Seed Treatment as a Means of Preventing Nematode Damage to Crop Plants

B. TRUELOVE, R. RODRIGUEZ-KABANA, and PEGGY S. KING[1]

Abstract: A procedure for treating crop seeds with aqueous solutions of the systemic nematicide oxamyl is described. Seedlings from treated seeds were more resistant to attack by parasitic nematodes. Leachate from treated seeds reduced the number of free-living nematodes in the surrounding soil. *Key Words:* control, oxamyl, bean, corn, cucumber, soybean, watermelon, *Helicotylenchus dihystera, Meloidogyne incognita, Meloidogyne arenaria, Paratrichodorus christiei, Rotylenchus* sp.

For some years, scientists have been investigating the possibility of introducing chemicals (including pesticides) dissolved in organic solvents, such as acetone or dichloromethane, into seeds (2, 3, 5, 6, 8). Introduction of chemicals into seeds in aqueous solutions has, however, generally been considered impractical (1). One reason is that the germination percentage for many seeds, particularly certain legumes, is drastically reduced after prolonged soaking in water. The cause of this sensitivity to soaking is unclear. It may be related to structural and biochemical changes and/or the development of anaerobic conditions within the seeds. However, the germinability of water-soaked seeds can often be restored by drying (4).

This paper describes a technique (patent pending) for introducing chemicals in aqueous solutions into seeds with the aim of conferring protection against nematodes on plants grown from the seeds. The method was developed by using the systemic nematicide oxamyl; however, any nonphytotoxic, systemic material which is water soluble or which can be formulated as a stable, aqueous suspension might be used in a similar manner.

MATERIALS AND METHODS

Seed treatment: The seeds used were: bean (*Phaseolus vulgaris* L. 'Black Valentine'), watermelon (*Citrullus vulgaris* L. 'Charleston Gray'), soybean (*Glycine max* (L.) Merr. 'Lee 58'), cucumber (*Cucumis sativus* L. 'Ashley'), and corn (*Zea mays* L. 'Pioneer 3369A').

In preliminary experiments, the length of time that seeds of each species could be soaked in water, and thereafter dried, without appreciably affecting either germination or seedling growth was determined. From a knowledge of the volume of water imbibed/gram of seed during a noninjurious soaking period, oxamyl solutions in water were prepared to introduce varying concentrations of the nematicide into seeds during a precisely timed soaking period.

For treatment, equal weights of seeds were added to a series of beakers containing an excess of each of the oxamyl solutions. The oxamyl concentration ranges used were: bean and watermelon, 0 to 10 gm/100 ml solution; cucumber, corn, and soybean, 0 to 24 gm/100 ml solution. The contents of the beakers were stirred gently several times during the imbibition period. The

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[1] Professors, and Laboratory Technician, respectively, Auburn University Agricultural Experiment Station, Department of Botany and Microbiology, Auburn, AL 36830. We thank W. H. Hatley and E. G. Ingram for excellent technical assistance. This work was supported in part by the Auburn University Grant-in-Aid of Research Program.

imbibition periods used were: soybean, 15 min; cucumber, 1 h; corn and watermelon, 3 h; and bean, either 3 or 6 h. Immediately upon completion of the imbibition period, the seeds were transferred to a sieve (2-mm mesh) and thoroughly but rapidly rinsed with running water to remove excess oxamyl solution. The imbibed and washed seeds were spread out on paper toweling and placed in a circulating air incubator (40 ± 2 C). The seeds were turned at intervals and left until dry. The drying period varied from 24 h, for the smaller seeded species such as soybean, to 48 h, for the larger seeded species such as bean. The dried seeds, which were indistinguishable from nontreated seeds, were stored in paper sacks at ambient temperature until planting time.

Greenhouse experiments: Oxamyl-treated and control seeds were planted in 10-cm diam plastic pots containing 500 gm quantities of soil at 0.5 bar. Depending on the species being tested, soils were selected for use which were known to contain heavy, natural infestations of particular plant-parasitic nematodes. Five treated seeds were planted per plot, and for each concentration used there were six pot replications. Pots were arranged in a randomized complete block design on the greenhouse bench. The plants were allowed to grow 3 to 5 weeks. Then a 50-cm^3 sample of soil was taken from each pot, and the numbers of soil nematodes present were determined by a flotation-sieving technique (7). Root systems were carefully washed free of soil and examined, when it was appropriate, for numbers of root-knot nematode galls and severity of galling by *Meloidogyne* species (9).

Data were subjected to statistical analyses by conventional analysis of variance techniques. Treatment means were compared for significant differences using Duncan's New Multiple Range Test.

RESULTS

Bean: Treated seeds were planted in two different samples of a sandy loam (sand 74%, silt 15%, clay 11%). Both samples were heavily infested with *Helicotylenchus dihystera,* but the soil used for the 3-h-treated seed had a greater nematode count. Plants were grown for 4 weeks, after which time the soil was examined for live nematodes. Soil from pots planted with oxamyl-treated seed showed a great reduction in *H. dihystera* (Fig. 1). Because 6-h-soaked seeds absorbed a larger volume of solution than 3-h-treated seeds, they showed a greater effect on the nematode count. Regardless of the length of the imbibition period, there was almost complete eradication of *H. dihystera* in soil planted with seeds treated with 4 gm or more of oxamyl/100 ml solution.

Watermelon: Treated watermelon seeds were tested in two soils. In the first test, 2 days after treatment and drying, seeds were planted in a sandy loam (sand 74%, silt 15%, clay 11%) heavily infested with *Meloidogyne incognita, H. dihystera,* dorylaimoid, and saprophagous nematodes. After 3 weeks, soil nematode counts were made and root systems were examined for galling (Table 1). The soil showed a drastic reduction in parasitic, dorylaimoid, and saprophagous nematodes. The root systems of plants from treated seed showed less galling than roots of control plants. A maximal effect was obtained with the lowest (2 gm/100 ml) oxamyl concentration used.

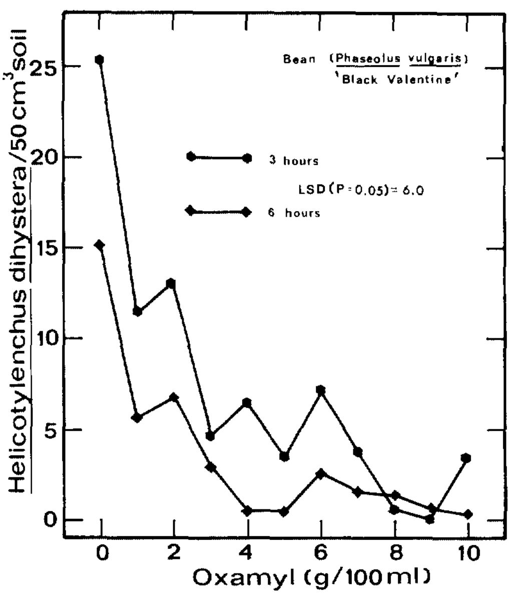

FIG. 1. Effects of soaking time and oxamyl concentration used in the treatment of beans on the *Helicotylenchus dihystera* content of soil. Values are the means of six replicates.

TABLE 1. Effects of oxamyl-imbibed seeds on the numbers of plant-parasitic and soil nematodes[y].

Plant	Oxamyl concentration (gm/100 ml solution)	Plant-parasitic nematodes				Soil nematodes (No./50 cm³ soil)	
		Nematode species	No. galls/gm root	Gall index[z]	No./50 cm³ soil	Dorylaimoid	Saprophagous
Watermelon		*Meloidogyne incognita*					
(sandy-loam)	0		20.3 a	4.2 a		6.0 a	92 a
	2		8.0 b	1.6 b		1.7 b	24 b
Watermelon		*Rotylenchus* sp.					
(sandy-clay-loam)	0				138.8 a	4.5 a	35 a
	2				9.2 b	0.3 b	15 b
	4				11.3 b	0.5 b	7 c
Soybean		*Meloidogyne arenaria*					
	0		17.4 a				168 a
	4		0.6 b				63 b
Cucumber		*Meloidogyne arenaria*					
	0		87.5 a			3.0 a	41 a
	12		1.5 b			0.7 b	11 b
Corn		*Helicotylenchus dihystera*					
	0				147.8 a	5.2 a	44.2 a
	5				27.0 b	0.0 b	44.0 a
		Paratrichodorus christiei					
	0				3.3 a		
	5				0.3 b		

[y]Each value represents the average from 6 pot replications. For each seed species, values within columns followed by different letters are different at $P = 0.05$ or better.

[z]Gall index: 5 = severe galling; 1 = none.

Watermelon seeds from this same treated batch were stored for 2 weeks before being planted in a sandy-clay-loam (sand 55%, silt 20%, clay 25%) heavily infested with *Rotylenchus* sp. After 4 weeks, soil from pots planted with oxamyl-imbibed seeds showed greatly reduced numbers of parasitic and nonparasitic nematodes (Table 1).

Soybean: Treated soybean seeds were stored for 4 weeks before being planted in a loamy sand (sand 80%, silt 15%, clay 5%) infested with *Meloidogyne arenaria.* After 4 weeks, soil was examined for live nematode content, and root systems were examined for severity of galling. In addition, the development of *Rhizobium* nodules was determined, and measurements were made of fresh weights of root and shoot systems. Oxamyl treatments suppressed development of root galls. Also, soil nematode populations were reduced (Table 1). The lowest oxamyl concentration used (4 gm/100 ml) was maximally effective.

The number of plants surviving/pot to the time of sampling, the numbers of *Rhizobium* nodules, and root and shoot growth were increased with concentrations of 8 gm or more oxamyl/100 ml of treatment solution (data not given).

Cucumber: Cucumber seeds treated 4 weeks earlier were planted in a loamy sand (sand 80%, silt 15%, clay 5%) infested with *M. arenaria.* After 4 weeks, the soil was examined for live nematodes and the root systems rated for severity of galling (Table 1). Oxamyl-treated seed showed excellent control of *M. arenaria* by suppressing development of root galls. In addition, the numbers of dorylaimoid, saprophagous, and predatory nematodes in the soil were reduced. Maximal control was obtained with the lowest oxamyl treatment (12 gm/100 ml solution).

Corn: Corn seeds were planted 1 week after treatment in a sandy clay loam (sand 55%, silt 20%, clay 25%) infested with *H. dihystera, Paratrichodorus christiei,* dorylaimoid, and saprophagous nematodes. After 24 days, *H. dihystera, P. christiei,* and dorylaimoid nematodes in the soil were considerably reduced in numbers (Table 1), but saprophagous nematodes were affected only by seed treatments with the two highest oxamyl concentrations (data not shown).

DISCUSSION

Our experiments demonstrated that the nematicide oxamyl, in aqueous solution, can be introduced into seeds and confers at least short-term protection upon plants grown from such seeds. Leachate from treated seeds reduces soil parasitic and free-living nematode counts. Occasionally, an obvious enhancement of plant performance was noted with the seed treatment. We believe this response was due to the prevention of nematode damage rather than to a direct effect of oxamyl on plant growth. Treated seeds can be stored for at least 4 weeks without loss of nematicidal activity.

Bean, soybean, watermelon, and cucumber seeds all have a high protein content and, therefore, absorb large quantities of a treatment solution. However, as we have shown, the technique can also be applied to a seed such as corn in which starch is the predominant storage reserve and which, therefore, absorbs relatively less water on soaking.

Introduction of a protective chemical into seeds presents certain obvious advantages: *(i)* because the material is inside the seed, it is concentrated at the site of attack; *(ii)* a precise, calculated, control rate of the chemical can be introduced into the soil; it thereby limits the amount of pesticide and minimizes environmental pollution; and *(iii)* it could conceivably permit the growing of nonresistant, but desirable plant varieties under conditions where adequate soil treatment might be too expensive or impractical.

If Lee soybean treated with the lowest (maximally effective) level of oxamyl used in these experiments were planted at the recommended rate of approximately 50 kg seeds/ha, less than 0.5 kg (a.i.)/ha of oxamyl would be introduced into the soil. The recommended field rate of application of the commercial formulation of oxamyl, when applied in the planting furrow, would require 1.1 to 1.7 kg (a.i.)/ha of oxamyl. If, instead of being applied in-furrow, the nematicide were incorporated into the soil prior to planting, or applied as a soil drench, the amount used would be increased several-fold.

How long the resistance conferred by seed treatment lasts is currently being investigated. The greenhouse experiments described never extended beyond 1 month. In a field situation, additional post-plant treatments may be required to maintain adequate control.

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