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Differential Sensitivity of *Meloidogyne* spp. and *Heterodera glycines* to Selected Nematicides[1]

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Abstract: Differential sensitivity of *Meloidogyne arenaria, M. hapla, M. incognita, M. javanica,* and *Heterodera glycines* races 1 and 5 to the nonfumigant nematicides aldicarb, ethoprop, and fenamiphos was evaluated using a 48-hour root-penetration bioassay. Generally, *H. glycines* was more tolerant of the nematicides, especially ethoprop, than were the *Meloidogyne* species. Among *Meloidogyne* species, *M. incognita* was most sensitive to aldicarb and fenamiphos, but its reaction to ethoprop was similar to the other three *Meloidogyne* species.

Key words: aldicarb, differential sensitivity, ethoprop, fenamiphos, *Glycine max, Heterodera glycines,* juvenile, *Meloidogyne arenaria, Meloidogyne hapla, Meloidogyne incognita, Meloidogyne javanica,* nematicide, nematode penetration, root-knot nematode, soybean, soybean cyst nematode.

Nonfumigant organophosphate and carbamate nematicides have been used for some 30 years to control plant-parasitic nematodes on many crops (17). Because of ease of application and handling, suitability for application at planting, and limited residues in foodstuffs, the use of these compounds on several crops in the United States has increased (8). These compounds control nematodes by impairing neuromuscular activity through the inhibition of acetylcholinesterase (4,18,19), which limits movement, invasion, feeding, rate of development, and reproduction (20).

Meloidogyne species exhibit differential sensitivity to nonfumigant organophosphate and carbamate nematicides under field conditions in flue-cured tobacco in North Carolina (2). *Meloidogyne arenaria* (Neal) Chitwood is relatively tolerant to ethoprop (an organophosphate) rates that suppress *M. hapla* Chitwood, *M. incognita* (Kofoid & White) Chitwood, and *M. javanica* (Treub) Chitwood (2). However, this compound is more effective against some *M. arenaria* populations than against *M. javanica* and *M. incognita* in Florida (9).

Meloidogyne spp. affect many crops worldwide, including soybean, *Glycine* max. (L.) Merr. *Heterodera glycines* Ichinohe is a significant economic pest of soybean, a major human and animal food produced on 52 million hectares (16). *Meloidogyne arenaria, M. hapla, M. incognita, M. javanica,* and races 1 and 5 of *H. glycines* are found in North Carolina (15), and often have to be controlled with nematicides. The objectives of this research were (i) to assess the sensitivity of the four common species of *Meloidogyne* and *H. glycines* races 1 and 5 to selected nematicides and (ii) to determine dosage response of the nematicides to each nematode.

Materials and Methods

Differential activity of aldicarb, ethoprop, and fenamiphos: This experiment was designed to evaluate the effects of aldicarb, ethoprop, and fenamiphos on nematode penetration into roots of the *H. glycines*- and *Meloidogyne* spp.-susceptible soybean 'Lee 68'. For inocula, *M. arenaria, M. hapla, M. incognita,* and *M. javanica* were obtained from the *Meloidogyne* collection at North Carolina State University and cultured on 'Rutgers' tomato, *Lycopersicon esculentum* Mill. Eggs were extracted from roots using a modified sodium hypochlorite extraction technique (7). Race 1 of *H. glycines* was cultured on Lee 68 and race 5 on 'Pickett 71' soybean. These two races were obtained from greenhouse cultures at North Carolina State University. Females and cysts were removed from roots using high water

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pressure. The cysts and females were then crushed with a 40-ml Ten-Broeck tissue grinder to release eggs (1). Eggs from each race and species were placed onto individual screens (25.5-μm-pore) for hatching. Second-stage juveniles (J2) that hatched during the first 24 hours were discarded. J2 hatching within the next 24 hours were suspended in water (100 J2/ml) and used to infest the soil.

Nematicides used in this test were technical aldicarb, ethoprop (Mocap® 6E), and fenamiphos (Nemacur® 3E). Aldicarb was applied at 0, 0.5, 1, and 2 μg a.i./g of soil. Ethoprop was used at rates of 0, 1, 4, and 8 μg a.i./g of soil. Rates of fenamiphos were 0, 1, 3, and 6 μg a.i./g of soil. Each rate of each chemical was replicated five times.

The experimental unit consisted of sty-

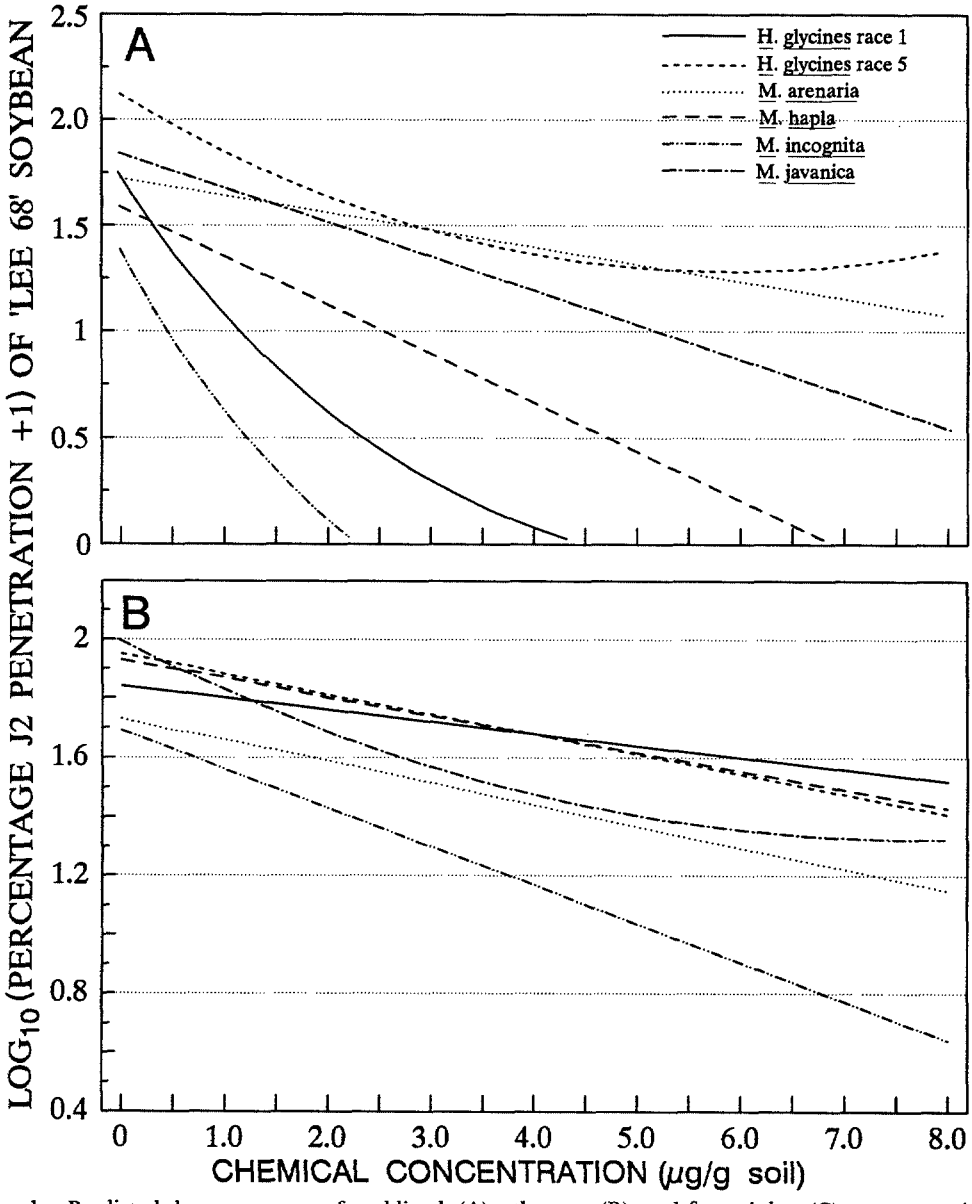

FIG. 1. Predicted dosage response for aldicarb (A), ethoprop (B), and fenamiphos (C) on penetration of *Meloidogyne arenaria, M. hapla, M. incognita, M. javanica* and races 1 and 5 of *Heterodera glycines* juveniles into bioassay plants during a 48-hour period (combined data from two runs).

rofoam containers (475 cm^3 capacity) filled with 300 g of loamy sand (92% sand, 6% silt, 2% clay, and 0.3% humic matter; bulk density = 1.5 g/cm^3). Lee 68 soybean seeds were soaked in water for 5 minutes and then allowed to germinate in a container of moist, sterilized vermiculite enclosed in a plastic bag and incubated at 27 C. One hundred grams of soil was removed from each container and a funnel-shaped depression was created. Single, 42-hour-old soybean seedlings with 3-cm-long radicles were selected and transplanted into the depression. Aliquants of 500 freshly hatched J2 in 5 ml water were pipetted over the roots and immediately covered with the soil that had been removed. Approximately 20 ml of water containing the respective nematicides was added to the soil surface in each container to bring the soil to field capacity (−0.03 MPa). Perlite (20 grams) was placed over the exposed soil surface to retard evaporation. The containers were then placed in the greenhouse with ambient air temperatures ranging from 25 to 31 C.

Roots were harvested at 48 hours after inoculation, and the amount of J2 penetration was determined. In order to enhance the contrast between the plant tissues and nematodes, the latter were stained with acid fuchsin (3). Roots were then placed in glycerin in 6-cm-d inverted plastic petri dishes, flattened with slight pressure, and examined with the aid of a stereomicroscope. Counts of J2 in roots were converted to percentages of initial inoculum.

Data were $\log_{10}$-transformed for analysis. For each nematode–nematicide combination, data from all five replicates in both experiments were pooled and the best-fit linear or quadratic regression was determined for the relationship between percentage penetration and nematicide rate.

Sensitivity of Heterodera glycines and Meloidogyne species: This experiment was modified from the first one in order to characterize the sensitivity of *M. arenaria, M. hapla, M. incognita, M. javanica,* and *H. glycine* races 1 and 5 to a greater range of concentrations of aldicarb and fenamiphos. The rates of application for each nematicide were 0, 0.125, 0.25, 0.50, 1.0, 2.0, 4.0, and 8.0 μg a.i./g of soil. The experimental design was a randomized complete block with five replicates. The entire experiment was repeated. Procedures used to isolate nematodes, inoculate plants, ap-

TABLE 1. Regression equations for response ($y = \log_{10}$ of % root penetration) of *Meloidogyne arenaria, M. hapla, M. incognita, M. javanica,* and *Heterodera glycines* races 1 and 5 to concentrations (x = μg/g) of aldicarb, ethoprop, and fenamiphos.

Nematode	Nematicide	Best-fit regression equation	Pr > |T|†
H. glycines (race 1)	Aldicarb	$y = 2.00 - 1.60x + 0.39x^2$	0.03
	Ethoprop	$y = 1.95 - 0.13x$	0.04
	Fenamiphos	$y = 2.0 - 0.11x$	0.03
H. glycines (race 5)	Aldicarb	$y = 1.96 - 0.49x$	0.07
	Ethoprop	$y = 2.11 - 0.9x$	0.15
	Fenamiphos	$y = 2.04 - 0.11x$	0.05
M. arenaria	Aldicarb	$y = 1.96 - 2.10x + 0.48x^2$	0.11
	Ethoprop	$y = 1.94 - 0.28x$	0.07
	Fenamiphos	$y = 2.13 - 0.17x$	0.05
M. hapla	Aldicarb	$y = 2.05 - 2.08x + 0.47x^2$	0.10
	Ethoprop	$y = 1.75 - 0.26x$	0.11
	Fenamiphos	$y = 1.96 - 0.25x$	0.01
M. incognita	Aldicarb	$y = 1.81 - 2.86x + 0.75x^2$	0.28
	Ethoprop	$y = 1.20 - 0.18x$	0.31
	Fenamiphos	$y = 2.00 - 0.31x$	0.03
M. javanica	Aldicarb	$y = 1.92 - 2.67x + 0.68x^2$	0.14
	Ethoprop	$y = 1.87 - 0.22x$	0.01
	Fenamiphos	$y = 1.80 - 0.33x$	0.04

† Pr > |T| = probability of a value greater than T.

ply nematicides, and analyze data were the same as described for Experiment 1 ("differential activity of aldicarb, ethoprop, and fenamiphos").

Results and Discussion

Differential activity of aldicarb, ethoprop, and fenamiphos: Sensitivity to aldicarb re-

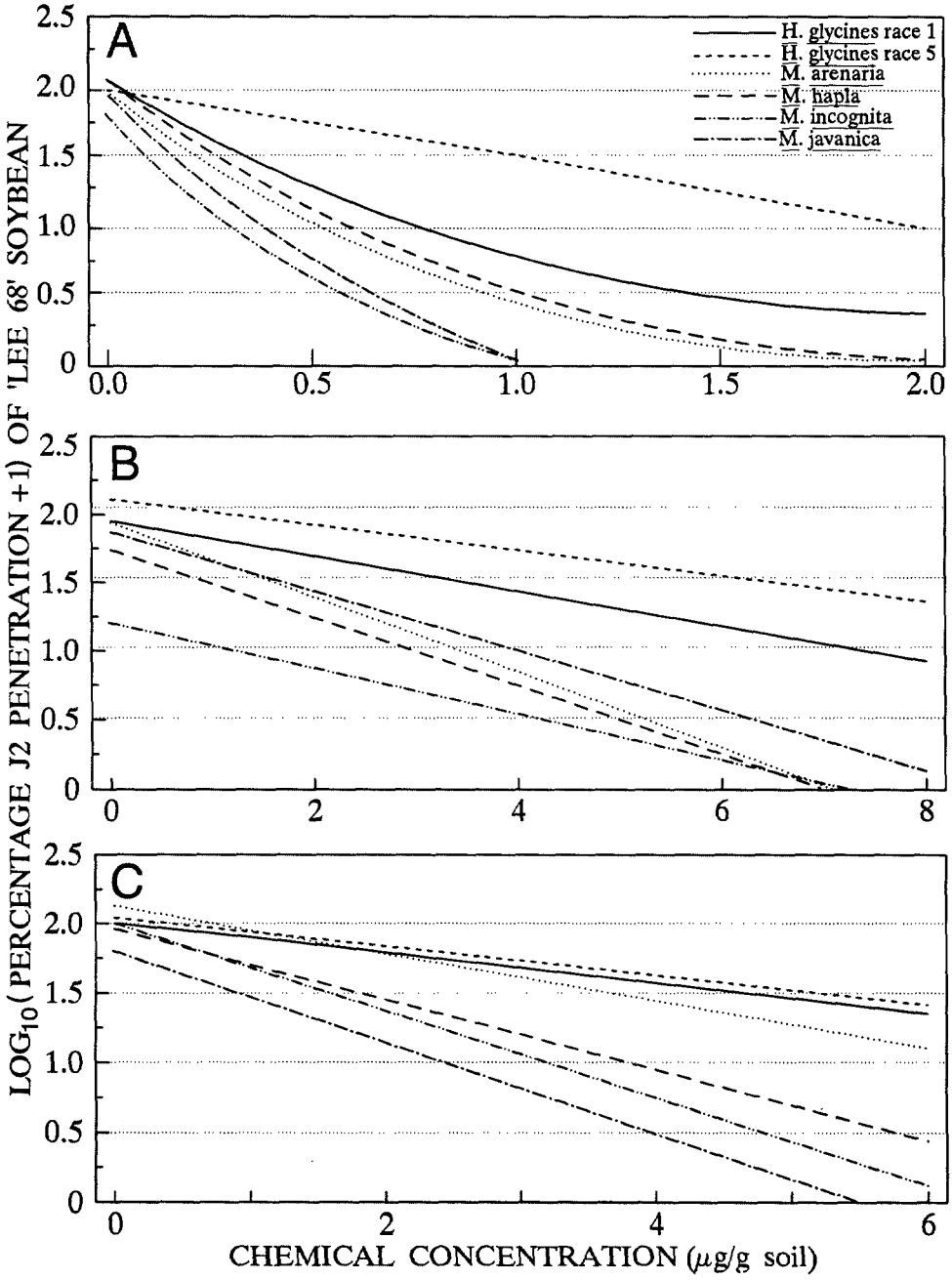

FIG. 2. Predicted dosage response for aldicarb (A) and fenamiphos (B) on penetration of *Meloidogyne arenaria, M. hapla, M. incognita,* and *M. javanica, Heterodera glycines* races 1 and 5 juveniles into bioassay plants during a 48-hour period (combined data from two runs).

TABLE 2. Regression equations for response (y = $\log_{10}$ of % root penetration) of penetration of *Meloidogyne arenaria, M. hapla, M. incognita, M. javanica,* and *Heterodera glycines* races 1 and 5 to a range of concentrations (x = 1 μg a.i. chemical/g soil) of aldicarb and fenamiphos.

Nematode	Nematicide	Best-fit regression equation	Pr > |T|†
H. glycines	Aldicarb	$y = 1.75 - 0.72x + 0.08x^2$	0.02
(race 1)	Fenamiphos	$y = 1.84 - 0.04x$	0.02
H. glycines	Aldicarb	$y = 2.12 - 0.3x + 0.03x^2$	0.01
(race 5)	Fenamiphos	$y = 1.95 - 0.07x$	0.01
M. arenaria	Aldicarb	$y = 1.74 - 0.08x$	0.02
	Fenamiphos	$y = 1.73 - 0.07x$	0.01
M. hapla	Aldicarb	$y = 1.59 - 0.24x$	0.01
	Fenamiphos	$y = 1.94 - 0.06x$	0.01
M. incognita	Aldicarb‡	$y = 1.37 - 0.81x + 0.11x^2$	0.01
	Fenamiphos	$y = 1.69 - 0.13x$	0.01
M. javanica	Aldicarb	$y = 1.84 - 0.16x$	0.01
	Fenamiphos	$y = 1.98 - 0.19x + 0.01x^2$	0.01

† Pr > |T| = probability of a value greater than T.
‡ Maximum concentration = 2.75 μg/g soil.

sulted in three groups of responses (Fig. 1A, Table 1). *Heterodera glycines* race 5 was most tolerant (tolerance to nematodes was arbitrarily designated by slope of the regression line) of aldicarb. *Heterodera glycines* race 1, *M. hapla,* and *M. arenaria* were less tolerant of this chemical. *Meloidogyne incognita* and *M. javanica* were the most sensitive of the six nematodes evaluated. *Meloidogyne* spp. were more sensitive to ethoprop than *H. glycines* (Fig. 1B, Table 1). *Heterodera glycines* and *M. arenaria* were the most tolerant of fenamiphos, and *M. javanica* the most sensitive (Fig. 1C, Table 1). *Meloidogyne hapla* and *M. incognita* were also sensitive to this nematode.

Sensitivity of Heterodera glycines and Meloidogyne species: This experiment confirmed that *Meloidogyne incognita* is sensitive to both aldicarb and fenamiphos (Fig. 2, Table 2). *Meloidogyne arenaria, M. javanica,* and *H. glycines* race 5 were tolerant to moderately tolerant of aldicarb (Fig. 2A, Table 2). *Meloidogyne hapla* and *H. glycines* race 1 were intermediate in their responses. Fenamiphos was most effective against *M. incognita* (Fig. 2B, Table 2). The other five nematodes were moderately tolerant of fenamiphos.

Host penetration by J2 of all nematodes examined was usually lower in aldicarb-treated soil than in either ethoprop or fenamiphos soil treatments. The former compound has been shown to be more effective than fenamiphos in inhibiting acetylcholinesterase (AChE) from *Helicotylenchus dihystera* (Cobb) Sher and other selected nematodes (13). The lower level of host penetration with aldicarb also may be related to delayed invasion by J2 (5), but this aspect was not investigated in our experiments.

Differential nematicidal activity on some nematode species has been documented. Juveniles of *Heterodera schachtii* Schmidt are more sensitive to aldicarb than is *M. javanica* (6). Acetylcholinesterase from *H. glycines* is 10 times less sensitive than *Meloidogyne* AChE to aldicarb and fenamiphos (12). No differences in sensitivity of AChE between *Aphelenchus avenae* Bastian and *H. dihystera* were observed when treated with either aldicarb or fenamiphos (13). Our experiments revealed that aldicarb, ethoprop, and fenamiphos were less effective in inhibiting penetration by *H. glycines* race 5 compared with *Meloidogyne* spp., especially *M. incognita.* Reactions of races 1 and 2 of *H. glycines* to aldicarb were similar (14).

The responses of certain *Meloidogyne* species to nonfumigant nematicides are variable (2,9,10). *Meloidogyne arenaria* was only partially controlled by ethoprop on

tobacco in North Carolina at rates that controlled other *Meloidogyne* species (2). Ethoprop has given variable results for other investigators as well (9,10).

Differences among species of *Meloidogyne* exposed to aldicarb or fenamiphos could be due to a number of physiological reactions in the nematode or even to test conditions. Species of *Meloidogyne* exposed to these nematicides vary in their behavior. For example, *M. javanica* consumes more oxygen than *M. incognita* and *M. arenaria* when treated with aldicarb (11). Nematicide inhibition of nematode respiration corresponds to the physical properties of chemicals. Aldicarb causes high initial respiratory inhibition, whereas the more lipophilic and less water soluble fenamiphos and ethoprop show lower initial activity. Respiratory inhibition corresponds to decreases in nematode motility (11). Opperman and Chang (12) reported no differences in AChE binding to aldicarb or fenamiphos for *M. incognita* or *M. arenaria*.

Proper identification of the target nematode species is crucial in order to maximize control with minimum chemical input because of differences in sensitivity to nematicides. Economic returns are likely to be realized in several crop–nematode combinations, once nematicide dosages are optimized.

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