Screening of alfalfa cultivars for resistance to fusarium wilt in northeastern Alberta

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Greenhouse and field tests were conducted to evaluate sixteen alfalfa cultivars for dry matter yields and resistance to fusarium wilt. All cultivars showed symptoms of fusarium wilt, but disease severity varied considerably among cultivars. Algonquin, Angus, Beaver, Drylander, Saranac, and Spredor were the cultivars least affected by ***Fusarium oxysporum*** f. sp. ***medicaginis;*** Anchor, Peace, Roamer, Rambler, and Trek were the most affected. Significant differences were found among cultivars for dry matter yields.

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Des essais au champs et en serre furent efectues pour evaluer les rendements en matière seche et la resistance à la fletrissure fusarienne de seize cultivars de luzerne. Tous les cultivars ont présenté des symptômes de fletrissure fusarienne mais avec une sévérité variant considérablement. Les cultivars Algonquin, Angus, Beaver, Drylander, Saranac'et Spredor furent les moins affectés par le ***Fusarium oxysporum*** f. sp. ***medicaginis.*** Les cultivars Anchor, Peace, Roamer, Rambler et Trek furent les plus affectes. Parmis les cultivars, on a trouve des differences significatives pour les rendements de la matiere seche.

Introduction

Fusarium wilt of alfalfa ***(Medicago sativa*** L.), caused by ***Fusarium oxysporum*** f. sp. ***medicaginis*** (Weimer) Snyd. and Hans., progresses slowly in natural alfalfa stands, but can cause considerable yield losses in a stand over a period of several years (2,3,4). In Alberta, winter survival is critical for successful production of alfalfa and survival depends largely on the storage of adequate food reserves in the roots and crowns during the fall (2,8,9). Fusarium wilt may predispose alfalfa to winterkill by affecting the accumulation of food reserves (12). The development of fusarium wilt-resistant alfalfa cultivars would offer the best possibility for the control of this disease, but more information on the responses of currently available cultivars to fusarium wilt is needed by plant breeders. The objective of this study was to evaluate alfalfa cultivars for resistance to fusarium wilt under greenhouse and field conditions.

Materials and methods

One single-spore isolate of ***Fusarium oxysporum*** f. sp. ***medicaginis*** was obtained from roots of symptomatic alfalfa seedlings collected in northeastern Alberta and identified based on descriptions by Booth (1) and Nelson ***et al.*** (11). The fungus was maintained on potato dextrose agar plates at 5°C. Conidial inoculum was prepared by placing a 9 mm-diameter mycelial disk of *F. oxysporum* f. sp. ***medicaginis*** in 250 mL conical flasks containing 100 mL sterile Kerr's (7) solution, which was shaken continuously at 200 rpm for 5 days at room temperature in natural light. The cultures were filtered through two layers of cheesecloth to remove mycelium and the filtrate was centrifuged at 1000 rpm for 10 min. The pellets of conidia were resuspended and diluted with sterile water to a concentration of 1.5 x 10^6 conidia/mL.

Seeds of each alfalfa cultivar were surface-sterilized in 70% ethanol for 2 min, followed by 2 min in 0.6% sodium hypochlorite, then rinsed three times in sterile distilled water, and sown in fiber flats (50x 30 x 10 cm) containing sterilized vermiculite. The seedlings were inoculated with ***Rhizobium meliloti,*** fertilized with 20-20-20 (N-P-K), and placed on a greenhouse bench at 22-26°C. Three months after seeding, alfalfa plants were lifted, and the tops were trimmed to about 4 cm from the crown while the roots were trimmed to about 12 cm from the crown. The plants were inoculated by immersing the roots in the conidial suspension for 30 min. For the greenhouse test, the inoculated plants were planted in 13-cm-diameter plastic pots containing a steam-sterilized mixture of sand, loam and vermiculite (1:1:1, v/v). Ten replicate pots (5 plants/pot) were used for each cultivar and all pots were arranged randomly on the greenhouse bench. For the field test, one field plot was established in the spring of 1989 at the Alberta Environmental Centre, Vegreville. A' pre-emergence herbicide, Eptan EC, at a rate of 4.5 L/ha along with 90 kg/ha of monoammonium phosphate (11-51-0), 20 kg/ha of potash (0-0-60) and 19 kg/ha of elemental sulphur (0-0-0-90) were incorporated into the soil. Inoculated plants were planted in a randomized complete block design with four replications. Each single cultivar plot consisted of 50 plants spaced 20 cm apart in a 10-m-long row. There was 1 m between cultivars and 2.5 m between replicates. When necessary inoculated plants were stored at 3-5°C in a container with 1-2 cm water to keep the roots moist until transplanting.

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Table 1. Comparative forage yield of alfalfa cultivars and their resistance to fusarium wilt under greenhouse and field conditions.

	Dry weight (g)		Disease Severity[y]		
Cultivar	Greenhouse	Field	Greenhouse	Field	$\bar{x}$ Field & Greenhouse
Algonquin	3.22 a[x]	394 abc	1.1 b	2.0 cde	1.6
Anchor	2.75 abcd	327 abcd	1.9 ab	2.9 ab	2.4
Angus	3.05 ab	294 abcd	1.6 b	1.5 e	1.6
Anik	1.37 g	57 d	1.7 ab	2.0 cde	1.9
Beaver	2.75 abcd	480 ab	1.0 b	1.6 e	1.3
Drylander	2.97 abc	181 cd	1.7 b	1.7 de	1.7
Peace	2.29 def	270 abcd	2.7 a	3.0 a	2.9
Rambler	3.34 a	283 abcd	2.0 ab	2.4 abcd	2.2
Rangelander	3.14 ab	334 abcd	1.7 b	2.0 cde	1.9
Roamer	1.97 f	203 bcd	2.7 a	3.0 a	2.9
Saranac	2.84 abcd	554 a	1.3 b	2.1 bcde	1.7
Spredor	2.62 bcde	516 a	1.6 b	1.7 de	1.7
Thor	2.80 abcd	469 abc	1.5 b	2.4 abcd	2.0
Trek	2.14 ef	326 abcd	1.8 ab	2.5 abc	2.2
Trumpetor	1.45 g	376 abc	1.5 b	2.3 abcd	1.9
Vernal	2.39 cdef	551 a	1.8 ab	2.4 abcd	2.1

[x] Values in a column followed by the same letter are not significantly different using Duncan's Multiple Range test (P=0.05).

[y] Disease severity was based on a scale of 0–5 where 0 = no discoloration in the root; 1 = small dark strands in the inner stele; 2 = small dark-brown arcs in the inner stele; 3 = larger dark-brown areas in the inner stele or partial dark-brown rings in the outer stele; 4 = the entire outer stele dark brown, plant alive; 5 = plant dead.

The plants were harvested three months after transplanting, dried at 70°C for 24 hr, and weighed. Tap roots of surviving plants were dug up, cross-sectioned, and rated for disease severity based on a previously described 0 to 5 scale (14): 0 = no discoloration in the root; 1 = small dark strands in the inner stele (Fig. 1); 2 = small dark-brown arcs or rings in the inner stele (Fig. 2); 3 = larger dark-brown areas, arcs or rings in the inner stele, or partial dark-brown rings in the outer stele (Fig. 3); 4 = the entire outer stele dark brown, plant alive (Fig. 4); 5 = plant dead. Sections approximately 1 µm thick were cut from the infected tap roots, mounted on glass slides, stained with toluidine blue, and examined and photographed with a light microscope (10).

ANOVA and Duncan's Multiple Range tests were used to statistically analyze the data on dry matter yield and disease severity of fusarium wilt.

Results and discussion

The sixteen cultivars were affected to varying degrees by F. oxysporum f. sp. medicaginis in both greenhouse and field tests (Table 1). The highest disease ratings were recorded for cvs. Anchor, Peace, Roamer, Rambler, and Trek (x for field and greenhouse of 2.9 to 2.2, respectively), whereas lowest disease ratings were observed for cvs. Algonquin, Angus, Beaver, Drylander, Saranac, and Spredor (x for field and greenhouse of 1.3 to 1.7, respectively). Disease severity ratings of the remaining cultivars were intermediate (Table 1). In the greenhouse test, highest dry matter yields (3.05 to 3.34 g/pot) were observed for cvs. Algonquin, Angus, Rambler, and Rangelander compared with cvs. Anik, Peace, Roamer, Trek, Trumpetor, and Vernal, which yielded the least (1.37 to 2.39 g/pot). Yields of the remaining six cultivars were intermediate (2.62 to 2.97 g/pot) (Table 1). In the field test, highest yields (469 to 554 g/plot) were observed for cvs. Beaver, Saranac, Spredor, Thor, and Vernal compared with cvs. Anik, Drylander, and Roamer, which yielded the least (57 to 203 g/plot). Yields of the remaining eight cultivars were intermediate (270 to 376 g/plot) (Table 1).

Crown and root rot is a complex disease that has been considered a major limiting factor in the production of alfalfa for a number of years (2,5,6). Selection for resistance to crown and root rot has been difficult because of the large number of causal organisms (4,5,6,13). Fusarium wilt appears to be an important component of the crown and root rot disease complex; in some years, the damage of fusarium wilt alone may be lethal to alfalfa (3). Moreover, according to Richard *et al.* (12), cold resistance of alfalfa is affected more by fusarium wilt than by fusarium root rot, because infection 'with F. oxysporum f. sp. medicaginis affects physiological processes that normally lead to hardening (8,12).

A combination of fusarium wilt disease and winter stress factor is believed most likely to cause stand decline and yield reduction in Alberta. The results of this study clearly demonstrate that Algonquin and Beaver are the known winter-hardy cultivars within the group of lowest disease severity and the other two tested winter-hardy cultivars, Anik and Peace, belong to the intermediate and greatest disease severity groups, respectively. Beaver is the only promising cultivar with high dry matter yield; Anik had the least yield in both the greenhouse and field tests.

Disease severity ratings in this study were not related to yields, but did indicate comparative resistance to fusarium wilt of different cultivars. The very close agreement of field and greenhouse disease evaluations in this study suggests that greenhouse testing can be an important supplement to field testing for resistance to fusarium wilt. It offers breeders the advantage of rapid progress in developing fusarium wilt-resistant alfalfa cultivars.

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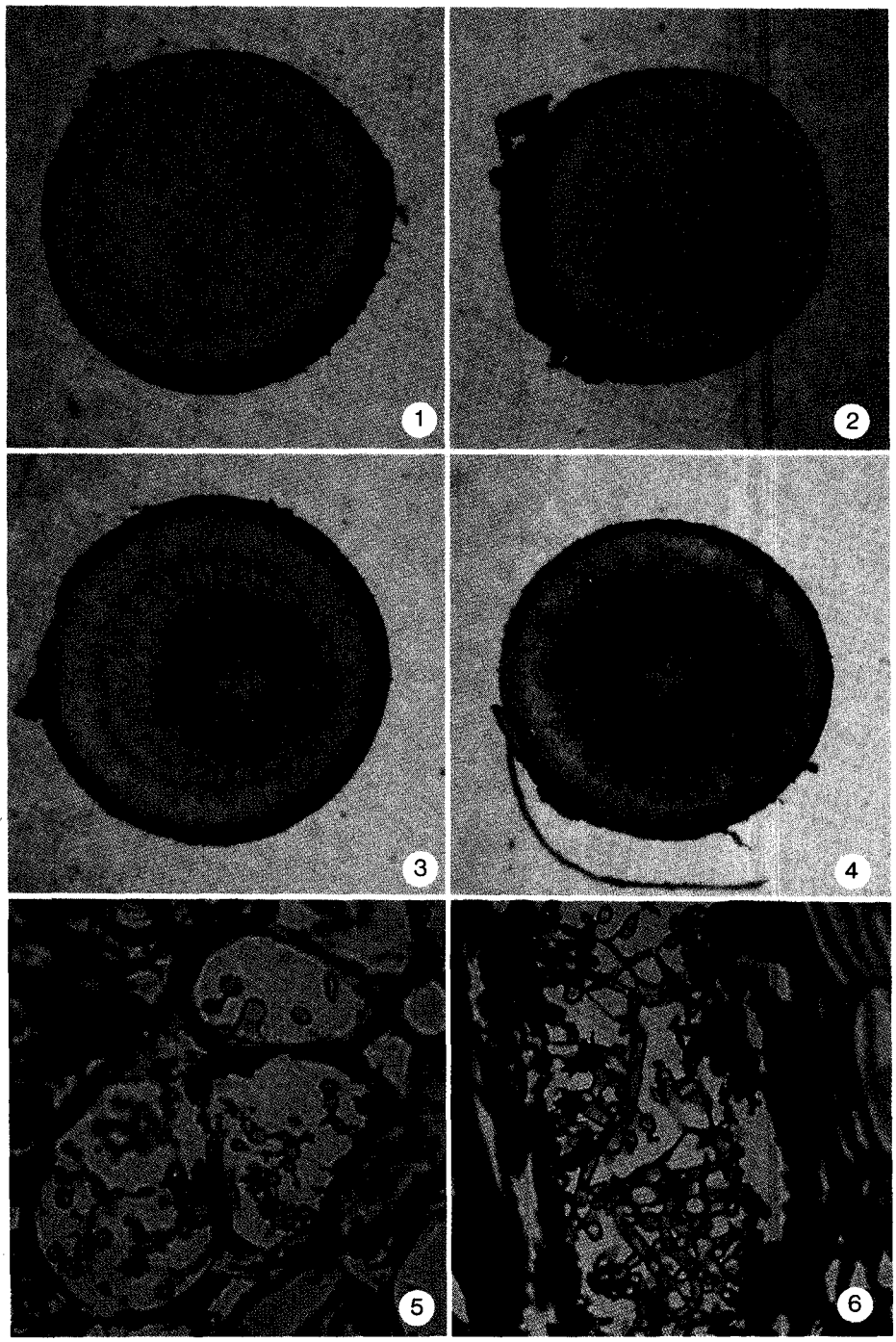

Figs. **1-4.** Cross section of alfalfa tap root infected with Fusariurn oxysporum f. sp. medicaginis.
Fig. 1. Small dark strands in the inner stele (disease severity= 1).
Fig. 2. Small dark-brown arcs in the inner stele (disease severity = 2).
Fig. **3.** Large dark-brown areas in the inner stele (disease severity = 3).
Fig. **4.** Entire outer stele dark brown (disease severity = **4).**

Fig. 5. A portion of a cross section through an alfalfa tap root infected with Fusariurn oxysporurn f. sp. medicaginis. Note the conidia in the xylem vessel element.

Fig. **6.** A portion of a longitudinal section through an alfalfa tap root infected with *Fusarium* oxysporurn f.sp. rnedicaginis. Note the hyphae in the xylem vessel element.