Effects of Selected Carbamate and Organophosphate Nematicides on Hatching and Emergence of Heterodera schachtii

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Abstract: Ethoprop, oxamyl, PP 156, fenamiphos, carbofuran, AC 64,475, Bunema M®, CG 12223, aldicarb, aldicarb sulfoxide, and aldicarb sulfone were tested for their effects on hatching and emergence of larvae from cysts of *Heterodera schachtii*. The oxime carbamates and carbofuran inhibited hatching, but this response was reversed by removing the chemical treatment. Inhibition of hatching by Bunema M and all organophosphates tested was irreversible. *Key Words:* chemical control, cyst nematodes, reversible toxicity, screening tests.

Recent awareness of the impact of high residual broad-spectrum pesticides on nontarget microorganisms has stimulated a search for pesticides of high specificity and low residual toxicity that can be effectively applied at minimal rates. The development of organophosphate and carbamate pesticides has been followed by investigations of their nematicidal properties and modes of action. At economic rates (4, 5, 8, 11) some of these materials are not nematicidal, but they appear to disrupt nematode movement and behavior (4, 8), an action which results in control. Use of sublethal rates of pesticides for limited periods of protection is attractive as a pest management tool, particularly with annual crops (such as sugarbeet) for which protection is critical during the first several weeks of growth but usually of less importance on older, well-established plants.

In most of the beet-growing areas of North America, the sugarbeet nematode, *Heterodera schachtii* Schmidt, is the major pest affecting production of sugarbeet (*Beta vulgaris* L.). Dormancy of *H. schachtii* larvae within soilborne cysts is broken by hatch-stimulating secretions of host roots. Hatched larvae emerge from cysts, invade plant roots, and there they complete their development as sedentary parasites. Protection of sugarbeet from nematode invasion during the first 6 to 8 weeks of growth greatly minimizes economic losses. Thus, control efforts directed toward unhatched larvae within cysts and newly hatched migrating larvae with applications of nonphytotoxic nematicides at planting time or early after planting are likely to succeed.

In my laboratory, coded chemicals are evaluated for their effects on the hatchability and viability of cyst contents (15, 16) as a prerequisite to selection for field trials. In this study, selected organophosphates and carbamates were examined for their immediate and residual effects on hatching of sugarbeet nematode larvae.

MATERIALS AND METHODS

Experimental nematicides (seven carbamates and four organophosphates) were evaluated in six tests for their effects on hatching and emergence of larvae from cysts of *Heterodera schachtii*. The following nematicides were evaluated in the first test: ethoprop, supplied by Mobil Chemical Co., Richmond, Virginia; oxamyl, supplied by E. I. duPont de Nemours and Co., Wilmington, Delaware; fenamiphos, supplied by Chemagro Corp., Kansas City, Missouri; and PP 156 (1,4-dithiepan-6-one *N*-[(methylcarbamoyl)oxy]oxime), supplied by ICI United States Inc., Goldsboro, North Carolina. Aqueous solutions prepared with technical grade materials were tested in dilutions of 100, 200, 500, and 1,000 μg/ml. Additional treatments of 250 and 2,000 μg/ml oxamyl were evaluated. Four replications of 20 cysts were treated 1 week in the chemical test solutions, after which the cysts were placed in fresh changes of tap water daily for a period of 4 days and then in sugarbeet root diffusate for a period of 4 weeks to evaluate the residual effects of chemicals on hatching.

The effects of carbofuran supplied by Niagara Chemical Div., FMC Corp., Middleport, New York, and AC 64,475 [2-(Di-

Received for publication 13 July 1976.

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ethoxyphosphinylimino)-1,3-dithietane] supplied by American Cynamid Co., Princeton, New Jersey were compared in a second test. Aqueous solutions of 0.1, 0.5, 1, 5, 10, 50, 100, and 500 μg/ml were evaluated by treating 5 replications of 20 cysts 1 week, and by following this treatment, as before, with 4 days in water and 4 weeks in sugarbeet root diffusate.

In a third test, 4 replications of 25 cysts were treated 2 weeks with 1, 5, 10, 50, 100, 500, and 1,000 μg/ml of potassium (hydroxymethyl) methyldithiocarbamate (supplied as Bunema M by Buckman Laboratories, Inc., Memphis, Tennessee). After being initially treated 2 weeks with the chemical solutions, the cysts were placed in 4 changes of tap water for several minutes and then immediately placed in sugarbeet root diffusate where they were kept for 2 weeks.

The fourth test evaluated hatching and emergence of larvae from cysts of *H. schachtii* treated with CG 12223, an organophosphate insecticide-nematicide supplied by Ciba-Geigy Corporation, Greensboro, North Carolina. Four replications of 20 cysts were treated 1 week with aqueous solutions of 1, 10, 50, 100, 250, and 500 μg/ml of CG 12223; these treatments were followed by daily changes into fresh water for 4 days to remove the chemicals, and then by 4 weeks in sugarbeet root diffusate to evaluate viability by hatching the contents of cysts.

The fifth and sixth tests evaluated the effects of 1, 10, 50, 100, 200, 500, and 1,000 μg/ml of aldicarb, aldicarb sulfoxide [2-methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)oxime] and aldicarb sulfone [2-methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)oxime]. Four replications of 20 cysts were treated 1 week in aqueous chemical solutions, taken through 4 changes of tap water during a period of 4 days, and then treated 4 weeks with sugarbeet root diffusate to induce hatching.

Each test included a control in which the cysts were placed in distilled water without chemicals where they remained during the initial treatment period. Except for tests 1, 5, and 6, cysts were also treated continuously during the entire test periods with sugarbeet root diffusate. All tests were conducted in an incubator at a constant temperature of 24 C. Larval hatches were analyzed for statistical significance by analysis of variance and Duncan's multiple range test. Other materials and procedures used in this study have been described elsewhere (14).

RESULTS AND DISCUSSION

All tested rates of ethoprop, oxamyl, fenamiphos, and PP 156 inhibited hatching and emergence of larvae from cysts of *H. schachtii* (Table 1). However, except in cysts treated with fenamiphos, hatching resumed after removal of the nematicides and subsequent treatment with sugarbeet root diffusate. Permanent suppression of hatching was obtained with 100-1,000 μg/ml fenamiphos even after cysts were removed from the chemical solutions.

Although complete suppression of hatching was obtained with 50-500 μg/ml carbofuran, hatch of cysts from which carbofuran was removed was higher than that of untreated cysts (Table 2). In contrast, 0.1 μg/ml carbofuran stimulated hatching, but hatches from cysts in diffusate after carbofuran was removed were not significantly different from those of cysts treated only with tap water. Retarded hatching was obtained with 50, 100, or 500 μg/ml of AC 64,475, but the highest concentration was the only one that permanently depressed hatching.

Treatments of 5 μg/ml or greater of Bunema M inhibited hatching of larvae, and this effect persisted after removal of cysts from the chemical solutions if the initial treatment contained 50 μg/ml of chemical (Table 3). Although 10 μg/ml of CG 12223 suppressed hatching, only 50 μg/ml or greater showed a pronounced residual effect (Table 4). When the pretreatment solution contained from 1 to 100 μg/ml CG 12223, the post-treatment emergence of larvae in sugarbeet root diffusate was proportional to the log concentration of nematicide. At concentrations of 250 μg/ml or greater, the effects on emergence were disproportionately greater. Almost no larvae hatched from cysts treated with aqueous solutions of 500 μg/ml and then by sugarbeet root diffusate. Treatments of 1-100 μg/ml of aldicarb, aldicarb sulfoxide,

TABLE 1. Influence of nematicides on emergence of larvae from cysts of Heterodera schachtii.[x]

Treatment Chemical	Concentration (μg/ml)	No. larvae in chemical[y]	No. larvae in water	No. larvae in diffusate	Larval emergence[z]
Check	0	165 a	5	2,023	2,193 c
Ethoprop	100	14 b	36	2,859	2,884 abc
	200	4 b	44	3,639	3,671 a
	500	4	4	2,516	2,526 c
	1,000	0 b	0	1,691	1,693 d
Oxamyl	100	3 b	52	3,025	3,080 abc
	250	0 b	41	2,532	2,574 c
	500	0 b	3	3,508	3,515 ab
	1,000	1 b	2	2,957	2,960 abc
	2,000	0 b	0	2,326	2,327 c
PP 156	100	1 b	59	3,426	3,486 ab
	200	0 b	6	2,810	2,817 abc
	500	2 b	8	2,657	2,667 bc
	1,000	0 b	14	2,373	2,387 c
Fenamiphos	100	1 b	0	15	16 e
	200	1 b	0	10	12 e
	500	0 b	0	9	9 e
	1,000	0 b	0	1	1 e

[x]Cysts treated 1 week in chemical(s), 4 days in water, and 4 weeks in sugarbeet root diffusate. Common letters indicate Duncan's multiple range groupings of treatments which do not differ significantly ($P = 0.05$).
[y]Mean numbers of larvae emerged from 4 replications of 20 cysts.
[z]Value includes larvae emerged in chemical(s), water, and diffusate.

TABLE 2. Influence of carbofuran and AC 64,475 on hatching and emergence of larvae from cysts of *Heterodera schachtii*.[x]

Treatment Chemical	Concentration (μg/ml)	No. larvae in chemical[y]	No. larvae in water[y]	No. larvae in diffusate[y]	Larval emergence[z]
Carbofuran	0.1	112 b	4	228	344 de
	0.5	67 bc	1	227	295 def
	1	29 bc	0	239	268 def
	5	19 c	2	258	279 def
	10	8 c	21	473	502 cd
	50	0 c	3	613	616 bc
	100	0 c	2	1,088	1,090 a
	500	0 c	1	799	800 b
AC 64,475	0.1	40 bc	0	158	198 ef
	0.5	80 bc	0	158	238 def
	1	63 bc	0	100	163 ef
	5	57 bc	8	228	293 def
	10	26 bc	7	260	293 def
	50	3 c	23	473	499 cd
	100	0 c	1	406	407 cde
	500	0 c	0	31	31 f
Tap water	—	18 c	3	191	212 def
Diffusate	—	500 a	63	41	603 bc

[x]Cysts treated 1 week in chemical(s), 4 days in water, and 4 weeks in sugarbeet root diffusate. Common letters indicate Duncan's multiple range groupings of treatments which do not differ significantly ($P = 0.05$).
[y]Mean numbers of larvae emerged from 5 replications of 20 cysts.
[z]Value includes larvae emerged in chemical(s), water and diffusate.

TABLE 3. Influence on hatching and emergence of larvae from *Heterodera schachtii* cysts treated for 2 weeks with Bunema M and then for 2 weeks with sugarbeet-root diffusate.

Concentration (μg/ml)	2 weeks in chemical[x]	2 weeks in diffusate	Total Hatch[x]
0[y]	1,228 b	2,478	3,721 a
1	996 bc	2,250	3,246 a
5	631 c	2,351	2,981 ab
10	630 c	1,457	2,086 b
50	16 d	13	29 c
100	21 d	1	22 c
500	24 d	2	26 c
1,000	4 d	1	5 c
Diffusate[z]	3,540 a	295	3,835 a

[x]Mean numbers of larvae emerged from 4 replications of 25 cysts. Different letters indicate Duncan's multiple groupings of treatments that differ ($P = 0.05$).
[y]Treated 2 weeks with tap water and then 2 weeks with diffusate.
[z]Treated 4 weeks with sugarbeet-root diffusate.

or aldicarb sulfone inhibited hatching of *H. schachtii,* but (except for treatments of 100 μg/ml aldicarb sulfone) this effect was reversed by removing the oxime carbamates (Table 5). Data of an experiment in which concentrations of 100, 200, 500, and 1,000 μg/ml of aldicarb, aldicarb sulfoxide, and aldicarb sulfone were tested are not presented in this report. However, all concentrations of each chemical equally but only temporarily inhibited hatching which resumed after cysts were removed from the chemical solutions and treated with sugarbeet root diffusate.

Both organophosphate and carbamate nematicides exhibited great variation in hatching effects. The oxime carbamates, oxamyl, PP 156, aldicarb, aldicarb sulfoxide, and aldicarb sulfone as well as the methylcarbamate (carbofuran) inhibit hatching. However, their effects were subsequently reversed by removal of the inhibiting compounds. The highest concentrations of dithiocarbamate, Bunema M, irreversibly inhibited hatching. Nabam has proven to have exceptionally strong hatching activity at 1,000 μg/ml, but 2,000 inhibited hatching (13). All organophosphorous compounds tested suppressed hatching but the effect was reversible only when cysts were treated with ethoprop.

Results of this study are in general agreement with reports on the biological activities of organophosphorous and carbamate compounds. Pesticides of both groups inhibit cholinesterase activity. However, the carbamate inhibition is rapidly reversible, whereas reversibility is not characteristic of organophosphates. Symptoms of poisoning of *Ditylenchus dipsaci* were reversible for oxamyl but less so for fenamiphos (1). Inhibition by an organophosphate is an irreversible chemical reaction in which the cholinesterase enzymes, primarily acetylcholinesterase and cholinesterases, are phosphorylated. Inhibition by oxycarbamates is due to competitive adsorption on the enzyme surface (2). The potency of these materials depends upon their intrinsic enzyme affinity, their anti-cholinesterase properties, and their solubility (6). In water contact tests of cell-free preparations of *Panagrellus redivivus* (12), aldicarb inhibited enzymatic acetylcholine hydrolysis ($I_{50} = 6.5 \times 10^{-6}$ M) at concentrations considerably below those required for nematicidal activity ($ED_{50} = 2.6 \times 10^{-3}$ M). Unfortunately, the effects of these materials on enzyme systems in plant-parasitic nematodes have not yet been explored.

Notwithstanding, potency in nematodes is probably related to concentration and

TABLE 4. Effects of CG 12223 on hatching and emergence of larvae from cysts of *Heterodera schachtii.*

Concentration[w] (μg/ml)	No. larvae in chemical[x]	No. larvae in water	No. larvae in diffusate	Larval emergence[xy]
0	95 a	26	1,661	1,782 a
1	95 a	43	1,887	2,025 a
10	3 b	12	1,474	1,488 a
50	2 b	0	1,084	1,086 b
100	8 b	0	862	870 b
250	1 b	0	102	104 c
500	1 b	0	2	3 c
Diffusate	951[z]	304	526	1,780 a

[w]Cysts treated 1 week in chemicals, 4 days in water, and 4 weeks in sugarbeet-root diffusate.
[x]Mean numbers of larvae emerged from 4 replications of 20 cysts. Common letters indicate Duncan's multiple range groupings of treatments which do not differ significantly ($P = 0.05$).
[y]Value includes larvae emerged in chemical(s), water, and diffusate.
[z]Not included in statistical analysis.

TABLE 5. Influence of aldicarb and its oxides on emergence of larvae from cysts of *Heterodera schachtii*.[x]

Treatment chemical	Concentration (μg/ml)	No. larvae in chemical[y]	No. larvae in water	No. larvae in diffusate (1,000's)	Larval emergence[z] (1,000's)
Check	0	1,479.3 a	23.5	1.7	3.2 a
Aldicarb	1	129.8 c	219.3	2.5	2.8 bcd
	10	9.5 c	7.3	3.0	3.0 bcd
	50	4.8 c	0.3	2.9	2.9 bcd
	100	4.5 c	0.3	1.8	1.8 cd
Aldicarb-sulfoxide	1	555.5 b	57.5	1.5	2.1 bcd
	10	4.0 c	121.5	2.0	2.1 bcd
	50	15.3 c	199.8	1.6	1.8 bcd
	100	2.5 c	49.3	3.1	3.1 bc
Aldicarb-sulfone	1	761.0 b	45.3	1.5	2.4 bcd
	10	255.5 c	116.3	1.4	1.8 cd
	50	9.5 c	177.3	1.7	1.9 bcd
	100	2.3 c	1.5	0.3	0.3 d

[x]Cysts were treated 1 week in chemical(s), 4 days in water, and 4 weeks in sugarbeet-root diffusate. Different letters indicate Duncan's multiple range groupings of treatments that differ significantly ($P = 0.05$). Tests separately analyzed for statistical significance.
[y]Value includes larvae emerged in chemical(s), water, and diffusate.
[z]Mean numbers of larvae emerged from 4 replications of 20 cysts.

the modifying effects of time, temperature, and factors affecting hydrolysis or oxidation of the materials in plants and in soil. Reports indicate that these exposure parameters may also influence reversibility of carbamate and phosphate inhibitors. Hatch of *H. rostochiensis* (Woll.) (9, 10) and *H. schachtii* (16) was inhibited by relatively low concentrations of aldicarb. Higher concentrations of aldicarb decreased hatching of *H. rostochiensis* (10) and hatchability of cyst contents of *H. schachtii* declined after exposure to 10 μg/ml for 45 days (17). The viability of hatched second-stage larvae was reduced in proportion to the concentration and duration of treatment with aldicarb (13), and migration of males did not occur with treatments of 0.01μg/ml aldicarb (3).

Results of this study show that, in general, hatch inhibition by carbamates is reversible, whereas the effects of organophosphates are not. However, reversibility is dependent upon exposure parameters and probability varies for each material.

LITERATURE CITED

1. BUNT, J. A. 1975. Effect and mode of action of some systemic nematicides. Meded. Landbouwhogesch. Wageningen 75 (10). 127 p.
2. FUKUTO, T. R. 1957. The chemistry and action of organic phosphorous insecticides. Pages 147-192 *in* R. L. Metcalf, ed., Advances in pest control research. Vol. 1, Interscientific Publications, New York.
3. HOUGH, A., and I. J. THOMASON. 1975. Effects of aldicarb on the behavior of Heterodera schachtii and Meloidogyne javanica. J. Nematol. 7:221-229.
4. MC LEOD, R. W., and G. T. KHAIR. 1975. Effects of oxime carbamate, organophosphate and benzimidazole nematicides on life cycle stages of root-knot nematodes, Meloidogyne spp. Ann. Appl. Biol. 79:329-341.
5. MILLER, P. M. 1970. Failure of several nonvolatile and contact nematicides to kill eggs in cysts of Heterodera tabacum. Plant Dis. Rep. 54:781-783.
6. MITCHELL, L. E. 1966. Pesticides: Properties and prognosis. Pages 1-22. *in* R. F. Gould, ed., Organic pesticides in the environment. Am. Chem. Soc. Wash. D.C. 309 pp.
7. MOTSINGER, R. E. 1961. Evaluation of an experimental nematicide 0,0-diethyl-0-2-pyrazinyl phosphorothioate. Plant Dis. Rep. 45:335-340.
8. NELMES, H. J., D. L. TRUDGILL, and D. C. M. CORBETT. 1973. Chemotherapy in the study of plant parasitic nematodes. Pages 95-112, *in* A. R. Taylor, and R. Muller, eds. Chemotherapeutic agents in the study of parasites. Vol. 2. Proc. Symp. Br. Soc. Parasitol., Oxford, England.
9. OSBORNE, P. 1973. Effect of aldicarb on the hatching of Heterodera rostochiensis larvae. Nematologica 19:7-14.

10. PAIN, B. F., and N. G. M. HAGUE. 1971. The effect of organophosphate and carbamoyl-oxime nematicides against the potato cyst eelworm, Heterodera rostochiensis Woll. Meded. Fac. Landbouwwet. Rijksuniv. Gent. 36:161-173.
11. REDDY, D. D. R., and A. R. SESHADRI. 1971. Studies on some systemic nematicides. I. Evaluation for systemic and contact action against the root-knot nematode, Meloidogyne incognita. Indian J. Nematol. 1:199-208.
12. SPURR, H. W. 1966. Nematode cholinesterases and their inhibition by nematocidal carbamates. Phytopathology 56:902-903 (Abstr.).
13. STEELE, A. E. 1961. The effect of nabam solution on the emergence of larvae from cysts of Heterodera schachtii. J. Am. Soc. Sugar Beet Technol. 11:528-532.
14. STEELE, A. E. 1976. Improved methods of hatching Heterodera schachtii larvae for screening chemicals. J. Nematol. 8:23-25.
15. STEELE, A. E. 1976. Effects of oxime carbamate nematicides on development of Heterodera schachtii on sugarbeet. J. Nematol. 8:137-141.
16. 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.
17. STEUDEL, W. 1972. Versuche zum Einfluss von Aldicarb auf den Schlüpvorgang bei Zysten von Heterodera schachtii nach längerer Einwerkung. Nematologica 18:270-274.