SHORT COMMUNICATION

A novel source of resistance to verticillium wilt in alfalfa

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Gossen, B. D. and Jefferson, P. G. 2004. **A novel source of resistance to verticillium wilt in alfalfa.**. Can. J. Plant Sci. **84**: 401–404. Verticillium wilt (VW) can cause substantial losses in yield and stand longevity in alfalfa. Resistant cultivars are available, but susceptible cultivars continue to be grown on the Canadian Prairies, where VW generally occurs only under irrigation. A study was conducted to assess the yield and persistence of 11 alfalfa lines under irrigation on three commercial fields in southern Saskatchewan. An epidemic of verticillium wilt developed at one site; the disease reaction of the susceptible *Medicago sativa* ssp. *falcata* line 'SC Mf3713' was similar to that of the resistant cv. Barrier. SC Mf3713 may carry a novel form of field resistance to VW, and merits further study.

Key words: *Medicago sativa* ssp. *falcata, Verticillium albo-atrum*

Gossen, B. D. et Jefferson, P. G. 2004. **Nouvelle source de résistance à la flétrissure verticillienne chez la luzerne**. Can. J. Plant Sci. **84**: 401–404**.** La flétrissure verticillienne (FV) entraîne parfois des pertes importantes au champ tout en réduisant la longévité des peuplements de luzerne. Il existe des cultivars résistants, mais, dans les Prairies canadiennes, on continue de cultiver les variétés sensibles là où la maladie ne survient que lorsqu'on irrigue. Les auteurs ont entrepris une étude pour évaluer le rendement et la persistance de 11 lignées de luzerne dans trois champs commerciaux irrigués du sud de la Saskatchewan. Une épidémie de flétrissure a vu le jour à un site; la variété sensible Medicago sativa ssp. falcata (lignée SC Mf3713) a réagi à la maladie de la même manière que le cultivar résistant Barrier. On en conclut que SC Mf3713 pourrait porter une nouvelle source de résistance à la FV et mériterait des recherches plus poussées.

Mots clés: *Medicago sativa* ssp. *falcata*, *Verticilium albo-atrum*

Verticillium wilt (VW) of alfalfa (*Medicago sativa* L.), caused by *Verticillium albo-atrum* Reinke & Berth., is an important constraint to production of irrigated alfalfa in the interior of British Columbia and southern Alberta. VW-resistant cultivars dominate the market because genetic resistance provides cost-effective disease management. In the small irrigation projects scattered across southwestern Saskatchewan, VW either does not occur, or develops more slowly (Gossen and Jesperson 1990; Gossen et al. 1995) than in the larger irrigation districts of southern Alberta. The large fetch of dryland agriculture surrounding each project results in an oasis effect and relatively low-humidity conditions. The water supply is often limited, and therefore the amount of water applied is often less than optimum; reducing the volume of irrigation water applied reduces disease spread in this region (Jefferson and Gossen 2002).

Most of the alfalfa acreage under irrigation in Saskatchewan is sown to VW-susceptible cultivars. The original research objective was to compare the agronomic performance of newer alfalfa cultivars with that of cultivars that were utilized in the region during the 1940s and 1950s, because producers in the region were sceptical about the agronomic merit of the newer cultivars, including those with VW resistance. When plants with VW were noted in the second year of the trial, our objective was modified to include assessment of field reaction to VW in these cultivars.

One test was seeded 1989 May 29 in a commercial alfalfa field at Miry Creek, Saskatchewan (50°0′N, 108°30′W), on a grumic Sceptre clay soil with orthic Willows soil intermixed. Irrigation water from the South Saskatchewan River was applied by the producer cooperator using field-scale side-roll sprinkler irrigation equipment. The slope of the field was minimal and soil salinity was low (electrical conductivity 2.0 mS cm^{-1}), but a heavy clay surface soil texture reduced the infiltration rate. The year prior to seeding, the site was cropped to oat (*Avena sativa* L.).

A second test was seeded 1989 May 23 at a commercial field at Ponteix, Saskatchewan (49°45′N, 107°28′W) on an orthic alluvium clay soil with an electrical conductivity of 2.8 mS cm^{-1} and a 2% slope. The site was flood-irrigated using a border-dyke system with water from the Notukeu irrigation district. The year prior to seeding, the site was cropped to oat. A third test was seeded on 1989 May 17 at the Semiarid Prairie Agricultural Research Centre at Swift Current, Saskatchewan (50°16′N, 107°44′W). The alluvial

Abbreviations: **VW**, verticillium wilt

clay loam soil at this site had an electrical conductivity of 0.9 mS cm^{-1} and zero slope. It was sprinkler irrigated with hand-set sprinklers from 1989 to 1991, and with field-scale side-roll sprinkler irrigation equipment from 1992 to 1994. The year prior to seeding, the site was fallowed.

Ten alfalfa cultivars (Algonquin, Anchor, Apica, Barrier, Beaver, Grimm, Heinrichs, Ladak, Roamer, Vernal) and one breeding line (SC Mf3713) were arranged in a randomized complete block experiment with six replications. Heinrichs and Roamer are creeping-rooted cultivars developed for dryland pastures in the semiarid region of southern Saskatchewan. Heinrichs has exhibited excellent cold tolerance under irrigation (Jefferson and Gossen 1992). Beaver, Grimm, Ladak, and Roamer are classified as winter-hardy for southern Saskatchewan (Heinrichs 1973). Algonquin has excellent winter hardiness in northern regions, and Anchor, Apica, and Vernal have faster regrowth but slightly less winter hardiness than cultivars recommended for southern Saskatchewan (Gossen et al. 1992). Barrier was the first cultivar with resistance to verticillium wilt that was developed for western Canada (Hanna and Huang 1987). SC Mf3713 is a synthetic breeding line of *M. sativa* ssp. *falcata* Arcengeli that exhibits excellent persistence under grazing (Bittman and McCartney 1994).

Plot dimensions were 1.8 m × 5.5 m, consisting of six rows spaced 0.3 m apart. The seeding rate was 8 kg ha^{-1}; 54 kg ha^{-1} of monoammonium phosphate fertilizer (11-51-0, N-P-K) was applied at seeding. No additional fertilizer was applied during the trial. Weeds were controlled in the establishment year by application of Cobutox (2,4-DB) at the recommended rate in late June 1989, followed by mowing in August. The combined growing-season (May to August) precipitation and irrigation at the Miry Creek site were 403, 440, 429, 400, 275, and 380 mm for 1990–1995, respectively. Combined precipitation and irrigation at Ponteix were 407, 410, 424, 472, and 520 mm for 1990–1994, respectively, and 352, 600, 659, and 674 mm for 1990–1993, respectively at Swift Current.

Forage dry matter yield was determined at the 10% flowering stage by harvesting 0.91 m × 5.5 m of each plot with a flail plot harvester. The fresh weight of forage was assessed in the field; a 300-g subsample was dried for 48 h at 60°C and re-weighed to determine the dry matter content of the forage, and forage dry matter yields were calculated. Harvest dates at Miry Creek were 1990 Jul. 04 and 1990 Aug. 24, 1991 Jul. 12 and 1991 August 30, 1992 Jul. 03 and Sep. 01, 1993 Jul. 02, 1994 Jun. 20, and 1995 Jun. 26. No second harvest was taken from 1993 to 1995; in 1993, the cooperator harvested the test area before sampling was complete; in 1994, irrigation water was not delivered after the first harvest due to a pumping system breakdown; in 1995, stand decline associated with the VW epidemic was so severe that a second harvest was not warranted. The trial was plowed down in the fall of 1995. Harvest dates at Ponteix were 1990 Jun. 28 and Aug. 21, 1991 Jul. 09 and Aug. 28, 1992 Jul. 07 and Sep. 02, 1993 Jun. 28 and Aug. 26, and 1994 Jun. 17 and Aug. 19. Harvest dates at Swift Current were 1990 Jun. 26 and Aug 15, 1991 Jul. 16 and Aug. 21, 1992 Jun. 25 and Aug. 13, and 1993 Jul. 12 and Aug. 25.

At Miry Creek, the number of alfalfa plants with visible VW symptoms in each plot were counted for the first time on 1991 Aug. 30. Stem pieces were collected from plants with symptoms; the pieces were surface-sterilized, split lengthwise under aseptic conditions, and incubated on water agar on a laboratory bench. The presence of *V. albo-atrum* was confirmed by microscopic evaluation of the resulting fungal cultures. No counts of infected plants were made in 1992 because characteristic symptoms were not evident. The number of alfalfa plants with visible symptoms was counted 1993 Oct 13. In 1994 and 1995, a substantial portion of the plants in most plots showed symptoms of VW, so wilt incidence per plot was recorded (1994 Jun 23, 1995 Aug. 25) as a percentage of the plants with visible symptoms. Alfalfa ground cover (percent soil surface area covered with living plants within plot boundary) was scored at Swift Current in 1994, and at Miry Creek and Ponteix in 1995. Three independent observers rated percent ground cover with alfalfa, and the mean percentage was used for analysis.

Analysis of variance was carried out on dry matter yield, ground cover, and VW incidence. Cultivar means were compared using Fisher's Least Significant Difference (LSD) when the cultivar effect was significant ($P \leq 0.05$). A two-factor model in JMP Software (SAS Institute Inc., Cary, NC) was used for analyses of variance and least square estimates of cultivar means reported.

At Miry Creek, first-cut forage yields were generally similar across cultivars; the *M. sativa* ssp. *falcata* line SC Mf3713 provided excellent first-cut yields, even under severe pressure from verticillium wilt (data not shown). Yield of Grimm, the oldest cultivar in the test, was generally lower than the other cultivars (Table 1). Beaver, an old cultivar that is still popular in Saskatchewan, produced the second highest forage yield from 1990 to 1995 (Table 1).

Barrier was the only VW-resistant cultivar in the trial. This cultivar has a high frequency of plants resistant to VW (Hanna and Huang 1987), but a portion of the population is susceptible, so a low level of disease is expected under disease pressure. At Miry Creek, VW occurred at very low levels when first observed. In 1991, the mean number of plants per plot with symptoms was slightly lower ($P < 0.05$) in SC Mf3713 (0.1 plants) than in Barrier (1.5) and the other cultivars (mean of 1.3 infected plants). In 1992, few or no wilted plants were observed. In 1993, incidence was lower ($P < 0.05$) in SC Mf3713 and Barrier (0.1 plants) than in the other cultivars (mean of 0.4). Disease incidence was much higher in 1994 and 1995 (Table 1). The reaction of SC Mf3713 to VW was similar to that of Barrier throughout the course of the trial. This observation was surprising because SC Mf3713 was selected for grazing tolerance (Jefferson unpublished), but not for disease resistance. Also, in trials under controlled conditions where roots of seedlings were clipped and soaked in a conidial suspension of *V. albo-atrum*, SC Mf3713 was susceptible to VW (Gossen, unpublished data). Variability in the disease assessments was very high, as expected when dealing with natural infection that spreads on harvest equipment from hot spots within the field. However, plots of SC Mf3713 and Barrier were visu-

Table 1. Verticillium wilt (VW) incidence, ground cover, and forage yield (Mg ha^{-1}) of 11 alfalfa cultivars grown with irrigation at Miry Creek from 1990–1995, compared with ground cover and forage yield at Swift Current and Ponteix sites, where VW did not occur

	Miry Creek					Swift Current		Ponteix	
	VW (%)		Cover (%)	Yield		Cover (%)	Yield	Cover (%)	Yield
Cultivar	1994	1995	1995	1995	1990–1994 Mean	1994	1990–1993 Mean	1995	1990–1994 Mean
Algonquin	13	38	28	2.89	5.29	41	9.64	24	6.64
Anchor	17	27	41	3.58	5.46	34	9.71	28	7.50
Apica	16	45	26	3.12	5.59	40	9.74	21	7.24
Barrier	4	19	44	3.58	5.53	14	9.46	27	6.32
Beaver	12	23	39	3.23	5.91	35	9.33	30	6.55
Grimm	14	45	19	2.46	4.90	19	8.53	10	6.01
Heinrichs	18	26	31	3.17	5.81	53	9.08	34	7.14
Ladak	14	26	25	2.59	5.38	17	8.95	15	6.85
Roamer	8	27	34	2.46	5.99	44	9.53	29	7.61
SC Mf3713	8	13	43	3.58	5.60	69	8.52	35	7.95
Vernal	12	37	23	2.69	5.57	28	9.40	25	7.71
$LSD_{(0.05)}$	8.0	12.5	14.7	0.45	0.50	21.3	0.53	12.6	0.84
CV (%)	55.6	36.3	39.1	12.5	7.7	50.9	4.9	39.9	9.3

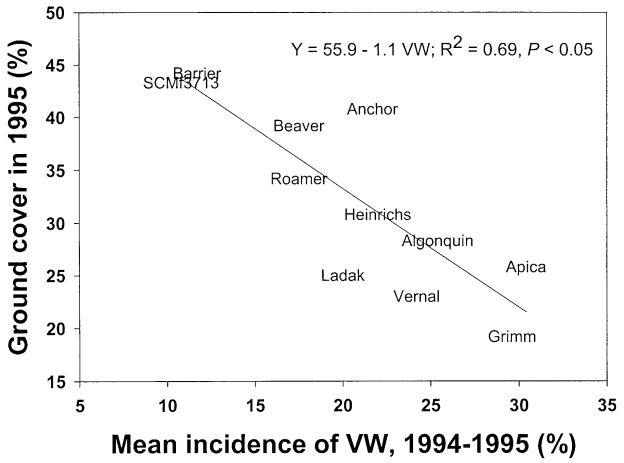

Fig. 1. Relationship between mean verticillium wilt rating from 1994 to 1995 and ground cover of 11 alfalfa cultivars in 1995 at Miry Creek. Cultivar names are used as data symbols.

ally distinct from the other cultivars in 1994 and 1995, when VW was present throughout the test at high levels.

Ground cover ratings at Miry Creek in 1995 differed among alfalfa cultivars (Table 1). Barrier and SC Mf3713 exhibited excellent persistence after several years of VW disease pressure, and percent ground cover was negatively correlated with VW incidence (Fig. 1). Other workers have previously reported a correlation between VW incidence and plant losses in cultivar trials infected with VW (Viands et al. 1992; Gray et al. 1992). Ground cover also differed among cultivars at Swift Current and Ponteix (Table 1), but there was no evidence of VW at either site. SCMf3713 was very persistent at all three sites. In contrast, persistence of Barrier at Swift Current was much lower than at Miry Creek or Ponteix. Barrier has previously suffered severe stand density loss in trials at Swift Current, associated with low soil temperatures during the winter (Jefferson, unpublished data).

Our observations indicate that SC Mf3713 may carry a novel source of resistance to VW. Resistance to several other diseases has been identified in other alfalfa lines with a high proportion of *M. sativa* ssp. *falcata* in their background (Elgin et al. 1988; Gossen et al. 1992; Gossen 1994), but this resistance has rarely been exploited because the slow regrowth of these lines limits their utilization in areas where multiple hay cuts are taken each year. In fact, resistance in SC Mf3713 may result from disease avoidance or tolerance associated with slow regrowth (and therefore a reduced potential for infection) following the first harvest, when the incidence of VW is often high. This resistance is unlikely to be effective under severe disease pressure associated with high levels of irrigation (Jefferson and Gossen 2002), but it clearly provided effective disease reduction in the low-humidity, low-irrigation environment of the Miry Creek irrigation project. Additional work is required to identify the mechanism of resistance and assess its stability under conditions that are conducive for development of VW.

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