Distribution and Persistence of Oxamyl in Relation to Root-Lesion Nematode Control following Seed Treatment of Corn

Mikio Chiba,* G. Julius Fulop,† Brian D. McGarvey, and John W. Potter

Research Station, Agriculture Canada, Vineland Station, Ontario, Canada L0R 2E0

Corn seeds, treated in oxamyl solutions at concentrations from 1 to 64 mg/mL, were grown in soil infested with root-lesion nematodes (*Pratylenchus penetrans*). All concentrations of oxamyl substantially reduced nematode numbers in roots, but concentrations of 16 mg/mL and higher were phytotoxic to plants. Lower concentrations reduced nematode populations for 28 days after sowing. The fate of oxamyl in seeds, soil, root, and foliage was also monitored. Oxamyl dissipated rapidly from the treated seeds after sowing, with only 0.2% of the applied amount found after 28 days. In contrast, high concentrations of oxamyl were found in soil and foliage; dissipation was slower from these sources than from seeds. Oxamyl oxime, a major degradation compound of oxamyl, was found in seeds and subsequently in soil, and *N,N*-dimethyl-1-cyanoformamide, another degradation compound, was found in seeds.

INTRODUCTION

Coating of seeds with nematicide for commercial crop production could be the least expensive and easiest method of nematicide application. Furthermore, it is the least contaminating for the environment. The potential benefit of seed coating with oxamyl has been suggested previously (McGarvey, 1982). An appropriate quantity of oxamyl nematicide, methyl *N,N*-dimethyl-*N*-[(methylcarbamoyl)-oxy]-1-thio-oxamimidate, coated on alfalfa seeds (Townshend and Chiba, 1987), protected plants well from root-lesion [*Pratylenchus penetrans* (Cobb)] and root-knot (*Meloidogyne hapla* Chitwood) nematodes in greenhouse studies and also enhanced plant growth. Applied to seed tubers, oxamyl also protected potatoes (Townshend and Olthof, 1988) against root-lesion nematodes in a greenhouse but not in field microplots (Olthof and Townshend, 1991).

Chiba et al. (1983) have reported quantitative studies of the redistribution of oxamyl after absorption onto peach seeds.

This paper presents studies on the fate and redistribution of oxamyl and its degradation compounds, oxamyl oxime and *N,N*-dimethyl-1-cyanoformamide (DMCF), into roots, foliage, and soil from oxamyl-treated corn seeds. Because corn is one of the most important crops in North America, sweet corn seeds were used in this study. The effects of oxamyl treatment on plant growth and on the control of nematodes were also studied.

MATERIALS AND METHODS

Materials and Apparatus. Analytical grade oxamyl and its oxime were obtained from E. I. du Pont de Nemours and Co. Inc., Wilmington, DE. DMCF was synthesized in-house (McGarvey et al., 1984). The commercial product Vydate L, a 24% formulation of oxamyl (w/v), was obtained from DuPont Canada, Toronto, ON.

Methanol and acetonitrile were of HPLC grade, and acetone and diethyl ether were of reagent grade, all from Caledon Laboratories Ltd., Georgetown, ON.

Nuchar-Attaclay and silane-treated glass wool were from Supelco Inc., Bellefonte, PA. AcroLC-13 syringe-tip filters, 0.45-μm pore size, were from Gelman Sciences Inc., Montreal, PQ.

† Brock University, St. Catharines, ON, Canada L2S 3A1. Present address: Environment Protection Laboratories Inc., Mississauga, ON, Canada L4V 1P1.

HPLC Instruments and Operating Conditions. Two systems were used: (1) a Milton Roy Model 396 minipump (flow rate 1.3 mL/min) with a Spectra Physics Model SP 8200 UV detector (254 nm) for seed, soil, and root samples; (2) a Hewlett-Packard 1090 LC (flow rate 1.0 mL/min) with a diode array UV detector monitoring 220 and 240 nm for seed samples. A Spherisorb ODS-2 column (5 μm, 25 cm × 4.6 mm) preceded by a Brownlee 3-cm RP-18 guard cartridge was used with a mobile phase of acetonitrile and water (15:85 v/v).

Foliage samples were analyzed using a Hewlett-Packard HP-1090 system with a single-stage postcolumn derivatization system. The mobile phase was acetonitrile and 0.0005 M sodium tetraborate in water (1:4 v/v), at a flow rate of 1.0 mL/min, passed through a Hamilton PRP-1 15 cm × 4.1 mm column followed by a 3 m × 0.5 m i.d. knitted Teflon delay tube (Supelco, Oakville, ON) immersed in a glass-bead bath at 130 °C. The derivatizing reagent, 10 mg of *o*-phthalaldehyde and 50 μL of 2-mercaptoethanol in 400 mL of 0.01 M aqueous sodium tetraborate, was delivered at 1.0 mL/min. Fluorescence was measured with a Kratos FS970 detector, at an excitation wavelength of 229 nm and emission wavelength of 418 nm.

Seed Coating. Five hundred milliliters each of aqueous solutions containing oxamyl at 0, 1, 2, 4, 8, 16, 32, and 64 mg/mL were prepared by diluting Vydate L commercial formulation with water. Forty-gram portions of dry corn seeds (Stokes Hybrid Seneca Chief UT125, Stokes Seed Co., St. Catharines, ON) were added to each solution and kept at room temperature (24 ± 1 °C) for 18 h. Treated seeds were centrifuged with dry sand at the bottom of the centrifuge bottle to absorb liquid. The seeds were spread over paper towels and air-dried for 72 h.

Plant Growth and Nematode Population. Delhi loamy sand (84% sand, 11% silt, 5% clay, 1.2% O.M.; pH 6.2) infested with root-lesion nematodes and air-dried Delhi loamy sand were used; the nematode population level in the soil was adjusted by mixing as required. Two experiments were conducted.

In one experiment, the effect of treating seed with an aqueous solution of oxamyl at 4 mg/mL was compared at two population levels of root-lesion nematode. Treated and control corn seeds, 20 seeds per 15-cm-diameter pot, were grown in 48 pots filled with 1.2 kg of soil containing 1.2×10^3 root-lesion nematodes and also in 20 pots containing air-dried soil. All pots were kept in a controlled growth room at 22 °C, 50% humidity, and 1500 ft-c of light intensity. The moisture level of the soil was maintained carefully by daily watering. For residue analysis, four replicate pots were randomly sampled 0, 1, 3, 7, 14, 21, and 28 days after sowing; plants were divided into seed, foliage, and root, and adhering soil was removed with a small paintbrush. Samples were weighed and kept frozen at −15 °C until extracted.

To assess nematode populations, five replicate pots per treatment were randomly sampled 7, 14, 21, and 28 days after sowing. A 50-g sample of soil was collected randomly from each

0021-8561/93/1441-2160$04.00/0 Published 1993 by the American Chemical Society

pot, and root-lesion nematodes were extracted by the modified Baerman pan method (Townshend, 1963) for 7 days. The fresh weights of tops and of roots from the combined 20 plants per pot were determined, and nematodes were extracted from the root mass by mist extraction (Ayoub, 1980) at 24 ± 1 °C for 14 days.

In another experiment, seeds were soaked in oxamyl solutions at concentrations of 0, 1, 2, 4, 8, 16, 32, and 64 mg/mL. Three replicate pots per concentration were seeded with 20 seeds in 1.2 kg of soil containing approximately 600 root-lesion nematodes per pot. The pots were kept in the growth room as previously, and all pots were sampled 10 days after sowing. Total numbers of seedlings were counted, and foliage and roots were weighed separately. Nematodes were extracted from a 50-g soil sample and from the total root per pot 10 days after sowing.

Extraction Procedures. (*1*) *Seeds.* Seeds were homogenized for 2 min with 140 mL of methanol using a Polytron homogenizer. The sample mixture was shaken for 30 min on a wrist-action shaker and filtered through a Whatman No. 1 filter paper. The filtrate volume was made up to 200 mL with methanol and stored at 4 °C until HPLC analysis. Because extraction of oxamyl from dry unplanted seeds was very inefficient, yielding only 65% of the total amount of oxamyl from a single extraction of wet seeds, dry seeds were soaked in 4 mL of water overnight and then extracted.

(*2*) *Soil.* One hundred grams of moist soil was extracted with 100 mL of methanol by tumbling for 1 h in a 900-mL Mason jar. The mixture was vacuum-filtered through a Whatman No. 1 filter paper, rinsing the flask and filter bed with 3 × 40 mL of methanol. The combined filtrates were made up to volume in a 250-mL volumetric flask and stored at 4 °C until HPLC analysis. The moisture content of soil was determined separately, and the concentrations of analytes were calculated on dry weight of soil.

(*3*) *Root.* Roots (5 g) in a 250-mL wide-neck Erlenmeyer flask were homogenized in 140 mL of methanol using a Polytron homogenizer for 45 s at speed 7. The homogenate was filtered and rinsed with 3 × 35 mL of methanol, and the combined extracts were analyzed as above.

(*4*) *Foliage.* Foliage was cut into small pieces and homogenized in 140 mL of methanol using a Polytron homogenizer. The homogenized mixture was allowed to stand with occasional swirling for 1 h and then filtered and analyzed as above.

Cleanup. The method of McGarvey et al. (1986) was modified as follows. The cleanup column, 0.2 g of Nuchar-Attaclay in a disposable pipet on top of a silanized glass wool plug, was washed with 2 mL of methanol, forcing elution with low air pressure without allowing the Nuchar layer to dry. A 2-mL aliquot of sample extract was added, and the eluate was collected in a 15-mL graduated centrifuge tube. The column was rinsed with 2 mL of methanol, and the combined eluate was added to the centrifuge tube. Methanol was evaporated just to dryness with swirling on a Vortex-Genie mixer under reduced pressure and made up to 2 mL with water for HPLC analysis.

Recovery Tests. Recovery tests were done by adding oxamyl to triplicate samples of soil, root, and foliage at a rate of 5 μg/g. Recoveries of oxamyl from the air-dried and nematode-infested soils were 86.0 ± 2.7% and 82.0 ± 1.8%, respectively. Recoveries from root and foliage were 93.0 ± 4.3% and 91.9 ± 5.2%, respectively.

RESULTS

Decline of Oxamyl Concentrations in Seeds after Sowing. Amounts of oxamyl in seeds steadily declined (Figure 1). The regression coefficient after log transformation of oxamyl concentration was $r^2 = 0.98$, indicating a constant decline at the first-order rate. Quantities of oxamyl oxime also declined, though more slowly (Figure 1). DMCF was also found in seeds; its concentration increased during the first week and then declined (Figure 1).

Distribution of Oxamyl to Soil, Root, and Foliage (Top Part of Plants). The oxamyl concentration in soil increased to 2.27 μg/g 3 days after sowing but slowly decreased thereafter (Figure 2). The concentration at 28 days after sowing was 1.1 mg/g, less than half of that present

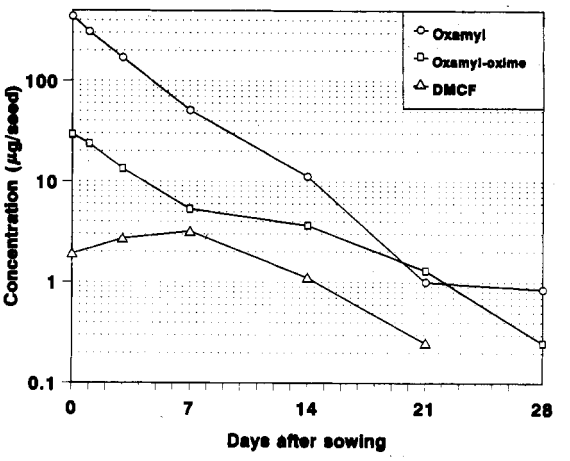

Figure 1. Changes in concentration of oxamyl, oxamyl oxime, and DMCF in corn seeds during 28 days after sowing.

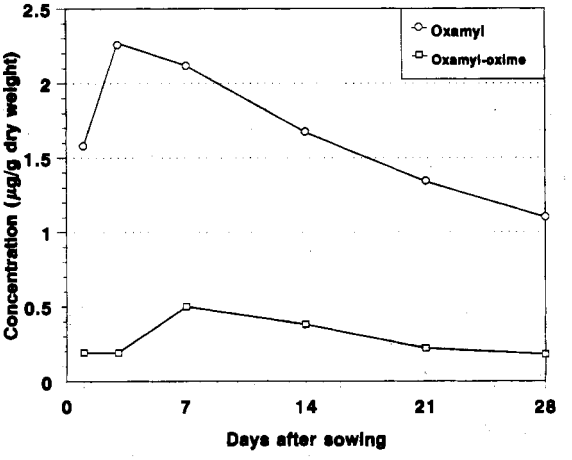

Figure 2. Changes in concentration of oxamyl and oxamyl oxime in soil by redistribution from seeds during 28 days after sowing.

Table I. Amounts and Concentrations of Oxamyl in Corn Roots and Foliage, Respectively, in Plants Grown from Oxamyl-Treated Seeds[a]

days after sowing	in root, μg/plant	in foliage, μg/g of foliage
7	0.6[b] (73.1)[c]	–[d]
14	1.2 (59.2)	24.1 (37.5)
21	1.2 (23.6)	14.6 (26.4)
28	1.0 (61.2)	9.7 (31.8)

[a] Treated in 4 mg/mL oxamyl solution. [b] Average of 4 replicate samples, each sample consisting of 20 roots. Total quantities found in 20 roots were divided by 20. [c] Coefficient of variation (%). [d] No leaves were grown yet.

3 weeks earlier. From day 3 when oxamyl concentrations in soil reached their peak, the linear regression of oxamyl concentration with time was highly significant ($r^2 = 0.99$). The concentration of oxamyl oxime increased more slowly to a maximum of 0.50 μg/g 7 days after sowing and gradually decreased thereafter.

Oxamyl was also found in roots at a concentration of 0.6 μg/plant at 7 days after sowing (Table I). Subsequently, the total quantity of oxamyl found in roots remained fairly constant at 1.0–1.2 μg/plant up to 28 days.

The concentrations of oxamyl in foliage declined slowly from 24.1 μg/g at 14 days to 9.7 μg/g at 28 days (Table I).

Nematode Control by Oxamyl. The effect of oxamyl seed treatment (4 mg/mL in treatment solution) on nematode populations up to 28 days is summarized in Table II. Nematode attack was substantially reduced by oxamyl. The treatments with oxamyl showed significant ($P = 0.05$) nematode reduction by 7 days; the mean number

Table II. Nematode Populations and Plant Growth (Weight of Root and Top) in the Air-Dried and Nematode-Infested Soils in Relation to Time after Sowing Oxamyl-Treated[a] or Untreated Corn Seeds

days after sowing	soil	oxamyl	nematode population[b] per g of root	nematode population[b] total per pot	plant growth root wt, g	plant growth top wt, g
7	+[c]	–[d]	26 a[e]	1240 a[e]	4.3 ab[e]	3.8 b[e]
	+	+	3 b	263 b	2.9 c	2.3 c
	–	–	4 b	1204 a	4.9 a	5.1 a
	–	+	1 c	73 c	4.1 b	3.4 b
14	+	–	108 a	890 a	5.2 b	8.7 ab
	+	+	7 c	190 b	4.9 b	6.6 b
	–	–	23 b	616 a	6.3 a	9.3 a
	–	+	4 c	50 c	5.2 b	7.9 ab
21	+	–	181 a	874 a	5.5 b	14.4 b
	+	+	8 bc	179 b	4.7 b	10.6 c
	–	–	47 b	840 a	7.8 a	17.5 a
	–	+	6 c	151 b	5.6 b	13.2 bc
28	+	–	143 a	1120 a	5.4 a	13.4 a
	+	+	10 c	257 ab	6.3 a	12.8 a
	–	–	22 b	750 a	7.7 a	13.4 a
	–	+	5 c	179 b	5.5 a	10.6 a

[a] Treated in a 4 mg/mL oxamyl solution. [b] Nematode counts transformed by $x = \log(x + 1)$ for analysis of variance. [c] "+" indicates nematode-infested soil was used for this treatment; "–" indicates air-dried soil was used. A low popuation of lesion nematodes persisted in the air-dried soil at initiation of the experiment. [d] "+" indicates oxamyl-treated seeds (in 4 mg/mL solution) were used; "–" indicates seeds were not oxamyl-treated. [e] Column means (within a week) followed by the same letter are not significantly different by LSD ($P = 0.05$).

Table III. Populations of Nematodes in Root and Soil of Plants, Grown 10 Days after Sowing Oxamyl-Treated Seeds in Nematode-Infested Soil

concn of oxamyl in seed treatment solution, mg/mL	population of nematodes in root/pot	population of nematodes in soil/pot	population of nematodes total no./pot
0	386 a[a]	191 a[a]	577 a[b]
1	25 b	251 a	276 bc
2	6 b	164 a	170 c
4	6 b	216 a	222 bc
8	1 b	320 a	321 bc
16	4 b	329 a	333 bc
32	1 b	199 a	200 bc
64	1 b	363 a	364 b

[a] Column means followed by the same letter are not significantly different ($P = 0.05$); data transformed by $\log(x + 1)$. [b] Column means followed by the same letter are not significantly different by LSD ($P = 0.05$).

of root-inhabiting nematodes at 7 days was significantly ($P = 0.05$) lower than those at the later three dates, which did not differ among themselves.

Top weights and root weights were not influenced by interactions of treatment factors but responded to main treatments (oxamyl, nematode, time). Greatest top and root weights were found from the check (low nematodes, no oxamyl) samples at each sampling date. Top and root weights were significantly lower in the presence of oxamyl and were lower in the early weeks compared with the later weeks.

In the second experiment, which was harvested after 10 days, the nematode population in roots was sharply reduced by the whole range of oxamyl concentrations (Table III). No significant linear regression was found; rather, the "0" oxamyl treatment was separated by ANOVA and 5% LSD from the rest, which were not significantly different. Similarly, the total nematode count per pot differed essentially between the presence and absence of oxamyl.

Phytotoxicity. The various concentrations of oxamyl were inversely correlated with plant root weight ($r^2 = 0.99$) and top weight ($r^2 = 0.90$), both in the presence ($r^2 = 0.98$) and in the absence ($r^2 = 0.96$) of nematodes (Table IV).

Table IV. Number of Seedlings and Weights of Root and Plant Top 10 Days after Sowing in Air-Dried and Nematode-Infested Soils, from 20 Seeds Treated in Vydate Solutions of Various Concentrations

concn of oxamyl in seed treatment solution, mg/mL	av no. of seedlings in a pot –N[a]	av no. of seedlings in a pot +N[a]	root wt, g –N	root wt, g +N	top wt, g –N	top wt, g +N
0	20.0 a[b]	19.7 a	9.7 a	7.8 a	14.4 a	9.2 a
1	19.7 a	19.3 a	10.2 a	7.7 a	14.0 ab	8.3 abc
2	19.7 a	19.7 a	9.5 a	7.9 a	11.7 c	8.6 ab
4	19.7 a	20.0 a	9.9 a	7.2 a	12.9 bc	7.9 bc
8	19.7 a	19.7 a	9.6 a	6.9 ab	13.4 ab	7.5 bc
16	19.7 a	19.3 b	10.2 a	5.9 b	11.7 c	7.1 c
32	18.3 b	17.7 b	7.2 b	4.0 c	8.2 d	4.9 d
64	3.0 c	3.0 c	0.8 c	0.2 d	0.4 e	0.2 e

[a] "–N" indicates air-dried (absence of nematode) soil used; "+N" indicates nematode-infested soil used. [b] Column values followed by the same letter are not significantly different by LSD ($P = 0.05$).

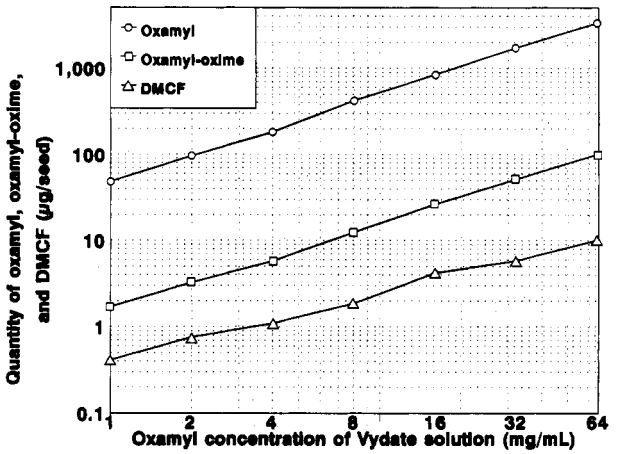

Figure 3. Quantity of oxamyl in corn seeds following seed treatment with various Vydate solutions.

Quantities (micrograms per seed) of oxamyl in corn seeds treated with oxamyl solutions of various concentrations and used for this study are shown in Figure 3. Treatment solution concentrations and resultant seed concentrations were positively correlated, linear, and highly significant, being $r^2 = 0.99$, 0.99, and 0.98 for oxamyl, oxamyl oxime, and DMCF, respectively.

The threshold of phytotoxicity on seeds is about 400–800 μg of oxamyl/seed, attained from Vydate solutions containing 8–16 mg of oxamyl/mL.

DISCUSSION

Distribution of Oxamyl from Seeds to Root, Foliage, and Soil. Oxamyl was translocated from seeds to soil, root, and foliage rapidly. As oxamyl in seeds was dissipated, concentrations of oxamyl in the soil increased. The rate of dissipation of oxamyl was substantially slower in soil than on seeds. This result and the reduction in nematode population in roots (Tables II and III) indicate that there is potentially good protection of roots from nematode attack. This is important particularly during the early stage of plant growth when the plant is most susceptible to nematodes.

Oxamyl was found in foliage also up to 24.1 μg/g (Table I); this demonstrates the systemic movement of oxamyl from seeds to foliage of Gramineae. This suggests the possibility that seed treatment of oxamyl may contribute to protection of foliage from insect attack.

During the course of this study, DMCF was found in seeds and oxamyl oxime in seeds and soil (Figures 1 and 2). However, since the nematocidal or nematostatic activity of oxamyl oxime is nil, and that of DMCF is weak compared to that of oxamyl (McGarvey et al., 1984), DMCF does not likely contribute to protecting plants from nematodes with the low concentration found in this study.

Nematode-Related Effects. At a high concentration of oxamyl (64 mg/mL), which causes phytotoxicity (Table IV), root growth is too slight to permit much nematode reproduction. Consequently, most of the nematodes remain in the soil (Table III). At low concentrations or in the absence of oxamyl (Table III), the nematode can invade roots and multiply, and this activity may also reduce plant growth. At oxamyl concentrations near but just below the phytotoxicity threshold, plant growth is not affected, but roots are protected from the root-lesion nematode attack (Tables II and IV).

Oxamyl can thus control lesion nematodes on corn when each seed contains 400–800 μg of oxamyl. This seed treatment approach has potential as an economical, easy, and efficient method of application of oxamyl for nematode control. Because oxamyl is highly toxic by ingestion [LD_{50} to rats 12 mg/kg of body wt (Spencer, 1981)] but is not readily absorbed through skin (LD_{50} 2960 mg/kg of 24% commercial formulation on rabbits), the coated seed may be dangerous mainly for bird ingestion (LD_{50} 4.18 mg/kg) for about 3–5 days after seeding.

ACKNOWLEDGMENT

Sincere appreciation is extended to L. Wainman for excellent technical assistance.

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Received for review June 8, 1993. Accepted September 7, 1993.®

® Abstract published in *Advance ACS Abstracts,* October 15, 1993.