Effects of *Cylindrocladium* gracile, Fusarium roseum and Plenodomus *meliloti* on crown and root rot, forage yield, and winterkill of alfalfa in north-eastern Alberta

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A field trial was conducted to compare the effects of three soil-borne pathogens on the incidence of crown and root rot, forage yield and winter survival of alfalfa. ***Cylindrocladiurngracile, Fusarium roseum*** and ***Plenodomus rneliloti*** were applied alone and in combination to test plots. There was no significant difference in fresh and dry matter yields among the treatments. Disease severity was significantly less in the control plots than in the treated plots. All treated plots were assessed an average disease severity rating of moderate. Percent winterkill in the plots inoculated with a combination of the three fungi was not significantly different than that of the other treatments except *F.* roseumalone.

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Un essai au champ a été fait pour comparer les effets de trois pathogenes du sol sur l'incidence de la pourriture du collet et de la racine, sur le rendement en fourrage et sur la survie en hiver de la luzerne. ***Cylindrocladium gracile, Fusarium roseum*** et ***Plenodomus rneliloti*** ont été appliques seul et en combinaison sur des parcelles expérimentales. Les rendements en poid frais et en matière sèche n'ont pas montre de difference significative entre les traitements. La sévérité de la maladie était significativement moins importante dans les parcelles témoins que dans celles traitées. L'on a estimé la sévérité de la maladie a modérée dans toutes les parcelles traitées. Le pourcentage de perte dû à l'hiver n'était pas significativement different entre les parcelles inoculées avec les trois pathogenes et les autres traitements, exception faite, de celles inoculées avec ***F. roseum.***

Introduction

The complex nature of crown and root deterioration in alfalfa is a product of an interaction of biological and environmental stress factors (3,5,14). In Alberta, crown and root rot has become a major limiting factor in the production of alfalfa two to three years after establishment (4). From surveys in central Alberta, ***Cylindrocladium gracile*** Bugn. (Boesew.) and ***Fusarium roseum*** (LK.) emend. Snyder and Hansen were recovered most frequently from three- and four-year old alfalfa with crown and root rot (3.8). Brown root rot, caused by ***Plenodomus meli-*** loti Mark.-Let., was also frequently observed in the fields (2,3,10). This indigenous fungus, which causes symptoms that are most common in alfalfa early in the spring, infects dormant and semidormant alfalfa.

Many forms of stress in nature may interfere with the hardening process and reduce alfalfa's full cold hardiness potential (6). In Alberta, winter survival is critical for the successful production of alfalfa. Evidence that diseases predispose alfalfa to winterkill is increasing (9.12.13). Undoubtedly, the cold hardiness potential of alfalfa after infection by C. ***gracile,*** F. ***roseum*** or ***P. meliloti*** would be considerably reduced, mainly because there is a reduction of food reserves in the rotted crown (11). This study was undertaken to determine the effect of these three soil-borne fungi alone and in all possible combinations on the incidence of crown and root rot and forage yield of alfalfa, and to determine their possible role in winterkill.

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Materiials and methods

Experimental plots were established in the spring of 1983 at the Alberta Environmental Centre, Vegreville. Eptam® EC was incorporated in the soil at a rate of 4.5 L/ha as a pre-emergence herbicide along with 90 kg/ha of monoammonium phosphate (11-51-0), 20kg/ha of potash (0-0-60) and 19 kg/ha of elemental sulphur (0-0-0-90). Eight treatments were arranged in a randomized complete block design with six replicates (Table 1). Each plot consisted of four 6 m rows spaced 30 cm apart. Treatments were spaced 1 m apart and replicates 2.5 m. Seeds of alfalfa (***Medicago sativa*** L.) cv. Beaver were seeded at 8 kg/ha and peat-based inoculant was used as a source of root-nodule bacteria. Due to poor stand establishment, gaps in rows were reseeded in the fall of 1983.

The substrate for fungus inoculum consisted of a mixture of rye, oats and distilled water which was autoclaved for 2 hrs. at 121"C in 2 L Erlenmeyer flasks. Suspensions of C. ***gracile, F. roseum*** and ***P. meliloti,*** grown on PDA in 9 cm culture plates and macerated in sterile water were added to the grain, once the flasks cooled. Autoclavable bags were filled with a mixture of rye, oats and distilled water and autoclaved as mentioned above. Infested grain from the Erlenmeyer flasks was used as a source of inoculum for further multiplication in the bags. During the summer of 1984, 9 kg of inoculated grain was incorporated into each plot. The treatments consisted of either a single fungus or a combination of fungi (Table 1). Sterilized grain without a fungus was used as a control.

Fresh and dry matter yields in each plot were recorded twice from the two centre rows in both 1984 and 1985. In the spring of 1986, winterkill was determined by counting the number of plants in the two middle rows of each plot without green shoots. Twenty randomly selected plants in total from the outside rows of each plot were dug up and the roots

Table 1. Inoculation Treatments.

Treatment Codes	Treatments	Amt. of inoculum/plot
C	*C. gracile*	9 kg
F	*F. roseum*	9 kg
P	*P. meliloti*	9 kg
C + F	*C. gracile* + *F. roseum*	4.5 kg + 4.5 kg
C + P	*C. gracile* + *P. meliloti*	4.5 kg + 4.5 kg
F + P	*F. roseum* + *P. meliloti*	4.5 kg + 4.5 kg
C + F + P	*C. gracile* + *F. roseum* + *P. meliloti*	3 kg + 3 kg + 3 kg
Control	Sterilized grain only	9 kg

Table 2. Effects of *C. gracile, F. roseum* and *P. meliloti* on crown and root rot, forage yield and winterkill of alfalfa.

Treatment	Fresh Weight (kg) 1984	Fresh Weight (kg) 1985	Dry Weight (kg) 1984	Dry Weight (kg) 1985	% Winterkill	Disease Severity[X]
C	11.80a[Y]	7.37a	4.37a	2.58a	45.0ab	2.07b
F	10.57a	6.80a	4.07a	2.37a	28.3bc	2.43ab
P	10.93a	8.40a	4.17a	2.82a	40.6ab	2.53a
C + F	11.30a	7.81a	4.37a	2.71a	42.8ab	2.10b
C + P	11.65a	8.47a	4.33a	3.00a	41.7ab	2.53a
F + P	10.91a	7.13a	4.16a	2.46a	50.0a	2.37ab
C + F + P	12.13a	7.14a	4.48a	2.43a	47.2a	2.23ab
Control	10.79a	7.08a	4.14a	2.41a	15.6c	1.27c

X Scores assigned: 0 = clean; 1, 2 and 3 = slight, moderate and severe crown and root rot, respectively.

Y Values in a column followed by the same letter are not significantly different (P = 0.05)

bisected longitudinally to assess the severity of crown and root rot. Severity scores assigned were 0, no disease; 1, slight; 2, moderate; 3, severe. ANOVA and Duncan's Multiple Range tests were used to statistically analyze the data on disease severity, forage yield, and percent winterkill.

Fungi were isolated and identified from ten randomly selected plants from plots inoculated with the mixture. One hundred pieces of crown and upper tap root tissue from the ten plants were surface disinfested in 0.6% sodium hypochlorite for 2 min., rinsed in sterile water, blotted dry and plated on acidified PDA (3.0 ml sterile 85% lactic acid per L of medium). After incubation for two weeks in darkness at 5°C, the plates were examined and all fungi identified.

Results

There were no significant differences (P=0.05) in fresh and dry matter weights of alfalfa from the various treatments in 1984 and 1985 (Table 2). Forage yields were not collected from the three-year-old stand in 1986 because of severe winter injury. Non-inoculated plots had significantly less winterkill than the inoculated plots. Winterkill in the plots inoculated with a mixture of the three fungi did not differ significantly from the other treatments except for *F. roseum* alone.

Disease severity was significantly less in the control plots than in the mixtures or singly pathogen-treated plots. All treated plots were assessed an average disease severity rating of moderate (2.07 to 2.53). *F. roseum* was most frequently isolated from diseased plant tissue; *P. meliloti* was recovered with moderate frequency and *C. gracile* was recovered with low frequency.

Discussion

Successful fungal colonists need great independence and phenotypic plasticity (1). The chlamydospores of *F. roseum* can germinate and retreat into chlamydospores if conditions become unfavorable, which gives it greater survival capability. *C. gracile* and *P. meliloti* have no such retreat mechanism; there are no replacements if the hyphae die. These two fungi have a much lower competitive saprophytic ability compared to *F. roseum*. Moreover, the broad tolerance to temperature changes of *F. roseum* undoubtedly contributes to its high frequency of isolation (4).

Pathogens had no apparent effect on forage yields, either singly or in combination in the first two years. The ability of alfalfa to survive the winter depends, in part, on the storage of adequate food reserves in the roots and crowns during the fall (7). The presence of any one or a combination of the pathogens increased the percent of winterkill three years after establishment. This increased winterkill may have been the result of reduced food reserves due to pathogen infection prior to the winter. Parasitized plants may not compete well in spring and this may also lead to reduced food reserves. Additional research would be useful to determine how the presence of crown and root rot fungi on alfalfa in early spring or in late fall influences the degree of alfalfa stand survival. The development of disease-resistant cultivars offers the best possibility for controlling crown and root rot of alfalfa. At present, all recommended cultivars of *M. sativa* are susceptible to crown and root rot (4).

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