Effects of Oxime Carbamate Nematicides on Development of Heterodera schachtii on Sugarbeet

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Abstract: Treatment of sugarbeet, *Beta vulgaris* L., with aldicarb, aldicarb sulfoxide, or aldicarb sulfone 10 days after plants were inoculated with *Heterodera schachtii* prevented development of the nematode, but second-stage larvae penetrated the roots. These chemicals had no measurable effects on nematodes in plants treated 15 days after inoculation. The tests established that soil treatments of aldicarb are directly or indirectly lethal to larvae developing within roots of sugarbeet. *Heterodera schachtii* failed to develop on root slices of red table beet grown in soil treated with aldicarb or aldicarb sulfoxide. Similar treatment of plants with aldicarb sulfone or oxamyl did not affect subsequent development of *H. schachtii* on root slices of treated plants. *Key Words:* oxamyl, aldicarb, sugarbeet nematode, culture, storage root slices.

Studies at this laboratory (7) have established that aldicarb [2-methyl-2(methylthio) propionaldehyde-*O*-(methylcarbamoyl) oxime], with or without hatching agents, temporarily inhibits hatching of *Heterodera schachtii* Schmidt. Normal rates of hatching occur when the aldicarb is removed. In-vitro treatment of second-stage larvae (L2) with aldicarb or aldicarb sulfoxide [2-methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)oxime] prevented invasion of the larvae, whereas aldicarb sulfone [2-methyl-2-(methylsulfonyl)propionaldehyde-*O*-(methylcarbamoyl)oxime] had no effect. Although the study showed that aldicarb acts as a systemic agent to control *H. schachtii,* the results did not reveal whether aldicarb is nematicidal or nemastatic within plants. This paper reports results of additional tests on the modes of action of aldicarb, aldicarb sulfoxide, aldicarb sulfone, and oxamyl [methyl N′-N′-dimethyl-N-(methylcarbamoyl)oxy-1-thiooxamimidate].

MATERIALS AND METHODS

Aldicarb and its by-products (aldicarb sulfoxide and aldicarb sulfone) were compared for their effects on development of *Heterodera schachtii* in roots of sugarbeet (*Beta vulgaris* L.). Analytical grades of these chemicals of 99% purity were utilized (Union Carbide Corporation, Salinas, California 93901). In the first study, sugarbeet seedlings germinated in steam-sterilized sand were transplanted to individual styrofoam cups containing 300 g of steam-sterilized sand-soil mixture (prepared by adding 1 part sand to 4 parts clay loam soil). At this time, 30 broken cysts, each with about 250 eggs and larvae, were added to the soil. Ambient temperatures in the growth chamber, in which the experiment was conducted, were regulated to maintain soil temperatures at 24C ± 0.5 during both 16 h of high intensity illumination (about 55,974 lux), and 8 h of darkness. After 10 and 15 days, the plants were removed, carefully washed, and placed in funnels containing either distilled water or a 5 μg/ml aqueous solution of each test chemical. The root system of each seedling was supported in

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the funnels by a wire screen placed just below the surface of the aqueous solutions. After 5 days, plants were carefully removed from their respective solutions; the roots were stained in acid fuchsin-lactophenol solution, and then fragmented with a Waring Blendor. Counts were made of all nematodes developing within plant roots and of L2 and adult males that migrated from the roots during the periods of exposure to chemicals. The arcsin transformations of percentages of populations existing as L2 were analyzed for statistical significance.

A second experiment was conducted to determine if aldicarb temporarily inhibited development or was lethal to *H. schachtii* larvae. Eighty sugarbeet seedlings were started from seed in sterilized sand, transplanted to styrofoam cups containing 300 g of sand-soil mixture, and grown 10 days in a growth chamber. The seedlings were then inoculated with the contents of 30 broken cysts/plant and again placed in the growth chamber. Ten and 15 days after inoculation, the seedlings were carefully removed from their containers; the roots were washed free of soil and either stained for evaluation of nematode populations or transplanted to nontreated soil or soil treated with 5 μg/g aldicarb for periods of 14 days, and then again transplanted to aldicarb-free soil for 27 days. An additional treatment consisted of transplanting plants that had been infected 10 and 15 days to soil treated with 5 μg/g aldicarb where they remained for a period of 41 days. At the end of the last treatment period, the roots of each plant were washed, stained in acid fuchsin-lactophenol, and examined to determine the developmental stages of nematodes adhering to or within the roots. Treatments of this experiment were replicated 10 times.

A third experiment was designed to compare the systemic effects of oxamyl, formulated as Vydate 10G® (E. I. du Pont de Nemours & Co., Wilmington, Delaware 19898), aldicarb formulated as Temik 10G®, aldicarb sulfoxide, and aldicarb sulfone (Union Carbide Corporation). The chemicals of analytical grade were incorporated, using a twin shell laboratory blender, into a steam-sterilized, sand-soil mixture. Each soil lot was mixed for 5 min after the addition of chemicals.

Red table beets, *Beta vulgaris* L. 'Detroit Dark Red', with storage roots varying from 4-6 cm in diam, were transplanted to 15-cm diam clay pots containing steam-sterilized soil either free of or treated with 6 μg/g of test chemicals. After 21 days, eight plants from each treatment were washed free of potting media, and the leaves, petioles, and lateral roots were removed from the storage roots. The storage roots were cut transversely midway between the crown and root tip. The surface of each root slice was inoculated with eggs and larvae of broken cysts with a hatch potential of about 250 larvae/cyst. The root slices were placed in loosely capped, crystallizing dishes and incubated in a chamber kept at 100% relative humidity and 24 C. After 25 days, the slices were removed and examined for nematodes in various developmental stages on the surfaces of the root slices. All of the second-stage larvae which had not penetrated the root slices were washed from the inoculated surfaces and counted. The total numbers of developing nematodes (L3, L4, and adults) for the various treatments were counted and statistically analyzed.

RESULTS AND DISCUSSION

The response of *H. schachtii* larvae to aldicarb and related compounds varied with test conditions and compounds. The percentage of the population existing as L2 in plants treated with aldicarb was higher than the percentage existing as L2 in plants treated with aldicarb sulfoxide or aldicarb sulfone. The sulfoxide and sulfone treatments, however, resulted in a greater percentage of L2 when the results were compared with populations in nontreated checks. Numbers of nematodes from sugarbeet seedlings grown 10 days in infested soil and treated 5 days in aqueous solutions containing 5 μg/ml of aldicarb, aldicarb sulfoxide, or aldicarb sulfone were not different from numbers of nematodes in nontreated plants in water without chemicals added (Table 1). Few L2 migrated from roots treated with chemical solutions or water. The mean numbers of L2 that migrated from roots during treatment with nematicides were: Aldicarb—3.2; aldicarb sulfoxide—5.6; aldicarb sulfone—5.0; and nontreated control—4.2. Although second-

stage larvae penetrated into the roots of plants treated with chemical solutions (Table 1), lower numbers of L3 and L4 were found, a result indicating that aldicarb and its by-products delayed or stopped the development of L2 into adults within roots of treated plants. Treatment of potato infected with *H. rostochiensis* Wollenweber with aldicarb solutions for 1 day caused L2 to migrate from roots (4). However, migration did not occur if infected roots were kept in nutrient solution 2 or more days before the aldicarb treatment, and underdeveloped L2 and L3 accumulated within the roots.

None of the chemicals appreciably affected development of larvae when plants were treated after growing 15 days in infested soil (Table 1). Failure of the chemicals in this experiment to affect development may have been due to cessation of feeding by nematodes at the time the plants were treated, or to ensheathment by larval integuments in the molting process which may have prevented absorption of chemicals. However, chemical treatments of 5 μg/ml aldicarb sulfoxide of aldicarb sulfone did not measurably affect emergence of males. Other investigators have shown that 10 μg/ml aldicarb totally suppressed migration of males, whereas migration was not affected by 1.0 μg/ml (1).

The first experiment established that L2 do not migrate (to a significant degree) from roots in the presence of aldicarb. In the second test, failure to find larval stages in the numbers observed at the time the aldicarb treatments were initiated (Table 2) indicates that degeneration of dead L2 precluded their recovery from treated plants. The few L2 found in check plants were probably progeny of the population initially inoculated.

Development of all larval stages was arrested by treating plants with aldicarb for only 14 days (Table 2). Since development did not continue when plants were removed from the aldicarb environment after 14 days, it can be concluded that aldicarb taken up by sugarbeet roots is either directly or indirectly lethal to developing larvae of *H. schachtii*.

Growing table beets for 21 days in soil containing 6 μg/g aldicarb or aldicarb sulfoxide significantly suppressed *H. schachtii* subsequently developing on storage root slices (Table 3). Nearly twice as many L2 were found on the surface of root slices of plants treated with aldicarb as were found for other treatments or nontreated checks (Table 3). This difference shows that aldicarb or its metabolites prevent invasion of the roots and that the toxicants were present in levels sufficient for bioactivity throughout the cross-sectioned areas of the storage roots. Soil treatments of aldicarb also prevented invasion of tobacco roots by *H. tabacum* Lownsbery & Lownsbery (2)

TABLE 1. Penetration and development of sugarbeet by *Heterodera schachtii* as affected by chemical treatments.

Chemical treatment[x]	L2	L3	L4	Adult males	% L2[z]
Plants treated 10 days after inoculation[y]					
Aldicarb	458	127	33	0	74.1 a
Aldicarb sulfoxide	358	271	79	0	50.6 b
Aldicarb sulfone	401	357	84	0	47.6 b
Nontreated check	231	381	172	0	29.5 c
Plants treated 15 days after inoculation[y]					
Aldicarb	32	98	312	46	6.6
Aldicarb sulfoxide	50	106	343	76	8.7
Aldicarb sulfone	18	80	342	61	3.6
Nontreated check	49	130	358	40	8.5

[x]Plants treated in vitro with 5 μg/ml of indicated chemical.
[y]Mean numbers of nematodes in five plant replicates. Means of individual stages and means of total numbers not significantly different.
[z]Means with unlike letters are significantly different ($P = 0.05$).

TABLE 2. Influence of aldicarb on development of *Heterodera schachtii* on roots of sugarbeet.

Treatment	Mean numbers/developmental stage/plant/harvest[v]					
	10 days after inoculation			15 days after inoculation		
	L2	L3 + L4	Adults	L2	L3 + L4	Adults
Harvested before treatment	86 a[z]	44 bc	0 c	55 b	149 a	26 c
Transplanted to nontreated soil[w]	6 c	48 bc	172 b	8 c	92 bc	225 a
Transplanted to soil with aldicarb[x]	0 c	4 c	0 c	0 c	8 c	9 c
Transplanted to nontreated soil after aldicarb[y]	0 c	11 c	1 c	0 c	13 c	16 c

[v]Plants were treated at indicated number of days after inoculation.
[w]Transplanted to nontreated soil for 14 days, then again to nontreated soil for 27 days, then examined for nematodes.
[x]Transplanted to soil with aldicarb for 41 days, then examined for nematodes.
[y]Transplanted to soil with aldicarb for 14 days, then again transplanted to soil for 27 days, then examined for nematodes.
[z]Figures given are means of 10 replications. Only means within the same developmental stage were compared. Means with unlike letters are significantly different ($P = 0.05$).

and potato roots by *H. rostochiensis* (4). Equivalent rates of aldicarb sulfone or oxamyl were not lethal; thus no systemic nematicidal activity was established for these chemicals. Nearly twice as many adult males were found on root slices of plants treated with aldicarb sulfoxide as were found on those treated with aldicarb, but means of total numbers of developing larvae for these treatments were not significantly different.

Studies indicate that nematicidal activity of certain carbamates may depend on their ability to effect cholinesterase inhibition, but enzymatic acetylcholine hydrolysis is also inhibited by concentrations of aldicarb considerably below those required for nematicidal activity (6).

Other studies have shown nemastatic properties in hatch inhibition and nematicidal action on migrating larvae (7). In addition, sublethal concentrations of aldicarb disrupt movement and behavior of larvae (3) and neutralize attraction of males by females (1). In parasitized plants which have taken up aldicarb, death of inactivated larvae could perhaps result from indirect effects on the syncytial complex. Interruption of nematode feeding and associated biochemical activities required for continuous functioning of the syncytia may initiate irreversible changes which lead to collapse of syncytia and ultimately to death of larvae. Finally, aldicarb may in some way create an unfavorable environment for parasitic nematodes by directly affecting

TABLE 3. Development of *Heterodera schachtii* on root slices of red table beet as influenced by chemical treatments.

Treatment[x]	Numbers/developmental stages[y]				Total number of developing nematodes[z]
	L2 (1000's)	L3 & L4	Adult males	Adult females	
Aldicarb	2.5	5	5	0	10 a
Aldicarb sulfoxide	1.5	7	10	3	20 a
Aldicarb sulfone	1.5	62	252	88	402 b
Oxamyl	1.2	86	400	157	643 c
Nontreated check	1.3	34	303	248	585 bc

[x]Red table beet grown 21 days in soil with 5 μg/g of indicated chemical treatment before storage roots were sliced and inoculated with viable cysts.
[y]Mean of 8 plants (16 slices).
[z]Means include L3, L4, and adults. Different letters indicate Duncan's multiple groupings of treatments that differ at $P = 0.05$.

host-plant physiology. Thus far, reports in literature do not favor this interpretation.

In-vitro treatment of newly hatched L2 with aldicarb sulfone has little effect on later invasion and development (5). Although aldicarb and aldicarb sulfoxide prevent development of larvae in roots of sugarbeet and table beet, aldicarb sulfone apparently has no such effect and probably does not play a significant part in systemic control or control by contact in soil.

LITERATURE CITED

1. HOUGH, A., and I. J. THOMASON. 1975. Effects of aldicarb on the behavior of Heterodera schachtii and Meloidogyne javanica. J. Nematol. 7:221-229.
2. 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.
3. NELMS, A. 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. Miller, eds. Chemotherapeutic agents in the study of parasites, Vol. 2 Proc. Symp. Br. Soc. Parasitol., Oxford, England.
4. OUDEN, H. DEN. 1971. De ontwikkeling van Heterodera rostochiensis larven in aardappelwortels bij behandeling met systemische nematiciden in het bijzonder aldicarb. Meded. Fac. Landbouwwet. Rijksuniv. Gent. 36:889-903.
5. PAIN, B. F., and N. G. M. HAGUE. 1971. The effect of organophosphate and carbamyl-oxime nematicides against the potato cyst eelworm Heterodera rostochiensis Woll. Meded. Fac. Landbouwwet. Rijksuniv. Gent. 36:161-173.
6. SPURR, H. W., JR. 1966. Nematode cholinesterases and their inhibition by nematicidal carbamates. Phytopathology 56:902-903 (Abstr.).
7. 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.