Journal of Nematology 21(4):472–476. 1989.

Effect of Carbamate, Organophosphate, and Avermectin Nematicides on Oxygen Consumption by Three *Meloidogyne* spp.[1]

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Abstract: Second-stage juveniles (J2) of *Meloidogyne arenaria* consumed more oxygen ($P \leq 0.05$) than *M. incognita* J2, which in turn consumed more than *M. javanica* J2 (4,820, 4,530, and 3,970 µl per hour per g nematode dryweight, respectively). Decrease in oxygen consumption depended on the nematicide used. Except for aldicarb, there was no differential sensitivity among the three nematode species. *Meloidogyne javanica* had a greater percentage decrease ($P \leq 0.05$) in oxygen uptake when treated with aldicarb, relative to the untreated control, than either *M. arenaria* or *M. incognita*. *Meloidogyne javanica* J2 had a greater degree of recovery from fenamiphos or aldicarb intoxication, after subsequent transfer to water, than did *M. incognita*. This finding may relate to differential sensitivity among *Meloidogyne* spp. in the field. Degree of respiratory inhibition and loss of nematode motility for *M. javanica* after exposure to the nematicides were positively correlated ($P \leq 0.05$).

Key words: aldicarb, avermectin, carbamate, carbofuran, ethoprop, fenamiphos, *Meloidogyne arenaria*, *Meloidogyne incognita*, *Meloidogyne javanica*, organophosphate, oxamyl, oxygen, root-knot nematode.

Oxygen consumption by an organism is an expression of its biological activity. Inhibition or uncoupling of an organism's respiratory functions will have a major influence on its energy metabolism and may represent vulnerable points of attack against pest organisms (4). Nematicides were shown to influence oxygen uptake in several free-living nematodes (2,6,11), but to our knowledge the effect of nonfumigant nematicides on oxygen consumption of plant-parasitic nematodes has never been described. Field observations, mainly on tobacco, in recent years have indicated that the three major root-knot nematode species—*Meloidogyne javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood, and *M. incognita* (Kofoid & White) Chitwood—respond differently to nonfumigant nematicides (1,8,9). Our objectives were to determine whether there is a possible differential effect of selected nonfumigant nematicides on the oxygen uptake of three *Meloidogyne* spp. and the reversibility of inhibitory effects on nematode respiration.

MATERIALS AND METHODS

Meloidogyne javanica, *M. arenaria*, and *M. incognita* were cultured in the greenhouse on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers). Eggs and second-stage juveniles (J2) were extracted from tomato roots using 0.5% sodium hypochlorite (12).

The nematicides used were technical formulations of aldicarb, avermectin B2a, carbofuran, ethoprop, fenamiphos, and oxamyl. Stock solutions (1,000 µg/ml) of each nematicide were prepared in acetone and stored at 5 C no longer than 14 days. Final concentrations in our experiments were 5 µg/ml except avermectin B2a which was tested at 0.5 µg/ml. Freshly hatched juveniles (no older than 48 hours) of each species (12) were exposed to the nematicides in 50-ml glass beakers for 24 hours at 28 C.

All nematode samples were washed thoroughly with sterile distilled water over an 8-µm-pore filter to reduce bacterial contamination immediately before determining their respiration. The rinse water was

Received for publication 4 January 1989.

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We thank E. I. DuPont de Nemours and Company, FMC Corp., Merck and Company, Mobay Chemical Corp., Mobil Chemical Co., and Union Carbide Agricultural Products Co. for providing technical formulations of nematicides and partial support of this investigation.

analyzed and showed no significant oxygen consumption. Oxygen consumption did not exceed 5 μl per hour per g nematode dry weight. In preliminary tests it was found that the use of antibiotics (streptomycin, penicillin) influenced oxygen uptake, thus they were omitted. The oxygen uptake was measured by a Gilson K-1C oxygraph equipped with a Clark electrode. The reaction chamber was cleaned thoroughly with acetone between each analysis.

Each sample consisted of 50,000 J2 concentrated in the 1-ml volume reaction chamber. During oxygen measurement, the nematode samples were stirred at a constant speed with a magnetic stirrer. The reaction chamber was inside a waterjacketed cell through which 28 C water was pumped at a constant rate. Before measurement, air was bubbled through each nematode sample repeatedly and left for 5 minutes for temperature equilibration. The cell was closed and the oxygen consumption was recorded for 5 minutes. The amount of oxygen consumed was expressed as microliters oxygen per hour per gram of nematode dry weight. The oxygen concentration at the beginning of an experiment was set at 100%, based on an air-saturated water control solution. To determine nematode dry weights for each species, 200,000 J2, no older than 48 hours, were concentrated in 2 ml distilled water and placed in an aluminum container. The samples were dried at 120 C for 48 hours and weighed immediately after drying. Containers with 2 ml distilled water served as controls.

To determine nematode motility, 100 freshly hatched *M. javanica* J2 were exposed to the individual nematicides for 24 hours and subsequently observed at 40× magnification on a microscope slide. All J2 that showed body movement within ca. 10 seconds were regarded as active. The speed of juvenile motility, relative to the untreated juveniles, was rated subjectively as regular, slow, very slow, or none.

To determine the reversibility of nematicidal action, *M. incognita* and *M. javanica* J2 were exposed to fenamiphos and aldicarb at 5 μg/ml for 24 hours. After determining the oxygen consumption, the nematicides were removed by quickly rinsing the nematodes with distilled water over an 8-μm-pore filter. The nematodes were then transferred to water for 24 hours and oxygen consumption was determined again after this period. Nematodes in sterile water served as untreated controls in all experiments. All experiments contained three replicates and were repeated at least twice. The data were subjected to analysis of variance and treatment means were compared by Duncan's multiple-range test.

TABLE 1. Decrease in oxygen uptake (%) by second-stage juveniles of *Meloidogyne javanica* relative to the untreated control after exposure to different nematicides for 1 hour and 24 hours.

Nematicide and final concentration	1 hr	24 hr
Carbofuran, 5 μg/ml	3.0 a	3.7 a
Aldicarb, 5 μg/ml	9.5 c	15.3 b
Ethoprop, 5 μg/ml	6.0 b	18.7 c
Fenamiphos, 5 μg/ml	7.0 b	26.7 d
Oxamyl, 5 μg/ml	20.0 d	26.7 d
Avermectin B2a, 0.5 μg/ml	44.0 e	60.0 e

Data are mean of three replicates. Means within a column with the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

RESULTS

Meloidogyne arenaria consumed 4,820 μl O_2 per hour per g nematode dryweight. This was greater ($P \leq 0.05$) than that consumed by either *M. incognita* (4,530 μl O_2 per hour per g) or *M. javanica* (3,970 μl O_2 per hour per g).

When *M. javanica* J2 were exposed to six different nematicides for 1 hour and 24 hours, a nematicide-specific decrease in oxygen consumption relative to the control was evident (Table 1). Carbofuran had little effect on oxygen uptake for either exposure period. Avermectin and oxamyl caused a greater reduction ($P \leq 0.05$) in oxygen uptake than either aldicarb, ethoprop, or fenamiphos after 1 hour, but after a 24-hour exposure only avermectin reduced oxygen uptake to a greater extent than the carbamates or organophosphates ($P \leq 0.05$). After 24 hours there was no

TABLE 2. Decrease in motility of second-stage juveniles of *Meloidogyne javanica* after exposure to different nematicides for 24 hours.

Nematicide and final concentration	Active (%)	Relative motility
Untreated	91 e	Regular
Carbofuran, 5 μg/ml	89 e	Regular
Aldicarb, 5 μg/ml	39 d	Slow
Ethoprop, 5 μg/ml	28 c	Slow
Fenamiphos, 5 μg/ml	11 b	Very slow
Oxamyl, 5 μg/ml	10 b	Slow
Avermectin B2a, 0.5 μg/ml	0 a	None

Data are mean of three replicates. Means with the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

TABLE 3. Decrease in oxygen uptake (%) by second-stage juveniles of three *Meloidogyne* spp. relative to the untreated control after exposure to different nematicides for 24 hours.

Nematicide and final concentration	*M. javanica*	*M. arenaria*	*M. incognita*
Carbofuran, 5 μg/ml	3.7 a	2.0 a	4.3 a
Aldicarb, 5 μg/ml	15.3 a	22.0 b	24.7 b
Ethoprop, 5 μg/ml	18.7 a	17.7 a	19.3 a
Fenamiphos, 5 μg/ml	26.7 a	28.7 a	29.3 a
Oxamyl, 5 μg/ml	26.7 a	24.7 a	26.7 a
Avermectin B2a, 0.5 μg/ml	60.0 a	62.0 a	61.3 a

Data are mean of three replicates. Means across columns with the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

difference in the reduction in oxygen uptake between fenamiphos or oxamyl, but both compounds reduced oxygen uptake greater than aldicarb or ethoprop ($P \leq 0.05$). After 1 hour the carbamates and avermectin had reduced oxygen uptake to 62–81% of the 24-hour inhibition rate, whereas after 1 hour the organophosphates reduced oxygen uptake to 26–32% of the 24-hour measurement.

When nematicides were compared for effects upon the motility of *M. javanica*, only carbofuran failed to reduce J2 motility below the untreated control ($P \leq 0.05$) (Table 2). The relative motility of J2 treated with aldicarb, ethoprop, and oxamyl was rated as slow, whereas fenamiphos treated J2 were rated as very slow. *Meloidogyne javanica* J2 treated with avermectin exhibited no activity. The relative motility of *M. javanica* J2 (Table 2) was correlated positively with the percentage of decrease in oxygen uptake for the individual nematicides (Table 1) ($r = 0.84$, $P \leq 0.05$).

No differences ($P \leq 0.05$) were observed among the three *Meloidogyne* species in their response to nematicides, except aldicarb, when the decrease in their oxygen uptake was compared (Table 3). Aldicarb induced a greater decrease ($P \leq 0.05$) in oxygen uptake by *M. javanica* J2 than by either *M. arenaria* or *M. incognita*. There was no difference between *M. arenaria* or *M. incognita*. Exposure of J2 of *M. javanica* and *M. incognita* to aldicarb or fenamiphos for 24 hours caused a decrease in their oxygen uptake (Table 4). When the nematodes were transferred to water for 24 hours, there was greater ($P \leq 0.05$) reactivation of oxygen uptake by *M. javanica* than by *M. incognita*.

DISCUSSION

All three *Meloidogyne* spp. showed different rates of oxygen consumption, but they did not differ in their response to different nematicides, except aldicarb. This is contrary to a finding with *Aphelenchus avenae* Bastian where three isolates showed similar rates of oxygen consumption but differed in their response to respiratory inhibitors (7).

We found that respiration rates of *Meloidogyne javanica* were affected differently by different nematicides. Similarly, nonfumigant nematicides each had a different effect upon the oxygen uptake of *Panagrellus redivivus* (L.) Goodey and *Rhabditis oxycerca* de Man (4,11). It seems, however, that the nematicide concentration needed to evoke an initial response had to be much higher for free-living nematodes than for *Meloidogyne* spp.

The differences in respiratory inhibition in *M. javanica* J2 after 1 hour and 24 hours exposures can probably be explained by the different rate of nematicide uptake by the nematodes. The ranking of the nematicides based on their effect on oxygen uptake corresponds with their physical properties (3). The rather hydrophilic and highly water soluble oxamyl and aldicarb

TABLE 4. Decrease in oxygen (%) uptake by second-stage juveniles of two *Meloidogyne* spp. relative to the untreated control after 24-hour exposure to two nematicides and consumption (%) relative to the nematicide treatment after transfer to distilled water for 24 hours.

Nematicide and final concentration	After nematicide		After water	
	M. javanica	*M. incognita*	*M. javanica*	*M. incognita*
Aldicarb, 5 μg/ml	15.3 a, x	24.7 b, x	11.0 a, x	21.0 b, x
Fenamiphos, 5 μg/ml	26.7 a, x	29.3 a, x	7.0 a, y	16.0 b, y

Data are mean of three replicates. Means across columns within a treatment (a, b) or within a nematode species (x, y) with the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

exhibited high initial inhibition, whereas the more lipophilic and less water soluble fenamiphos and ethoprop showed lower initial activity.

The degree of respiratory inhibition in our study paralleled a decrease in nematode motility. All nematicides producing nematode paralysis also inhibited oxygen uptake. Although avermectin B2a induced total paralysis, it did not completely inhibit respiration. All nematicides except carbofuran caused nematode paralysis that would probably prevent the nematode from migrating to its host plant. Carbofuran-treated J2 appeared able to move freely, as has been shown for *Pratylenchus vulvus* Allen & Jensen when carbofuran was tested at a concentration similar to that reported herein (5). The nematicide probably blocked chemoreceptor sites necessary for host finding. Carbofuran at 5 μg/ml also inhibits nematode penetration into host roots (10).

This study does not provide evidence to explain why *M. javanica* appears to be less sensitive than other *Meloidogyne* spp. to some nonfumigant nematicides in the field (8,9). When *M. javanica* and *M. incognita* were subjected to aldicarb or fenamiphos and were subsequently transferred to water, *M. javanica* J2 recovered from respiratory inhibition to a greater degree than *M. incognita*. The greater recovery may enable *M. javanica* to approach and penetrate host plants more rapidly than *M. incognita*. This may become important in field situations where heavy rainfalls occur immediately after nematicide applications or during the first 4–6 weeks following such applications. With the reduction in toxicant levels through dilution and (or) leaching from heavy rains, a nematode that recovers quickly would have a competitive advantage over one that doesn't recover quickly. The ability to recover from respiratory inhibition does not seem to be related to total oxygen consumption. *Meloidogyne javanica* J2 consumed less oxygen than the other two species.

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