Effect of Verticillium Wilt on Forage Yield of Alfalfa in Southern Alberta

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ABSTRACT

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The incidence of Verticillium wilt and the forage yield of 12 cultivars of alfalfa (*Medicago sativa*) were compared in an irrigated field naturally infested with *Verticillium albo-atrum* near Lethbridge, Alberta. The alfalfa cultivars were seeded in 1986 and examined annually for Verticillium wilt and forage dry matter yield during 1987–1993. The incidence of wilt varied from year to year, and averaged over the years, the resistant cultivars Barrier, AC Blue J, Pioneer 5444, and Vertus had a significantly ($P < 0.01$) lower incidence than the moderately resistant cultivars Maris Kabul, Admiral, and Trumpetor and the susceptible cultivars Excalibur, WL316, Apollo II, Beaver, and Pacer. Differences in yield were not significant ($P > 0.05$) until the third year. During the 7-yr trial, the total forage yields of resistant cultivars Pioneer 5444, Barrier, and AC Blue J (87,143, 86,850, and 85,398 kg/ha, respectively) were significantly ($P < 0.01$) higher than those of the susceptible cultivars Beaver and Pacer (75,420 and 73,519 kg/ha, respectively). The moderately resistant cultivars Maris Kabul, Admiral, and Trumpetor had intermediate yields. Although the cultivar Vertus was resistant to the disease, its total forage yield was significantly ($P < 0.01$) lower than that of resistant cultivars Pioneer 5444, Barrier, and AC Blue J. These results illustrate the impact of Verticillium wilt on alfalfa production in southern Alberta.

Verticillium wilt, caused by *Verticillium albo-atrum* Reinke & Berthier, is an important disease of alfalfa (*Medicago sativa* L.) in Canada (2,24). The disease has been found in most provinces, including British Columbia (24), Alberta (12,14), Saskatchewan (7,8), Ontario (4, 25), Quebec (3,22), New Brunswick (19), Nova Scotia (2), and Prince Edward Island (18,19). In southern Alberta, the disease occurs mainly on irrigated alfalfa (12).

Verticillium wilt can cause a major reduction of forage yield and shorten stand life (1,10,20,21,27). Growing resistant cultivars of alfalfa is the only effective method to reduce losses due to Verticillium wilt (1,20,27). Two Verticillium wilt-resistant cultivars of alfalfa, Barrier (11) and AC Blue J (formerly line VW-34-2; S. N. Acharya and H. C. Huang, *unpublished*), have been developed at the Agriculture and Agri-Food Canada Research Centre, Lethbridge, Alberta, and licensed for production in Canada. The objectives of this study were to compare alfalfa cultivars from Canada, the United States, and Europe for resistance to Verticillium wilt and to determine forage yield and stand life of each cultivar in the presence of disease.

MATERIALS AND METHODS

The experiment was conducted in a field naturally infested with *V. albo-atrum* near Lethbridge, Alberta. Twelve alfalfa cultivars were seeded on 9 July 1986 in plots arranged in a randomized complete block design with four replications. Ten of the cultivars were known to have some resistance to Verticillium wilt and two, Beaver and Pacer, were known to be susceptible. Each plot was 6 m in length and contained eight rows of alfalfa spaced 0.18 m apart. One row of the cultivar Anik was seeded between plots as a plot divider. The seeding rate for each cultivar was 600 viable seeds per 6-m row. The field was irrigated on the schedule used for an adjoining commercial alfalfa field.

Alfalfa was harvested as forage for 7 yr (1987–1993) with two cuts in each year except for 1988, when three cuts were taken. The center six rows of each plot were harvested with a forage harvester and weighed. To obtain dry matter yields at each harvest, a 250-g sample of fresh alfalfa from each plot was dried at 60 C for 2–3 days and reweighed. During 1987–1992, any weeds that encroached on the plots were not separated from the alfalfa. In 1993 (the seventh production year), the relative weed and alfalfa biomass was estimated before the first cut in all plots and before the second cut in two plots per cultivar. Two representative 0.25-m^2 areas were hand-harvested and separated into alfalfa and weed components. Dry weight of the components was used to determine the composition of the samples.

Prior to each cut in 1987, 1988, and 1989, stems of alfalfa plants with wilt symptoms were collected from each plot and stem segments were plated on selective medium (6) to verify infection by *V. albo-atrum* and to determine the incidence of diseased plants per plot. During these years, a colored marker was placed near the base of each diseased plant to avoid repeat counting of diseased plants. No counts of diseased plants were made in 1990, and during 1991–1993, the number of diseased plants in each plot was estimated by visual symptoms and diseased plants were not marked. The total number of plants in each plot was determined in 1990 and 1993.

Analyses of variance for a randomized block design (26) were done on the number of diseased plants, plant stand, yield, and weed data for each year. The number of diseased plants and percentage of stand diseased in 1993 were log-transformed to stabilize the variances. The cultivars were clustered for similarity of their mean response within years (23). Analyses of variance were also done over years by incorporating years as an additional split-plot factor. For the yield and disease response data, cluster analyses were done to reveal subsets of the cultivars that had similar mean disease levels and yield responses over the years (method 1 of Lin and Butler [15] and Lin et al [16]). On the basis of these results, cultivars were grouped by disease levels.

RESULTS

During the first 3 yr (1987–1989), there was a significant ($P < 0.001$) effect of year on the number of Verticillium wilt infection loci; more newly diseased plants were found in the second year than in the first or third (Table 1). Differences between cultivars ($P < 0.001$) in numbers of diseased plants were similar from year to year for 1987–1989 and 1991–1993. When the cultivars were clustered for similarity of their mean response to the disease and change over years, three groups were formed: resistant = Barrier, AC Blue J, Pioneer 5444, and Vertus; moderately resistant = Maris Kabul, Admiral, and Trumpetor; and susceptible = Excalibur, WL316, Apollo II, Pacer, and Beaver (Table 2, Fig. 1). The effect of cultivar on alfalfa population was not significant ($P > 0.05$) in 1990 (the fourth year of production) but was significant ($P < 0.001$) in 1993 (the seventh production year) (Table 1). The decrease in alfalfa plant numbers between the fourth and seventh production years differed with cultivar ($P < 0.001$), with the resistant cultivars Barrier, AC Blue J, and Pioneer 5444 showing a smaller decrease than the eight

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Table 1. Comparison of 12 cultivars of alfalfa for resistance to Verticillium wilt (1987–1989, 1991–1993) and life of stand (1990 and 1993) in a field naturally infested with *Verticillium albo-atrum*

	Number of newly diseased plants[x]/plot						Alfalfa plants/plot[y]		
Cultivar	1987	1988	1989	1991	1992	1993	1990	1993	Decrease (%)
Barrier	1.1 c	1.6 c	0.0 b	5.8 c	4.4 c	19.1 c	777 a	686 a	11.4 b
AC Blue J	2.8 c	1.5 c	0.4 b	6.4 c	6.0 c	40.7 b	875 a	637 a	27.0 b
Pioneer 5444	1.6 c	1.8 c	0.5 b	13.1 b	14.7 b	42.2 b	876 a	624 a	24.6 b
Vertus	2.7 c	2.9 c	1.2 a	13.3 b	15.5 b	54.6 a	851 a	271 d	66.9 a
Maris Kabul	5.4 b	7.9 b	2.1 a	10.5 b	9.3 b	64.2 a	748 a	304 d	59.3 a
Trumpetor	6.6 b	16.9 a	5.0 a	18.7 b	11.4 b	42.6 b	847 a	338 c	59.3 a
Admiral	3.9 c	6.5 b	2.8 a	14.6 b	21.6 a	73.1 a	826 a	431 b	47.1 a
Apollo II	10.2 a	20.4 a	5.1 a	35.3 a	30.5 a	58.7 a	837 a	349 c	57.4 a
Excalibur	5.6 b	7.5 b	2.5 a	35.6 a	33.0 a	85.3 a	816 a	359 c	55.5 a
WL316	6.9 b	16.9 a	3.8 a	38.7 a	43.2 a	86.7 a	807 a	325 c	59.2 a
Beaver	10.8 a	20.8 a	8.3 a	62.3 a	30.8 a	72.8 a	717 a	270 d	61.9 a
Pacer	14.4 a	22.5 a	8.6 a	61.7 a	38.1 a	112.0 a	738 a	263 d	64.1 a
SE (33 df)[z]	0.1003	0.1079	0.1252	0.0933	0.0955	0.0773	46	24	4.5
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	>0.05	<0.001	<0.001

[x]Means for number of diseased plants are backtransformed values following a $\log_{10}(X + 1)$ transformation. Means followed by the same letter within a column indicate cultivars are homogeneous according to cluster analysis (23) using the 5% significance level. No diseased plant data were obtained in 1990.
[y]Each plot consists of eight rows of alfalfa, 6 m long and 0.18 m between rows.
[z]Standard error of a cultivar mean in $\log_{10}$ units for number of diseased plants.

Table 2. Verticillium wilt resistance level and forage yield of 12 cultivars of alfalfa

		Present study[z]	
Cultivar	Previous reports[y]	Verticillium wilt	Forage yield
Barrier	Resistant, Huang and Hanna (13)	Resistant	High
AC Blue J	Resistant, Huang and Hanna (13)	Resistant	High
Pioneer 5444	Resistant, Woodward et al (28)	Resistant	High
Vertus	Resistant, Graham et al (9), Huang and Hanna (13)	Resistant	Low
Maris Kabul	Resistant, Graham et al (9), Huang and Hanna (13)	Moderately resistant	Moderately high
Admiral	Moderately resistant, Huang and Hanna (13)	Moderately resistant	Moderately high
Trumpetor	Moderately resistant, Gray et al (10), Huang and Hanna (13), Viands et al (27)	Moderately resistant	Moderately high
Excalibur	Resistant, Viands et al (27)	Susceptible	Moderately high
WL316	Moderately resistant, Huang and Hanna (13) Resistant, Gray et al (10), Viands et al (27)	Susceptible	Low
Apollo II	Moderately resistant, Gray et al (10), Huang and Hanna (13), Viands et al (27)	Susceptible	Low
Pacer	Susceptible, Huang and Hanna (13)	Susceptible	Low
Beaver	...	Susceptible	Low

[y]See Literature Cited.
[z]Category based on cluster analysis of data (15).

Table 3. Dry forage yield of 12 cultivars of alfalfa in a field naturally infested with *Verticillium albo-atrum* (1987–1993)

	Dry forage yield[y] (kg/ha)							
Cultivar	1987	1988	1989	1990	1991	1992	1993	Total
Barrier	13,038 a	14,789 a	14,480 a	11,580 a	11,789 a	12,599 a	8,544 a	86,850 a
AC Blue J	12,677 a	14,457 a	13,356 b	11,852 a	11,632 a	13,244 a	8,150 a	85,398 a
Pioneer 5444	12,676 a	16,307 a	14,601 a	11,123 a	11,984 a	12,664 a	7,846 a	87,143 a
Vertus	12,170 a	13,295 a	12,639 b	9,891 c	10,005 b	10,593 a	5,417 b	74,040 b
Maris Kabul	12,144 a	14,722 a	13,447 b	10,530 b	11,024 a	12,196 a	6,806 b	80,812 a
Admiral	12,734 a	14,987 a	13,075 b	10,596 b	11,304 a	11,913 a	8,104 a	82,742 a
Trumpetor	13,112 a	13,848 a	13,079 b	10,600 b	10,147 b	11,726 a	7,066 b	79,520 a
Excalibur	11,470 a	15,352 a	14,000 a	10,428 b	10,274 b	12,682 a	7,118 b	81,353 a
WL316	12,048 a	14,277 a	13,264 b	8,895 c	9,766 b	9,667 a	6,046 b	73,906 b
Apollo II	11,735 a	14,402 a	12,620 b	9,348 c	9,502 b	10,506 a	6,851 b	74,906 b
Pacer	11,800 a	14,274 a	12,697 b	9,395 c	8,380 c	10,585 a	6,360 b	73,519 b
Beaver	12,328 a	13,996 a	12,506 b	9,606 c	8,651 c	11,368 a	6,848 b	75,420 b
SE[z]	426	742	430	355	484	792	528	2,450
P	>0.05	>0.05	<0.05	<0.001	<0.001	<0.10	<0.01	<0.001

[y]Means followed by the same letter within a column indicate cultivars that are homogeneous according to cluster analysis (23) using the 5% significance level.
[z]Standard error of a cultivar mean based on 32 df; 1 df was accounted for by a covariate used to adjust for plot moisture (wet or dry).

cultivars that were moderately resistant or susceptible. Although Vertus appeared to be resistant to Verticillium wilt during the first two production years (1987 and 1988), the eventual decline of Vertus stands was similar to that of susceptible cultivars.

Forage yields of all cultivars were similar ($P > 0.05$) in the first two production years (1987 and 1988), but differences were evident thereafter (Table 3). From 1990 to 1993, Barrier, AC Blue J, and Pioneer 5444 had the highest yields and were in the same cluster group in each of these years. When cultivars were clustered for similarity of their mean yield and difference among years, three groups were formed: high yield = Barrier, AC Blue J, and Pioneer 5444; moderate yield = Excalibur, Maris Kabul, Admiral, and Trumpetor; and low yield = WL316, Vertus, Apollo II, Pacer, and Beaver (Table 2, Fig. 2). Between 1987 and 1993, the difference in mean yield of high- and low-yielding groups increased from 6.1 to 22.9%. Although Barrier and Vertus were both resistant to Verticillium wilt (Fig. 1), the yield of Barrier was significantly higher than that of Vertus for 1989 (third year, $P < 0.01$), 1990 ($P < 0.01$), 1991 ($P < 0.05$), 1992 ($P < 0.10$), and 1993 (seventh year, $P < 0.001$) and for the entire 7-yr period (1987–1993, $P < 0.001$). The total yields for Barrier and Vertus were 86,850 and 74,040 kg/ha, respectively (Table 3). An initial increase in yield from the first to the second production year was followed by a general decrease from the third to the seventh production year (Fig. 2).

In the seventh production year (1993), differences between cultivars in the proportion of alfalfa and weed biomass were more pronounced for the first cut than for the second cut (Table 4). The plots of wilt-resistant cultivars Barrier, AC Blue J, and Pioneer 5444 had higher alfalfa yields and lower weed yields than those of moderately resistant or susceptible cultivars. The weed component in the first cut was mainly dandelion, with some quackgrass, whereas the weed component in the second cut was mostly quackgrass, with a small amount of dandelion. The correlation of total yield (both alfalfa and weed components) and alfalfa-only yield in 1993 was high ($r = 0.92$, $P < 0.001$), and the cultivars ranked similarly for both variables ($r = 0.80$, $P < 0.01$). Because weeds tended to encroach less on plots with resistant cultivars (Table 4), differences among the cultivars were greater for the alfalfa-only yield. For example, in 1993 the difference between Barrier and Vertus was 37% for total forage yield (alfalfa and weed) and 49% for alfalfa-only yield.

DISCUSSION

The results from this study indicate that Verticillium wilt can have a significant effect on forage yield and quality of alfalfa in southern Alberta. Previous reports indicating that the cultivars Barrier, AC Blue J, Pioneer 5444, and Vertus were resistant (11,13,17,21,28) are confirmed. Maris Kabul, Excalibur, and WL316 have also been reported to be resistant to Verticillium wilt (5,9,10, 13,27), but in this study Maris Kabul proved to be only moderately resistant and Excalibur and WL316 were almost as susceptible as Beaver and Pacer. Apollo II, which has been reported to be moderately resistant (13), also proved to be susceptible.

Although an increase in wilt incidence and a decline in forage yield are usually associated with increasing age of alfalfa stands, these trends were not pronounced in this trial, in part because of favorable weather conditions during 1991–1992. Forage yields in the seventh production year (1993) were lower and disease levels were higher than expected because of an unusually wet and cool summer.

Our study also confirms previous

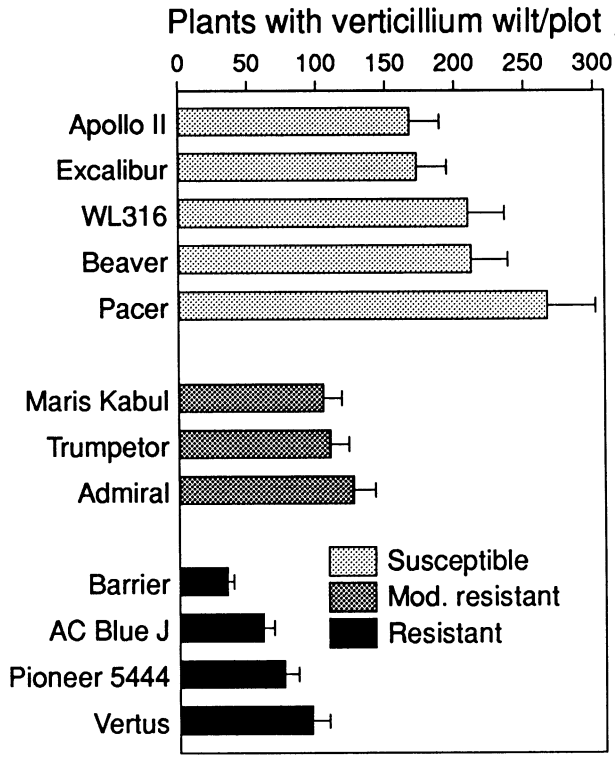

Fig. 1. Incidence of Verticillium wilt in 12 alfalfa cultivars in a field naturally infested with *V. albo-atrum*. Geometric means and approximate standard errors for the total number of diseased plants per plot during 1987–1989 and 1991–1993 for the three resistance groups.

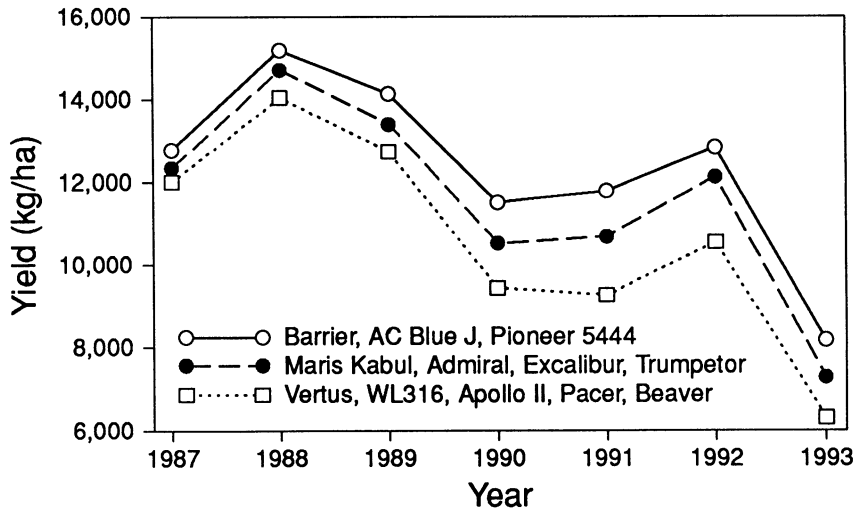

Fig. 2. Effect of Verticillium wilt on forage yield of 12 alfalfa cultivars in a field naturally infested with *V. albo-atrum*. Based on cluster analysis of data (15), cultivars are high yielding (○), moderately yielding (●), and low yielding (□).

Table 4. Alfalfa and weed yields in the seventh production year (1993) of 12 cultivars of alfalfa in a field naturally infested with *Verticillium albo-atrum*

Cultivar	Cut 1[y]			Cut 2[y]		
	Alfalfa yield (kg/ha)	Weed yield (kg/ha)	Percent weeds	Alfalfa yield (kg/ha)	Weed yield (kg/ha)	Percent weeds
Barrier	3,990 a	514 b	12.0 b	4,027 a	270 a	6.4 a
AC Blue J	3,846 a	538 b	12.4 b	4,081 a	150 a	3.3 a
Pioneer 5444	3,626 a	610 b	16.1 b	3,638 a	60 a	1.2 a
Vertus	1,801 b	1,029 a	37.5 a	2,309 a	829 a	25.2 a
Maris Kabal	2,317 b	1,213 a	35.2 a	2,902 a	521 a	15.2 a
Admiral	3,201 a	1,197 a	26.2 a	3,867 a	327 a	9.2 a
Trumpetor	2,207 b	1,418 a	38.5 a	3,292 a	503 a	13.8 a
Excalibur	2,701 b	993 a	27.1 a	2,965 a	360 a	12.5 a
WL316	1,935 b	1,093 a	36.5 a	3,088 a	431 a	12.7 a
Apollo II	2,562 b	1,031 a	28.0 a	3,125 a	479 a	13.8 a
Pacer	2,558 b	1,091 a	29.8 a	2,809 a	273 a	8.5 a
Beaver	2,905 b	1,008 a	24.7 a	2,538 a	424 a	14.9 a
SE[z]	333	198	5.0	513	150	4.3
P	<0.001	<0.10	<0.01	>0.05	>0.05	<0.10

[y] Means followed by the same letter within a column indicate cultivars that are homogeneous according to cluster analysis (23) using the 5% significance level.
[z] Standard error has 32 df for cut 1 and 10 df for cut 2.

findings that yield decline in alfalfa is negatively correlated with level of cultivar resistance to Verticillium wilt (10,20, 21,27). For the first 6 yr of this study, the alfalfa and weed components of forage yield were not separated. Separation of weeds in the seventh year indicated that cultivar differences were greater when alfalfa yield alone rather than total yield was considered. Therefore, differences among the cultivars in the actual alfalfa yield for the previous years are likely to have been at least as large as those obtained for the total yield. Page et al (20) conducted a 6-yr study in Wyoming and found that in the final year, Verticillium wilt-resistant cultivars had a higher alfalfa yield than susceptible cultivars in the first cut but not in the second cut; this result agrees with that of our study.

Cultivar adaptation also affects forage yield of alfalfa. The decline in forage yield between the third and seventh production years was less drastic in the resistant cultivars Barrier and AC Blue J from Canada and Pioneer 5444 from the United States than in the resistant cultivar Vertus from Europe. Both resistance and regional adaptation trials are vital to the development of alfalfa cultivars with high yield and long-term persistence in areas where Verticillium wilt is a threat.

This study indicates that under irrigated conditions in southern Alberta, forage yield of alfalfa starts to decline in the third production year and the yield loss is greater with susceptible cultivars than with resistant cultivars. The progressive thinning of stands with wilt-susceptible cultivars may result in severe weed encroachment. This 7-yr field study shows that the use of wilt-resistant alfalfa cultivars with good regional adaptation prolongs stand life while maintaining forage yields and quality.

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