Irrigation Increases Verticillium Wilt Incidence in a Susceptible Alfalfa Cultivar

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ABSTRACT

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Verticillium wilt (VW) caused by *Verticillium albo-atrum* occurs in many irrigated alfalfa (*Medicago sativa*) fields in southwestern Saskatchewan, Canada, but is very rare in dryland stands. A trial was established in 1994 to determine if incidence of VW is affected by the amount of irrigation water applied. A line-source irrigation system was used to produce an irrigation water gradient on two adapted alfalfa cultivars, Beaver (VW-susceptible) and Barrier (VW-resistant). The irrigation gradient was subdivided into six irrigation level treatments within each cultivar plot. Irrigation plus precipitation from April to August averaged 265, 291, 309, 330, 351, and 353 mm for irrigation levels one to six, respectively, over 3 years. A 1-m-wide strip through the center of each subplot was inoculated with a spore suspension of *V. albo-atrum* before the first harvest in 1995. Plant density (ground cover) and incidence of VW-diseased plants per subplot were recorded and forage yield was assessed twice per year. Beaver had more VW-infected plants in 1995 and 1996 than Barrier, and the number of diseased plants of Beaver increased with irrigation level. Ground cover of Beaver declined in 1997 and this response was correlated to VW-diseased plant counts from 1995 and 1996. There was no significant correlation between VW-diseased plant counts and ground cover for Barrier. Forage yield increased linearly with irrigation level for both cultivars in two harvests during 1995. However, yield of Beaver did not respond to irrigation during cut 2 in 1996. Beaver forage yield declined with irrigation level at cut 1 in 1997 because ground cover had declined by 40% at irrigation level six compared with 22% at irrigation level one. The ground cover of Beaver at irrigation level one was similar to that of Barrier at all irrigation levels. We conclude that VW will affect susceptible alfalfa cultivars on irrigated stands in proportion to the amount of irrigation water applied. Irrigated alfalfa producers should adopt VW-resistant cultivars even when irrigation is limited.

Additional keywords: forage yield, forage quality, management, *Medicago sativa*, *Verticillium albo-atrum*

Verticillium wilt (VW) of alfalfa (*Medicago sativa* L.), caused by *Verticillium albo-atrum* Reinke & Berthold, was first reported in the northwestern United States in the early 1970s and subsequently spread into most of the alfalfa production areas of North America in the 1970s and 1980s (5). It can cause major stand and yield losses in susceptible cultivars (6). The pathogen requires periods of high humidity for spore dispersal and infection; therefore, it rarely causes severe damage in semiarid, dryland environments (5). Controlled environment studies have shown that water stress reduces the rate of symptom development compared with well-watered controls (19). This relationship is supported by observations from field studies (10,24), but has not been confirmed by studies of variable irrigation rates under field conditions.

VW is an important constraint to production of irrigated alfalfa in the interior of British Columbia and southern Alberta, Canada. VW-resistant cultivars dominate the market, because genetic resistance provides cost-effective disease management. In southern Saskatchewan, however, irrigated alfalfa is still sown to VW-susceptible cultivars because the disease has not yet occurred in most irrigation districts (9).

VW occurred in several irrigated alfalfa fields in Saskatchewan during the early 1980s, but cultivation and crop rotation prevented spread within the local irrigation district (8). However, from 1991 to 1994, the disease was found in several districts that had previously been free of VW (9). In most irrigation districts, VW occurred at low levels and spread appeared to be slower (8,9) than in the large irrigation districts in southern Alberta (14).

In southwestern Saskatchewan, irrigation developments or districts are generally <1,000 ha and are widely scattered with large surrounding regions of dryland agriculture. Hot, dry winds reduce relative humidity of the irrigated alfalfa canopy in these districts. Most districts utilize surface water sources and the supply is often limited; therefore, the amount of water applied is frequently less than the target of 300 mm for rainfall plus irrigation. This situation provided an excellent opportunity to test the hypothesis that the incidence of VW is influenced by irrigation level in alfalfa plants.

One way to achieve differential water stress in alfalfa stands in this semiarid region is to apply different amounts of irrigation water. However, it is almost impossible to apply different amounts of irrigation to various treatments in a randomized small-plot layout. Differential irrigation is possible using very large plots, each with its own irrigation system, but it is difficult to achieve adequate replication in such large plots, and the large size required would introduce confounding factors associated with irrigation time, uniformity of application, and variability in soil parameters across the site. Fortunately, variable irrigation water application can be achieved in a systematic fashion using a line source sprinkler design (11; Fig. 1). This system delivers a gradient of irrigation water perpendicular to a line of overhead sprinklers, so that high application subplots are located adjacent to the line and increasingly lower application subplots are progressively further from the line. This technique has been used to study the impact of water stress on alfalfa forage yield, physiology, and genetics (16,20,21). The objective of this study was to determine the impact of irrigation level on VW incidence on VW-susceptible and -resistant cultivars of alfalfa.

MATERIALS AND METHODS

A trial was seeded on 19 April 1994 on a previously summer-fallowed field immediately north of Swift Current, SK (50° 16′ N, 107° 44′ W). VW had been observed in fields adjacent to this site in 1992 (9). Treated sewage effluent water was used for irrigation throughout the course of this study. Previous research results had indicated effluent irrigation had no significant impact on alfalfa yield or plant density (15). The soil is an alluvial clay loam (2) or alluvial typic Haplustoll (United States

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Department of Agriculture classification). The experimental design was a split plot design with six replications. Two cultivars were the main plot treatments and six irrigation levels were systematic subplot treatments. The cultivars utilized were Beaver, a VW-susceptible cultivar that is widely used for both dryland and irrigated alfalfa production in the region (4), and Barrier, a VW-resistant cultivar recommended for irrigation in southern Alberta (13) and adapted in southwestern Saskatchewan. The seeding rate was 8 kg ha^{-1}, with 0.3 m between rows; 20 kg of P_2O_5 ha^{-1} fertilizer was banded with the seed to promote seedling establishment. Main plot dimensions were 11 by 6 m and subplots were 1.8 by 6 m. Clipping was used to control annual weeds during the establishment year.

A line-source irrigation system (11) was used to produce an irrigation gradient for the subplot treatments (irrigation levels). Irrigation pipes with 90-cm risers to the impact sprinklers were placed between replications so that half the replications were north of the irrigation line and half were south of the line (Fig. 1). This system delivers a gradient of water application perpendicular to the line, so that high-application subplots were located adjacent to the line and low-application subplots were located most distant from the line (Fig. 2). Plastic rain gauges with a 5-cm-diameter opening were placed perpendicular to the irrigation system at 0.60-m intervals and used to record the water applied for each irrigation level (Fig. 2). The low-irrigation treatment (level 1) received natural precipitation (rainfall) and a very low level of irrigation. The six irrigation level subplots were separated by 0.9 m of border area of alfalfa that was not sampled. Irrigation was applied at the same time as adjacent commercial alfalfa fields were irrigated by the site cooperator. The precipitation plus irrigation applications for irrigation level 1 to irrigation level 6, respectively, were 330, 367, 404, 437, 461, and 479 mm in 1995; 281, 305, 320, 337, 351, and 333 mm in 1996; and 189, 199, 207, 217, 242, and 244 mm in 1997. In 1996 and 1997, only limited volumes of water were available for irrigation at this site. As a result, water gradient differences were less than planned.

Two isolates of *V. albo-atrum* (De 9302 and De 9303) from alfalfa in Saskatchewan were grown on commercial potato dextrose agar (PDA) and assessed for purity and uniformity. Vigorously growing cultures were transferred onto PDA. After 10 days, spores were harvested and suspended in sterile water. Harvested spores of the two isolates were combined, and the concentration was measured using a hemacytometer. The suspension was stored overnight at 5°C and diluted to 1×10^5 spores ml^{-1} prior to application. On 13 June 1995, prior to the first harvest, a 1-m-wide strip (parallel with the irrigation line) was cut through the center of each subplot with a flail mower. The alfalfa stem bases in the cut strip then were sprayed to run-off with the spore suspension, applied with a backpack sprayer.

All plants with VW symptoms (i.e., flagging leaves, V-shaped necrosis on leaflets, necrotic leaflets with green stems) in each subplot were counted on 26 June and 19 August 1996 and 12 August 1997, and visual assessment of symptoms was confirmed by isolation onto PDA for a subsample of diseased plants.

Plant density was estimated visually in spring of each year by three or more experienced independent observers and recorded as a percentage of ground cover.

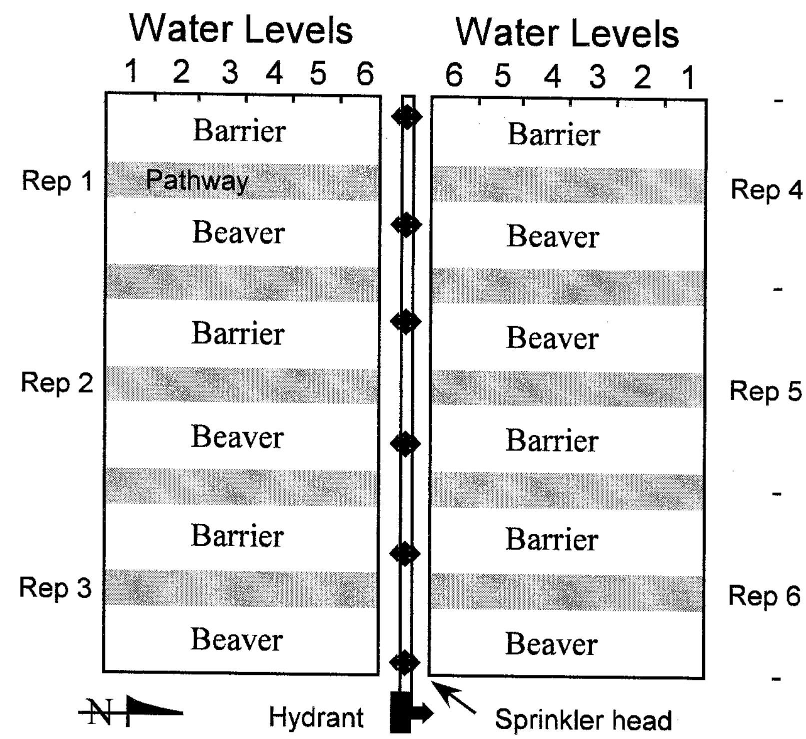

Fig. 1. Plot schematic of the trial with line-source sprinkler, irrigation levels, and cultivars shown.

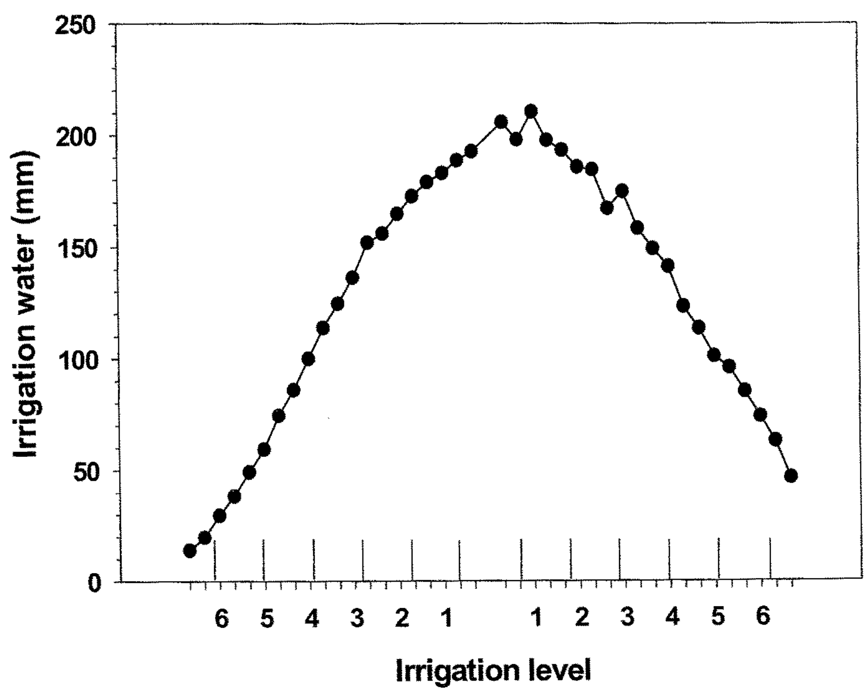

Fig. 2. Irrigation water volume applied in 1995 on each side of the line-source gradient irrigation system. Vertical bars mark sampling points used for the six irrigation levels.

Observations were averaged for each subplot. Establishment was excellent in all subplots with ground cover of 100% in spring 1995. Ground cover decline in 1997 and 1998 was calculated as 100 – (percent ground cover) observed in each spring. Weed invasion did not occur until the 1998 growing season and no forage yield data was collected after 1997.

Forage yield was determined by harvesting 0.91 by 5.5 m of each subplot at early flowering (10% of stems with an open flower). At cut 1 in 1995, the area harvested for forage yield was adjacent to but did not include the area cut and inoculated with VW spore suspension. All harvests after cut 1 in 1995 included the area where plants had been inoculated. Harvest dates were 27 June and 20 August 1995, 2 July and 15 August 1996, and 26 June and 18 August 1997. The June and July harvest dates are referred to as cut 1 and the August dates are referred to as cut 2. A 300-g sample was dried for 48 h at 60°C and reweighed to determine the moisture content of the forage, and forage yields were calculated on a dry matter basis. Dried forage samples from 1997 were ground to pass through a 1-mm screen and stored in glass jars. Forage quality was assessed using acid detergent fiber, neutral detergent fiber (7), and nitrogen content (1) for each sample. Phosphorous content was colorimetrically determined after the sample was digested with sulfuric acid (23).

Analysis of variance (ANOVA) followed the model proposed by Hanks et al. (12) as modified by Jefferson et al. (16) to adjust for the systematic application of irrigation treatments by a line-source sprinkler system. The model provides a valid F statistic for cultivar effects and cultivar–irrigation level interactions, but not for the irrigation level main effect. ANOVA analyses was performed using the General Linear Models procedure of SAS (SAS Institute Inc., Cary, NC), and single degree of freedom contrasts were used to examine the cultivar–irrigation level interaction. Some variables were transformed prior to analysis: ground cover using the arcsine square root transformation, and numbers of diseased plants using the square root of (X + 0.5) (22). Means were back-transformed for presentation.

RESULTS AND DISCUSSION

In 1996, VW incidence was higher in the susceptible cv. Beaver than in the resistant cv. Barrier (P <0.05) (Table 1). VW incidence increased linearly with irrigation level for Beaver in 1996 and 1997. There were a small number of Barrier plants with VW in all of the subplots, but incidence did not change with irrigation level in 1996. However, in 1997, VW incidence increased linearly with irrigation level for Barrier. Disease incidence levels were lower in the trial in 1997 than in previous years, and there were no differences between Beaver and Barrier. Lower-than-planned levels of irrigation were applied to the site in 1996 and 1997 due to limited availability of water. This almost certainly reduced the potential for severe disease in the plots, because VW incidence in Beaver declined from 1996 to 1997. Spore contamination on harvesting equipment moves V. *albo-atrum* from infected to healthy plants (5) and, thus, we expected the counts of VW-diseased plants to increase with time. Barrier contains about 56% resistant plants, representing a high level of resistance to VW (13). Therefore, the higher counts of VW-diseased plants in Barrier in 1997 at irrigation levels 4 to 6 than in the two previous years was unexpected, and the factors responsible for it are not well understood.

Ground cover decreased with irrigation level in the spring of 1997, but losses in ground cover were much greater for Beaver than for Barrier (Table 1). Ground cover decline of Beaver was 50% higher than that of Barrier at the lowest irrigation level (level 1) and nearly twice that of Barrier at the highest irrigation level (level 6). Ground cover decline of Beaver in 1997 was correlated to VW incidence from spring (r = 0.84, P < 0.05) and summer (r = 0.90, P < 0.01) of 1996, but there was no significant correlation between these variables for Barrier (r = 0.35, not significant [NS] and r = 0.30, NS, respectively).

Ground cover declines in 1998 were similar to those from 1997, but this was expected because they include the ground cover losses that occurred previously in 1997 (Table 1). Ground cover decline for Beaver in 1998 increased with irrigation level more than for Barrier. Ground cover for Beaver at level 1 did not decline in 1998 but it did decline at level 6. This loss was correlated with VW incidence from 1997 for Beaver (r = 0.82, P < 0.05), but not for Barrier (r = 0.47, NS). This supports previous reports that ground cover decline is associated with VW infection in alfalfa (10,24). Our results show that, as irrigation increased, VW incidence increased and ground cover decreased, but the magnitude of response was greater for the susceptible cv. Beaver than for the

Table 1. Incidence of Verticillium wilt (VW) and ground cover decline in alfalfa cvs. Barrier (resistant) and Beaver (susceptible) grown at six irrigation levels near Swift Current, SK, Canada[a]

	VW-diseased counts of plants ha^{-1}			Ground cover decline (%)[b]	
Cultivar, irrigation level applied[c]	June 1996	August 1996	August 1997	1997	1998
Barrier					
1	700	300	1,000	13	18
2	500	600	700	15	22
3	400	100	1,300	16	28
4	1,400	1,900	4,100	15	29
5	1,300	1,200	6,400	21	33
6	900	1,000	6,200	21	29
Beaver					
1	4,600	4,500	800	23	22
2	6,900	6,700	1,200	31	35
3	7,400	7,700	1,400	40	42
4	11,000	8,800	3,300	38	48
5	12,600	8,400	5,000	39	52
6	14,100	10,500	6,600	40	49
Contrasts					
Barrier, linear	NS	NS	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$
Barrier, quadratic	NS	NS	NS	NS	NS
Beaver, linear	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$
Beaver, quadratic	NS	NS	NS	$P \leq 0.01$	$P \leq 0.01$
SEM (any 2 means)[d]	2,000	1,200	2,000	4.2	5.5

[a] Means were transformed for analysis and back-transformed for presentation. NS = not significant.
[b] Ground cover decline = 100 – ground cover.
[c] Irrigation levels from 1 to 6, where 1 = precipitation only and 6 = the most water applied.
[d] SEM = standard error of the mean.

resistant cv. Barrier. Although VW incidence and associated losses in ground cover were greatest under intensive irrigation, these results also demonstrate that the potential for ground cover decline caused by VW exists even with low levels of irrigation. Therefore, VW-resistant cultivars should be recommended for irrigated alfalfa production in southwestern Saskatchewan, where irrigation level is frequently restricted by water availability.

Forage yield increased with irrigation level in 1995 for both cultivars at both harvests (Table 2). In 1996, Barrier exhibited a yield response to increased irrigation at cut 2 while Beaver did not. Note that this was 1 year after VW-infected plants were observed in the plots, but 1 year before ground cover decline was observed. This suggests that the impact of VW on yield of susceptible cultivars occurred before the impact on plant density was apparent. In 1997, yield of Beaver declined with irrigation level at cut 1 but increased with irrigation level at cut 2, whereas Barrier forage yield increased with irrigation level at both harvests.

In the spring of 1997, ground cover in Beaver was 60% at level 6 compared with 77% at level 1. In contrast, ground cover of Barrier was 79% or more for all irrigation levels. This indicates that the difference in yield response to irrigation level between these two cultivars was caused by differences in ground cover; ground cover of Barrier was sufficient to produce increased yield in response to irrigation level but the ground cover of Beaver at irrigation level 6 was too low to produce a positive response to irrigation. This is consistent with a previous report that the yield potential of irrigated alfalfa stands declines when ground cover falls below a threshold value (17).

There was no impact of irrigation level on N concentration in 1997 (*data not shown*). Irrigation level increased P concentration of Barrier at cut 1 and for both cultivars at cut 2 (Table 3). The P concentration of the effluent used for irrigation was not determined but may have contributed to this increase in forage P concentration. The neutral detergent fiber and acid detergent fiber concentrations of Beaver at cut 2 increased with irrigation level. These results are consistent with previous reports about the impact of irrigation level on forage quality with exception of N concentration (3,18). Generally, N concentration and digestibility decline, while P concentration and fiber increase with increased irrigation level (18).

The ground cover decline observed in our study was similar to that observed in VW-inoculated, irrigated alfalfa cultivars in Wyoming (10). In that study, ground cover decline over a 3-year period was much greater for VW-susceptible cultivars than for resistant cultivars, and VW ratings were correlated to forage yield in each year. In the Wyoming report, Vernal, the VW-susceptible cultivar, had 53% ground cover compared to 65% for Apollo II, the VW-resistant cultivar in the third year. These values are similar to our ground cover values for Beaver (51%) and Barrier (71%) at irrigation level 6 in the third year after VW symptoms first appeared.

At higher levels of irrigation, increased incidence of VW in the susceptible cultivar was associated with ground cover decline and lower yield. These results are consistent with results from controlled environment conditions using VW-infected alfalfa clones, where well-watered control plants exhibited higher disease rating than water-stressed plants (19). Translocation of spores in the transpiration stream was reduced in water-stressed plants, but there was a flush of VW infection when they were rewatered after water stress (19).

These results demonstrated that ground cover and forge yield losses due to VW of alfalfa increased with irrigation level, despite the relatively small gradients of irri-

Table 2. Forage dry matter yield of two alfalfa cultivars grown at six levels of irrigation water application near Swift Current, 1995 to 1997[a]

	Forage yield tonne ha^{-1}					
	1995		1996		1997	
Cultivar, irrigation level[b]	**Cut 1**	**Cut 2**	**Cut 1**	**Cut 2**	**Cut 1**	**Cut 2**
Barrier						
1	3.69	2.60	4.24	2.09	5.79	0.97
2	4.19	2.56	4.47	2.37	6.32	0.74
3	4.26	3.01	4.71	2.61	6.06	0.91
4	4.48	2.85	4.50	2.54	6.07	1.05
5	4.66	3.21	4.24	2.58	6.18	1.26
6	4.79	3.29	4.29	2.80	6.84	1.54
Beaver						
1	3.98	2.64	3.98	2.60	5.30	1.06
2	4.53	2.55	3.90	2.78	5.45	1.01
3	4.26	2.88	3.93	2.93	5.05	1.10
4	4.52	2.70	3.90	2.68	5.14	1.21
5	4.65	2.60	3.91	2.73	5.01	1.38
6	5.28	3.00	4.17	2.86	4.51	1.52
Contrasts						
Barrier, linear	$P \leq 0.01$	$P \leq 0.01$	NS	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$
Barrier, quadratic	NS	NS	NS	NS	NS	$P \leq 0.01$
Beaver, linear	$P \leq 0.01$	NS	NS	NS	$P \leq 0.01$	$P \leq 0.01$
Beaver, quadratic	NS	NS	NS	NS	NS	NS
SEM (any 2 means)[c]	0.19	0.10	0.18	0.10	0.22	0.08

[a] NS = not significant.
[b] Irrigation levels from 1 to 6, where 1 = precipitation only and 6 = the most water applied.
[c] SEM = standard error of the mean.

Table 3. Phosphorus (P), neutral detergent fiber (NDF), and acid detergent fiber (ADF) concentrations in two alfalfa cultivars grown at six irrigation levels near Swift Current, SK, Canada for over two harvests in 1997[a]

	P g kg^{-1}		NDF g kg^{-1}		ADF g kg^{-1}	
Cultivar, irrigation level[b]	**Cut 1**	**Cut 2**	**Cut 1**	**Cut 2**	**Cut 1**	**Cut 2**
Barrier						
1	2.3	2.3	344	244	305	190
2	2.2	2.3	380	203	335	156
3	2.2	2.4	382	251	341	194
4	2.0	2.6	400	237	362	179
5	2.0	3.0	400	244	344	189
6	2.0	2.8	385	247	345	192
Beaver						
1	2.4	2.3	369	268	328	211
2	2.3	2.3	380	244	333	191
3	2.3	2.5	417	260	346	201
4	2.4	2.6	414	260	359	204
5	2.4	2.8	365	282	319	222
6	2.3	2.8	390	281	333	226
Contrasts						
Barrier, linear	$P \leq 0.01$	$P \leq 0.01$	NS	NS	NS	NS
Barrier, quadratic	NS	NS	NS	NS	NS	NS
Beaver, linear	NS	$P \leq 0.01$	NS	$P \leq 0.01$	NS	$P \leq 0.01$
Beaver, quadratic	NS	NS	NS	NS	NS	NS
SEM (any two means)[c]	0.1	0.1	18	11	14	9

[a] NS = not significant.
[b] Irrigation levels from 1 to 6, where 1 = precipitation only and 6 = the most water applied.
[c] SEM = standard error of the mean.

gation in 1996 and 1997. Although VW was present in Beaver (susceptible) at the lowest irrigation level (nearly equivalent to dryland growing conditions), stand loss was much less than at higher levels of water application. This supports the observation that a VW-susceptible cultivars are generally not at risk from VW under dryland conditions in a semiarid environment (5). The present study is the first report of the impact of variable water application in the field, and it confirms that increasing irrigation increases VW disease incidence in a susceptible cultivar.

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