-range test.

tions larvae may have hatched from eggs but very few (4.80 µg/ml) or none (48.00 µg/ml) entered roots. Again the higher tolerance of *H. schachtii* was demonstrated and larvae hatched from eggs exposed to 4.80 µg/ml and entered roots of sugarbeet seedlings. The injury at 48.00 µg/ml did not appear to be reversible.

Infectivity: All phenamiphos concentrations tested almost completely depressed the infectivity of second-stage larvae of *M. javanica* (Fig. 1d). A few *H. schachtii* (1.8%) were found inside roots treated with 1 µg/ml, the highest concentration. In the control, 34.2% of *M. javanica* and 17.2% of *H. schachtii* were infective.

Movement: Movement of larvae in the columns was random in the untreated control and at 0.1 µg/ml phenamiphos for both species of nematodes (Fig. 2a). In the controls 37.3% of the *M. javanica* larvae were found in the lowest section, and only 13.2% of *H. schachtii*. At all other treatment levels, more larvae were found in the upper section near the point of introduction.

Movement of males of *H. schachtii*: More males were found in the top section of the untreated control columns when females were present (Fig. 2b). More males were found in the lower sections of the untreated columns in which no females were added. Concentrations of phenamiphos up to 1

$\mu\text{g/ml}$ seemed to have impaired male orientation toward females but not movement, since males were distributed at random in the columns. Phenamiphos at $10 \mu\text{g/ml}$ depressed movement, since 80.7% of collected males were found in the central section where they had been inoculated.

Field experiments: At least 5 kg phenamiphos/ha was required to depress infection by *H. schachtii* (Table 3). Higher amounts did not achieve better control. Frequent sprinkler irrigations, applying an average of 4.8 mm of water per day, and relatively high soil temperatures (28 C at

planting time) during the test, may have reduced the effectiveness of the nematicide.

DISCUSSION

Phenamiphos has been shown to be more effective against *M. javanica* than *H. schachtii*. To achieve the same nematocidal activity against *H. schachtii* as against *M. javanica*, significantly higher doses of the chemical are required. It is difficult to explain the difference between the two species in sensitivity to phenamiphos though we suggest that ability to penetrate the egg and/or larval cuticle, as well as larval metabolic activity, may be involved. Hough and Thomason (4) noted that aldicarb was more effective against eggs in cysts of *H. schachtii* than eggs in egg masses of *M. javanica*. McLeod and Khair (8) tested many nematicides against three species of *Meloidogyne* and found them not to be equally effective against all species. It is evidently necessary to have a separate determination of the action of phenamiphos on each economically important species to determine the amount of this nematicide required for field control.

Table 3. Effect of phenamiphos on the infection of sugarbeet roots by *Heterodera schachtii* under field conditions.

Phenamiphos (kg a.i./ha)	Cysts per 3 g of feeder roots ^a
0.00	131 a
1.25	56 bc
2.50	111 ab
5.00	24 c
7.50	32 c
10.00	25 c
12.50	15 c

^aAverages followed by different letters differ ($P = 0.05$) using Duncan's multiple-range test.

Figs. 1D and 2A suggest that in these tests untreated larvae of *M. javanica* may

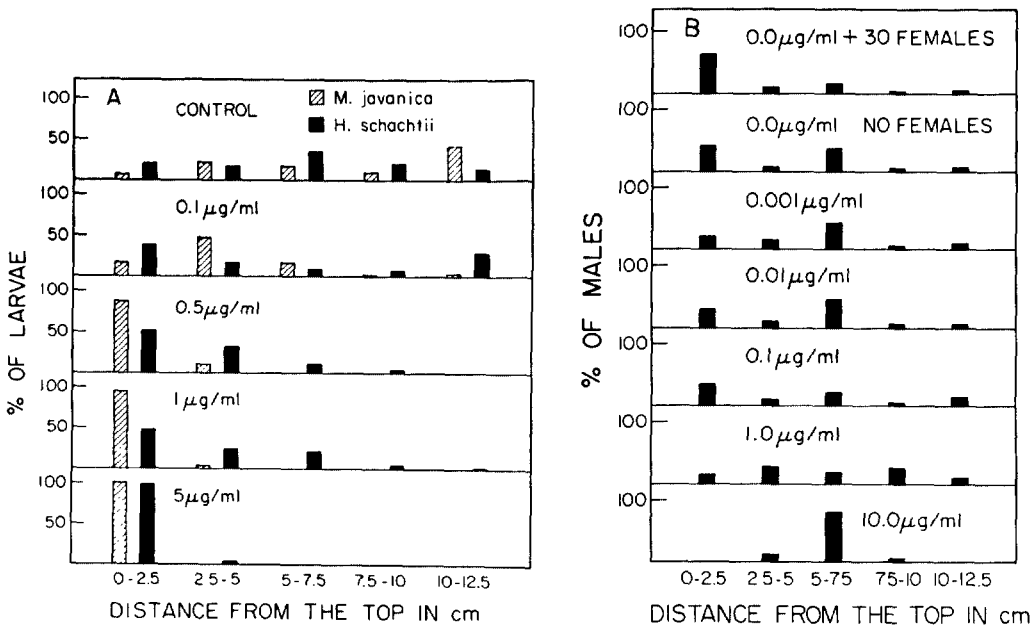


Fig. 2. Effect of different concentrations of phenamiphos on: A) the movement of the larvae of *Meloidogyne javanica* and *Heterodera schachtii*; and B) the movement of males of *H. schachtii*.

be more vigorous and have a higher infection potential on tomato seedlings than did *H. schachtii* larvae on sugarbeet. Perhaps both species should have been tested on the common host, sugarbeet. It is possible, but not likely, that the differences in response to phenamiphos were due to inherent vigor of test populations. Some fungus parasitism of *H. Schachtii* eggs occurred but this was not deemed a serious problem especially in the second experiment.

At certain concentrations ($\sim 0.48 \mu\text{g/ml}$ for *M. javanica* and $4.8 \mu\text{g/ml}$ for *H. schachtii*) the effect of phenamiphos on eggs is largely nematostatic and some eggs resume hatching once the nematicide has either degraded or flushed away. Higher concentrations are apparently lethal. Different life stages or behavioral activities of the nematodes are affected differently by phenamiphos. While $0.48 \mu\text{g/ml}$ did not affect the hatching of *H. schachtii* eggs, $0.01 \mu\text{g/ml}$ was enough to depress significantly the infectivity of second-stage larvae of both *H. schachtii* and *M. javanica*. In addition, $0.5 \mu\text{g/ml}$ was required to reduce movement. So, in field conditions, it is doubtful that the nematicide at normal rates would kill eggs inside the cysts. It has been found (7) that when most of the nematicide is dissipated, the eggs hatched and larvae infected the roots, so that at harvest time the egg count in soil of treated plots was as great as in untreated plots, if not greater.

An understanding of the effectiveness of phenamiphos will require further investigations on the diffusion and breakdown in field conditions and on the concentrations found in the soil in the root rhizosphere during seedling plant development. Knowledge of the effects of soil moisture as influenced by soil type, rainfall, and irrigation would also be helpful in determining use patterns.

LITERATURE CITED

1. Cetas, R. C. 1971. Evaluation of granular formulation of systemic nematicides for the control of *Pratylenchus penetrans* on potatoes. *Phytopathology* 61:887.
2. Di Vito, M., and F. Lamberti. 1976. Efficacia di nematocidi sistemici nella lotta contro *Heterodera gottingiana* su pisello. *Nematol. Medit.* 4:63-69.
3. Griffin, G. D. 1978. Aldicarb post-plant control of the sugarbeet cyst nematode *Heterodera schachtii*. *Pl. Dis. Reprtr.* 62:1027-1030.
4. Hough, A., and I. J. Thomason. 1975. Effects of aldicarb on the behavior of *Heterodera schachtii* and *Meloidogyne javanica*. *J. Nematol.* 7:221-229.
5. Hough, A., I. J. Thomason, and W. J. Farmer. 1975. Behavior of aldicarb in soil relative to control of *Heterodera schachtii*. *J. Nematol.* 7:214-221.
6. Kerry, B. R., and D. H. Crump. 1977. Observation on fungal parasites of females and eggs of the cereal cyst-nematode, *Heterodera avenae*, and other cyst nematodes. *Nematologica* 23:193-201.
7. Kontaxis, D. G., and I. J. Thomason. 1978. Chemical control of *Heterodera schachtii* and sugarbeet production in Imperial Valley, California. *Pl. Dis. Reprtr.* 62:79-82.
8. McLeod, R. W., and G. T. Khair. 1975. Effects of oximecarbamate, organophosphate and benzimidazole nematicides on life cycle stages of root-knot nematodes, *Meloidogyne* spp. *Ann. Appl. Biol.* 79:329-341.
9. Miller, P. M., and G. S. Taylor. 1970. Nematicidal control of *Heterodera tabacum*. *Phytopathology* 60:411-414.
10. Moss, S. R., D. Crump, and A. G. Whitehead. 1975. Control of potato cyst nematode, *Heterodera rostochiensis* and *H. pallida*, in sandy, peaty and silt loam soil by oximecarbamate and organophosphate nematicides. *Ann. Appl. Biol.* 81:359-365.
11. Ouden, H. Den, and R. F. Van de Veer. 1977. The effect of some systemic nematicides on the control of *Heterodera rostochiensis* in the field. *Netherlands J. Plant Pathol.* 85:129-137.
12. Steele, A. E., and L. R. Hodges. 1975. In vitro and in vivo effects of aldicarb on survival and development of *Heterodera schachtii*. *J. Nematol.* 7:305-312.
13. Steele, A. E. 1977. Effect of oxime carbamate nematicides on development of *Heterodera schachtii* on sugarbeet. *J. Nematol.* 9:149-154.