Effects of Herbicides on Sclerotinia Crown and Stem Rot of Alfalfa

S. L. Reichard, Graduate Research Assistant, **R. M. Sulc,** Assistant Professor, Department of Horticulture and Crop Science, **L. H. Rhodes,** Associate Professor, Department of Plant Pathology, and **M. M. Loux,** Associate Professor, Department of Horticulture and Crop Science, The Ohio State University, Columbus 43210

ABSTRACT

Reichard, S. L., Sulc, R. M., Rhodes, L. H., and Loux, M. M. 1997. Effects of herbicides on Sclerotinia crown and stem rot of alfalfa. Plant Dis. 81:787-790.

This study was conducted to determine whether herbicides and adjuvants registered for postemergence use in alfalfa have an effect on Sclerotinia crown and stem rot (SCSR). In a controlled environment, disease severity index (DSI) of alfalfa seedlings was reduced by pronamide and 2,4-DB compared with the untreated control, whereas bromoxynil and 13% sethoxydim + petroleum-based adjuvant (PBA) increased DSI. In the field, disease severity in all herbicide treatments was similar to that in untreated alfalfa. In a second controlled-environment study, pronamide and 2,4-DB reduced DSI compared with the no herbicide control when seedlings were inoculated 1 day after herbicide application, but this protective effect was not observed when seedlings were inoculated 8 days or longer after herbicide application. The results demonstrate that several herbicides are capable of suppressing or enhancing SCSR severity in a controlled environment if seedling inoculation occurs soon after herbicide application; however, the residual effect of these herbicides on SCSR appeared to be much shorter than the 4- to 6-week infection period occurring in the field.

Additional keywords: *Medicago sativa, Sclerotinia trifoliorum*

Sclerotinia crown and stem rot (SCSR), caused by *Sclerotinia trifoliorum* Eriks., is one of the most destructive diseases of forage legumes in the eastern United States (10). Infection by *S. trifoliorum* occurs in the fall, although symptoms may not be evident until the following spring. In alfalfa (*Medicago sativa* L.), stands seeded in late summer or early fall can be completely destroyed by SCSR. Older stands are affected less than seedling stands. Sulc and Rhodes (11) recently reported that SCSR severity (percentage of stand affected) was 4, 12, 23, and 41% for spring, early August, mid-August, and late August alfalfa plantings, respectively, demonstrating that even 2-week delays in seeding dates during August resulted in increases in disease severity.

Herbicide–pathogen interactions have been noted for many diseases, including those caused by *Sclerotinia* species (6). Herbicides can increase disease severity by inhibiting plant growth, injuring plants, or stimulating growth and reproduction of the pathogen. A decrease in disease severity can be a result of herbicide toxicity to the pathogen or an alteration in the metabolism of the plant (1,6). Herbicides have been reported to retard mycelial growth, reduce sclerotium weight, and hinder carpogenic germination of *S. sclerotiorum* (Lib.) de Bary or *S. minor* Jagger, whereas other herbicides stimulated these fungi or had no effect (3,4,7,8). There are no published reports on the effect of herbicides on disease severity caused by *S. trifoliorum*. Some of the herbicides currently being used on alfalfa, especially those used on seedling stands in the fall, may affect SCSR incidence or severity. Such effects could influence herbicide recommendations made to growers. The objective of our research was to determine the effects of various herbicides and adjuvants on SCSR severity in alfalfa. All herbicides and adjuvants tested are currently registered for postemergence use on seedling alfalfa. Studies were conducted in controlled environments and in the field.

Corresponding author: L. H. Rhodes
E-mail: rhodes.2@osu.edu

Salary and research support provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center and The Ohio State University, and by a seed grant provided by the Office of Research of The Ohio State University. Journal Article 132-95.

Accepted for publication 31 March 1997.

Publication no. D-1997-0430-04R

MATERIALS AND METHODS

Controlled-environment experiments. The seedling test used in the controlled environment was a modified version of the technique described by Rhodes (9). Plants were grown in 7.0-cm-diameter × 6.5-cm-deep pots in a 1:1:1 (vol/vol/vol) mixture of Crosby silt loam soil, peat, and vermiculite. A solution of 90 g of KH_2PO_4 + 210 g of KCl in 5 liters of water was added to 0.34 m^3 of the soil mixture. The mixture was adjusted to pH 7 with $CaCO_3$ and steam-sterilized at 180°C for 3 h. Approximately 25 seeds of Armor alfalfa, inoculated with *Rhizobium meliloti*, were planted in each pot and covered with 6 mm of soil mix. The pots were placed under intermittent mist for 2 days and then moved to a greenhouse bench, where the temperature was maintained at 20°C with supplemental light provided by 400-W high-pressure sodium bulbs. Seven days after planting, plants were thinned to 15 per pot, and each pot was fertilized with 30 ml of Hoagland's nutrient solution no. 2 (5). The experimental unit in all experiments consisted of 15 alfalfa seedlings in one pot.

For inoculum production, 12 g of Difco potato dextrose broth powder was suspended in 500 ml of distilled water. The broth was autoclaved at 121°C for 20 min, then allowed to cool to ca. 25°C. Ten 1-cm-diameter plugs from the colony margin of a 3- to 5-day-old plate culture of *S. trifoliorum*, designated Ohio isolate 3-8A (American Type Culture Collection no. 76619), were added to the broth, and flasks were placed on an orbital shaker at 120 rpm in a dark incubator at 15°C. After 7 days, spherical colonies, ca. 2.5 cm diameter, had developed. Three colonies (ca. 40 cm^3 of mycelium) were removed from the broth, rinsed with tap water for 1 min, placed in a Waring blender with 100 ml of distilled water, and blended for 2 s. An additional 150 ml of distilled water was added, and the mixture was blended for 2 to 3 s. The mycelial mixture was strained through a 250-µm-aperture sieve to remove large mycelial fragments.

In experiment 1, seedlings were placed in a mechanized spray chamber (Allen Machine Works, Midland, MI) and sprayed with 11 herbicide and/or adjuvant treatments (Table 1) 17 days after planting. Commercial grade herbicides and adjuvants were used at rates recommended for alfalfa. An untreated control (no herbicide) was also included for a total of 12 treatments. The alfalfa seedlings had two to three trifoliolate leaves when treatments were applied. After treatments were applied, seedlings were returned to the greenhouse for 24 h to allow drying of the spray deposits on foliage. The next day, seedlings were either inoculated with *S. trifoliorum* or uninoculated. Inoculated seedlings were first misted with water and then sprayed with the mycelial suspension (described previously) at 5 ml per 15

plants. Uninoculated seedlings were misted with water alone. Each pot was immediately placed in a plastic bag that was closed with a twist tie and incubated for 7 days at 15°C with a 12-h photoperiod. Photosynthetically active radiation levels (400 to 700 nm) were 30 to 47 $\mu E \cdot m^{-2} \cdot s^{-1}$. The 12 (herbicide–adjuvant) × 2 (*S. trifoliorum*) factorial treatment combinations were arranged in eight randomized complete blocks. The experiment was repeated once; the first and second runs were designated experiments 1A and 1B.

Based on the results of experiment 1 and the experiments described under "Field evaluations," a second controlled-environment experiment was conducted. Plants were grown and herbicide treatments were applied as in experiment 1. A factorial combination of four dates of inoculation with *S. trifoliorum* and five herbicide treatments and an untreated control (Table 1) was evaluated in experiment 2. The four dates of seedling inoculation were 1, 8, 15, and 22 days after herbicide application. Corresponding seedling ages at time of inoculation were 18, 25, 32, and 39 days after planting. The first inoculation date was the same protocol used in experiments 1A and 1B. Treatments were arranged in eight randomized complete blocks. The experiment was repeated once; the first and second runs were designated experiments 2A and 2B.

In all controlled-environment experiments, each plant was rated visually for disease severity 7 days after inoculation as follows: 1 = no disease symptoms; 2 = one or more small lesions present; 3 = upper leaves dead, stem lesions present, and wilting of the plant; 4 = plant mostly rotted but lower stem still green; 5 = dead plant. A disease severity index (DSI) was calculated for each experimental unit as the mean of the 15 individual plant ratings within each pot. Plant fresh weight was recorded for all treatments in experiments 1A and 1B.

Field evaluations. Plots were seeded in 1994 and 1995 at Columbus, Ohio, in grass-clover (*Trifolium* spp.) sods. Separate fields were used for the two seedings. To prepare the sites, the grass-clover sods were inoculated in April the year of seeding with a mixture of wheat (*Triticum aestivum* L.) and oat (*Avena sativa* L.) grain (1:1) that had been colonized by *S. trifoliorum*. Nine isolates of *S. trifoliorum* collected in Ohio were used to colonize the grain. Grain inoculum was broadcast uniformly on the sod at 50 ml/m^2. Clover plants were colonized by mycelium of *S. trifoliorum*, and sclerotia were formed. Fertilizer and lime applications were made according to soil test recommendations for alfalfa seedings.

The grass-legume sod was killed 3 weeks before planting with glyphosate at 3.4 kg a.i./ha in a water volume of 187 liters/ha. Armor alfalfa was no-till seeded at 18 kg/ha on 7 September in both 1994 and 1995. Five herbicide–adjuvant treatments (Table 1) were applied on 25 October 1994 and 30 October 1995, to coincide with the initial flush of apothecia. Alfalfa canopy height was ca. 15 to 20 cm at the time of herbicide application in both years. The herbicide–adjuvant treatments were applied with a CO_2 sprayer equipped with flat fan 8002 spray nozzles. The sprayer was set to maintain a pressure of 3.5 kg/cm^2 and to deliver 187 liters/ha. The herbicide–adjuvant treatments plus an untreated control were arranged in four randomized complete blocks. Plot size was 1.8 × 5.5 m. Disease severity was assessed in April the year after seeding, when symptoms of SCSR were most evident. Visual ratings of percentage of the stand affected by SCSR (diseased and dead plants) in each plot were based on visible symptoms as described by Rhodes and Gilbert (10). Ratings were recorded independently by two of the authors and averaged before analysis.

Data analysis. Data were subjected to analysis of variance with SAS (SAS Institute, Cary, NC) software. In experiment 1, analysis of variance was conducted to determine the effect of herbicide–adjuvant treatment on DSI of inoculated plants only, because uninoculated plants were not infected with *S. trifoliorum* and showed no symptoms of SCSR. All data were included in the remaining analyses. Except for the fresh weight data in experiment 1, all data are presented separately for the repetitions of each experiment because there were experiment repetition × treatment interactions. Means were separated with Fisher's protected LSD at $P = 0.05$.

RESULTS AND DISCUSSION

Large differences in disease severity ($P < 0.05$) were found among herbicide–adjuvant treatments in a controlled environment (Table 2). In both experiments 1A and 1B, pronamide and 2,4-DB reduced DSI compared with the inoculated, no-herbicide control treatment. In contrast, DSI of plants treated with bromoxynil and 13% sethoxydim + PBA was greater than that of the untreated control in both repetitions of experiment 1. Several other treatments increased DSI compared with the no herbicide control in experiment 1A, including PBA, 13% sethoxydim, and non-

Table 1. Herbicide, adjuvant, and herbicide + adjuvant treatments applied to alfalfa seedlings in three experiments

Treatment	Concentration (kg a.i./liter)	Rate (kg a.i./ha)	Experiment		
			1	2	Field
No herbicide control	…	…	X[u]	X	X
Bromoxynil	0.48	0.56	X	X	X
2,4-DB	0.24	1.12	X	X	X
Pronamide	50%[v]	0.84	X	X	X
Petroleum-based adjuvant (PBA)[w]	99%	2.34[x]	X	…	…
13% sethoxydim	0.12	0.21	X	X	…
13% sethoxydim + PBA	0.12 …	0.21 2.34[x]	X	…	X
18% sethoxydim	0.18	0.31	X	…	…
Nonionic surfactant (NIS)[y]	…	0.25%[z]	X	…	…
Urea ammonium nitrate (28% UAN)	…	2.34[x]	X	…	…
Imazethapyr	0.24	0.07	X	…	…
Imazethapyr + NIS + 28% UAN	0.24 … …	0.07 0.25%[z] 2.34[x]	X	X	X

[u] Designates treatment used in experiment.
[v] Active ingredient concentration by weight of product.
[w] Petroleum-based adjuvant was Dash HC from BASF Corp., Research Triangle Park, NC 27709.
[x] Rate given in liters of product per ha.
[y] Nonionic surfactant was Ortho X-77 Spreader from Valent USA Corp., Walnut Creek, CA 94596.
[z] Rate given as volume of product to total volume of solution applied.

Table 2. Effect of herbicides and adjuvants on severity of Sclerotinia crown and stem rot of alfalfa seedlings in a controlled environment

Treatment	DSI[y]	
	Exp. 1A	Exp. 1B
Bromoxynil	4.6 ab[z]	4.0 a
13% sethoxydim + PBA	4.7 a	3.3 b
PBA	4.5 abc	2.9 bc
13% sethoxydim	4.5 abc	2.7 c
18% sethoxydim	4.2 bcd	2.6 c
NIS	4.4 abc	2.6 c
Imazethapyr	4.2 bcd	2.5 cd
Imazethapyr + NIS + 28% UAN	4.1 cd	2.7 c
No herbicide	3.8 d	2.7 c
28% UAN	3.8 d	2.1 de
2,4-DB	3.0 e	2.0 e
Pronamide	2.0 f	1.1 f
Mean	4.0	2.6
LSD (0.05)	0.5	0.5

[y] Disease severity index 7 days after inoculation, based on the following scale: 1 = no disease symptoms; 2 = one or more small lesions present; 3 = upper leaves dead, stem lesions present, and wilting of the plant; 4 = plant mostly rotted but lower stem still green; 5 = dead plant.
[z] Means followed by the same letter within a column are not significantly different according to Fisher's protected LSD at $P = 0.05$.

ionic surfactant (NIS). The detection of more significant differences among treatments in experiment 1A than in 1B probably resulted from greater disease pressure in experiment 1A, as indicated by the mean DSI for that experiment (Table 2); however, plants treated with bromoxynil, 13% sethoxydim + PBA, 2,4-DB, and pronamide had DSI consistently different from those of the untreated controls in both experiments. Seedlings treated with imazethapyr, imazethapyr + NIS + 28% urea ammonia nitrate (UAN), and 18% sethoxydim had DSI similar to those of untreated plants in both experiments (Table 2).

There was an herbicide × inoculation treatment interaction (P = 0.01) for seedling fresh weight; thus, simple effects of herbicide–adjuvant treatments were evaluated. Fresh weight of inoculated seedlings was influenced by herbicide treatment (Table 3). Fresh weight of inoculated seedlings was inversely related to DSI (r = –0.83, P = 0.0001), and the differences among inoculated treatments for seedling fresh weight (Table 3) reflected the differences among treatments for disease severity (Table 2). Herbicide–adjuvant treatments did not affect fresh weight of uninoculated seedlings compared with the no herbicide control (Table 3), indicating that the herbicides and adjuvants had negligible effects on plant growth.

In the field, alfalfa stand densities were uniformly excellent in all plots at the time of herbicide application in late October. Visual symptoms of SCSR were not observed until late winter and early spring (February to March), which is typical for Ohio. Although plants are infected in October through early December, plants usually do not begin to exhibit visual symptoms until late winter or early spring. Disease severity was rated in April, when symptoms of SCSR were most evident. Substantial alfalfa stand loss to SCSR occurred in the field trials in both years (Table 4), indicating that both inoculum level and environmental conditions were suitable for disease development. Plants

Table 3. Effect of herbicides and adjuvants on fresh weight of alfalfa seedlings inoculated or not inoculated with *Sclerotinia trifoliorum* in a controlled environment

	Fresh weight (mg)[y]	
Treatment	**Inoc.**	**Uninoc.**
Bromoxynil	641 f[z]	1,413 b
13% sethoxydim + PBA	762 ef	1,538 ab
13% sethoxydim	835 def	1,602 ab
PBA	908 cde	1,579 ab
18% sethoxydim	921 cde	1,640 a
NIS	902 cde	1,635 a
Imazethapyr + NIS + 28% UAN	942 cde	1,570 ab
Imazethapyr	990 cd	1,594 ab
No herbicide	994 cd	1,563 ab
28% UAN	1,086 bc	1,557 ab
2,4-DB	1,204 ab	1,510 ab
Pronamide	1,311 a	1,658 a
Mean	958	1,572
LSD (0.05)	199	

[y] Fresh weight determined 7 days after inoculation.

[z] Means followed by the same letter within a column are not significantly different according to Fisher's protected LSD at P = 0.05.

Table 4. Effect of fall-applied herbicide treatments on Sclerotinia crown and stem rot severity in field-grown alfalfa

	Area affected (%)[z]	
Treatment	**1995**	**1996**
No herbicide	63	86
Bromoxynil	46	90
13% sethoxydim + PBA	55	86
Imazethapyr + NIS + 28% UAN	50	75
2,4-DB	60	85
Pronamide	51	84
P value	0.29	0.08
CV (%)	19.6	7.6

[z] The percentage of plot area with dead and dying alfalfa plants in April of the year following an early September no-till seeding into a sod infested with sclerotia of *Sclerotinia trifoliorum*.

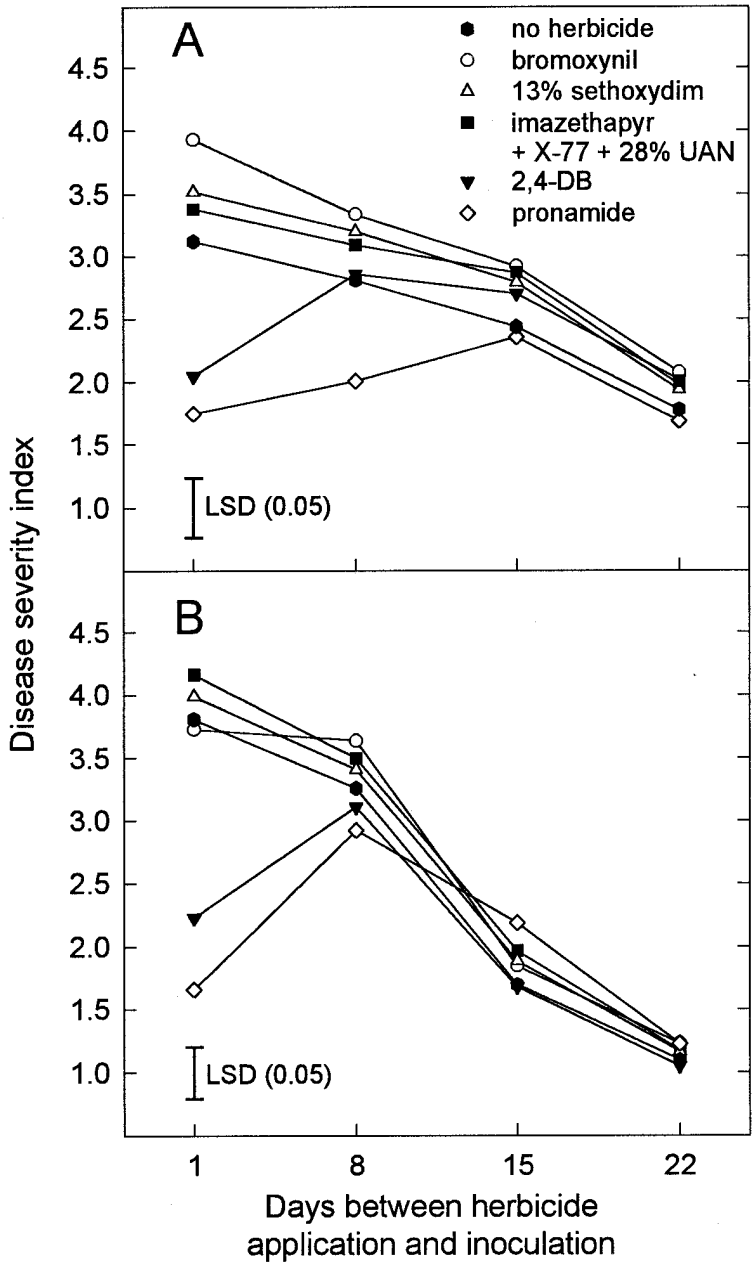

Fig. 1. Disease severity index for alfalfa seedlings inoculated with *Sclerotinia trifoliorum* at four intervals from application of herbicide treatments in **(A)** the first run and **(B)** the second run of the experiment. Disease severity index is the average of 15 individual plant ratings using the following scale: 1 = no disease symptoms; 2 = one or more small lesions present; 3 = upper leaves dead, stem lesions present, and wilting of the plant; 4 = plant mostly rotted but lower stem still green; and 5 = dead plant. LSD is for comparing any two treatment means.

showing symptoms of SCSR in the field were nearly always eventually killed; therefore, visual ratings essentially combined both disease incidence and severity and for all practical purposes equated to stand loss.

The herbicide treatment effects on SCSR severity observed in controlled environments were not observed in the field. Disease severity in all herbicide treatments was similar to that of untreated alfalfa in the field (Table 4). The protective effect of pronamide and 2,4-DB in the controlled environment (Table 2) was not observed in the field, nor did bromoxynil and 13% sethoxydim + PBA increase disease severity in the field.

The second controlled-environment experiment was devised in an effort to better understand the lack of herbicide treatment effects on SCSR severity in field-grown alfalfa. An herbicide treatment × inoculation date interaction (P = 0.0001) was found in both experiments 2A and 2B (Fig. 1). The greatest herbicide treatment effects on disease severity occurred when plants were inoculated 1 day after herbicide application (the protocol used in experiments 1A and 1B), and the differences among treatments progressively narrowed as the period between herbicide application and inoculation increased. The DSI of plants treated with pronamide and 2,4-DB followed a different pattern across inoculation dates compared with that of the other treatments. On the first inoculation date, the plants treated with pronamide and 2,4-DB had lower DSI than that of untreated plants, as in experiments 1A and 1B. When plants were inoculated 8 days after herbicide application, DSI of plants treated with pronamide was lower than that of the untreated control plants in experiment 2A; whereas DSI of plants treated with 2,4-DB was similar to that of the untreated control plants in both experiments. There were no treatment effects on DSI in either experiment when plants were inoculated 15 or 22 days after herbicide application.

Based on our data, pronamide and 2,4-DB can reduce SCSR severity in alfalfa seedlings in a controlled environment; however, this protective effect was relatively short-lived. The effect was lost within the first or second week for pronamide and within the first week for 2,4-DB. The relatively short residual effect of these herbicides on SCSR in a controlled environment appears to explain the lack of herbicide effects on SCSR severity in the field. Apparently, the residual effect of these herbicides on SCSR severity was not long enough to adequately prevent infection and growth of *S. trifoliorum* in field-grown alfalfa. Herbicide application in the field was timed to coincide with the initial flush of apothecia, but apothecia emergence continued for several more weeks, during which time any herbicide effects were apparently lost. This illustrates that care must be exercised when comparing data obtained in controlled environments with those obtained under field conditions, where many other factors influence disease severity and the interaction between pathogens and hosts (6).

Disease severity of untreated seedlings decreased as the period between herbicide application and inoculation increased in experiments 2A and 2B (Fig. 1). Similarly, disease severity in most of the herbicide treatments also decreased with delayed inoculation. The only exceptions were the pronamide and 2,4-DB treatments, because of their effect in reducing disease severity at the first inoculation date. The general trend of decreasing disease severity associated with delayed inoculation can probably be attributed to the increasing age of the plants at the time of inoculation with *S. trifoliorum*. Plant age at the first to fourth inoculation dates was 18, 25, 32, and 39 days after planting, respectively. Brune (2) reported that SCSR severity in alfalfa seedlings decreased exponentially as plant age increased from 14 to 56 days. After plants reached 56 to 70 days of age, the disease ratings remained consistently low. Similarly, Sulc and Rhodes (11) reported that delays in planting date, and the consequent decrease in plant age, resulted in increased severity of SCSR in field-grown alfalfa. In the current study, herbicide effects were gradually lost as inoculation was delayed, and increasing plant age apparently became a factor in reducing disease severity in all treatments.

Our study demonstrates that several herbicides registered for use in late summer–fall establishment of alfalfa can suppress or increase SCSR severity in a controlled environment; however, the residual effect of these herbicide treatments on SCSR severity in alfalfa were short-lived in a controlled environment and were not observed in the field. Sclerotinia crown and stem rot severity in the field was neither reduced nor increased by the herbicides tested. Therefore, herbicide recommendations need not be altered for late summer–fall seedings of alfalfa in regions where SCSR is a risk.

ACKNOWLEDGMENTS

We thank Bert Bishop, Senior Statistician at Ohio Agricultural Research and Development Center, for his helpful advice regarding data analysis.

LITERATURE CITED

1. Altman, J., and Campbell, C. L. 1977. Effect of herbicides on plant diseases. Annu. Rev. Phytopathol. 15:361-385.
2. Brune, P. D. 1988. A histological study of the relationship between plant age and severity of Sclerotinia crown and stem rot of alfalfa. M.S. thesis. The Ohio State University, Columbus.
3. Casale, W. L., and Hart, L. P. 1986. Influence of four herbicides on carpogenic germination and apothecium development of *Sclerotinia sclerotiorum.* Phytopathology 76:980-984.
4. Cerkauskas, R. F., Verma, P. R., and McKenzie, D. L. 1986. Effects of herbicides on *in vitro* growth and carpogenic germination of *Sclerotinia sclerotiorum.* Can. J. Plant Pathol. 8:161-166.
5. Hoagland, D. R., and Arnon, D. I. 1950. The water-culture method of growing plants without soil. Univ. Calif. Agric. Exp. Stn. Circ. No. 347.
6. Katan, J., and Eshel, Y. 1973. Interactions between herbicides and plant pathogens. Residue Rev. 45:145-177.
7. Porter, D. M., and Rud, O. E. 1980. Suppression of Sclerotinia blight of peanuts with dinitrophenol herbicides. Phytopathology 70:720-722.
8. Radke, V. L., and Grau, C. R. 1986. Effects of herbicides on carpogenic germination of *Sclerotinia sclerotiorum.* Plant Dis. 70:19-23.
9. Rhodes, L. H. 1995. Sclerotinia crown and stem rot resistance. Section D-10 in: Standard Tests to Characterize Alfalfa Cultivars. 3rd ed. C. C. Fox, R. Berberet, F. A. Gray, C. R. Grau, D. L. Jessen, and M. A. Peterson, eds. North American Alfalfa Improvement Conference, Beltsville, MD.
10. Rhodes, L. H., and Gilbert, R. G. 1990. Sclerotinia crown and stem rot. Pages 26-28 in: Compendium of Alfalfa Diseases. 2nd ed. D. L. Stuteville and D. C. Erwin, eds. American Phytopathological Society, St. Paul, MN.
11. Sulc, R. M., and Rhodes, L. H. 1997. Planting date, fungicide, and cultivar effects on Sclerotinia crown and stem rot severity in alfalfa. Plant Dis. 81:13-17.