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Control of *Pratylenchus penetrans* and *Meloidogyne hapla* and Yield Response of Alfalfa Due to Oxamyl Seed Treatments

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Abstract: Alfalfa (*Medicago sativa* L. cv. Saranac) seed were soaked for 20 minutes in water, acetone, or methanol containing 10 or 50 mg/ml of oxamyl (Vydate L) or coated with a 2% aqueous cellulose solution containing the same amounts of oxamyl. Seed were analyzed for oxamyl by HPLC immediately after treatment and after 9 and 26 months of storage. Oxamyl content of alfalfa seed did not decline after 26 months of storage. The effects of seed treatment on growth of alfalfa and nematode control were examined using soils infested with *Pratylenchus penetrans* and *Meloidogyne hapla*. Germination was not affected by any of the seed treatments. Twenty-one days after sowing, the total growth of alfalfa seedlings grown from seed treated with 50 mg/ml of oxamyl in *P. penetrans*-infested soils had increased by 62% over controls. Nodulation per pot increased by as much as 267%, and the densities of *P. penetrans* per gram of root were reduced by as much as 73% compared to control plants. In *M. hapla*-infested soils, increases in plant growth (32%) and nodulation (71%) also occurred with oxamyl-treated seeds. Root gall reduction (86%) was also substantial due to oxamyl seed treatment.

Key words: HPLC analysis, *Medicago sativa*, alfalfa, *Meloidogyne hapla*, northern root-knot nematode, oxamyl, Vydate, *Pratylenchus penetrans*, root-lesion nematode, seed treatment.

A number of plant-parasitic nematodes are associated with forages in eastern Canada (10,12). The root-lesion nematode, *Pratylenchus penetrans* Cobb and the northern root-knot nematode, *Meloidogyne hapla* Chitwood are two of the more destructive nematodes on legume forage crops (7,11). Seedling stand, plant weight, and long-term survival are reduced by *M. hapla* and the pin nematode, *Paratylenchus projectus* Jenkins (9). Fumigation is economically impractical because of its high cost. Also, each component of a forage mixture or of a cereal rotation is a preferred host of one of the associated nematodes (6). Consequently, the rotation crops and (or) forage components support all nematodes commonly associated with forages.

In Australia, oxamyl, *N′,N′*-dimethyl-2-methyl-carbamoyloxyimino-2-(methylthio) acetamide, is applied to wheat seed as a dressing for protection against the cereal cyst nematode, *Heterodera avenae* (1). Thus seed treatment with a nematicide that is more target oriented appeared to be a practical means of control for *P. penetrans* and *M. hapla* on alfalfa. Further, a preliminary study indicated that infection of alfalfa seedlings by *P. penetrans* was reduced when seed were treated with oxamyl (8).

This paper reports the effects of oxamyl seed treatment on nematode control and growth of alfalfa in *P. penetrans*- and *M. hapla*-infested soils sown with oxamyl-treated seed.

Materials and Methods

Seed treatments and analysis: Three lots of 50 g alfalfa (*Medicago sativa* L. cv. Saranac) seed were soaked for 20 minutes in 200 ml water, acetone, or methanol each containing 10 or 50 mg/ml oxamyl. Oxamyl for seed treatment was prepared from Vydate L, 24% a.i. The solutions were decanted after soaking, and the seed placed between two fine mesh sieves (15.5 cm d, 850-μm opening). The seed were dried initially with a stream of air until the seed tumbled freely and then dried further overnight on sheets of paper towelling. A fourth lot of seed was placed in a tumbler, and 5 ml of 2% aqueous cellulose solution (Hercules cellulose gum Type 7MP) containing 10 or 50 mg/ml oxamyl was slowly applied to the seed with a pipette as the tumbler was rotated for 2–3 minutes. The coated seed were then dried on a screened

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trough in which a hair drier was inserted. The coating procedure was repeated immediately after the first application. Comparable lots of control seed (without oxamyl) were prepared with individual solvents and cellulose. A fifth lot of seed was the untreated control. In all, 13 lots of seed were prepared. A portion of this seed was used immediately, and the remainder was stored in paper cups at room temp (25 C) for up to 26 months.

Seed (10 g) were soaked in 20 ml methanol in a 50-ml volumetric flask; the flask was agitated on a wrist action shaker for 30 minutes. The methanol solution was decanted and kept for HPLC analysis, as described subsequently. The rinsing procedure was repeated three more times with fresh methanol to complete the surface rinsing. The methanol solutions were combined, diluted with water, and analyzed by HPLC. Analyses were conducted immediately after treatment, and 9 and 26 months after the oxamyl treatment of seed.

The seed stored for 9 and 26 months were rinsed in methanol as previously described, then treated in a Polytron homogenizer (Brinkmann Instruments Co.) in 50 ml methanol for 2 minutes to complete the extraction of oxamyl. The extract obtained from the homogenate by sintered glass filtration was diluted with water and analyzed by HPLC.

A Spectra-Physics Model SP-8000 HPLC equipped with Specra-Physics autoinjector and Spectra-Physics SP-8300 UV-vis fixed wavelength detector (at 254 nm) was used for final analysis. The analytical column used was a Regis ODS column, 5 μm, 4.6 mm (i.d.) × 15 cm. The mobile phase used was 25% methanol in water; the flow rate was 1.0–1.2 ml/minute, and temperature was kept at 30 C.

Nematode control and plant growth: Pratylenchus penetrans and *M. hapla* used in this study were collected from the Niagara Peninsula in southern Ontario. The former nematode was increased on sweet corn (*Zea mays* L. cv. Earlivee) in a greenhouse groundbed, and the latter was reared on celery (*Apium graveolens* Pers. cv. Utah 15) in large plastic tubs (46 × 46 × 27 cm). Both hosts were grown in Vineland silt loam. Infested soils were diluted to the desired inoculum density with steam-sterilized Vineland silt loam which had been exposed to the air for 3–4 weeks to develop a microflora prior to use.

Soils infested with *Pratylenchus penetrans* and *M. hapla* were sown with seed lots in a growth room held at 17 C (day) and 14 C (night), and with a light intensity of 11,000 lux during the 16-hour day. Styrofoam pots (11 cm d × 7.5 cm high) with 8-mm drainage holes were aerated for 2 weeks to dissipate any possible toxic substance. Each pot was filled with 425 ml soil infested with either *M. hapla* or *P. penetrans* (8,550/pot); 20 seed sites (8 mm deep) were impressed into the soil with a multi-point dibble, and a single alfalfa seed was placed in each site. A commercial preparation of the nodulating bacterium, *Rhizobium meliloti* Rangeard, was dusted along the seed rows which were filled with 30 ml infested soil. A randomized block design was used with five replications. Seedling emergence was evaluated at 3-day intervals. Plants were harvested 21 days after sowing, tops and roots were weighed, and nodules and *M. hapla* galls were counted. *Pratylenchus penetrans* was extracted from roots by the Baermann pan method (4) at 21 days after planting and counted. All data were subjected to a factorial analysis of variance and LSD were determined at $P = 0.05$. Growth experiments were carried out 1 week after the treatment of seed and repeated after the seed had been stored over 18 months.

Results

Similar results were obtained whether the alfalfa seed were used for experimentation 1 week or 18 months after treatment, but the results from the 1-week experiments were incomplete. Therefore, the results reported in this paper are those from experiments using nematode-infested soil planted with seed stored for 18 months.

M. hapla: An analysis of variance re-

TABLE 1. Control of *Meloidogyne hapla* and growth of Saranac alfalfa seedlings as affected by oxamyl-treated seed sown in infested soil at 21 days.

	Concentration of oxamyl (mg/ml)			
	0	10	50	$LSD_{5\%}$
Seedlings/pot	19	19	18	1.0
Tops, g/pot	1.49	1.75	1.90	0.13
Roots, g/pot	1.27	1.51	1.75	0.14
Total, g/pot	2.76	3.26	3.65	0.18
Nodules/pot of roots	82	98	140	11.3
Nodules/g root	65	65	82	9.1
Galls/pot of roots	145	57	33	9.3
Galls/g root	145	39	20	8.1

vealed no differences among the five controls (four application method controls and the untreated control) in each of the eight parameters measured. For example, total fresh plant weight ranged from 2.75 g to 2.84 g, nodules per pot from 78 to 89, and root-knot nematode galls per pot from 123 to 147.

In the factorial analyses, excluding the untreated control, the partition of the treatment sum of squares revealed that concentration of oxamyl applied to the seed had a significant effect at the 5% level on the degree of nematode galling and alfalfa growth and accounted for an average of 70% of the total treatment variation. Methods of application did not have significant effects on plant growth or nematode galling. At 3 weeks, plant stands were 18–19 seedlings/pot; fresh top weights, 1.68–1.78 g/pot; fresh root weights, 1.50–1.52 g/pot; total weights 3.19–3.31 g/pot; nodules, 93–116/pot of roots; nodules, 63–77/g of root; *M. hapla* root-knot galls, 72–87/pot; and root-knot galls, 64–73/g of root.

The concentrations of oxamyl applied to the seed revealed significant differences in seven of the eight parameters measured at the 5% level (Table 1). There were no differences in seedling stand at 3 weeks. Top, root, and total fresh weight of seedlings per pot increased as the concentration of oxamyl applied to the seed increased. Also the numbers of nodules per pot and nodules per gram of root increased as the concentration of oxamyl applied to seed increased. There was one exception—the number of nodules per gram of root on seedlings from seed treated with oxamyl at 10 mg/ml was the same as the control. The number of root-knot galls per pot of roots and per gram of root declined dramatically as the concentration of oxamyl applied to the seed increased.

P. penetrans: No significant differences were found among the five controls in each of the eight parameters when the control data were subjected to a simple analysis of variance. For example, total fresh plant weight ranged from 1.97 g to 2.11 g, and *P. penetrans* per pot of roots from 1,240 to 1,600.

In the factorial analyses, again excluding the untreated control, the partition of the treatment sum of squares also revealed that concentration of oxamyl had a significant effect at the 5% level on plant growth and nematode control and accounted for an average of 69% of the total treatment variation. The method of application did not effect plant growth or nematode control. At 3 weeks after planting, stands were 18–19 seedlings/pot; fresh top weights, 1.42–1.61 g/pot; fresh root weights, 1.18–1.29 g/pot; total weights, 2.66–2.91 g/pot; nodules, 115–169/pot of roots; nodules, 94–135/g of root; *P. penetrans,* 1,040–1,200/pot of roots; and *P. penetrans,* 1,010–1,280/g of root.

Concentration of oxamyl applied to seed showed significant differences in seven of the eight parameters measured at the 5% level (Table 2). Seedling stand was not dif-

TABLE 2. Control of *Pratylenchus penetrans* and growth of Saranac alfalfa seedlings as affected by oxamyl-treated seed sown in infested soil at 21 days.

	Concentration of oxamyl (mg/ml)			
	0	10	50	$LSD_{5\%}$
Seedlings/pot	19	19	19	0.9
Tops, g/pot	1.25	1.62	1.77	0.15
Roots, g/pot	0.79	1.39	1.53	0.19
Total, g/pot	2.04	3.02	3.31	0.26
Nodules/pot of roots	61	140	224	28
Nodules/g root	72	105	164	30
P. penetrans/pot of roots	1,450	1,230	684	189
P. penetrans/g root	1,840	1,000	490	290

ferent at 3 weeks. Top, root, and total fresh weight of seedlings increased as concentration of oxamyl on seed increased. The numbers of nodules per pot of roots, and densities of nodules per gram of root increased as the concentration of oxamyl applied to seed increased. The number of *P. penetrans* per pot of roots and densities of *P. penetrans* per gram of root declined as the concentration of oxamyl applied to the seed increased.

When oxamyl was applied to seed in acetone, methanol, or 2% aqueous cellulose solution, the quantity of oxamyl extracted by rinsing was stable up to 26 months after seed treatment and most of the residual oxamyl was recovered by rinsing (Table 3). In contrast, when seed were soaked in water containing oxamyl, the amounts of oxamyl recovered by rinsing 9 and 26 months after seed treatment were substantially lower than that found immediately following seed treatment. High quantities of oxamyl, however, were found in extracts of the homogenized seed.

DISCUSSION

Germination was not affected by any of the seed treatments nor by the oxamyl dissolved in the solvents even when the relatively high concentration of 50 mg/ml oxamyl was used. Total plant growth increased by 32% and 62% above the control when seed were treated with oxamyl at 50 mg/ml and planted in soil infested with *M. hapla* and *P. penetrans*, respectively. Nodulation per pot also increased 71% in *M. hapla*-infested soil and 267% in *P. penetrans*-infested soil, compared with the control, when planted with seed treated with the high concentration of oxamyl. Increased nodulation was not merely a reflection of increased root growth but an

TABLE 3. Oxamyl concentrations (mg/g) in or on alfalfa seed immediately after oxamyl treatment, and 9 and 26 months later.

		Oxamyl (mg/g seed)				
		Immediately	After 9 months		After 26 months	
Solution	Oxamyl treatments (mg/ml)	Surface rinsing	Surface rinsing	Grinding	Surface rinsing	Grinding
Water†	10	1.85	0.71	2.45	0.81	1.13
	50	11.13	7.92	7.10	8.64	4.82
Acetone	10	1.30	1.29	0.03	1.24	0.04
	50	5.17	4.81	0.20	5.03	0.20
Methanol	10	1.06	1.02	0.04	0.95	0.03
	50	5.97	5.77	0.22	5.99	0.23
Cellulose	10	0.66	0.51	0.81	0.59	0.48
	50	8.12	7.85	0.66	7.67	0.38

† No oxamyl was found in untreated seed or in those treated with the solvent alone.

increase in terms of nodules per gram of root. This phenomenon has been observed in other studies with alfalfa (5).

The densities of *M. hapla* root-knot galls per gram of root and *P. penetrans* per gram of root of alfalfa seedlings were reduced by 86% and 73%, respectively, when infested soils were planted with seed treated at concentrations of 50 mg/ml. Oxamyl in or on alfalfa seed provides an acceptable degree of nematode control that enables alfalfa seedlings to become better established in the first 21 days.

In these experiments, oxamyl was applied at relatively high rates to alfalfa by soaking seed in appropriate solvents or by coating without causing phytotoxicity. In an earlier study, however, at 50 mg/ml oxamyl was phytotoxic when applied as a seed treatment to wheat, rye, oats, and ryegrass; germination and plant height were reduced (2,3). Townshend and Potter (8) found toxicity at 16 mg/ml when alfalfa seed were soaked for 17 hours in water. These studies indicate that phytotoxicity is related to duration of treatment, oxamyl concentration, and species of plant.

Degradation of oxamyl or reduction in efficacy did not occur after storage of oxamyl-treated seed up to 18 months. In addition, oxamyl was stable even after 26 months of storage. Furthermore, the data indicate that oxamyl when applied in water was absorbed into the seed during the first 9 months of storage.

The evidence presented suggests that the use of oxamyl-treated alfalfa seed is an effective method for control of *M. hapla* and *P. penetrans* on alfalfa seedlings. In addition, plants from oxamyl-treated seed grow better as the result of early nematode control.

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