Crown and root rot of alfalfa in southern Alberta

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Twenty-seven alfalfa fields in southern Alberta were surveyed in 1983 for the incidence and severity of crown and root rot disease. Mean disease incidence and severity were 61% and 0.80 (on a scale of 0-3), respectively. Four species of *Fusarium* (*F. solani, F. tricinctum, F. avenaceum* and *F. oxysporum*), *Pythium irregulare,* and two unidentified isolates of *Pythium* were found to be associated with crown and root rot of alfalfa.

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Vingt-sept luzernières du sud de l'Alberta ont fait l'objet d'une étude en 1983 pour determiner la fréquence et la gravité de la pourriture du collet et des racines. La fréquence et la gravité moyenne sont de 61 % et de 0,80 (sur une échelle de 0 à 3) respectivement. Quatre espbces de *Fusarium* (*F. solani, F. tricinctum, F. avenaceum* et *F. oxysporum*), *Pythium irregulare,* et deux isolats non identifies de *Pythium* s'avèrent associés à la maladie de la luzerne.

Introduction

Crown and root rot of alfalfa (*Medicago sativa* L.), a chronic and potentially devastating disease in most production areas (4, 13, 15, 19, 22), has been of major concern to alfalfa growers in southern Alberta for a number of years. This disease not only causes plants to develop asymmetrically because of the eventual death of buds and young shoots near the soil surface, but also prevents development of adequate cold tolerance in the fall, as a result of reduced food reserves in the rotted crown area (8, 17). Alfalfa stands are capable of surviving for 10 years or more, but because of this disease, the majority of the fields show a progressive deterioration after the second year (7).

Different species and strains of *Fusarium* and *Pythium* have been closely associated with crown and root rot of alfalfa in different regions of the world (1, 2, 3, 5, 6, 7, 13, 14, 19, 20). To obtain more information on crown and root rot of alfalfa in southern Alberta, a comprehensive field survey was carried out to determine its incidence and severity; to isolate and identify the species of *Fusarium* and *Pythium* associated with the disease; and to determine the pathogenicity of the organisms isolated from diseased crowns and roots of alfalfa.

Materials and methods

Twenty-seven alfalfa fields in southern Alberta were surveyed in 1983 for the incidence and severity of crown and root rot disease. Twenty-five plants dug at random with a sharpshooter shovel were shaken free of soil, placed in a paper bag, and stored in a cooler until processing. Plants were rinsed with tap water and split longitudinally to visually assess the severity of crown and root rot. Severity scores assigned were 0, no disease; 1, slight; 2, moderate; 3, severe.

For *Fusarium* isolation, ten pieces (0.5 × 0.5 cm) of crown and upper tap root tissue were taken from each of ten randomly selected plants from each of the 27 fields sampled. The tissue pieces were surface sterilized in 0.6% sodium hypochlorite for 2 minutes, rinsed in sterile water, blotted dry, and placed on pentachloronitrobenzene (PCNB) medium (11). After incubation for one week at room temperature under fluorescent light, the hyphal tips of fungi growing out of the tissue pieces were cut and transferred to potato dextrose agar slants and carnation leaf agar plates for identification (12).

Pythium spp. could not be isolated from below-ground portions of mature plants due to loss of rootlets and necrotic root tips when the plants were removed from the soil. Therefore, rhizosphere soil samples were randomly collected from 94 alfalfa fields to estimate the number of *Pythium* and *Fusarium* propagules present in the field soils. A soil dilution series was prepared for each air-dried soil sample using 0.2% water agar, then 1 mL of the soil dilution was spread onto a PCNB plate, which is selective for *Fusarium,* and onto a pimaricin-vancomycin agar medium (MPVM) with rose bengal (0.01 g/L), which is selective for *Pythium* (10). Four plates were used for each dilution of each soil sample. The PCNB plates were incubated under fluorescent light at room temperature and the number of *Fusarium* colonies recorded after 7 days. The MPVM plates were incubated for 48 h in darkness at room temperature, then washed under a slow stream of water to remove materials other than *Pythium* colonies which had grown into the medium. The number of *Pythium* colonies was recorded and the morphologically different colony types were transferred to cornmeal agar for further study.

The pathogenicity of the *Fusarium* isolates was evaluated on 4-week-old greenhouse-grown alfalfa plants, cv. Anchor. Roots were carefully washed and immersed in spore suspensions (10^5 conidia/mL) for 5 minutes before repotting in steam-sterilized soil. Roots immersed in distilled water were used for controls. The isolates were considered to be pathogenic if the length of the vascular discoloration of the split tap root exceeded 10 mm. The pathogenicity of the *Pythiurn* isolates was evaluated by growing them on a cornmeal and sand mixture (15 g cornmeal, 485 g sand, 120 mL distilled water) and thoroughly mixing each inoculated mixture with steam-sterilized soil at a rate of 300 propagules/g soil. Ten

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Table 1. Incidence and severity of crown and root rot of alfalfa in southern Alberta in 1983.

Location	No. of fields surveyed	Incidence %	Severity* rating
M. D. of Kneehill	1	95	0.21
Special Area No. 2	1	91	1.57
Co. of Wheatland	6	74	1.15
Co. of Mountain View	6	46	0.74
Co. of Newell	6	42	0.59
M. D. of Rocky View	4	42	0.64
Special Area No. 3	3	39	0.67
Total/average	27	61	0.80

*Crown and root rot severity rating scale: 0 = clean; 1 = slight, **1-20%** of the crown and root discolored; 2 = moderate, **21-50%** of the crown and root discolored; 3 = severe, **51-100%** of the crown and root discolored.

surface-sterilized seeds of alfalfa, cv. Anchor, were planted in each of five 15-cm-diameter plastic pots containing each of four isolates of *Pythium*-inoculated soil. Seeds sown in pots which had received *Pythium*-free cornmeal and sand mixtures served as controls. The pots were maintained in the greenhouse at 20°C for 30 days, after which the percentage of seedling damping-off was recorded.

Results

Crown and root rot was found in all of the alfalfa fields surveyed. Average disease incidence and severity of crown and root rot were 61% and 0.8, respectively (Table 1). Soils from fields of alfalfa with crown and root rot had populations of *Fusarium* ranging from 23×10^3 to 58×10^3 propagules/g soil and of *Pythium* from 26 x 10 to 76 x 10 propagules/g soil (Table 2).

Four species of *Fusarium* were identified from crown- and root-rot-affected alfalfa plants. *F. solani* was the most abundant at 47% of the total isolates, while *F. tricinctum, F. avenaceum,* and *F.* oxysporum were found at 25, 21, and 7%, respectively. *F. tricinctum* and *F. solani* were most virulent with 82% and 60% of infected alfalfa seedlings showing vascular discoloration of the tap roots, followed by *F.* oxysporum (42%) and *F. avenaceum* (25%). Seedlings infected with P. *irregulare* AH-14 and AH-1, and unidentified *Pythium* AB-6 and AG-13 showed 41%, 54%, 62% and 33% damping-off, respectively. Control seedling damping-off was 8%.

Table 2. Populations of Fusarium spp. and Pythium spp. isolated from the rhizosphere soil of alfalfa plants in southern Alberta.

Location	No. of fields sampled	Propagules/g air-dried soil	
		Fusarium ($\times 10^3$)	Pythium ($\times 10$)
Co. of Newell	10	33	26
Co. of Mountain View	10	27	44
Co. of Vulcan	6	47	53
Co. of Wheatland	10	53	41
M. D. of Foothills	10	23	39
M. D. of Rocky View	10	30	76
M. D. of Starland	10	28	52
M. D. of Taber	9	58	39
Special Area No. 2	10	53	62
Special Area No. 3	9	31	32
Total/average	94	38	46

Discussion

Crown and root rot of alfalfa was widespread in southern Alberta. Mean disease severity rating was not very high, but that could increase rapidly, particularly if the plants are damaged by frost allowing fungi to enter. The isolation and infection studies showed that *Fusarium* solani, F. *tricinctum, F.* avenaceum, *F.* oxysporum and *Pythium irregulare* were the principal pathogens of crown and root rot. However, it could not be determined which of the organisms was more active in the early spring, such as was reported for Plenodomus *meliloti* and *Cylindrocladium* gracile which were found to be parasitic early in the development of crown and root of alfalfa in central Alberta (4, 18, 21). Additional work is required to assess the seasonal effect on the incidence of fungal isolation.

The ability of alfalfa to survive the winter depends, in part, on the storage of food reserves in the roots and crowns during the fall (8). Infection with *Fusarium* no doubt affects physiological processes of alfalfa and reduces its potential to achieve maximum cold hardiness (17). Unfortunately, all recommended varieties of alfalfa are susceptible to crown and root rot (9). Selection and breeding for resistance to crown and root rot will probably be difficult, mainly because the disease is associated with many causal organisms and alfalfa is predisposed to nutritional and environmental stress factors (16). Primary consideration should be given to management practices, such as selection of winter-hardy varieties and proper fertilization and cutting that promote vigorous growth of alfalfa (8).

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