Residues of Aldicarb and its Oxides in Beta vulgaris L. and Systemic Control of Heterodera schachtii

ARNOLD E. STEELE[1]

Abstract: Aldicarb residues in foliage of *Beta vulgaris* L. 21 days after transplanting to soil treated with 1–5 μg aldicarb/g soil were proportional to residues in storage roots, but 20 times as great. Initial concentrations of residues in roots 21 days after treatment were proportional to applied rates but declined by 50% when roots were stored 25 days at 24 C. Mean respective concentrations of aldicarb, aldicarb sulfoxide, and aldicarb sulfone were 8.7, 81.6, and 9.8% of the total residues. In separate tests, equivalent concentrations of toxic carbamates in roots resulted in similar levels of control of *Heterodera schachtii*. Systemic levels that completely suppressed development of females and males on sectioned roots were respectively 0.35 and 0.8 μg/g of root tissue. *Key Words:* aldicarb sulfoxide, aldicarb sulfone, chemical assay, bioassay, sugarbeet nematode, beets.

Investigations at this laboratory have shown that aldicarb [2-methyl-2-(methylthio) propionaldehyde-O-(methylcarbamoyl) oxime] and its toxic oxygenated analog, aldicarb sulfoxide [2-methyl-2-(methylsulfinyl)-propionaldehyde-O-(methylcarbamoyl) oxime], act in several ways to control the sugarbeet nematode, *Heterodera schachtii* Schmidt (7, 9). In those tests, aqueous solutions of aldicarb inhibited hatching and emergence of second-stage larvae (L2) but hatching resumed if treated cysts were then transferred to hatching agents. Survival and development of treated L2 were found to be inversely proportional to duration (0–14 days) and concentration, while soil treatments of aldicarb depressed development of *H. schachtii* and the effect was proportional to application rates (9).

Aldicarb systemic within *B. vulgaris* prevents or delays the development of *H. schachtii* on fibrous roots and on storage root slices of plants first grown in soil containing aldicarb (9). However, treating *H. schachtii* L2 with aldicarb sulfone failed to control its development on sugarbeet. Soil treatments with this material had no effect on parasitism of sugarbeet or the development of this nematode on beet root slices. In field studies, aldicarb sulfone at 4 lb ai/A, incorporated as a wettable powder at planting, failed to control *H. schachtii* on sugarbeet (author's unpublished data), whereas equivalent rates of aldicarb applied as Temik 15G gave effective control (8). Those observations suggest that when soil or plants contain high concentrations of the sulfone analog with low concentrations of aldicarb and/or aldicarb sulfoxide, total residues may not be indicative of the level of nematicidal or nemastatic activity. The two tests reported herein were undertaken to establish concentration-effect parameters for aldicarb and its oxygenated analogs by correlating data obtained on systemic components by chemical and biological assay.

MATERIALS AND METHODS

Cultures of *H. schachtii* on sugarbeet root slices (5) used in previous studies of effects of aldicarb and its oxides (7, 9) were also used here.

The first test was designed to compare the total toxic aldicarb residues in foliage and roots of 'Detroit Dark Red' table beet, *B. vulgaris*, with the development of *H. schachtii* cultured on root slices of treated and untreated plants. A twin-shell blender was used to incorporate technical-grade aldicarb of 99% purity (Union Carbide Corporation, South Charleston, West Virginia) in steam-sterilized potting-soil mixture of 1 part sand and 2 parts clay loam soil. Soil was mixed 5 min and added to 15-cm-diam clay pots (about 1500 cm^3 capacity) at 2.3 kg/pot, after which 500 ml water was carefully added to the soil surface of each pot. To induce the formation of new rootlets, foliage and fibrous roots were removed from the 4-6-cm-diam storage roots, which were then transplanted to individual pots. Each treatment (Table 1) was replicated eight times and arranged in a randomized design on greenhouse benches.

Received for publication 30 May 1978.

[1] Nematologist, Nematology Investigations, U. S. Department of Agriculture, Science and Education Administration, Federal Research, P. O. Box 5098, Salinas, California 93915. No endorsements are implied herein.

TABLE 1. Initial concentrations of aldicarb in soil and soil water after wetting.

μg/plant	Soil (μg/g)	Soil water (μg/gml)
First test[a]		
0	0	0[b]
2.34	1.0	4.6
4.65	2.0	9.3
9.30	4.0	18.4
11.62	5.0	23.2
Second test[c]		
0	0[d]	0
0.73	0.33	1.8
1.47	0.67	3.7
2.20	1.00	5.5
2.93	1.33	7.3
3.67	1.67	9.8
4.40	2.00	11.0
5.13	2.33	12.8
5.87	2.67	14.7
6.60	3.00	16.5

[a]Technical-grade aldicarb added to 2.3 kg dry soil, mixed, and 500 ml water drenched on the surface of the potted soil.
[b]Figures given are calculated concentrations in soil water assuming complete solution of aldicarb.
[c]Technical-grade aldicarb dissolved in distilled water and 400 ml of solution drenched on the soil surface of pots containing 2.2 kg of soil.
[d]Figures given are calculated concentrations in soil assuming complete wetting of the soil with aldicarb solution.

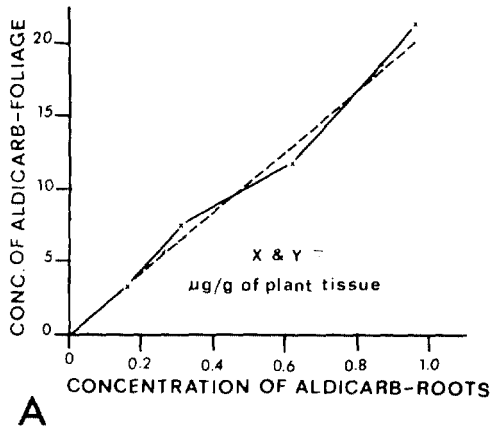
The greenhouse was maintained at 20–26.5 C for 21 days, and the watering regime was regulated to prevent leaching of aldicarb during this phase of the experiment.

To evaluate systemic concentrations of total toxic aldicarb residues and their effects on the development of *H. schachtii*, the beets were removed from soil and washed. After foliage and fibrous roots were removed, the remaining storage root was dipped in 80% ethanol, flamed, and sliced longitudinally to obtain 2 sections of about equal size. One section and the foliage of each plant were placed in separate plastic bags, frozen, and taken to Morse Laboratories, Sacramento, California, for residue analysis by gas-liquid chromatography (GLC). The aldicarb and aldicarb sulfoxide residue fractions were extracted from 100 g of each composited sample, and the toxic carbamates were simultaneously oxidized to aldicarb sulfone with a 1:1 mixture of chloroform and acetone containing peracetic acid by methods described previously (1). An equal amount of storage root sample was extracted and oxidized in a 20% chloroform and 80% acetone solution to which peracetic acid was added. The eluate of each sample was freed from interfering substances by passage over a Florisil column, and analysis was by GLC; peak heights were quantitated by comparison with a standard curve derived by analysis of concentrations of aldicarb sulfone (1). These methods can accurately detect residues of 0.005 μg/g, but 0.01 μg was selected as a practical limit of sensitivity. Duplicate composite samples of foliage were analyzed for residues, whereas single root samples were analyzed.

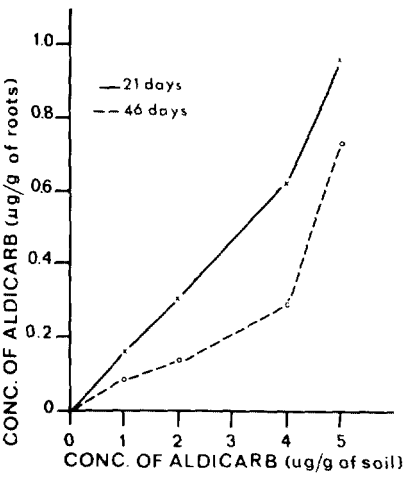
The sliced surface of each root section not immediately processed for residue analysis was inoculated with eggs and larvae of 50 hand-selected cysts previously broken open to remove their contents. The hatch potential of the inoculum was about 150 larvae/cyst. The inoculated root sections were placed in loosely capped crystallizing dishes of 170 cm^3 capacity and incubated in a chamber kept at 100% relative humidity and 24 C. After 25 days, the sections were removed and examined for adult male and female nematodes. Immediately after nematode counts, the sections saved for bioassay were frozen and taken to Morse Laboratories for analysis of total toxic aldicarb residues.

The second test was designed to obtain additional data on the systemic effects of concentrations of aldicarb on nematode development and to determine the effects of aldicarb, aldicarb sulfoxide, and aldicarb sulfone on the nematode. In this test, aqueous solutions of aldicarb, prepared by adding technical aldicarb to distilled water, were drenched into potted soil mix. Table 1 lists the concentrations of aldicarb in soil and soil water at the time the beets were transplanted. Four hundred ml of distilled water or aldicarb solution was added to the soil surface of each 15-cm-diam pot containing 2.2 kg of steam-sterilized dry soil mix, and single beets were immediately transplanted to individual pots. Treatments were replicated 10 times.

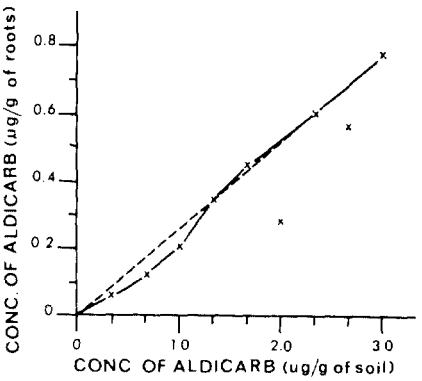
Twenty-one days after transplanting, the storage roots were sliced longitudinally and one section of each plant was inoculated


CONC. OF ALDICARB-FOLIAGE
20
15
10
5
0
X & Y =
µg/g of plant tissue
0
0.2
0.4
0.6
0.8
1.0
CONCENTRATION OF ALDICARB-ROOTS

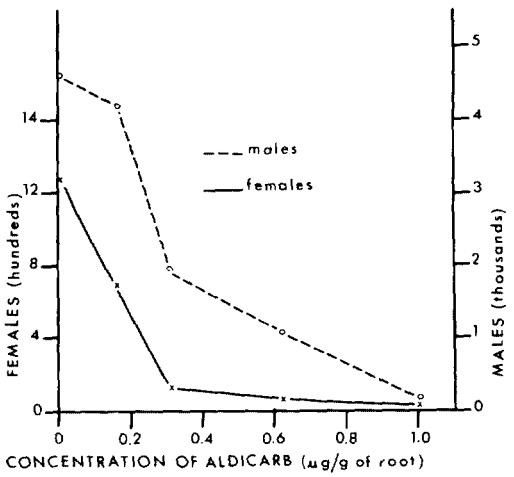
A


CONC. OF ALDICARB (µg/g of roots)
1.0
0.8
0.6
0.4
0.2
0
21 days
46 days
0
1
2
3
4
5
CONC. OF ALDICARB (ug/g of soil)

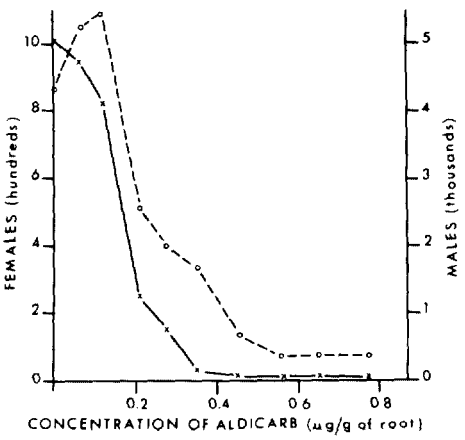
B


CONC. OF ALDICARB (µg/g of roots)
0.8
0.6
0.4
0.2
0
0
1.0
2.0
3.0
CONC OF ALDICARB (ug/g of soil)

C


FEMALES (hundreds)
14
12
8
4
0
MALES (thousands)
5
4
3
2
1
0
males
females
0
0.2
0.4
0.6
0.8
1.0
CONCENTRATION OF ALDICARB (µg/g of root)

D


FEMALES (hundreds)
10
8
6
4
2
0
MALES (thousands)
5
4
3
2
1
0
0.2
0.4
0.6
0.8
CONCENTRATION OF ALDICARB (µg/g of root)

E

with nematodes for bioassay of systemic toxins. The remaining section was immediately frozen and stored for later residue analysis. To obtain residue data on individual oxime components, each component was selectively eluted from a Florisil column and oxidized to sulfone with peracetic acid. An additional Florisil column was used to remove nontoxic oxime and nitrite metabolites. The samples were analyzed by vapor-phase chromatography and quantified by comparison with standard curves. The method employed proved valid over a range of 0.21-21.1 μg/l. Duplicate 50-g samples of root tissue were analyzed for residues. In the second experiment, residue analysis was conducted only on the root sections that were not bioassayed.

Except as detailed, all methods were identical in the first and second test.

RESULTS AND DISCUSSION

In the first experiment, total toxic carbamate residues in foliage of table beet were proportional to residues in root tissues, but about 20 times as great (Fig. 1A). The relationship between residues in roots and foliage suggests that residues in foliage might be used to quantify residues in storage roots. The consistency of the relationship first needs verification, however.

Residue levels of storage roots in the first test (Fig. 1B) were proportional to applied rates except for 5 μg/g. That relation was observed again in the second test, although equivalent application rates gave much higher residues (Fig. 1C). The higher concentration may stem from larger fibrous root systems with greater absorptive capacities; decreased metabolization or degradation of carbamates in roots or soil; or the immediate availability of higher carbamate concentrations for absorption by application of aldicarb solutions, rather than incorporation of technical aldicarb in dry soil.

As expected, residues in root samples were less after bioassay (25 days after inoculation and 46 days after transplanting) than before (Fig. 1B). Concentrations of total toxic residues in storage roots of plants grown in soil treated with aldicarb at 1, 2, or 4 μg/g of soil declined by about 50% during 25 days of incubation at 24 C during bioassay. However, samples obtained before and after bioassay from plants treated with aldicarb at 5 μg/g of soil contained disproportionately higher concentrations of carbamates, suggesting that this highest concentration exceeded levels that can be effectively metabolized.

Except for applied rates of 1.67 and 2.00 μg/g of soil, the proportions of aldicarb, aldicarb sulfoxide, and aldicarb sulfone were similar for application rates greater than 0.67 μg/g (Table 2). Aldicarb sulfoxide amounted to nearly 82% of the total toxic residues.

Equivalent concentrations of total toxic residues in roots evaluated in both tests gave about the same effect on the development of *H. schachtii* cultured *in vitro* on root sections (Figs. 1-D,E). Extrapolation of the curves in Figs. 1-D,E suggests that systemic levels of total toxic residues of about 0.35 μg/g will suppress development to the extent that few or no adult females can be found 25 days after inoculation. In this test, however, two of 26 sections having residues exceeding 0.35 μg/g supported more than 10 adult females; 10 sections had no adult females, and only one adult female was found on each of 5 sections. Not all tissues in storage roots may be equally able to take up or metabolize aldicarb. This, together with continuous upward transport of absorbed materials, may preclude equal

FIG. 1 (A). First test. Relationship of residues (aldicarb, aldicarb sulfoxide, and aldicarb sulfone) in storage roots and foliage of *Beta vulgaris* 21 days after transplanting to treated soil. Data are mean residues of 10 plants at each treatment rate applied. Broken line is the line of best fit ($r = 0.977$).

FIG. 1 (B-C). Influence of selected rates of aldicarb applied to soil on total residues in storage roots of *B. vulgaris* 21 days after transplanting. *B)* First test. Data were obtained 21 and 46 days after transplanting, and are means of 8 plants. Broken line records residues in storage root sections 25 days after harvesting and inoculating with *H. schachtii*. *C)* Second test. Mean residues of duplicate storage root samples composited from 10 plants for each treatment rate. The solid line represents the observed relationship but excludes two coordinates of data that are not reliable estimates of the treatment-residue parameter.

FIG. 1 (D-E). Total numbers of adult male and female *H. schachtii* 25 days after inoculation on root slices of 10 *B. vulgaris* plants as influenced by total aldicarb residues. *D)* First test. *E)* Second test.

TABLE 2. Residues of aldicarb and its sulfoxide and sulfone in storage roots of *Beta vulgaris* 21 days after transplanting to treated soil.

Soil treatment (μg/g)	Total root weight (g)	Aldicarb (μg/g)	Aldicarb sulfoxide (μg/g)	Aldicarb sulfone (μg/g)	Total residues (μg/g)
0	461.9[a]	—0.01[a]	—0.01[a]	—0.01[a]	—0.01[a]
0.33	453.1	—0.01	0.06	—0.01	0.06
0.67	396.9	—0.01	0.11	0.01	0.13
1.00	425.3	0.01	0.17	0.03	0.21
1.33	368.6	0.02	0.30	0.03	0.35
1.67	311.9	0.14	0.29	0.06	0.49
2.00	396.9	—0.01	0.25	0.03	0.28
2.33	425.3	0.04	0.56	0.05	0.65
2.67	368.6	0.06	0.46	0.04	0.56
3.00	340.2	0.10	0.60	0.07	0.77
Mean percent concentration[b]		8.6	81.6	9.8	

[a]Figures given are means of duplicate analysis of composite samples each including 10 root slices.
[b]Computations of means do not include values given for application rate of 0.33 μg/g.

distribution of residues throughout the storage root, thereby providing areas of low biological activity, where nematode development is relatively unimpaired. Until analytical methods are available which will evaluate residues in small cell groups, levels which give predictable biological activity must be viewed in a statistical context.

Although there was a high male-to-female ratio in both treated and untreated root sections (probably characteristic of ratios obtained with root-slice culture), the proportionate decrease in adult males with increasing concentrations of residue was about the same as that obtained for females. Only roots initially having total toxic residue greater than 0.80 μg/g completely suppressed the development of males, and that concentration is nearly equivalent to twice the level required for total suppression of females. However, the apparent differences in response of males and females to similar levels of aldicarb may be in some way related to differences in nutritional uptake. Females feed for longer periods than males, require a continuous supply of food for maximum egg production (2), and have nutritional requirements many times those of males (3, 4). Also, males typically begin to emerge from roots several days before females reach maturity (6). On the other hand, if aldicarb is nemastatic rather than nematicidal, the observed differences in these tests may be due to earlier emergence of males than females in response to declining levels of toxic residues rather than to differential toxicity.

LITERATURE CITED

1. ANDRAWES, N. R., H. F. LYKINS, and R. R. ROMAINE. 1978. The metabolism and associated residues of Temik aldicarb pesticide in sugarbeet plants. J. Agr. Food Chem. (In press).
2. COOK, R. 1978. The relationship between feeding and fecundity of females of Heterodera avenae. Nematologica 23:403-410.
3. JOHNSON, R. N., and D. R. VIGLIERCHIO. 1969. Sugarbeet nematode (Heterodera schachtii) reared on axenic Beta vulgaris root explants. II. Selected environmental and nutritional factors affecting development and sex ratio. Nematologica 15:144-152.
4. SENGBUSCH, R. von. 1927. Beltrag zur biologie des rubennematoden Heterodera schachtii. Z. Pflanzenkrankh. 37:86-102.
5. STEELE, A. E. 1972. Development of Heterodera schachtii on large rooted crop plants and the significance of root debris as substratum for increasing field infestations. J. Nematol. 4:250-256.
6. STEELE, A. E. 1975. Population dynamics of Heterodera schachtii on tomato and sugarbeet. J. Nematol. 7:105-111.
7. STEELE, A. E. 1976. Effects of oxime carbamate nematicides on development of Heterodera schachtii on sugarbeet. J. Nematol. 8:137-141.
8. STEELE, A. E., W. H. HART, and G. W. WHEATLEY. 1977. Results of field tests of nematicides for control of Heterodera schachtii on sugarbeet. Fungicide and Nematicide Tests 32:214. Am. Phytopath. Soc.
9. STEELE, A. E., and L. R. HODGES. 1975. In-vitro and in-vivo effects of aldicarb in survival and development of Heterodera schachtii. J. Nematol. 7:305-312.