

Economic Impact and Management of Verticillium Wilt on Irrigated Alfalfa Hay Production in Wyoming

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ABSTRACT

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Loss in irrigated alfalfa (*Medicago sativa*) attributable to Verticillium wilt (caused by *Verticillium albo-atrum*) was determined by comparing yields of resistant and susceptible cultivars grown in the presence of Verticillium wilt. This loss, in combination with the estimated number of hectares infested with *V. albo-atrum* and planted to susceptible cultivars, was used to calculate the economic impact of Verticillium wilt on alfalfa hay production. Test plots were established on 13 May 1982 near Dayton, WY. Verticillium wilt was first detected in the test plots before the third harvest in 1984. Resistant cultivars had less disease, higher yield, and higher plant stands, beginning in 1984, than susceptible cultivars. Average annual yield loss (average difference between the two cultivar groups from 1984 to 1987) attributed to Verticillium wilt was 0.88 Mg/ha. Verticillium wilt was found in nine of the 13 Wyoming counties surveyed. Goshen County had the highest incidence of infested fields (65%). The disease was not found in Fremont County, which has the most hectares of irrigated alfalfa in the state. Approximately 32,877 ha of alfalfa hay grown under irrigation in Wyoming were infested with *V. albo-atrum*. Of the total infested hectares, an estimated 85% (27,946 ha) was planted to susceptible cultivars, of which Ranger was the most frequently planted. With an average hay price of \$70.46 per megagram, the annual loss in Wyoming hay production was estimated to be \$1,732,786. Regression analyses were used to develop equations for predicting the year when alfalfa fields, grown in areas infested with Verticillium wilt, would fall below an acceptable yield (yield threshold) or plant stand (plant stand threshold) level. The use of these equations provides the basis for making economically sound decisions for managing alfalfa.

Verticillium wilt of alfalfa (*Medicago sativa* L.), caused by *Verticillium albo-atrum* Reinke & Berthier, was first reported in the United States in 1976 (13). The fungus has since spread in seed (6) to much of the northern alfalfa-growing region of the United States. It also has been reported in British Columbia, Canada (3), and most recently in two locations in California (9,12). The fungus, which causes a severe wilting and eventual death of alfalfa plants, devastated alfalfa production in Europe (23,24) until resistant cultivars were developed (27).

Verticillium wilt of alfalfa was reported for the first time in Wyoming in 1981 (20). At that time, approximately 800 ha of irrigated alfalfa were found to be infested with *V. albo-atrum* on one

ranch in north central Wyoming. In 1984, Verticillium wilt was found in several fields in southeastern Wyoming, more than 480 km from the original site of infestation. Because of the potentially devastating nature of this disease (2,13) and the known ability for rapid spread of the pathogen (6,8,22,29), studies were conducted to determine to what degree the pathogen had spread and the economic impact of the disease on irrigated alfalfa in Wyoming. Yield loss was estimated by comparing resistant and susceptible cultivars grown in the presence of Verticillium wilt. This, in combination with the calculated hectares infested with *V. albo-atrum* and planted to susceptible alfalfa cultivars, was used to estimate the economic impact of Verticillium wilt on alfalfa hay production in the state. Using regression analyses, equations were developed to assist growers in predicting when resistant or susceptible alfalfa cultivars would fall below a designated yield or plant stand threshold once Verticillium wilt appeared in a field.

MATERIALS AND METHODS

Verticillium wilt survey. Counties in six geographically distinct areas of Wyoming where large hectares of irrigated alfalfa are grown were surveyed for Verticillium wilt between 5 May and 15 June 1987 and between 15 September and 10 October 1988 using a technique previously described for the alfalfa stem nematode (17), with slight modifications. Areas surveyed included the Wind River Basin, the Big Horn Basin, the eastern slopes of the Big Horn Mountains, the North Platte River Valley from Casper to Torrington, southeastern Wyoming, and the Star Valley in west central Wyoming.

Randomly selected fields that were 2.4 ha or larger were surveyed along existing roads. Plants were observed for typical symptoms of Verticillium wilt (26) along a W-shaped pattern through the field. Each field was surveyed until Verticillium wilt was found or until the entire field had been examined. Because of the rapid spread of Verticillium wilt through mowing and baling equipment (29), the entire field was recorded as infested if one or more plants had symptoms of Verticillium wilt. This differed from the previously mentioned survey for the alfalfa stem nematode in which designated sites were sampled and a percent infestation of the field was calculated. Initially, *V. albo-atrum* was confirmed by the presence of the typical verticillate branched conidiophores and conidia (13) on stems or petioles placed on 15% Bacto agar medium (7) and observed at $\times 100$ magnification after 24–48 hr. However, once confidence was gained in visual diagnosis, isolations and microscopic observations were made only where symptoms were questionable.

Estimating yield loss from Verticillium wilt. Loss attributed to Verticillium wilt was estimated from differences in yield between resistant and susceptible cultivars selected from a previous cultivar

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evaluation test (19) and was compared for 4 yr (1984–1987) following the year *Verticillium* wilt was detected in the test. The cultivar test was established in the spring of 1982 near Dayton, WY, to evaluate alfalfa for field reaction to *Verticillium* wilt. A total of 22 entries, including cultivars and experimental lines, were included. The area receives an average of 30.5 cm of precipitation annually (rain and snow), and the test site was flood irrigated after each harvest. Entries were seeded at a rate of 4.54 kg of germinable seed per hectare. Because none of the entries had 100% germination, seed weights were adjusted relative to the percent germination of each entry. Plots (0.8 × 4.6 m) had five rows with a 15-cm spacing. Each entry was replicated four times in a randomized complete block design.

Although *Verticillium* wilt was present in nearby alfalfa fields, plots were sprayed with an aqueous spore suspension of *V. albo-atrum* immediately after the second harvest in 1983 to ensure uniform disease (19). Symptoms of *Verticillium* wilt (7,13) were first observed in the fall of 1984. Plots were rated every spring before the first harvest for disease severity on a scale of 1–5 where 1 = no disease, 2 = slight (1–10% of plants diseased), 3 = moderate (11–30% diseased), 4 = severe (31–50% diseased), and 5 = very severe (>50% diseased). The density of alfalfa plant stands (percent of plant stand remaining compared to a perfect stand of 100%) was rated 2 mo after planting in 1982 and every spring thereafter. Plots were harvested once in 1982 and either twice or three times each year thereafter (three times in 1983, twice in 1984, and three times in 1985, 1986, and 1987), depending on the duration of each

growing season. Subsamples were taken for moisture determination and yield was adjusted to 12% moisture. Plots were maintained and data were collected for 6 yr.

The seedling blight phase of *Phytophthora* root rot caused by *Phytophthora megasperma* Drechs. f. sp. *medicaginis* T. Kuan & D. C. Erwin, previously reported from Wyoming (16), also was found in the test site. Although loss from this disease in the cultivar test appeared to be minimal, only cultivars having resistance to *Phytophthora* root rot were selected for the comparison.

Alfalfa cultivars currently are rated for insects and disease as either susceptible (S = 0–5% of plants are resistant) or resistant where low resistance (LR) = 6–14% of plants are resistant, moderately resistant (MR) = 15–30%, resistant (R) = 31–50%, and highly resistant (HR) = >50% of plants are resistant (11). Cultivar WL 316 (W-L Research, Inc.), which has an R and MR rating for *Verticillium* wilt and *Phytophthora* root rot, respectively, and cultivar Apollo II (ABI alfalfa, AgriPro, Shawnee Mission, KS), which has an MR and HR rating for *Verticillium* wilt and *Phytophthora* root rot, respectively, were designated as the controls resistant to *Verticillium* wilt. WL 312 (W-L Research, Inc., Bakersfield, CA), which has an S and MR rating for *Verticillium* wilt and *Phytophthora* root rot, respectively, and cultivar Wrangler (University of Nebraska, Lincoln), which has an LR and HR rating for *Verticillium* wilt and *Phytophthora* root rot, respectively, were designated as the controls susceptible to *Verticillium* wilt.

All cultivars have a fall dormancy rating of 4 (alfalfa cultivars are rated on a scale of 1–9 where 1 = most dormant

and 9 = least dormant) except Wrangler, which has a rating of 2. Annual yield loss attributed to *Verticillium* wilt from 1982 through 1987 was calculated by subtracting the bulked yields of the susceptible controls (WL 312 and Wrangler) from the bulked yields of the resistant controls (WL I316 and Apollo II). Apollo II, WL 312, WL 316, and Wrangler have yielded similarly in variety trials conducted in Wyoming at locations and during years where *Verticillium* wilt, *Phytophthora* root rot, and the stem nematode (*Ditylenchus dipsaci* (Kühn) Filipjev) were not present (14,25). Apollo II and WL 316 both have ratings of MR whereas WL 312 and Wrangler both have ratings of S to the stem nematode. Comparisons of the resistant and susceptible cultivars also were made for disease and spring plant stands during the 6-yr period. In 1987, weeds and alfalfa were hand-clipped and separated from a 1-m area in each plot, and the percent alfalfa composition was determined on a dry-weight basis. All data from the two resistant cultivars or the two susceptible cultivars were bulked and compared by analysis of variance; means were separated by the least significant difference test (32).

Calculating the economic impact. Calculations of the overall economic impact from *Verticillium* wilt were made from the information on the hectares of susceptible cultivars infested with *V. albo-atrum* collected during the survey and from average yield differences of the resistant and susceptible cultivar groups during the 4 yr after *Verticillium* wilt became established in the cultivar test (1984–1987). The average price for alfalfa hay was obtained from Wyoming Agricultural Statistics (31).

Table 1. *Verticillium* wilt (VW) survey of irrigated alfalfa conducted in Wyoming between 5 May 1987 and 15 September 1988

Major alfalfa growing areas surveyed	County ^a	Hectares surveyed	Surveyed hectares with VW ^b	Percent surveyed hectares with VW	Total hectares of irrigated alfalfa	Percent total hectares surveyed ^c	Estimated hectares with VW ^d
Big Horn River Basin	Big Horn	151	0	0	10,521	1.4	0
	Washakie	146	31	21	6,475	2.3	1,360
	Hot Springs	57	16	28	7,284	0.8	2,040
	Park	179	25	14	14,569	1.2	2,040
Wind River Basin	Fremont	295	0	0	23,472	1.3	0
Eastern Slope of Big Horns	Johnson	204	17	8	9,713	2.1	777
	Sheridan	568	329	58	10,522	5.4	6,103
Star Valley	Lincoln	164	16	10	16,188	1.0	1,619
Platte River Valley	Natrona	46	0	0	5,666	0.8	0
	Converse	256	0	0	11,736	2.2	0
	Goshen	425	278	65	15,378	2.8	9,996
	Platte	316	136	43	8,094	3.9	3,480
Southeastern Wyoming	Laramie	143	77	54	10,117	1.4	5,463
Average				23		2.0	
Total		2,950	925		149,735		32,878

^aCounties listed are those with more than 4,000 ha of irrigated alfalfa and counties where *Verticillium* wilt has been previously identified.

^bPlants were observed for typical symptoms of *Verticillium* wilt along a W-shaped pattern through the field. Each field was surveyed until *Verticillium* wilt was found or until the entire field had been examined.

^cThe percent of total hectares surveyed was obtained by dividing the number of hectares surveyed for each county by the total hectares of irrigated alfalfa in each county.

^dValues were obtained by multiplying the percent alfalfa hectares infested with *Verticillium* wilt by the total number of hectares of irrigated alfalfa in each county.

Developing yield and stand threshold equations. To provide a predictive tool for *Verticillium* wilt management in irrigated alfalfa, yield and percent alfalfa plant stand (dependent variables) were regressed on year after planting for the resistant and susceptible cultivar groups. Data from the year of planting (1981) for both yield and stand was considered to be abnormal and was excluded from the calculations. For the purpose of standardization, as well as ease in calculation, years after planting were converted to consecutive numbers beginning with 1.0. Ordinary least squares (35) then were used to estimate the y -intercepts and slopes for the regression of yield on year after planting, whereas logistic regression

(10) and maximum likelihood algorithms (21) were used to estimate the y -intercepts and slopes for the regression of percent stand on year after planting. Logistic regression, with maximum likelihood estimates, must be used for the regression of percent stand on year after planting because ordinary least squares is an inappropriate method to use when the dependent variable is in percentage scale (35).

The y -intercepts and slopes for all regressions were tested for a significant difference from zero using a two-tailed t test (35). In addition, the coefficient of determination (r^2) was calculated for the ordinary least squares regressions, but not for the logistic regressions

because this statistic is not available for the latter (35).

RESULTS AND DISCUSSION

Verticillium wilt survey. Nine of the 13 counties surveyed had fields infested with *V. albo-atrum* (Table 1). Goshen, Sheridan, Laramie, and Platte counties had the highest levels of infestation (65, 58, 54, and 43%, respectively). *Verticillium* wilt was not detected in Big Horn, Fremont, Natrona, or Converse counties. A total of 32,877 ha were estimated to be infested with *V. albo-atrum*.

The survey indicated that only 12% of the respondents identified the cultivars grown. Because of limited time for the survey, many land owners could not be located, and others had no record of the cultivars planted. Of those who listed the cultivars grown, 16 were identified, of which 13 were susceptible to *Verticillium* wilt, representing 85% of the hectareage. Ranger, which is susceptible to *Verticillium* wilt, was the most frequently planted cultivar. Using this percentage, an estimated 27,946 ha of the total hectares surveyed were planted to susceptible cultivars.

Estimating yield loss from *Verticillium* wilt. Resistant cultivars showed a trend toward less disease beginning in 1984, when *Verticillium* wilt first appeared, through 1987 than susceptible cultivars (Fig. 1). A significant difference ($P < 0.05$) was obtained only in 1986 but approached significance ($P < 0.01$) in 1987. Disease ratings in 1986 were higher ($P < 0.05$, $LSD_{0.05} = 0.2$) in both cultivar groups than in any other year (Fig. 1). Resistant cultivars had significantly ($P < 0.05$) higher yield than the susceptible cultivars in all years from 1984, when *Verticillium* wilt first appeared, through 1987, when the test was terminated (Fig. 2). Although the resistant cultivars outyielded the susceptible cultivars, yields in both groups were beginning to decline in the fifth year after planting.

In a 6-yr study conducted in Riverton, WY, in which alfalfa was evaluated for reaction to *Phytophthora* root rot (17), yields of resistant cultivars were still increasing, whereas those of susceptible cultivars were decreasing when the study was terminated. Therefore, declining yields of the cultivars resistant to *Verticillium* wilt in the sixth year of this study were unexpected and could not be explained. Yield loss between the two cultivar groups, attributed to *Verticillium* wilt for 1984–1987, was 0.8, 1.2, 0.6, and 0.5 Mg/ha, respectively. The average annual yield loss directly attributed to *Verticillium* wilt (overall difference between the two groups from 1984 to 1987) was 0.88 Mg/ha.

The resistant cultivar group showed a trend of higher percent plant stands from 1982 (the year of seeding) through 1987 when the test was terminated, than the susceptible cultivar group (Fig. 3). How-

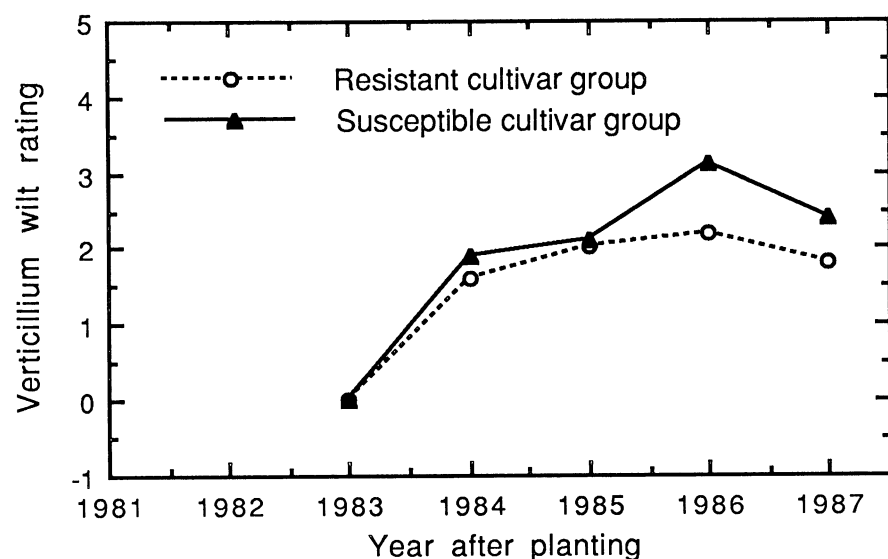


Fig. 1. Development of *Verticillium* wilt in resistant and susceptible cultivars planted near Dayton, WY. *Verticillium* wilt was rated each spring on a scale of 1–5 where 1 = no disease, 2 = slight, 3 = moderate, 4 = heavy infestation, and 5 = majority of plants diseased. Disease ratings of the two cultivars in each group (mean of four replicates) were bulked. Data points for 1986 are different ($P < 0.05$).

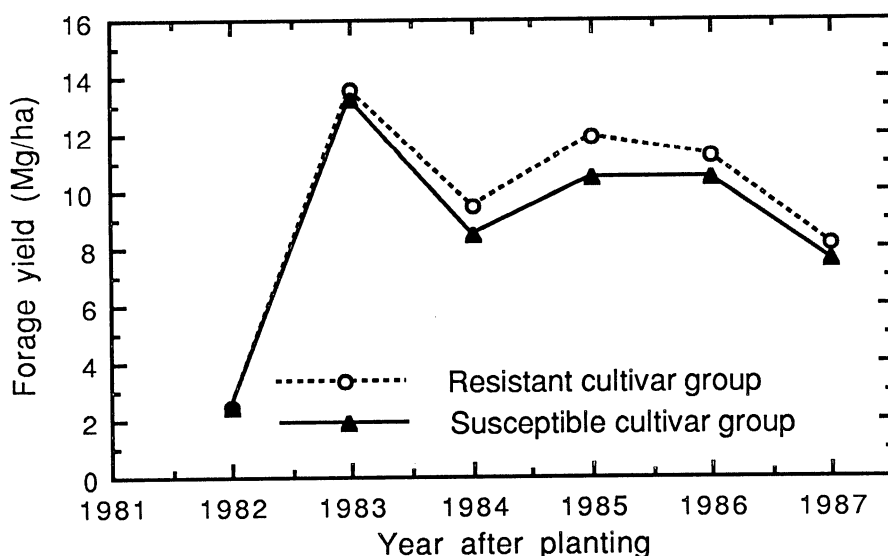


Fig. 2. Yield loss attributed to *Verticillium* wilt in resistant and susceptible cultivar groups grown near Dayton, WY. Mean forage yield (four replicates and two or three harvests) of the two cultivars representing each group were combined. Values are the average forage yield for the two cultivars. Data points for 1984–1987 are different ($P < 0.05$).

ever, significance was obtained only in 1986, and only then at the $P < 0.10$ level of probability. Plant stands in both groups were relatively stable from 1982 to 1984, followed by a gradual decline from 1985 through 1987. A significant ($P < 0.05$) decline occurred in both cultivar groups in 1986 and again in the resistant group in 1987.

The resistant cultivar group had a significantly ($P < 0.05$) higher percentage of alfalfa in the harvested forage than the susceptible cultivar group in the first cutting but not in the second cutting of 1987. Percent alfalfa composition for the resistant and susceptible cultivar groups was 87.8 and 66.4 for the first cutting and 95.0 and 90.1 for the second cutting, respectively. Overall, the resistant cultivar group showed a trend of less disease, better spring plant stand, higher forage yield, and a higher composition of alfalfa in harvested forage in the last harvest year than the susceptible cultivar group.

Calculating the economic impact. The average price of alfalfa hay in Wyoming during 1982–1986 was \$70.46 per megagram (31). Using the estimated per hectare annual yield reduction of 0.88 Mg/ha obtained from the cultivar comparison and an average alfalfa hay price of \$70.46 per megagram, the estimated annual dollar loss per hectare attributable to Verticillium wilt is \$62 per hectare.

According to a statewide survey, there are 32,877 hectares with Verticillium wilt. Multiplying that by 85%, the proportion of hectareage planted to susceptible cultivars, there are 27,946 hectares of susceptible cultivars. Multiplying that number by 0.88, the average annual yield reduction attributable to Verticillium wilt, the total estimated yield reduction is 24,593 megagrams. Again using \$70.46 as the average alfalfa hay price per megagram, the estimated annual dollar loss in Wyoming attributable to Verticillium wilt is \$1,732,786.

The assessment only accounted for the loss in hay yield and did not include possible losses caused by lower hay value attributable to weeds (which were more abundant in the susceptible cultivars), possible lower cash receipts or revenues resulting from 2- to 3-yr rotations with other less valuable crops, or from reseeded caused by more frequent replacement of stands. Had these factors been included, the overall loss would have been much greater. Also, the yield loss assessment was based on differences between resistant and susceptible cultivars. Cultivars used in the resistant category (WL 316 and Apollo II) were rated as resistant (31–50% of plants resistant) and moderately resistant (15–30% of plants resistant), respectively. Had both cultivars been highly resistant (cultivars are now available with >50% of plants resistant) the difference in yield between the resistant and susceptible cultivars may have been even greater. In

similar studies conducted in the presence of Verticillium wilt, the highly resistant cultivar Oneida VR yielded 4.04 Mg/ha more than the susceptible cultivar Saranac AR in the third production year in New York (34).

Another means of assessing disease loss is with the use of plots sprayed with fungicide or unsprayed. Loss assessment of several leaf and stem diseases of alfalfa has been reported using this method (4,5,18,28,33,36,37). However, the use of fungicides to assess loss from vascular wilt diseases, including Verticillium wilt of alfalfa, has been less successful. A recent study conducted in Wyoming found that the fungicides benomyl (applied at 10.3 kg a.i./ha in 4 L of water) and chlorothalonil (applied at 4.6 L a.i./ha in 4 L of water) applied alternately and immediately after harvest, significantly reduced but did not eliminate Verticillium wilt (30). Although the use of alfalfa cultivars with different parentages may not be the best method for assessing loss of alfalfa attributable to Verticillium wilt, it may be the best method currently available. The use of isogenic lines varying only in resistance to Verticillium wilt, in combination with systemic fungicide sprays such as benomyl, would offer a better method. Although several systemic fungicides active against *V. albo-atrum* are available, desirable isogenic lines of alfalfa are not.

Fremont County was the only large irrigated alfalfa-producing area where Verticillium wilt was not found. Because of the transmission of *V. albo-atrum* in seed (3) and its rapid spread from harvesting equipment (26) and insects (22), it is expected to spread eventually to this county, as well as other areas in the state where irrigated alfalfa is grown.

Verticillium wilt has not previously been reported in dryland alfalfa in Wyoming and it was not detected in an identical test grown under dryland conditions at the Sheridan Research and Extension Center, located only 40 km from the irrigated test at Dayton (M. S. Page and F. A. Gray, unpublished data). Therefore, *V. albo-atrum* is not expected to infest and cause yield loss in alfalfa fields grown in the dryland areas of the state.

Developing yield and stand threshold equations. All coefficients for the regression of yield and percent stand on year after planting for the resistant and susceptible cultivar groups were different from zero ($P < 0.05$) (Table 2). Therefore, these equations were algebraically manipulated to predict the number of years after planting that a resistant or susceptible alfalfa cultivar will produce a designated, minimally acceptable yield or percent plant stand. For the case in which a minimally acceptable yield has been determined, the equation takes the general form: years(yield – y -intercept)/slope, where years are the predicted number of years after planting that a given field will be productive, yield is the given minimally acceptable alfalfa yield (Mg/ha), and y -intercept and slope are the appropriate regression estimates for the resistant or susceptible cultivar groups found in Table 2. For the case in which a minimally acceptable percent plant stand has been determined, the equation takes the general form: years = $\{-\ln[1/(\% \text{ stand}/100) - 1] - y\text{-intercept}\}/\text{slope}$, where years are the predicted number of years after planting that a given field will be productive, \ln is the Napierian logarithm, stand is the minimally acceptable level of alfalfa plants considered to be ideal for maximum forage production,

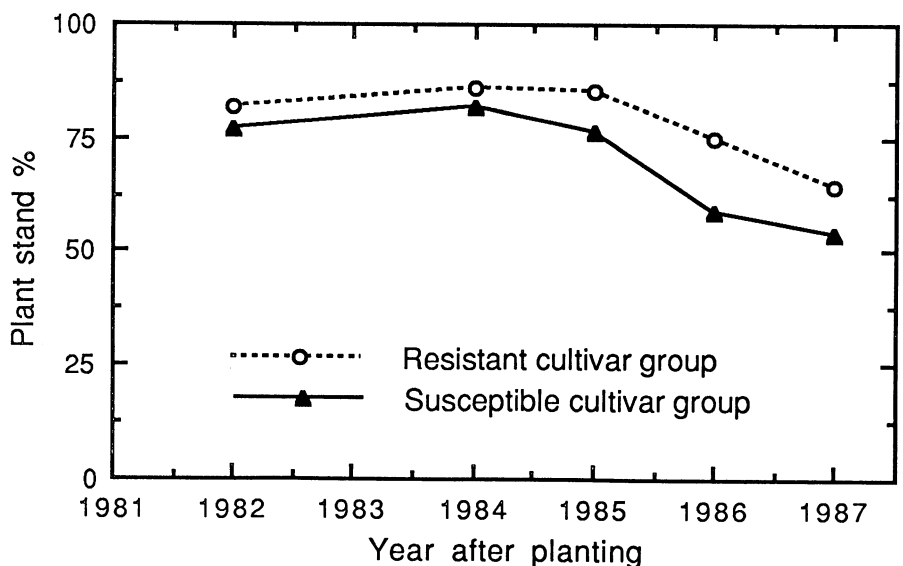


Fig. 3. Impact of Verticillium wilt on density of plant stands of resistant and susceptible alfalfa cultivars grown near Dayton, WY. Plant stands were rated each spring and are the percent of plants remaining compared to a perfect stand rated at 100%. Means of the two cultivars (four replicates each) representing each group were combined. Stand counts were not taken in 1983. Data points for 1986 are different ($P < 0.05$).

and y -intercept and slope are the appropriate logistic regression estimates for the resistant and susceptible cultivar groups found in Table 2.

The usefulness of these equations for management purposes is best illustrated through example. Suppose that a producer is anticipating planting alfalfa in an area infested with *Verticillium* wilt and has determined that a minimally acceptable alfalfa yield for the farming operation is 7.5 Mg/ha per cutting. He must decide whether to purchase seed of a more expensive, certified, resistant cultivar or that of a less expensive, non-certified, cultivar susceptible to *Verticillium* wilt. Using the equation developed for yield and the appropriate data from Table 2, yield would drop below the acceptable level (yield threshold) after 5.79 yr for the susceptible cultivar but not until after 6.66 yr for the resistant cultivar. Planting this field to a resistant cultivar would provide the grower an additional 0.87 productive years to the farming operation. Once this information is known, using estimated future hay and seed prices, the decision as to which seed to purchase could then be made. Similarly, a prediction of the number of acceptable production years can be made from a minimally acceptable alfalfa plant stand. However, if other stand decline diseases are present or suspected of being present, the equation developed for yield is recommended.

Although 100 certified alfalfa cultivars with a moderate or higher level of resistance to *Verticillium* wilt (1) are now available, the use of these cultivars in Wyoming appears to be relatively low. Meanwhile, *Verticillium* wilt continues to spread throughout the alfalfa-producing regions of Wyoming and the United States. Although cultivars with resistance are being produced at a rapid pace by the private sector, losses in the United States attributable to *Verticillium* wilt will continue to increase if grower-educational programs on *Verticillium* wilt are

not expanded.

Additional studies should address the effect of level of resistance on yield loss attributable to *Verticillium* wilt. Most cultivars having a highly resistant rating have only 50–60% resistant plants in their populations. Studies must determine if maximum yield benefit is obtained in these cultivars or if a higher level of resistance (>60%) is needed.

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Table 2. Regression y -intercepts and slopes, number of data pairs, and r^2 for regressions of yield (Mg/ha) and percent alfalfa stand on year after planting for cultivar groups resistant and susceptible to *Verticillium* wilt^a

Cultivar group	y -intercept	Slope	Data pairs	r^2
Regression of yield ^b				
Resistant ^c	13.528* ^d (0.6037) ^e	-0.9047* (0.1820)	40	0.39
Susceptible ^f	12.8250* (0.6509)	-0.9197* (0.1963)	40	0.37
Regression of percent alfalfa stand ^g				
Resistant	2.1979* (0.1016)	-0.2879* (0.0283)	40	...
Susceptible	1.8837* (0.0904)	-0.3386* (0.0256)	40	...

^a Years after planting were consecutive beginning with the number 1.

^b Ordinary least squares.

^c Apollo II and WL 316.

^d Coefficient was significantly different from zero (student's t test).

^e Numbers in parentheses are calculated standard errors.

^f WL 312 and Wrangler.

^g Logistic regression parameters.