Journal of Nematology 21(2):184–188. 1989.

A Nematicide Seed Treatment to Control *Ditylenchus dipsaci* on Seedling Alfalfa[1]

F. A. Gray[2] and D. H. Soh[3]

Abstract: Three nematicides were evaluated as seed treatments to control the alfalfa stem nematode (*Ditylenchus dipsaci*) on seedling alfalfa. Alfalfa seeds were soaked for 10 hours in a 0.5% (formulated by weight) concentration of either carbofuran, phenamiphos or oxamyl in acetone with no adverse effect on seed germination. All three treatments decreased nematode damage and increased survival of 'Ranger' (susceptible) and 'Lahontan' (resistant) alfalfa plants, when seeds were planted in soil infested with *D. dipsaci.* Mean live plant counts after 6 weeks in the untreated control, acetone alone, carbofuran, phenamiphos, and oxamyl treatments, respectively, were 4.3, 6.3, 19.0, 19.8, and 19.0 for Lahontan and 4.5, 1.5, 18.5, 19.3, and 18.0 for Ranger from 20 seeds/pot. Nematicide seed treatments resulted in significantly healthier Ranger alfalfa plants 4 months after planting. The combination of seed treatment and host resistance may provide a means of establishing alfalfa in an alfalfa monocropped system where soil populations of *D. dipsaci* are high.

Key words: alfalfa, carbofuran, *Ditylenchus dipsaci,* host resistance, *Medicago sativa,* oxamyl, phenamiphos, seed treatment, stem nematode.

The alfalfa stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev, is a major pathogen of alfalfa in the western United States (4,5,10). It is controlled by rotation of fields with nonhost crops and planting of resistant cultivars (4). In fields where the stem nematode becomes established, stands of susceptible alfalfa cultivars may decline rapidly (5). Many of these fields are either renovated (harrowed and interseeded with alfalfa) or plowed and reseeded. If this is done when environmental conditions are favorable for infection of *D. dipsaci,* severe injury or death of seedlings may occur before or after emergence. If fields are reseeded with a susceptible cultivar, stands may be severely reduced or lost. Severe damage may occur even if a resistant cultivar is planted because nematode penetration and injury occurs equally in resistant and susceptible plants (7,8,11,18). Although stands of resistant cultivars usually recover during the second year, yield may be nil the first year and severely reduced the second year.

The feasibility of using nematicides as a seed treatment in controlling parasitic nematodes has been investigated on several annual crop species (12–14,17,19). Infection of seedling alfalfa by the lesion nematode, *Pratylenchus penetrans* (Cobb) Filipjev & Schuumans Stekhoven, and the northern root-knot nematode, *Meloidogyne hapla* Chitwood, was reduced by treating seeds with oxamyl (15,16). Plants grown from seed treated with oxamyl produced 62% more forage in *P. penetrans*-infested soil and 32% more in soil infested with *M. hapla,* as compared with plants grown from untreated seeds. Infection by *P. penetrans* and root galls caused by *M. hapla* were also significantly reduced. Oxamyl content of treated seeds did not decline after 26 months of storage.

The purpose of this study was to determine the optimum nematicide concentration and soak time for alfalfa seeds that would provide maximum control against high populations of *D. dipsaci* without adversely affecting seed germination. A preliminary report has been published (6).

Materials and Methods

Nematicide concentration: In experiment 1 seeds of 'Ladak' alfalfa were soaked for 0.5 minutes in either water or acetone containing carbofuran (40.64% a.i.), phenamiphos (36% a.i.), and oxamyl (24% a.i.) at concentrations of: 0, 0.25, 0.50, 1.25, 1.50, 2.50, and 5.0% (formulated by weight).

Received for publication 4 April 1988.
[1] Part of an M.S. Thesis submitted to the University of Wyoming, Laramie, WY 82071.

[2] Associate Professor, Department of Plant, Soil and Insect Sciences, University of Wyoming, Laramie, WY 82071.
[3] Former Graduate Assistant, Department of Plant, Soil and Insect Sciences, University of Wyoming, Laramie, WY 82071. Present address: 509 North Hyland #3, Ames, IA 50010.

Seeds were treated under a vented hood at the rate of 1 ml nematicide solution/g seed. After soaking, seeds were air dried for 12 hours on absorbent paper and stored until used. Seeds were germinated in plastic petri dishes (100 seeds per dish, three dishes per treatment) on Whatman #1 filter paper, moistened with 5 ml sterile distilled water. Petri dishes were stacked in plastic bags and stored in the laboratory at 23–27 C, and germination was determined after 10 days. The experiment was a two factorial with 28 treatments and three replications.

Time of exposure to nematicide: In experiment 2, seeds of Ladak and 'Lahontan' alfalfa were soaked in acetone containing 5.0% (formulated by weight) carbofuran, oxamyl, or phenamiphos for 30 seconds, 1.0 minute, 1 hour or 10 hours. The experiment was a three factorial with 24 treatments and four replications.

Experiment 3 was similar to experiment 2, but 'Ranger' alfalfa (also susceptible to *D. dipsaci*) replaced Ladak. Seeds were soaked for 30 seconds, 1, 10 or 24 hours and seeds were soaked in water alone and acetone alone in addition to the three nematicide-acetone solutions (total of 40 treatments).

Nematicide efficacy: In experiment 4, seeds of Ranger and Lahontan alfalfa were soaked for 10 hours in a 5.0% (formulated by weight) concentration of carbofuran, oxamyl, or phenamiphos in acetone; soaked in acetone alone, or were untreated. After treatment, seeds were pregerminated in petri dishes containing sterile, moistened filter paper and sowed in rows in 10-cm plastic pots filled with an autoclaved soil (Rock River Series, 67% sand, 13% silt, 20% clay, 2.1% organic matter; pH 7.6): sand (1:1, v:v) mixture. Stem nematode inoculum (adults and juveniles) was produced in a greenhouse on a stem nematode-susceptible Ladak alfalfa clone and extracted from infected stem buds as previously described (2). Seeds were sprinkled with a commercial preparation of *Rhizobium meliloti* Rangeard, inoculated with an aqueous suspension of nematodes (1 ml containing 200 nematodes/seedling) and then covered with soil. An equal amount of water was applied to the uninoculated controls. There were two furrows in each pot and 10 seeds in each furrow. Pots were placed in a controlled-environment chamber set at 21 C with a 16-hour photoperiod. Lights were off for the first 8 hours after planting to minimize soil water evaporation and increase nematode infection. The experiment was a three factorial with 20 treatments; it was placed in a randomized complete block design with three replications.

Stand counts were taken weekly for 5 weeks and at 16 weeks when the experiment was terminated. Seedlings were examined after 1 week for distortion of cotyledons and swelling of the cotyledonary node region and after 16 weeks when plants were removed, washed free of soil, and observed for swollen, distorted, and stunted stems and stem buds. Although nematode reproduction is a more direct criterion for susceptibility, symptoms in both seedling and older plants have been positively correlated with nematode infection and represent an indirect criterion for plant susceptibility (1,18).

An analysis of variance (ANOV) test was conducted to determine significant data sets. When *F* values were significant, means were separated using the least significant determination test.

Results

Nematicide concentration: There were no significant differences among nematicides, concentrations, or their interaction (nematicides × concentrations) in experiment 1. Germination of Ladak seed ranged from 66.7 to 95.0% and was unaffected by either of the three nematicides in either water or acetone, even at the highest concentration.

Time of exposure to nematicide: There were significant ($P = 0.05$) differences among cultivars and nematicides but not among soak times or their interaction (cultivar × nematicide, cultivar × soak time, nematicide × soak time) in experiment 2. Mean germination was 81.4 for Ladak and 72.7

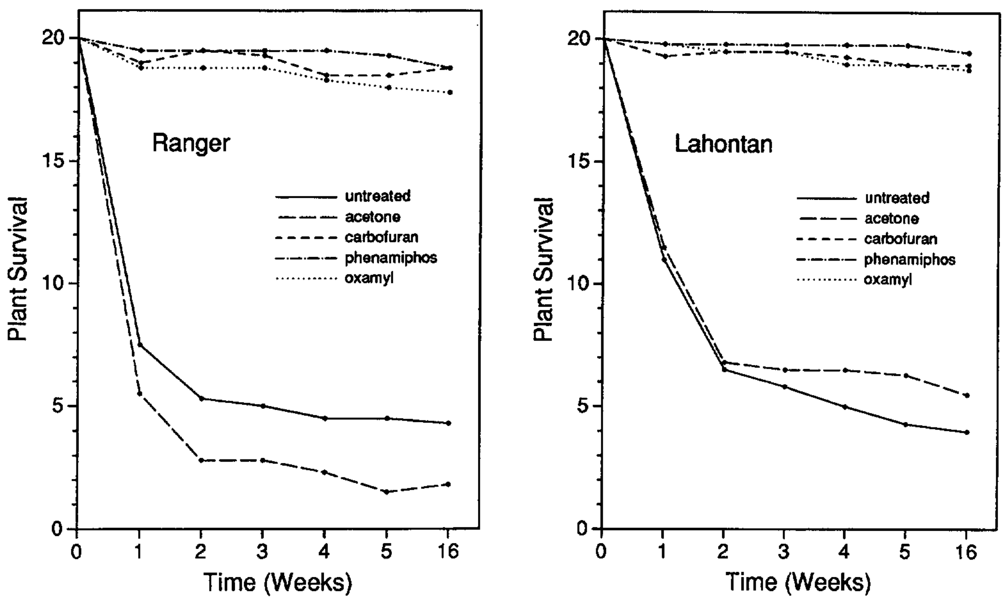

FIG. 1. Efficacy of three nematicides, applied in acetone as a seed treatment, in controlling *Ditylenchus dipsaci*. Ranger is susceptible and Lahontan is resistant to *D. dipsaci*. Data points are the number of surviving plants per pot of 20 total and are the mean of three replications.

for Lahontan. Mean germination for the three nematicide-acetone treatments was 74.7 for carbofuran, 75.1% for phenamiphos, and 79.3% for oxamyl. The germination rate of carbofuran-treated and phenamiphos-treated seeds were significantly lower ($P = 0.05$) than for oxamyl-treated seeds but were not different from each other. Soaking seeds up to 10 hours did not adversely affect seed germination of either cultivar.

There were highly significant ($P = 0.01$) differences among cultivars but not among nematicides or soak times in experiment 3. The cultivar × nematicide and cultivar × soak time interaction were also significantly different. Combined means of the four soak times were significantly different in Lahontan but not in Ranger. Lahontan had significantly lower germination (61.8%) when seeds were treated with water alone, compared with all other treatments, and germination in Lahontan was lower than germination in Ranger in all treatments. Means for the water, acetone, carbofuran, phenamiphos, and oxamyl treatments, respectively, were 81.5, 81.8, 84.3, 78.3, and 81.3 for Ranger and 61.8, 72.0, 70.0, 72.5, and 70.3 for Lahontan. When means of the five seed treatments were combined, the 10-hour and 24-hour soak times significantly reduced germination in Lahontan, compared with the 1-hour soak time, but not the 30-second soak time. Means for the 30-second, 1-hour, 10-hour, and 24-hour soak times, respectively, were 79.4, 81.4, 83.4, and 81.4 for Ranger and 68.4, 73.4, 67.4, and 68.0 for Lahontan. Again, germination in Lahontan was significantly lower than in Ranger.

Nematicide efficacy: Survival of both inoculated Lahontan and Ranger plants from week 1 through week 16 was significantly ($P = 0.05$) better for seeds treated with any of the three nematicides than the untreated or from the acetone alone treated controls (Fig. 1). There was no difference between cultivars or nematicides. When the stem nematode was not present, emergence in either cultivar was unaffected by treatments. Based on symptoms produced by *D. dipsaci*, a high level of infection had occurred after 1 week in seedlings of both Ranger (93%) and Lahontan (91%) in the

TABLE 1. Percentages of Ranger and Lahontan alfalfa plants exhibiting symptoms of parasitism by *Ditylenchus dipsaci* at 1 and 16 weeks following treatment with nematicides.

	Ranger		Lahontan	
	1 wk	16 wk	1 wk	16 wk
Untreated	93	53	91	0
Acetone alone	100	13	79	4
Carbofuran-acetone	47	20	34	0
Phenamiphos-acetone	32	10	17	13
Oxamyl-acetone	19	11	21	15
$LSD_{0.05}$	19	NS	28	NS
$LSD_{0.10}$	14	38	23	NS

Values are the mean of four replications. Ranger is susceptible and Lahontan is resistant to *D. dipsaci*.

untreated controls (Table 1). All three nematicides reduced ($P = 0.05$) damage associated with nematode infection (1,17). A lower percentage of plants in both cultivars had symptoms at 16 weeks than at 1 week. In the susceptible cultivar Ranger, the percentage of plants showing symptoms at 16 weeks was still lower ($P = 0.05$) in each of the three nematicide treatments than in the untreated control, but was not different from the acetone alone treatment. There were no differences among treatments in the resistant cultivar Lahontan after 16 weeks.

DISCUSSION

Previous studies have provided strong evidence of the feasibility of using a nematicide seed treatment to protect plants from plant-parasitic nematodes (12–17,19). Our results are in agreement with those of Townshend and Potter (15,16) that their results showed oxamyl seed treatment to be efficacious in controlling root-parasitic nematodes on alfalfa; we showed carbofuran, phenamiphos, and oxamyl to be efficacious in controlling a stem and crown parasite.

Significant differences in seed germination of Lahontan and Ranger in experiments 2 and 3 were due to differences in seed lots before seed treatment and therefore were not due to the treatment effect. Generally, we found that soaking seeds for 10 hours in a 5.0% (formulated by weight) concentration of either nematicide had no detrimental effect on seed germination. Although the nematicide seed treatments provided similar protection to both resistant and susceptible cultivars, use of a susceptible cultivar would not be recommended because infection would eventually occur under field conditions after the nematicide had dissipated. Seed treatment of the resistant cultivar (moderate level of resistance or higher) should provide growers a means of establishing and maintaining a stand of alfalfa in fields having a high population of *D. dipsaci*. Studies are presently underway in Wyoming to evaluate the efficacy of these seed treatments under field conditions.

Carbofuran, a broad spectrum carbamate, is presently registered for application on established alfalfa stands for control of the alfalfa weevil, *Hypera postica* (Gyllenhal). The possibility of extending its label as a seed treatment to aid in the control of *D. dipsaci* should be pursued. Oxamyl is presently being used as a seed treatment in Australia to control the cereal cyst nematode, *Heterodera avenae* Woll., in wheat (3) which provides evidence of the commercial feasibility of such a control practice.

The potential use of this seed treatment is applicable in controlling *D. dipsaci* not only on alfalfa, but also on nonhost crops planted in fields following alfalfa. Seedling loss in sugarbeets and tomatoes can also be caused by penetration damage from the alfalfa stem nematode (9). Even though *D. dipsaci* (alfalfa race) is host specific relative to reproduction, it is nonspecific relative to penetration.

LITERATURE CITED

1. Barker, K. R., and J. N. Sasser. 1959. Biology and control of the stem nematode, *Ditylenchus dipsaci*. Phytopathology 49:664–670.

2. Boelter, R. H., F. A. Gray, and R. H. Delaney. 1985. Effect of *Ditylenchus dipsaci* on alfalfa mortality, winterkill, and yield. Journal of Nematology 17:140–144.

3. Brown, R. H. 1984. Cereal cyst nematode-registered nematicides and how to apply them. Agdex 1101683 Australia.

4. Graham, J. H., F. I. Frosheiser, D. L. Stuteville,

and D. C. Erwin. 1979. A compendium of alfalfa diseases. St. Paul, MN: American Phytopathological Society Press.

5. Gray, F. A., R. H. Boelter, and G. P. Roehrkasse. 1984. Alfalfa stem nematode (*Ditylenchus dipsaci*) in Wyoming. Plant Disease 68:620–623.

6. Gray, F. A., and D. H. Soh. 1986. Efficacy of a nematicide seed treatment in controlling seedling blight of alfalfa incited by *Ditylenchus dipsaci* and *Meloidogyne hapla*. Phytopathology 76:1139 (Abstr.).

7. Griffin, G. D. 1968. The pathogenicity of *Ditylenchus dipsaci* to alfalfa and the relationship of temperature to plant infection and susceptibility. Phytopathology 58:929–932.

8. Griffin, G. D. 1975. Parasitism of nonhost cultivars by *Ditylenchus dipsaci*. Journal of Nematology 7: 236–238.

9. Griffin, G. D., and W. W. Waite. 1971. Attraction of *Ditylenchus dipsaci* and *Meloidogyne hapla* by resistant and susceptible alfalfa seedlings. Journal of Nematology 3:215–219.

10. Griffin, G. D. 1986. The biology and control of parasitic nematodes on alfalfa. Utah Science 42: 131–135.

11. Griffin, G. D. 1987. Interaction of *Ditylenchus dipsaci* and *Meloidogyne hapla* on resistant and susceptible plant species. Journal of Nematology 19:441–446.

12. Hoveland, C. S., R. Rodríquez-Kábana, and R. L. Haaland. 1977. Phytotoxicity and efficacy of nematicide seed treatment on wheat, rye, oats and ryegrass. Agronomy Journal 69:837–839.

13. Prasad, K. S. K., and Y. S. Rao. 1976. Chemotherapy of the root-knot nematode (*Meloidogyne graminicola*) in rice. 1. Effects of soaking seeds in nematicide solutions. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 83:665–668.

14. Rodríquez-Kábana, R., D. Hoveland, and R. L. Haaland. 1977. Evaluation of a seed-treatment method with acetone for delivering systemic nematicides with wheat and rye. Journal of Nematology 9:323–326.

15. Townshend, J. L., and J. W. Potter. 1979. Inhibiting infection of alfalfa seedlings by *Pratylenchus penetrans* by treating seed with oxamyl. Canadian Journal of Plant Science 59:519–520.

16. Townshend, J. L., and M. Chiba. 1987. Control of *Pratylenchus penetrans* and *Meloidogyne hapla* and yield response of alfalfa due to oxamyl seed treatments. Journal of Nematology 19:454–458.

17. Truelove, B., R. Rodríquez-Kábana, and P. S. King. 1977. Seed treatment as a means of preventing nematode damage to crop plants. Journal of Nematology 9:326–330.

18. Wynne, J. C., and T. H. Busbice. 1968. Effects of temperature and incubation period on the expression of resistance to stem nematode in alfalfa. Crop Science 8:179–183.

19. Weaver, C. F., and R. Rodríquez-Kábana. 1987. Nematicide seed treatments for control of soybean nematodes. Journal of Nematology 19:561 (Abstr.).