Effects of VA Mycorrhizae and Metalaxyl on Growth of Alfalfa Seedlings in Soils from Fields with "Alfalfa Sickness" in Alberta

S. F. HWANG, Plant Pathologist, Alberta Environmental Centre, Vegreville, Alberta, Canada T0B 4L0

ABSTRACT

Hwang, S. F. 1988. Effects of VA mycorrhizae and metalaxyl on growth of alfalfa seedlings in soils from fields with "alfalfa sickness" in Alberta. Plant Disease 72:448-452.

The causes of poor alfalfa seedling growth in "alfalfa sickness" (AS) soils were studied in a growth chamber. The growth of alfalfa seedlings was increased in sterilized AS soils but not as vigorously as seedlings grown in "healthy" or sterilized "healthy" soils. Of the *Pythium* spp. isolated from stunted alfalfa seedlings, *P. paroecandrum* caused preemergence damping-off and stunting similar to that observed in AS fields. Seed treatment with metalaxyl increased seedling survival in AS soils but did not improve plant growth. Seedlings inoculated with VA mycorrhizae, however, grew more rapidly than nonmycorrhizal seedlings. Metalaxyl had a negligible effect on VA mycorrhizal infection of alfalfa root segments but reduced the VA mycorrhizal colonization of root length. This study suggested that a combination of metalaxyl and VA mycorrhizae could be used to produce healthy alfalfa seedlings in AS soils.

"Alfalfa sickness" (AS) was first described in 1962 in Alberta. Yields of alfalfa grown in fields previously cropped with alfalfa were lower than yields from fields not previously cropped with alfalfa (29). Plants grown in AS soils are stunted and chlorotic and eventually become flaccid. Poor seed germination and the death of plants result in irregular bare patches in the field. Alfalfa grown in sterilized AS soils does not show these symptoms, suggesting that the primary cause of the AS condition is a biotic agent(s) (9). The pathogen responsible for AS, however, has not been conclusively identified. *Phytophthora megasperma* f. sp. *medicaginis* (7), the nematode *Pratylenchus projectus* Jenkins (14), *Pythium* spp. (26), *Cylindrocarpon gracile*, and *Fusarium roseum* (4,22) often in association with low fertility have all been implicated as causes.

In California, growth of citrus trees can be reduced in soil where citrus has been grown for a long time. This condition is known as "soil sickness" (18). Stunting of seedlings following soil fumigation and soil sterilization to eliminate "soil sickness" was attributed to inadequate nutrition caused by killing mycorrhizal fungi during fumigation and sterilization (16,23,27,28). Inoculation of stunted citrus seedlings with mycorrhizal fungi increases their growth. In addition, mycorrhizal fungi protect roots from certain root-infecting fungi (1,3,8,11, 15,25). Disease tolerance in mycorrhizae-

Accepted for publication 8 December 1987.

inoculated seedlings is attributed to improved nutrient uptake, the production of antibiotics, altered root exudates, and changes in the microbial rhizosphere populations (6).

Metalaxyl (Apron, 28.35% a.i.), a systemic fungicide of the acylalanine class, controls root rot incited by Oomycetes (12). Nemec (19) observed that sour orange seedlings have more vesicular-arbuscular (VA) mycorrhizal colonization when grown in metalaxyl-treated soil than in untreated soil. This suggested that it might be possible to grow healthy, vigorous alfalfa in fields with AS by infesting the soil with VA mycorrhizal fungi and planting fungicide-treated alfalfa seeds. The objectives of this study were to determine the cause of poor alfalfa seedling growth in "sick" soil and to evaluate the effect of metalaxyl seed treatment and VA mycorrhizae on germination and growth of alfalfa seedlings in "sick soil."

MATERIALS AND METHODS

Growth conditions and VA mycorrhizal cultures. All studies were conducted in 13-cm-diameter pots containing the autoclaved (121 C for 1 hr) and nonautoclaved soils. Pots were randomly arranged in a growth chamber at 20 C (16-hr day) and 15 C (8-hr night); light intensity of 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ was provided by cool-white fluorescent tubes. Plant height was measured from the soil surface to the top leaf. Fresh weights and dry weights (dried at 70 C for 24 hr) were also established. Data were analyzed by analysis of variance and means were separated by Duncan's multiple range test. A $P = 0.05$ level of significance was employed for all statistical tests.

Glomus fasciculatus (Thaxter) Gerd. & Trappe and *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe were supplied by B. Mosse, Rothamsted Experimental Station, England, and *Glomus* spp. were obtained from alfalfa roots collected near Edmonton. They were maintained on roots of onion (*Allium cepa* L.) seedlings grown in a steam-sterilized mixture of sand and loam in plastic pots in a growth chamber. Two months after inoculation, the fibrous roots were collected, chopped, and mixed with the remaining soil. The mixture of soil and segmented roots was air-dried without free water, packed in plastic bags, and stored at 4 C until used.

Comparison of field soils. Three soils were compared: soil A and soil B from Spruce Grove, 28 km west of Edmonton, known as "sick" soil, and soil C, from Vegreville, 90 km east of Edmonton, known as "healthy" soil. Within each field, soil was collected from several random locations to a depth of 15 cm, then thoroughly mixed and passed through a 35-mesh screen, bulked, and stored at 4 C. The respective properties of sandy loam soils A, B, and C were: saturated paste pH value (using 0.01 M $CaCl_2$) of 5.5, 6.6, and 6; 3.69, 3.37, and 4.55% organic matter; 20.8, 34, and 28 ppm P; 121, 175, and 214.7 ppm K; and 28.3, 15, and 10.3 ppm Na. Part of each soil sample was autoclaved at 121 C for 1 hr prior to planting. Seeds of Anchor alfalfa (*Medicago sativa* L.) were surface-sterilized in 70% ethanol for 2 min followed by 2 min in 0.6% sodium hypochlorite, then rinsed three times in distilled water. Ten seeds were planted 2 cm deep in the sterilized and nonsterilized soils, and there were 10 replicate pots for each treatment. The experiment was repeated using seeds of Beaver alfalfa. Percent seedling survival was recorded 14 days after seeding and plant height was measured 1 mo after seeding. Roots of stunted Anchor alfalfa seedlings grown in nonsterilized soils A and B were washed free from soil, surface-sterilized by a 5- to 7-sec dip in a 1:1 mixture of 70% ethanol and 0.6% sodium hypochlorite, and plated on dilute potato-dextrose agar (PDA) (40 g of fresh potato, 5 g of dextrose, and 20 g of agar per liter, with 100 $\mu g/L$ of streptomycin added to inhibit bacteria). Portions of hyphal tips from mycelia that grew from root tissue were transferred to fresh plates of dilute PDA with streptomycin, and the representative fungal cultures were sent to the Biosystematics Research Centre in Ottawa for identification. These isolates were used in subsequent pathogenicity studies.

Pathogenicity studies. Five 6-mm-diameter agar disks from 4-day-old cultures of 18 different *Pythium* isolates were added to an autoclaved (45 min) mixture of cornmeal (5 g), sand (485 g), and water (120 ml) in 1,000-ml Erlenmeyer flasks. Inoculated flasks were incubated at 20 C for 2 wk and shaken periodically to ensure complete colonization of the mixture. The inoculum was then mixed with an autoclaved sand and loam mixture (1:1, v/v) at 1:3 and 1:7, referred to as 1/4 and 1/8 inoculum dilutions, respectively. Uninoculated autoclaved cornmeal and sand culture was used as a control. Soil of each of the 38 treatments (2 dilutions × 18 isolates plus controls) was placed in 13-cm-diameter plastic pots. Ten surface-sterilized seeds of Beaver alfalfa were planted in each of five replicate pots of each treated soil. Percent seedling survival and plant height were recorded 1 mo after seeding.

Metalaxyl seed treatment. Thirteen-cm-diameter plastic pots were filled with either of two dilutions of inoculated sand and loam containing each of seven pathogenic isolates of *P. paroecandrum* Drechsler. An uninoculated autoclaved cornmeal and sand culture was used as a control. Surface-sterilized Beaver alfalfa seeds (10/pot) were treated with metalaxyl at 1 ml/kg of seed. Four treatment combinations were used: 1) 1/8 dilution of *P. paroecandrum*, metalaxyl-treated alfalfa seeds; 2) 1/8 dilution, untreated seeds; 3) 1/4 dilution, metalaxyl-treated seeds; and 4) 1/4 dilution, untreated seeds. Eight replicate pots were used for each treatment. Percent seedling survival and plant height were recorded 1 mo after seeding.

VA mycorrhizae in *P. paroecandrum*-infested soil. The effect of species of *Glomus* in reducing the disease severity caused by *P. paroecandrum* was tested in two studies. In the first study, metalaxyl-treated seeds of Beaver alfalfa were sown in sterilized vermiculite, and after 1 wk five seedlings were transplanted into pots of *P. paroecandrum*-infested soil (1/4 inoculum dilution of seven pathogenic isolates) that had received a layer of inoculum (10 ml) of *G. fasciculatus, G. mosseae, Glomus* spp., or roots of onion without mycorrhizal infection as a control. Ten replicate pots were used for each treatment. Two months after transplanting, fresh and dry weights and shoot heights were determined. In the second study, metalaxyl-treated or untreated seeds of Anchor alfalfa were sown in pots (10/pot) with soil A that had received a layer of *Glomus* spp. inoculum placed 3 cm beneath the seeds. Pots that had received a layer of VA mycorrhizae-free onion roots served as controls. The four treatments were *Glomus* spp. only, metalaxyl only, *Glomus* spp. and metalaxyl, and control. After emergence, the seedlings were thinned to five per pot. After 2 mo, the seedlings were harvested and fresh and dry weights of shoots and roots were determined. Cores (10-mm diameter) of soil were removed from each pot after harvest and 50 1-cm-long root segments were randomly selected from each pot. Percentage of root length and

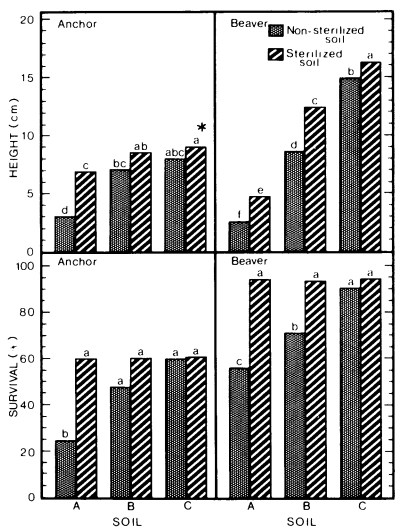

Fig. 1. Percent survival and shoot height of Anchor and Beaver alfalfa seedlings grown in sterilized and nonsterilized "sick" soils (soils A and B) and soil collected from "healthy" soil (soil C). *Means within alfalfa varieties followed by the same letter are not significantly different using Duncan's multiple range test.

of root segments colonized by VA mycorrhizae were determined by microscopically examining five sites on each root segment after clearing and staining the roots (10,20).

RESULTS

Comparison of field soils. Alfalfa seedlings of both Anchor and Beaver grown in pots of nonsterilized "sick" soils (soils A and B) were stunted and resembled those with AS, whereas alfalfa seedlings grown in sterilized A and B soils and in "healthy" soil (soil C) were not stunted. Beaver alfalfa plants were taller and survival was greater in soil C than in soils A and B (Fig. 1). There was no significant difference in plant growth of Anchor alfalfa between soils B and C. Sterilization of soil A significantly improved plant height and percent survival of both alfalfa cultivars, whereas sterilization of soil B significantly improved plant height and percent survival of Beaver only. Sterilization of soil C had no effect on plant height or percent survival in each cultivar.

Fungal isolation and pathogenicity. Two species of *Pythium, P. paroecandrum* and *P. sylvaticum* Campbell & Hendrix, were identified from 18 isolates obtained from root tips of alfalfa seedlings grown in "sick" soils. The virulence of the 18 *Pythium* isolates to Beaver alfalfa is shown in Table 1. Some *P. paroecandrum* isolates (S-2, S-6, S-8, S-16, S-21, S-22, and S-24) caused a reduction in seedling emergence and seedling growth typical of that observed in AS fields.

Metalaxyl seed treatment. Variability among the seven isolates of *P. paroecandrum* occurred for seedling survival and plant height of seeds not treated with metalaxyl (Table 2). After seed treatment with metalaxyl, there was no significant difference in percent seedling survival between the control and infested soil with the exception of isolate S-6 in the 1/4 dilution treatment. The improvement in plant height after seed treatment with metalaxyl was not as great as the improvement in percent seedling survival.

Effects of VA mycorrhizae. In the first study, VA mycorrhizal inoculation of Beaver alfalfa seedlings grown in *P. paroecandrum*-infested soil increased shoot height and fresh and dry weights of shoots compared with nonmycorrhizal seedlings (Fig. 2). Alfalfa inoculated with *Glomus* spp. produced more plant growth than those inoculated with either *G. mosseae* or *G. fasciculatus*. In the

Table 1. Pathogenicity of different *Pythium* isolates from alfalfa roots to Beaver alfalfa seedlings[y]

		Survival (%)		Plant height (cm)	
		Inoculum dilution (v/v)		Inoculum dilution (v/v)	
Pythium species	Isolate	1/8	1/4	1/8	1/4
P. paroecandrum	S-2	ND[z]	2	ND	0.4
	S-6	ND	0	ND	0
	S-8	16	14	1.6	1.6
	S-16	8	16	1.4	1.6
	S-21	18	12	2.1	1.5
	S-22	8	8	2.1	0.7
	S-24	2	3	0.5	0.3
P. sylvaticum	S-3	80	84	2.7	2.1
	S-10	40	54	3.2	2.9
	S-17	40	50	2.2	2.5
	S-20	80	82	2.4	2.1
Pythium spp.	S-1	88	78	2.4	2.3
	S-9	68	78	2.1	1.9
	S-15	64	70	2.6	2.3
	S-18	48	74	2.1	1.4
	S-19	94	78	2.5	2.0
	S-23	68	84	3.0	2.4
	S-25	72	72	2.9	2.4
Control		76	88	2.0	2.1
LSD (0.05)		22	18	1.3	0.8

[y] Different inoculum mixed with an autoclaved sand and loam mixture; 10 seeds per pot, five pots per treatment. Percent seedling survival and plant height recorded 1 mo after seeding.
[z] ND = not done.

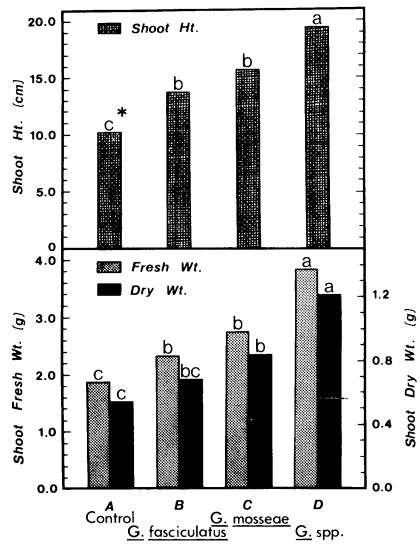

Fig. 2. Effect of inoculation with three mycorrhizal fungi on shoot height and fresh and dry shoot weights of Beaver alfalfa grown in sand and loam mixture infested with *Pythium paroecandrum* at 1/4 inoculum dilution. Four treatments were control, *Glomus fasciculatus*, *G. mosseae*, and *Glomus* spp. *Means followed by the same letters are not significantly different using Duncan's multiple range test.

Table 2. Effect of metalaxyl seed treatment on seedling survival and shoot height of Beaver alfalfa in soils infested with *Pythium paroecandrum*[y]

	Survival (%)				Plant height (cm)			
	Inoculum dilution (v/v)				Inoculum dilution (v/v)			
	1/8		1/4		1/8		1/4	
Isolate	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
S-2	85 ab[z]	53 bcd	83 ab	58 b	3.4 d	3.3 bc	4.8 bc	4.6 b
S-21	94 a	71 ab	90 a	54 bc	4.6 cd	4.9 ab	5.1 bc	3.8 bc
S-6	79 b	56 bcd	73 b	53 bc	6.3 ab	5.5 a	4.0 c	3.7 bc
S-16	93 a	61 bc	88 ab	46 bc	5.4 bc	5.5 a	5.6 b	3.9 bc
S-22	94 a	39 d	90 a	41 c	4.5 cd	4.4 abc	4.4 bc	3.3 c
S-24	89 ab	55 bcd	86 ab	28 d	3.5 d	2.6 c	2.5 d	1.8 d
S-8	90 a	45 cd	93 a	19 d	6.9 ab	4.2 abc	4.6 bc	3.6 bc
Control	87 ab	85 a	89 a	89 a	7.6 a	6.4 a	8.5 a	8.5 a

[y] Ten seeds per pot, eight pots per treatment.
[z] Means within a column followed by the same letter are not significantly different using Duncan's multiple range test ($P = 0.05$).

second study, the combined treatment of metataxyl and *Glomus* spp. increased shoot fresh and dry weights of alfalfa compared with the control and with metalaxyl only (Fig. 3). The combined treatment of metalaxyl and *Glomus* spp. increased root fresh and dry weights compared with *Glomus* spp. inoculation. There were no differences between root fresh and dry weights of the combined treatment and the control or metalaxyl treatments.

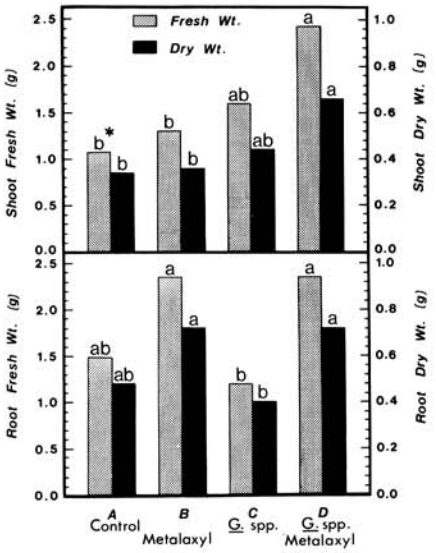

Fig. 3. Effect of vesicular-arbuscular mycorrhizae and metalaxyl alone or combined on shoot and root weights of 2-mo-old Anchor alfalfa seedlings grown in "sick" soil. Four treatments were control, metalaxyl alone, *Glomus* spp. alone, and metalaxyl and *Glomus* spp. *Means within fresh or dry weights followed by the same letter are not significantly different using Duncan's multiple range test.

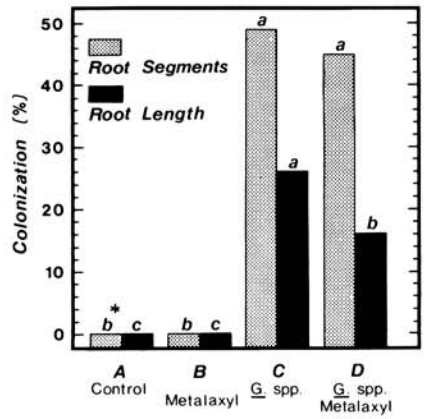

Fig. 4. Effect of vesicular-arbuscular mycorrhizae and metalaxyl alone or combined on mycorrhizal colonization of roots of 2-mo-old Anchor alfalfa seedlings grown in "sick" soil. Four treatments were control, metalaxyl alone, *Glomus* spp. alone, and metalaxyl and *Glomus* spp. *Means within root classes followed by the same letter are not significantly different using Duncan's multiple range test.

The percentage of root segments infected with VA mycorrhizae in nonsterilized "sick" soil was high in all pots of soil inoculated with *Glomus* spp. at the end of the experiment (Fig. 4) and was not affected by the metalaxyl treatment. However, the percentage of root length colonized was reduced from 26 to 16% by metalaxyl treatment. Alfalfa seedlings receiving the combined treatment of metalaxyl and *Glomus* spp. grew faster and had larger, darker green leaves than nonmycorrhizal seedlings (Fig. 5). No mycorrhizal colonization was detected in plants not inoculated with *Glomus* spp.

DISCUSSION

Alfalfa growth was increased in "sick" soil by autoclaved sterilization, but the increased growth was not comparable to that of plants in sterilized or nonsterilized "healthy" soil. This confirms the observation that heat sterilization eliminated AS in soils (29).

Previous studies reported a significant difference in dominant *Pythium* species between field sites and suggested that rootlet infection of alfalfa could directly affect forage yields and that chronic stresses could indirectly contribute to stand decline (2,13,24). The present investigation in support of the above observations revealed that *P. paroecandrum* is a cause of poor emergence, damping-off, and stunting of alfalfa seedlings grown in "sick" soil. This species has been reported as a seedling pathogen of alfalfa in Ohio (24) and California (13). *P. sylvaticum* was less pathogenic than *P. paroecandrum*. In Iowa, *P. sylvaticum* has been shown to cause rapid necrosis of germinating seed and seedlings of alfalfa and to retard seedling development through root infection (2). Metalaxyl provided some control of AS, as it increased seedling survival in soil infested with *Pythium* spp. However, considerable variability in sensitivity to metalaxyl exists among and even within species and strains of *Pythium* spp. (5).

Alfalfa growth is stimulated by infection with mycorrhizal fungi in "sick" soil. It is unlikely that "sick" soil had no VA mycorrhizae. More likely, the indigenous mycorrhizal fungi in "sick" soil were less effective in root infection or in stimulating plant growth than the mycorrhizae used in this study (17,21). The lack of response may also reflect low spore densities in "sick" soil.

These results indicate that VA mycorrhizae can not only stimulate alfalfa growth in "sick" soils but also can reduce the severity of damage from *P. paroecandrum* infection. Moreover, as shown in previous studies (12,19), metalaxyl had a negligible effect on VA mycorrhizal infection of alfalfa root segments. The percentage of root length colonized was quite reduced, however. It may be possible to reclaim "sick" soil by artificially inoculating it with mycorrhizal fungi and treating seeds with metalaxyl. Further field studies on this aspect are required.

ACKNOWLEDGMENTS

I thank L. J. Piening, D. Gaudet, B. Gossen, B. Bolwyn, H. Philip, and D. Orr for valuable criticism and suggestions and also D. Aiello and M. Herbut for technical assistance.

Fig. 5. Effect of vesicular-arbuscular mycorrhizae and metalaxyl alone or combined on the growth of 2-mo-old Anchor alfalfa seedlings in "sick" soil. Four treatments were control (pot A), metalaxyl alone (pot B), *Glomus* spp. alone (pot C), and metalaxyl and *Glomus* spp. (pot D).

LITERATURE CITED

1. Baltruschat, H., and Schonbeck, F. 1975. The influence of endotrophic mycorrhiza on the infestation of tobacco by *Thielaviopsis basicola*. Phytopathol. Z. 84:172-188.
2. Buchholtz, W. F. 1942. Influence of cultural factors on alfalfa seedling infection by *Pythium debaryanum* Hesse. Iowa Agric. Exp. Stn. Res. Bull. 296.
3. Chakravarty, P., and Mishra, R. R. 1986. Influence of endotrophic mycorrhizae on the Fusarial wilt of *Cassia tora* L. Phytopathol. Z. 115:130-133.
4. Chang, K. F., and Blenis, P. V. 1987. Pathogenicity testing of three fungi recovered from roots and crowns of diseased alfalfa in central Alberta. Can. Plant Dis. Surv. 67:11-13.
5. Cook, R. J., and Zhang, B.-X. 1985. Degrees of sensitivity to metalaxyl within the *Pythium* spp. pathogenic to wheat in the Pacific Northwest. Plant Dis. 69:686-688.
6. Cooper, K. M. 1984. Physiology of VA mycorrhizal associations. Pages 155-186 in: VA Mycorrhiza. C. L. Powell and D. J. Bagyaraj, eds. CRC Press, Inc., Boca Raton, FL. 234 pp.
7. Damirgi, S. M., Cook, F. D., and Webster, G. R. 1978. Incidence of a root rot fungus in a diseased alfalfa soil of central Alberta. Can. J. Soil Sci. 58:229-236.
8. Davis, R. M., and Menge, J. A. 1981. *Phytophthora parasitica* inoculation and intensity of vesicular-arbuscular mycorrhizae in citrus. New Phytol. 87:705-715.
9. Faechner, T. R., and Bolton, J. L. 1978. Chemical control of alfalfa sickness. Can. J. Plant Sci. 58:891-892.
10. Giovannetti, M., and Mosse, B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84:489-500.
11. Graham, J. H., and Menge, J. A. 1982. Influence of vesicular-arbuscular mycorrhizae and soil phosphorus on take-all disease of wheat. Phytopathology 72:95-98.
12. Groth, D. E., and Martinson, C. A. 1983. Increased endomycorrhizal infection of maize and soybeans after soil treatment and metalaxyl. Plant Dis. 67:1377-1378.
13. Hancock, J. G. 1985. Fungal infection of feeder rootlets of alfalfa. Phytopathology 75:1112-1120.
14. Hawn, E. J., and Kozub, G. C. 1978. Influence of *Pratylenchus projectus* on alfalfa sickness in Alberta. Can. Plant Dis. Surv. 58:1-4.
15. Kaye, J. W., Pfleger, F. L., and Stewart, E. L. 1984. Interaction of *Glomus fasciculatum* and *Pythium ultimum* on greenhouse-grown poinsettia. Can. J. Bot. 62:1575-1579.
16. Kleinschmidt, G. C., and Gerdemann, J. W. 1972. Stunting of citrus seedlings in fumigated nursery soils related to the absence of endomycorrhizae. Phytopathology 62:1447-1453.
17. Lambert, D. H., Cole, H., Jr., and Baker, D. E. 1980. Variation in the response of alfalfa clones and cultivars to mycorrhizae and phosphorus. Crop Sci. 20:615-618.
18. Martin, J. P. 1948. Effect of fumigation, fertilization, and various other soil treatments on growth of orange seedlings in old citrus soils. Soil Sci. 66:273-288.
19. Nemec, S. 1980. Effects of 11 fungicides on endomycorrhizal development in sour orange. Can. J. Bot. 58:522-526.
20. Phillips, J. M., and Hayman, D. S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158-161.
21. Powell, C. L. 1984. Field inoculation with VA mycorrhizal fungi. Pages 205-222 in: VA Mycorrhiza. C. L. Powell and D. J. Bagyaraj, eds. CRC Press, Inc., Boca Raton, FL. 234 pp.
22. Reeleder, R. D. 1982. Fungi recovered from diseased roots and crowns of alfalfa in north central Alberta and the relationship between disease severity and soil nutrient levels. Can. Plant Dis. Surv. 62:21-27.
23. Schenck, N. C., and Tucker, D. P. H. 1974. Endomycorrhizal fungi and the development of citrus seedlings in Florida fumigated soils. J. Am. Soc. Hortic. Sci. 99:284-287.
24. Schmitthenner, A. F. 1964. Prevalence and virulence of *Phytophthora, Aphanomyces, Pythium, Rhizoctonia*, and *Fusarium* isolated from diseased alfalfa seedlings. Phytopathology 54:1012-1018.
25. Schonbeck, F., and Dehne, H. W. 1977. Damage to mycorrhizal and nonmycorrhizal cotton seedlings by *Thielaviopsis basicola*. Plant Dis. Rep. 61:266-267.
26. Stelfox, D., and Williams, J. R. 1980. *Pythium* species in alfalfa fields in central Alberta. Can. Plant Dis. Surv. 60:35-36.
27. Timmer, L. W., and Leyden, R. F. 1978. Stunting of citrus seedlings in fumigated soils in Texas and its correction by phosphorus fertilization and inoculation with mycorrhizal fungi. J. Am. Soc. Hortic. Sci. 103:533-537.
28. Timmer, L. W., and Leyden, R. F. 1978. Relationship of seedbed fertilization and fumigation to infection of sour orange seedlings by mycorrhizal fungi and *Phytophthora parasitica*. J. Am. Soc. Hortic. Sci. 103:537-541.
29. Webster, G. R., Khan, S. U., and Moore, A. W. 1967. Poor growth of alfalfa (*Medicago sativa*) on some Alberta soils. Agron. J. 59:37-41.