Effects of Rhizobia, Metalaxyl, and VA Mycorrhizal Fungi on Growth, Nitrogen Fixation, and Development of Pythium Root Rot of Sainfoin

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ABSTRACT

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The potential for *Pythium* species to reduce sainfoin (*Onobrychis viciifolia*) seedling growth and for rhizobia and vesicular-arbuscular (VA) mycorrhizal fungi to enhance seedling growth were studied under controlled conditions. Seedling emergence and shoot dry weights of cvs. Nova and Melrose in pasteurized soils infested with each of 18 isolates of *Pythium* spp. were reduced. Seedlings inoculated with arctic rhizobia grew more rapidly than noninoculated seedlings. Plant growth, nodule dry weight, nitrogenase activity, and mycorrhizal colonization of sainfoin dually inoculated with *Rhizobium* plus either *Glomus fasciculatus* or *G. intraradices* were greater than those of uninoculated plants or those inoculated with either rhizobia or mycorrhizal fungus alone; nitrogen content was higher in plants concomitantly inoculated with *Rhizobium* plus *G. intraradices*. Seedling survival and growth in *Pythium*-infested soil increased for seeds treated with metalaxyl. Interactions of VA mycorrhizal fungi and two isolates of *Pythium* were investigated under controlled conditions. Shoot dry weights of sainfoin plants inoculated with VA mycorrhizal fungi significantly exceeded those of nonmycorrhizal plants. Seedlings inoculated with VA mycorrhizal fungi had a lower incidence of root rot than nonmycorrhizal ones. This study suggested that a combination of rhizobia, metalaxyl, and VA mycorrhizal fungi could be used to improve the establishment and vigor of sainfoin in *Pythium*-infested soils.

Additional keyword: fungicide

Sainfoin (*Onobrychis viciifolia* Scop.) is a long-lived, deep-rooted perennial legume that has been cultivated as a forage crop in Europe and Asia for several centuries (26). Despite having several advantages over alfalfa (*Medicago sativa* L.), sainfoin has never become a widely grown crop in Alberta (22). Although many factors undoubtedly contribute to stand and yield decline problems (22, 23,38), one of the major constraints restricting sainfoin cultivation in Alberta has been poor seed germination and seedling growth, attributed to Pythium root rot as well as to poor nodulation (22). Previous studies reported that species of *Pythium,* which are ubiquitous in cultivated soil, are also the primary causal agents of poor emergence, damping-off, and stunting of alfalfa seedlings (15,20). Severe root rot reduces the number of surviving roots available for symbiotic nodulation (39).

Many legumes benefit from the synergistic effect of dual inoculation with *Rhizobium* and vesicular-arbuscular (VA) mycorrhizal fungi (4,27,33). Nodules formed by *Rhizobium* on legumes fix atmospheric nitrogen, whereas VA mycorrhizal fungi improve phosphate uptake, which indirectly enhances the nitrogen status of the legumes (4,17). Nodulated and mycorrhizal plants are therefore adapted to cope with nutrient-deficient situations. *Rhizobium* and VA mycorrhizal fungi also have been reported to protect roots from certain root-infecting fungi (5–8, 10,20,24,40); however, the effect on plant disease is very difficult to demonstrate because the interactions involving *Rhizobium* and VA mycorrhizal and root-infecting fungi vary with the species of *Rhizobium* and mycorrhizal fungi, and with plant cultivar (11,36,37).

Metalaxyl (Apron, 28.35% a.i.), a systemic fungicide of the acylalanine class, controls Pythium root rot (9) and has a negligible effect on rhizobial and mycorrhizal infection of roots of legumes (20,34). Published information on the influence of rhizobia, VA mycorrhizal fungi, and metalaxyl on Pythium root rot of sainfoin does not exist. Consequently, studies were conducted under controlled environment conditions to 1) isolate and identify *Pythium* species causing poor seed germination and seedling growth, 2) assess the combined effects of rhizobia and VA mycorrhizal fungi (*Glomus fasciculatus* (Thaxter) Gerd. & Trappe and *G. intraradices* Schenck & Smith) on nodulation and growth, and 3) investigate the integrated effects of metalaxyl, rhizobia, and VA mycorrhizal fungi on germination and growth of sainfoin in *Pythium*-infested soils. Preliminary work has been reported (21).

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MATERIALS AND METHODS

All studies were conducted in 15-cm-diameter fiber pots containing a steam-pasteurized (121 C for 2 hr) mixture of loam, sand, and vermiculite (3:1:1, v/v). For all tests, seed was surface-disinfested in 70% ethanol for 2 min followed by 2 min in 0.6% sodium hypochlorite, and then rinsed three times in distilled water. Seeds were planted 1.5-cm deep in each pot. All pots were randomly arranged in a controlled environment growth chamber at 20 C (16-hr day) and 15 C (8-hr night); light intensity of 300 $\mu E \cdot s^{-1} \cdot m^{-2}$ was provided by cool-white fluorescent tubes and incandescent bulbs. During all tests, adequate spacing was left between pots, and plants were watered carefully to prevent cross contamination between treatments.

Isolation of *Pythium* species and pathogenicity studies. *Pythium* spp. were isolated from rootlets and necrotic root tips collected from several sainfoin fields in southern Alberta with poor seedling establishment, either on 0.2% water agar or on a *Pythium*-selective Mircetich's (29) pimaricin-vancomycin agar medium with rose bengal at 0.01 g/L. The plates were incubated 48 hr in darkness at 20 C. Portions of hyphal tips from mycelia that grew from root tissue were transferred onto cornmeal agar, and the resulting representative fungal cultures were identified and used in subsequent pathogenicity studies.

One colonized 6-mm-diameter agar disk, taken from 4-day-old cultures of each of the 18 different *Pythium* isolates, was grown on a 20% V8 juice agar plate for 10 days. The inoculum was prepared by comminuting fungal culture from a 90-mm petri plate with 200 ml of distilled water for 15 sec in a Waring Blendor; 25 ml of the inoculum of each of the 18 isolates was poured over plastic pots containing a steam-pasteurized soil mixture. Noninoculated V8 juice agar culture was used as a control. After applying the inoculum, 5 cm of steam-pasteurized soil mixture was added on top of the inoculated soil. Twenty-five seeds of sainfoin cv. Melrose were planted in each of eight replicate inoculated pots. Pots were randomized in a growth chamber, and the experiment was repeated using seeds of

sainfoin cv. Nova. Percent seedling survival was recorded 14 days after seeding, and plant height was measured one month after seeding.

Rhizobial inoculation. Rhizobial cultures of N-10, N-31, and SM-2 isolated from arctic legumes (32), and of 116A-12, 116A-15, and 116A-17 obtained from Nitrogen Sales Corp. (Milwaukee, WI) were maintained on yeast-extract manitol agar slants (41). Inoculum for each rhizobial isolate was produced in 250-ml conical flasks containing 100 ml of sterile yeast-extract manitol broth. Flasks were incubated for 6 days at room temperature (25 ± 2 C) on a rotary shaker (200 rpm). The culture of each flask was centrifuged at 8,000 rpm for 20 min, and the bacterial cells were resuspended in sterile distilled water to a concentration of 10^8 cells/ml.

Ten seeds of sainfoin cv. Melrose were planted, and after emergence the seedlings were thinned to five plants per pot. Plants were inoculated with each of the six rhizobial isolates (100 ml/pot) twice, 10 and 40 days after planting. Autoclaved rhizobial inoculum was applied to control pots. Every two weeks, pots were fertilized (100 ml/pot) with N-free Hoagland's nutrient solution (19). Ten replicate pots were used for each treatment, and the experiment was repeated using seeds of sainfoin cv. Nova. Plants were harvested 2 and 3 mo after seeding, and fresh and dry shoot weights (dried at 70 C for 24 hr) were recorded.

Dual inoculation with rhizobia and VA mycorrhizal fungi. Two species of VA mycorrhizal fungi, *G. fasciculatus* and *G. intraradices,* were used in this study and maintained on roots of onion (*Allium cepa* L.) seedlings as previously described by Hwang et al (24). Just before inoculation, the suspensions of three isolates (N-10, N-31, and SM-2) were mixed in equal volumes and added to surface-disinfested seeds of sainfoin for 2 days on moistened filter paper in petri plates. A layer of inoculum (50 ml) of *G. fasciculatus* or *G. intraradices* was placed 5 cm below the soil surface in pots before sowing to produce the mycorrhizal plants. There were six treatments: *G. fasciculatus, G. intraradices, Rhizobium, G. fasciculatus* plus *Rhizobium, G. intraradices* plus *Rhizobium,* and control. The control treatment received only uninoculated onion roots. There were four seedlings of sainfoin cv. Nova per pot and 10 pots per treatment. Pots were fertilized once each week with quarter-strength Long Ashton nutrient solution (18). Nitrogenase activity of the plants was determined by the acetylene reduction test (16). After 10 wk of growth, the roots were placed in a jar, 10 ml of air was removed with a syringe and replaced with 10 ml of acetylene, and the samples were then incubated at room temperature for 30 min in the dark. Duplicate gas samples of 0.3-ml volume were collected with a syringe from each jar and analyzed using a Hewlett Packard gas chromatograph equipped with a flame ionization detector and a column packed with Poropak-R, 80-100 mesh. Total nitrogen content of the dried sample was measured by a micro-Kjeldahl method (42). Mycorrhizal colonization was evaluated on roots cleared and stained with 0.1% Chlorazol Black E (43). Percent root length infected and frequency of arbuscule of *G. fasciculatus* and *G. intraradices* were determined by the observative scale measurement method (28). At the end of the experiment, dry weights of nodules, roots, and shoots were recorded. The experiment was repeated, and since the results were nearly identical, the data were pooled to represent the average of the two experiments.

Metalaxyl seed treatment. The cornmeal-sand inocula containing nine virulent *Pythium* isolates were produced according to the method described by Hwang (20) and mixed with the steam-pasteurized soil mixture to a rate of 500 propagules per gram of soil mixture. Uninoculated autoclaved cornmeal and sand culture were used as a control. A 10 × 2 factorial design was used with separate pots filled with uninoculated mixture and inoculated with each of nine pathogenic *Pythium* isolates as one factor and two levels of seed treatments (with and without metalaxyl at a rate of 0.35 ml a.i./kg of seed) as the other factor. Ten seeds of sainfoin cv. Nova were planted in each pot, and 10 replicate pots were used for each treatment. The experiment was repeated with seeds of cv. Melrose. Percent seedling survival was recorded 14 days after seeding, and plant height and shoot dry weights were measured 1 mo after seeding.

Integrated effects of metalaxyl, rhizobia, and VA mycorrhizal fungi in *Pythium*-infested soils. Ten metalaxyl-treated or untreated seeds of sainfoin cv. Nova were planted in each pot containing the soil infested with *Pythium* isolate P-16 with a layer of mycorrhizal inoculum (50 ml) 5 cm below the soil surface. Each seed was inoculated in the planting hole with 2 ml of the mixed rhizobial cell suspension (10^8 cells/ml). The following treatments were employed: *G. fasciculatus, G. intraradices,* metalaxyl, *G. fasciculatus* plus metalaxyl, *G. intraradices* plus metalaxyl, and control. There were eight replicate pots per treatment, and the experiment was repeated with isolate P-18. Percent seedling survival was recorded 2 wk after seeding. Seedlings were harvested 2 and 3 mo after seeding, and shoot dry weights were recorded. At the end of the experiment, the root systems were carefully washed free of soil, and root dry weights were determined.

Data analysis. Data were subjected to analysis of variance, and means were compared using Duncan's new multiple range test or a least significant difference test at the $P = 0.05$ level of significance on SAS software (35).

Table 1. Pathogenicity of different *Pythium* isolates from sainfoin roots to sainfoin cvs. Nova and Melrose seedlings[z]

Pythium sp. Isolate	Nova		Melrose	
	Survival (%)	Plant height (cm)	Survival (%)	Plant height (cm)
P. irregulare				
P-3	25	6.85	10	9.71
P-7	51	8.97	22	9.24
P-8	31	6.92	21	8.08
P-12	30	9.46	31	8.38
P-13	38	8.42	34	8.89
P-16	13	5.91	7	5.12
P. paroecandrum				
P-1	32	7.56	19	7.46
P-2	40	8.25	16	7.86
P-18	15	4.98	9	5.81
P. sylvaticum				
P-5	60	8.83	37	8.84
P-9	57	8.32	46	9.14
P-19	70	9.26	73	10.29
P-20	65	11.18	80	10.15
P-21	78	10.10	57	8.55
Pythium spp.				
P-4	51	8.97	45	9.37
P-10	70	9.29	72	9.91
P-11	64	10.33	76	10.57
P-14	71	8.72	46	9.96
Control	75	10.14	64	9.97
LSD (0.05)	19	2.58	18	2.56

[z] Twenty-five seeds per pot, eight replicate pots per treatment. Percent seedling survival recorded 2 wk after seeding and plant height measured 1 mo after seeding.

RESULTS

Fungal isolation and pathogenicity. Three species of *Pythium, P. irregulare* Buisman, *P. paroecandrum* Drechs., and

P. sylvaticum W. A. Campbell & J. W. Hendrix, were identified from 18 isolates obtained from sainfoin roots collected from fields with poor seedling establishment. There was considerable variation in pathogenicity among isolates (Table 1). Generally, *P. irregulare* and *P. paroecandrum* isolates were more pathogenic than were *P. sylvaticum* isolates. Lowest percent seedling survival and plant heights were recorded with isolates P-16 of *P. irregulare* and P-18 of *P. paroecandrum* (Table 1).

Rhizobial inoculation. Sainfoin plants treated with rhizobia had abundant nodulation and were free from root rot symptoms. For cv. Melrose, all rhizobial strains had higher fresh and dry weights than those of the control on both harvest dates (Table 2). For cv. Nova, isolates SM-2 and 116A-15 had higher fresh and dry weights than the control for both harvest dates. Isolates N-10, N-31, 116A-12, and 116A-17 had higher fresh and dry weights than the control at second harvest but not at the first (Table 2).

Dual inoculation with rhizobia and VA mycorrhizal fungi. Shoot and root dry weights were higher in sainfoin co-inoculated with *Rhizobium* plus *G. fasciculatus* or *Rhizobium* plus *G. intraradices* than in sainfoin inoculated singly with *Rhizobium, G. fasciculatus,* or *G. intraradices* (Table 3). Nodule dry weight was higher in plants inoculated with *Rhizobium* plus *G. fasciculatus* or *G. intraradices* than in plants inoculated with *Rhizobium* alone. There was no nodulation in noninoculated plants and or plants inoculated only with *G. fasciculatus* or *G. intraradices* (Table 3).

Nitrogen content was significantly higher in plants inoculated with *Rhizobium* in combination with *G. intraradices* or *G. fasciculatus* than with *G. fasciculatus, G. intraradices,* or *Rhizobium* alone (Table 3). The highest nitrogen content was recorded following concomitant inoculation with *Rhizobium* plus *G. intraradices.* Nitrogenase activity also was higher when sainfoin was inoculated with *Rhizobium* in combination with *G. fasciculatus* or *G. intraradices* than with *Rhizobium* alone (Table 3). The highest nitrogenase activity was recorded with the combination of *Rhizobium* plus *G. intraradices.* Acetylene reduction activity was not detected in plants inoculated with *G. fasciculatus* or *G. intraradices* or controls.

Both percent root length infected and arbuscule frequency of *G. fasciculatus* and *G. intraradices* were significantly greater when they were co-inoculated with *Rhizobium* (Table 3). *G. intraradices* colonized sainfoin roots with greater efficiency by infecting a higher percentage of the root length and higher arbuscule frequency than *G. fasciculatus* in both types of inoculation: alone or in combination with *Rhizobium* (Table 3). Root colonization by mycorrhizal fungi was not observed in control and *Rhizobium*-inoculated plants.

Metalaxyl seed treatment. For both sainfoin cvs. Nova and Melrose, plants with metalaxyl treatment had greater percent survival and plant dry weights ($P = 0.01$), but only cv. Nova had greater plant height ($P = 0.05$) (Table 4). Percent survival ($P = 0.05$) and dry weight ($P = 0.01$) of cv. Melrose varied with *Pythium* isolate. No significant isolate × metalaxyl interaction was found for all collected parameters with the exception of percent seedling survival for cv. Nova (Table 4). The average percent seedling survival and dry weight of sainfoin cv. Melrose inoculated with nine isolates of *Pythium* are shown in Table 5. The average effect of chemical on percent seedling survival and dry weight for both cvs. Melrose and Nova and on plant height for cv. Nova were significant (Table 6).

Integrated effects of metalaxyl, rhizobia, and VA mycorrhizal fungi in *Pythium*-infested soils. VA mycorrhizal inoculation of sainfoin cv. Nova grown in *Pythium*-infested soils increased percent seedling survival and shoot and root dry weights compared with those of the control treatments (Table 7). Both *P. irregulare* and *P. paroecandrum* significantly reduced the survival of sainfoin seedlings. Seedling survival was 54% for *P. irregulare* (P-16) and 58% for *P. paroecandrum* (P-18) (Table 7). There was no significant difference between percent seedling survival of the combined metalaxyl and VA mycorrhizal fungi treatments and the metalaxyl treatment alone. The combined treatments of metalaxyl and VA mycorrhizal fungi increased shoot and root dry weights of sainfoin compared with metalaxyl or VA mycorrhizae alone. Combined treatments of metalaxyl and VA mycorrhizal fungi produced the greatest total shoot dry weights (Table 7).

Table 2. Effect of rhizobia on the growth of sainfoin cvs. Melrose and Nova[z]

	Melrose				Nova			
	Cut 1		Cut 2		Cut 1		Cut 2	
Strains	Fresh wt	Dry wt	Fresh wt	Dry wt	Fresh wt	Dry wt	Fresh wt	Dry wt
SM-2	1.32	0.34	3.02	1.10	1.83	0.47	4.25	1.50
N-10	1.28	0.35	3.26	1.22	1.35	0.36	3.87	1.33
N-31	1.00	0.28	3.31	1.11	1.22	0.35	4.54	1.44
116A-12	1.09	0.29	3.14	1.11	1.13	0.32	3.75	1.35
116A-15	1.37	0.36	3.20	1.17	1.73	0.46	3.94	1.46
116A-17	1.00	0.28	2.92	1.00	1.14	0.31	4.56	1.45
Control	0.79	0.23	2.90	0.98	1.11	0.30	3.09	0.98
LSD (0.05)	0.20	0.05	0.49	0.16	0.28	0.07	0.51	0.19

[z] Ten seeds per pot, 10 pots per treatment. Shoot fresh and dry weights recorded 2 (cut 1) and 3 (cut 2) mo after seeding.

Table 3. Effects of dual inoculation of rhizobia and VA mycorrhizal fungi on growth of sainfoin cv. Nova[x]

	Dry wt				C_2H_4		Colonization[y]	
Treatment	Shoot (g/pot)	Root (mg/plant)	Nodule (mg/plant)	% N in shoots	μmol/hr per plant	μmol/hr per gram of nodule dry wt	Root length (%)	Arbuscule frequency (%)
Control	2.83 b[z]	206 e	0.0	3.18 f	0.0	0.0	0.0 e	0.0 e
Glomus fasciculatus	3.06 b	288 c	0.0	3.77 e	0.0	0.0	36.5 d	15.0 d
G. intraradices	3.02 b	260 d	0.0	3.96 d	0.0	0.0	55.4 c	34.0 c
Rhizobium	3.02 b	312 b	100.0 c	4.41 c	11.3 b	114.2 c	0.0 e	0.0 e
Rhizobium + *G. fasciculatus*	4.04 a	468 a	107.6 b	4.73 b	17.9 a	137.0 b	62.5 b	48.5 b
Rhizobium + *G. intraradices*	4.20 a	466 a	132.5 a	5.31 a	18.4 a	167.0 a	69.0 a	53.0 a

[x] Four seedlings per pot and 20 replicate pots pooled from two experiments. Values of dry weights of shoot and of roots and nodules represent means of 20 replicate pots and 25 seedlings, respectively.

[y] Values represent means of 250 root segments randomly collected from 25 seedlings.

[z] Means followed by the same letter in columns for a particular parameter are not different significantly ($P = 0.05$) from each other by Duncan's new multiple range test.

Table 4. Analysis of variance for the effect of *Pythium* and metalaxyl on growth of sainfoin cvs. Melrose and Nova[y]

Variable / Source of variation	Melrose df	Melrose SS	Melrose F^z	Nova df	Nova SS	Nova F
Survival, %						
Isolate	9	56.6	2.27*	9	24.9	1.29
Metalaxyl	1	101.2	36.60**	1	189.1	88.13**
Isolate × metalaxyl	9	39.0	1.57	9	66.3	3.43**
Residual	60	166.0	...	60	128.8	...
Dry weight, g						
Isolate	9	0.050	3.18**	9	0.019	1.29
Metalaxyl	1	0.036	20.78**	1	0.099	60.25**
Isolate × metalaxyl	9	0.021	1.36	9	0.017	1.13
Residual	60	0.104	...	60	0.135	...
Plant height, cm						
Isolate	9	8.10	0.62	9	15.01	1.36
Metalaxyl	1	1.36	0.93	1	6.96	5.66*
Isolate × metalaxyl	9	4.34	0.33	9	11.10	1.00
Residual	60	87.39	...	60	33.07	...

[y] Ten seeds per pot, 10 pots per treatment. Percent seedling survival recorded 2 wk after seeding, and plant height and shoot dry weight measured 1 mo after seeding.
[z] Values with one asterisk exceed the 0.05 probability level; values with two asterisks exceed the 0.01 probability level.

DISCUSSION

The present investigation demonstrated that *P. irregulare* and *P. paroecandrum* isolated from naturally infected sainfoin fields in southern Alberta can reduce seedling emergence and vigor when reinoculated to sainfoin under controlled conditions. *P. sylvaticum* was less pathogenic than *P. irregulare* and *P. paroecandrum*. These species were reported previously as seedling pathogens of alfalfa and were implicated as causal agents that could directly affect forage yield and produce chronic stresses that could indirectly contribute to stand decline (15,20).

Results of this study also indicated a distinct advantage from inoculating sainfoin with effective isolates of *Rhizobium* spp. to obtain the maximum benefits from biological nitrogen fixation to ensure the establishment of seedlings under poor soil conditions. On the prairies during cold phases of the growing season, temperature is a limiting factor for both bacterial growth and nitrogenase activity; therefore, the adaptation of arctic isolates to low temperatures could be advantageous.

Sainfoin growth and nitrogen-fixation can be enhanced significantly when coinoculated with VA mycorrhizal fungi and rhizobia. Plant yield, nodulation, nitrogenase activity, and total shoot nitrogen content were greater in dually inoculated sainfoin plants than in plants receiving a single treatment or the control plants. Rhizobial inoculation is known to increase the yields of several legumes by increasing root nodulation and biomass of root and shoot (33). The increased root system might facilitate more effective colonization by *G. fasciculatus* and *G. intraradices*; mycorrhizal plants produced significantly more nodules than nonmycorrhizal ones. Previous studies (4,27) suggested that colonization with efficient mycorrhizal fungi significantly improves phosphorus nutrition and consequent nodulation and nitrogen fixation by *Rhizobium*. The affinity of *Rhizobium* for VA mycorrhizal fungi may mutually benefit nutrient uptake. Enhancement of phosphate uptake by VA mycorrhizal fungi has been established (2,3). VA mycorrhizal fungi may supply *Rhizobium* with an adequate phosphate source in exchange for a nitrogen source. This association also may contribute to the increase of growth and yield of the host plant. Legumes require a high level of phosphorus for effective growth, nodulation, and nitrogen fixation (4). Mosse (30) indicated that the principal effect of VA mycorrhizal fungi on nodulation and nitrogen fixation is phosphorus mediated; phosphorus also is known to help other processes involved in nodulation and nitrogen fixation such as photosynthate production, trace element uptake, or plant hormone production. However, Niemi and Eklund (31) reported that mycorrhizal plants fix more nitrogen than nonmycorrhizal plants regardless of soil phosphorus content. Ames et al (1) and Kessel et al (25) found that VA mycorrhizal fungal hyphae are able to translocate labeled nitrogen from the soil to the plant. In our study, the greater plant growth, nodule dry weight, and nitrogenase activity in dually inoculated plants under similar phosphorus regimes further support these observations.

Metalaxyl increased sainfoin seedling survival in soil infested with *Pythium* spp. and consequently provided some control of *Pythium* root rot. The use of metalaxyl seed treatment to control seed and root disease should therefore be considered for further testing under field conditions.

VA mycorrhizal fungi not only stimulated sainfoin growth in *Pythium*-infested soils but also reduced the severity of damage from *Pythium* spp. infection. It is evident, therefore, that VA mycorrhizal fungi act to some degree as biocontrol agents against *Pythium* root rot of sainfoin. The mechanism of tolerance appears to be due not only to improved plant nutrition by the mycorrhizal fungi but also to other factors associated with VA mycorrhizal fungi (12,13). Dehne and Schönbeck (12) observed that mycorrhizal roots were more lignified and contained more polysaccharides than nonmycorrhizal ones, especially in the stellar tissue. This may be responsible for the restriction of the mycorrhizal fungi to the root cortex. The

Table 5. Average effect of *Pythium* isolates on growth of sainfoin cv. Melrose[z]

Isolates	Survival (%)	Dry wt (g)
P. paroecandrum		
P-1	53	0.09
P-2	43	0.08
P. irregulare		
P-3	38	0.07
P-7	56	0.10
P-8	56	0.13
P-12	58	0.12
P-13	43	0.09
P-16	58	0.13
P-18	56	0.09
Control	69	0.15
LSD (0.05)	17	0.04

[z] Ten seeds per pot, 10 pots per treatment. Percent seedling survival recorded 2 wk after seeding, and shoot dry weight measured 1 mo after seeding.

Table 6. Effects of metalaxyl on percent seedling survival, dry weight, and plant height of sainfoin cvs. Nova and melrose averaged over isolates[z]

Treatment	Survival (%)	Dry weight (g)	Plant height (cm)
cv. Nova			
Metalaxyl	76.0	0.155	7.05
Control	45.0	0.085	6.46
LSD (0.05)	6.6	0.018	0.50
cv. Melrose			
Metalaxyl	64.0	0.126	6.93
Control	42.0	0.083	6.67
LSD (0.05)	7.4	0.019	0.54

[z] Ten seeds per pot, 10 pots per treatment. Percent seedling survival recorded 2 wk after seeding, and plant height and shoot dry weight measured 1 mo after seeding.

Table 7. Effects of vesicular-arbuscular mycorrhizal fungi and metalaxyl alone and in combination on seedling survival and shoot and root weights of sainfoin cv. Nova grown in *Pythium*-infested soils[y]

Treatment	*P. irregulare* (P-16)					*P. paroecandrum* (P-18)				
	Survival (%)	Shoot dry wt (g)			Root dry wt (g)	Survival (%)	Shoot dry wt (g)			Root dry wt (g)
		Cut 1	Cut 2	Total			Cut 1	Cut 2	Total	
Control	54 c[z]	1.30 c	1.72 c	3.02 c	8.84 c	58 b	1.11 c	1.41 d	2.52 d	6.81 c
G. fasciculatus	78 ab	1.45 bc	4.74 b	6.19 b	13.30 b	76 a	1.41 b	4.59 ab	6.00 b	11.55 b
G. intraradices	74 b	1.51 bc	4.89 b	6.40 b	14.83 ab	74 a	1.42 b	4.36 b	5.78 b	11.90 b
Metalaxyl	84 ab	1.46 bc	4.66 b	6.12 b	11.49 bc	86 a	1.41 b	3.52 c	4.93 c	10.05 bc
Metalaxyl + *G. fasciculatus*	80 ab	1.64 b	5.63 a	7.27 a	15.17 ab	80 a	1.73 a	5.20 a	6.93 a	16.67 a
Metalaxyl + *G. intraradices*	86 a	1.91 a	5.51 a	7.42 a	17.26 a	84 a	1.65 a	5.17 a	6.82 a	16.12 a

[y] Ten seeds per pot, 10 pots per treatment. Shoot fresh and dry weights recorded 2 (cut 1) and 3 (cut 2) mo after seeding. Root dry weight determined 3 mo after seeding.

[z] Means followed by the same letters in columns for a particular parameter are not significantly ($P = 0.05$) different from each other by Duncan's multiple range test.

same mechanisms of resistance may be effective against parasitic soilborne pathogens invading the host root. Roots colonized by mycorrhizal fungi also exhibit greater chitinolytic activities. These enzymes can be inhibitory against certain fungal pathogens (14). Further studies are needed to investigate the possible production of antimicrobial compounds produced by VA mycorrhizal fungi or their hosts and the environmental conditions that could enhance disease resistance.

According to Hayman (17), species of VA mycorrhizal fungi differ in their root colonization abilities. It is unlikely that sainfoin fields with poor seedling establishment had an absence of VA mycorrhizal fungi. More likely, the indigenous mycorrhizal fungi in the sainfoin fields were less effective in root infection or in stimulating plant growth than the mycorrhizae used in this study. The lack of response also may reflect low spore densities in sainfoin fields. These results indicate that VA mycorrhizal fungi and rhizobia can not only stimulate sainfoin growth in *Pythium*-infested soils but also reduce the severity of damage from *Pythium* infection.

The exclusive use of any systemic fungicide risks selection of resistant pathogens and threatens the long-term usefulness of such chemicals. Considerable variability in sensitivity to metalaxyl has occurred among and even within species and strains of *Pythium* spp. (9). The synergistic phenomenon involved in integrated control using fungicides and biocontrol agents may be more efficient and prolonged than the control achieved through biocontrol agents or fungicides alone (24). It may be possible to produce healthy sainfoin seedlings in *Pythium*-infested soils by inoculation with mycorrhizal fungi and rhizobia and seed treatment with metalaxyl. For successful integration of biological and chemical control of root pathogens, the systems must be compatible. As shown in previous studies (20,34), metalaxyl had a negligible effect on VA mycorrhizal and rhizobial infection of alfalfa root segments. Studies under field conditions are necessary to determine the effectiveness of VA mycorrhizae alone or in combination with metalaxyl at different rates on the suppression of sainfoin root rot and to establish the potential economic benefit to be derived from the various treatments.

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