Relationship of Verticillium Wilt with Pink-Eye of Potato in Maine

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ABSTRACT

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In 1988 and 1989, the effect of Verticillium wilt on the incidence and severity of pink-eye of potato was studied. Six cultivars and 12 advanced breeding lines (clones) of *Solanum tuberosum* with varying degrees of resistance to Verticillium wilt were selected. Entries were inoculated with either *Verticillium albo-atrum, V. dahliae,* or a combination of both species; the control was noninoculated. Significant differences in the severity of Verticillium wilt symptoms were found for different clones and pathogens, and there was a significant year-by-clone interaction. Verticillium wilt symptoms were more severe in 1989 than in 1988. Pink-eye-infected tubers were found in all treatments, and the clones differed significantly in both incidence and severity of the disease. Significant positive correlations were found between the severity of Verticillium wilt with *V. albo-atrum* or the combination of species, and the incidence or severity of pink-eye. The incidence of pink-eye was enhanced by the presence of *Verticillium,* but the presence of *Verticillium* was not necessary for pink-eye to develop.

Additional keywords: potato early dying

Verticillium albo-atrum Reinke & Berthier and *V. dahliae* Kleb. are two fungal pathogens which cause Verticillium wilt of potato (*Solanum tuberosum* L.) (10). Both pathogens cause the characteristic leaf symptoms, as described by Isaac and Harrison (7), of recoverable true wilting, unilateral permanent wilting, and unilateral chlorosis and necrosis. Infection by these *Verticillium* species reduces the growth rates of tubers, stems, and leaves (1,5,9). Tubers of some cultivars with symptoms of Verticillium wilt develop a vascular discoloration which decreases their marketability. Infection by *Verticillium* species is one of the causes of premature maturation or senescence, which is commonly referred to as potato early dying (9).

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Verticillium wilt has been associated with the pink-eye disease complex (2,3,12). The specific causal organism for pink-eye has not been isolated and identified; however, *Pseudomonas fluorescens* (Trevisan) Migula has been suggested (2,3).

For several years we have rated advanced potato germ plasm lines for their reaction to pink-eye. Each year pink-eye was observed on white-, red-, and russet-skinned lines. On white-skinned lines, pink-eye symptoms ranged from superficial pink spots to complete coverage of the tubers. On red-skinned lines, pink-eye was expressed as a raised, purplish, discolored lesion of the infected areas. On deeply russet-skinned lines, pink-eye symptoms consisted of raised, pink, water-soaked areas surrounding the eyes. Preliminary storage studies indicated that tubers with pink-eye symptoms had a higher incidence of soft rot and tuber end rot. This suggests that the injury caused by pink-eye predisposes tubers to these diseases.

The objective of this study was to determine if there is a correlation between Verticillium wilt, caused by either *V. albo-atrum, V. dahliae,* or a combination of these two pathogens, and the incidence of tuber pink-eye in potato cultivars and breeding lines. A preliminary report on a portion of this research has been published (4).

MATERIALS AND METHODS

Verticillium isolates were recovered from potato plants with symptoms of Verticillium wilt. The inoculum was prepared by growing the isolates in 100- × 15-mm plastic petri dishes containing 25 ml of a medium consisting of 17.5 g of Difco Czapek Dox broth, 19.5 g of Difco potato-dextrose agar, and 10 g of Difco agar per liter of distilled water. After 21 days of incubation at 20–22 C, the cultures were comminuted in a blender, and the resultant slurry was diluted with distilled water to a final concentration of 10^9 conidia per milliliter.

Six cultivars and 12 breeding lines (clones) were selected for their known reaction to Verticillium wilt. Each seed tuber was cut into seed pieces weighing 40–60 g, and seed pieces were immersed in the inoculum or in Difco Czapek Dox broth for 1 min immediately prior to planting. Seed pieces were hand-planted in rows 0.76 m apart, with 0.3-m spacing between plants, on 9 June 1988 and 14 June 1989 at Aroostook State Farm in Presque Isle, Maine. Seed pieces were covered with soil immediately after planting. Potatoes had not been grown in the test plot for more than 5 yr prior to this trial.

Clones and pathogens were arranged in a randomized complete block design with four replications of five hills per plot. The Horsfall-Barratt scheme (6)

was used to evaluate the severity (1 = 0% wilt...12 = 100% wilt) of Verticillium wilt. Individual plants in each plot were rated for disease approximately 90 days after planting, and mean disease severity of Verticillium wilt was calculated for each plot.

At harvest, approximately 120 days after planting, all tubers from each plot were individually scored for severity of pink-eye symptoms by the Horsfall-Barratt rating system. A disease-severity index was used in which the number of tubers per Horsfall-Barratt disease class was multiplied by the disease class, summed over disease classes, and divided by the total number of tubers per plot. In addition, incidence of pink-eye was determined for each plot as the proportion of pink-eye-infected tubers.

Severity of Verticillium wilt, severity of pink-eye, and the arcsine transformation of the incidence of pink-eye were subjected to analysis of variance. Main effect (clone and pathogen) means were separated by LSD.

Years, replications within years, clones, and pathogens were all handled as random effects in the analyses. Satterthwaite's approximation (11) was used to synthesize an F test of the clonal source of variation as $F = MS_c/(MS_{cy} + MS_{cp} - MS_{cpy})$, where MS_c = clonal mean square, MS_{cy} = clone × year mean square, MS_{cp} = clone × pathogen mean square, and MS_{cpy} = clone × pathogen × year mean square.

Similarly, an approximate test for the pathogen source of variation was synthesized as $F = MS_p/(MS_{cp} + MS_{py} - MS_{cpy})$, where MS_p = pathogen mean square, MS_{cp} = clone × pathogen mean square, MS_{py} = pathogen × year mean square, and MS_{cpy} = clone × pathogen × year mean square.

Correlation coefficients between 1) severity and incidence of pink-eye, 2) severity of Verticillium wilt and pink-eye, and 3) severity of Verticillium wilt and incidence of pink-eye were calculated for each pathogen for each year (13).

RESULTS AND DISCUSSION

There were significant differences between years, among clones, and among pathogens; and there was a significant year-by-clone interaction for the severity of Verticillium wilt (Table 1). Verticillium wilt was significantly more severe in 1989 than in 1988. The severity of Verticillium wilt in 1989 may have been affected by increased moisture early in the growing season. According to National Oceanic and Atmospheric Administration (NOAA) records for Presque Isle, Maine, there was 2.6 cm more precipitation in June 1989 (6.8 cm) than in June 1988 (4.2 cm). This is in agreement with the work of Powelson (8), who reported that increased moisture following planting had considerable influence on the development of Verticillium wilt caused by *V. dahliae* in Oregon.

Two cultivars and five breeding lines (Abnaki, Reddale, B0169-56, B0177-20, B0179-3, B0184-30, and B0240-11) were significantly more resistant to Verticillium wilt than were any of the other clones. One breeding line, B0214-9, was significantly more susceptible to Verticillium wilt than were the susceptible cultivars Cherokee and Superior (Table 2).

Inoculation of potatoes with *V. albo-atrum,* alone or in combination with *V. dahliae,* produced significantly more severe early-dying symptoms than inoculation with *V. dahliae* alone (Table 2). The average ambient air temperature and the soil temperature at 13 cm were less than 23 C during the growing season. This may explain why the severity of Verticillium wilt was greater in plants inoculated with *V. albo-atrum.* Rowe et al (10) reported that *V. albo-atrum* grows optimally at 21 C, whereas the optimal growth temperature for *V. dahliae* may be as high as 27 C. The lack of a significant clone-by-pathogen interaction suggests that the clonal response to the different pathogens is one of degree.

There were significant differences among clones, and there were significant year-by-clone and year-by-pathogen interactions for both severity and incidence of pink-eye (Table 1). The incidence, but not the severity, of pink-eye was significantly greater in 1989 than in 1988 (Table 3). There was 5.0 cm more precipitation in September 1989 than in

Table 1. Analysis of variance on the severity of Verticillium wilt and the severity and incidence of pink-eye

Source	df	Severity of Verticillium wilt[a]	Pink-eye	
			Severity[b]	Incidence[c]
Year	1	488.12**[d]	1.08	0.59*
Reps(year)	6	1.58	1.04**	0.07
Clone	17	87.30**	3.47**	0.38*
Pathogen	2	31.49*	1.95	0.21
Year × clone	17	7.70**	0.61*	0.14**
Year × pathogen	2	1.24	2.16**	0.25**
Clone × pathogen	34	2.43	0.37	0.05
Year × clone × pathogen	34	2.21	0.30	0.04
Error	317	1.26	0.28	0.04
Total	430			

[a] Mean squares from the analysis of variance. Rated on the Horsfall-Barratt rating system (1 = 0% wilt ... 12 = 100% wilt) (6).
[b] Mean squares from the analysis of variance. Rated on the Horsfall-Barratt rating system (6).
[c] Mean squares from the analysis of variance on the arcsine transformation. Rated as proportion of tubers with pink-eye.
[d] * = Significant at the 5% level; ** = significant at the 1% level.

Table 2. Severity of Verticillium wilt in potato clones inoculated with *Verticillium albo-atrum* (Vaa), *V. dahliae* (Vd), and a combination of the two, by year and overall (all inoculated plots for both years), and in the noninoculated control[a]

Clone	Vaa		Vd		Vaa + Vd		Overall severity	Control	
	1988	1989	1988	1989	1988	1989		1988	1989
Abnaki	1.0	2.2	1.0	1.5	1.0	1.7	1.4	1.0	1.1
BelRus	2.5	7.0	1.8	4.9	1.8	5.3	3.9	1.0	1.0
B0169-56	1.0	2.1	1.0	2.2	1.0	2.2	1.6	1.0	1.0
B0172-22	3.5	6.3	1.3	5.2	3.2	6.9	4.4	1.0	1.2
B0177-20	1.4	2.8	1.0	2.9	1.0	2.5	1.9	1.0	1.0
B0178-35	1.8	6.1	1.2	4.5	3.0	4.8	3.5	1.0	1.5
B0179-3	1.6	2.6	1.0	1.8	1.0	2.4	1.7	1.0	1.0
B0183-25	1.5	4.9	1.9	3.3	1.5	3.1	2.7	1.0	1.0
B0184-30	1.6	2.1	1.0	2.4	1.0	2.2	1.7	1.0	1.0
B0209-1	1.0	5.4	1.0	3.5	1.2	4.2	2.7	1.0	1.0
B0214-9	9.4	8.8	3.3	7.6	8.6	8.4	7.7	1.0	1.3
B0233-1	1.0	5.5	1.0	4.1	2.3	5.8	3.3	1.0	1.2
B0240-11	1.0	2.5	1.0	2.4	1.0	1.5	1.6	1.0	1.0
B0243-10	1.1	5.2	1.2	2.8	1.3	4.9	2.7	1.0	1.0
Cherokee	6.0	7.1	3.8	6.8	5.0	6.9	5.9	1.0	1.0
Reddale	1.2	1.1	1.0	1.3	1.0	2.1	1.3	1.0	1.0
Russette	1.0	5.3	1.0	3.4	1.0	3.7	2.5	1.0	1.0
Superior	7.2	8.5	3.9	8.0	5.5	7.9	6.8	1.0	1.7
LSD (0.05)							0.6		
Mean	2.5	4.7	1.6	3.8	2.3	4.2	2.1[b] 4.2[c]	1.0	1.1
Overall mean[d]	3.6		2.7		3.3		3.2	1.1	

[a] Severity rated on the Horsfall-Barratt rating system (1 = 0% wilt ... 12 = 100% wilt) (6).
[b] 1988.
[c] 1989.
[d] Least significant difference for comparing pathogen main effect means was 0.3. Figures are for both years.

Table 3. Severity and incidence of pink-eye in potato clones inoculated with *Verticillium albo-atrum* (Vaa), *V. dahliae* (Vd), and a combination of the two, by year and overall (all inoculated plots for both years), and in the noninoculated control

	Severity[a]									Incidence × 100[b]								
	Vaa		Vd		Vaa + Vd			Control		Vaa		Vd		Vaa + Vd			Control	
Clone	1988	1989	1988	1989	1988	1989	Overall	1988	1989	1988	1989	1988	1989	1988	1989	Overall	1988	1989
Abnaki	1.5	2.0	1.4	1.6	1.6	1.3	1.6	1.3	1.5	11.0	29.0	10.3	16.7	12.2	6.9	14.3	8.9	16.0
BelRus	1.0	1.0	1.0	1.0	1.2	1.0	1.0	1.3	1.1	0.0	0.0	0.0	0.0	3.9	2.1	1.0	4.6	2.8
B0169-56	1.7	1.1	1.3	1.0	1.8	1.0	1.3	1.9	1.0	19.6	5.0	4.2	0.0	19.2	0.0	8.0	17.6	0.0
B0172-22	1.4	1.3	1.3	1.3	1.8	1.5	1.4	1.1	1.3	9.4	13.5	8.6	26.2	20.1	25.8	17.3	2.9	10.0
B0177-20	1.7	1.4	1.1	1.3	1.2	1.4	1.4	1.1	1.3	18.7	17.3	3.6	14.0	5.6	14.8	12.3	3.7	14.2
B0178-35	1.5	1.5	1.2	1.2	1.9	1.2	1.4	1.2	1.1	11.0	25.2	5.3	4.3	17.3	12.2	12.6	5.3	2.8
B0179-3	1.8	1.5	1.4	1.4	1.8	1.3	1.5	1.3	1.0	15.9	24.5	8.9	9.7	15.1	8.0	13.7	7.9	2.0
B0183-25	1.6	1.3	1.2	1.7	2.4	1.0	1.5	1.2	1.3	11.0	19.7	6.1	39.4	25.4	0.0	16.5	3.5	22.7
B0184-30	1.3	1.2	1.7	1.0	1.5	1.0	1.3	1.2	1.0	7.3	8.3	15.3	0.0	10.6	2.4	7.3	5.1	0.0
B0209-1	1.8	1.5	1.5	1.1	2.1	1.5	1.6	1.1	1.0	20.3	30.0	11.3	9.5	22.1	15.6	18.1	6.3	0.0
B0214-9	1.6	2.4	1.4	1.8	1.4	1.5	1.5	1.2	1.3	23.7	56.2	7.8	33.3	12.2	34.5	28.0	4.5	15.4
B0233-1	1.7	2.1	1.2	1.3	1.6	1.3	1.5	1.0	1.0	14.5	45.7	6.8	15.8	13.2	11.4	17.9	0.6	2.1
B0240-11	1.4	1.3	1.3	1.3	1.4	1.2	1.3	1.0	1.5	7.8	16.9	8.5	28.4	10.3	12.5	14.0	1.7	29.0
B0243-10	1.7	1.7	1.5	1.3	1.9	1.8	1.7	1.6	1.3	16.8	23.7	12.9	5.9	19.8	18.5	16.3	14.7	6.6
Cherokee	1.5	1.3	1.2	1.2	2.7	1.7	1.4	1.2	1.4	10.6	21.7	4.4	6.9	35.0	27.1	17.6	3.9	23.1
Reddale	1.0	1.0	1.0	1.0	1.1	1.0	1.0	1.1	1.0	1.4	0.0	0.0	0.0	3.0	0.0	0.7	2.1	0.8
Russette	1.0	1.0	1.1	1.1	1.1	1.0	1.1	1.2	1.0	0.0	0.0	2.2	9.4	3.4	0.0	2.5	8.6	0.0
Superior	3.3	3.4	1.5	3.7	2.4	2.3	2.8	1.5	1.2	45.8	68.4	12.4	78.8	27.3	37.5	45.0	10.2	6.5
LSD (0.05)							0.3									11.3		
Mean	1.6	1.6	1.3	1.4	1.7	1.3	1.5[c] 1.4[d]			13.6	22.5	7.1	16.6	15.2	12.7	12.0[c] 17.3[d]	6.2	8.6
Overall mean[e]	1.6		1.4		1.5		1.5			18.1		11.9		14.0		14.7	7.4	

[a] Rated on the Horsfall-Barratt rating system (1 = 0% pink-eye ... 12 = 100% pink-eye) (6).
[b] Rated as proportion of tubers with pink-eye.
[c] 1988.
[d] 1989.
[e] Figures are for both years.

Table 4. Correlation coefficients between the incidence and the severity of pink-eye in potatoes for plots inoculated with *Verticillium* species, and for the noninoculated control[a]

Pathogen	1988	1989
V. albo-atrum	0.95**[b]	0.85**
V. dahliae	0.96**	0.82**
V. albo-atrum + *V. dahliae*	0.96**	0.76**
Control	0.92**	0.82**

[a] Incidence rated as proportion of tubers with pink-eye; severity rated on the Horsfall-Barratt rating system (1 = 0% pink-eye ... 12 = 100% pink-eye) (6).
[b] Significant at the 1% level.

Table 5. Correlation coefficients between the severity of Verticillium wilt and the incidence or the severity of pink-eye in potato for plots inoculated with *Verticillium* species and for the noninoculated control[a]

	Incidence		Severity	
Pathogen	1988	1989	1988	1989
V. albo-atrum	0.37**[b]	0.34**	0.32**	0.37**
V. dahliae	0.12 NS	0.38**	0.14 NS	0.38**
V. albo-atrum + *V. dahliae*	0.31**	0.46**	0.26*	0.38**
Control	0.00 NS	−0.03 NS	0.00 NS	0.10 NS

[a] Severity rated on the Horsfall-Barratt rating system for wilt (1 = 0% wilt ... 12 = 100% wilt) and for pink-eye (1 = no pink-eye ... 12 = 100% pink-eye) (6); incidence rated as proportion of tubers with pink-eye.
[b] * = Significant at the 5% level, ** = significant at the 1% level, and NS = not significant.

September 1988, which may be one cause for the greater incidence of pink-eye in 1989.

Reddale, Russette, and BelRus were highly resistant to pink-eye. Superior was significantly more susceptible to pink-eye than any of the other clones tested (Table 3).

The incidence and severity of pink-eye were not significantly affected by inoculation with *V. albo-atrum, V. dahliae,* or both *Verticillium* species. Overall, the correlation between incidence of pink-eye and severity of pink-eye was very high (Table 4), indicating that the less laborious method of scoring for incidence can be used to provide valid results.

Regardless of the disease-rating system used for pink-eye, correlation coefficients between the severity of Verticillium wilt and of pink-eye were significant in potatoes inoculated with *V. albo-atrum* and the combination of *V. albo-atrum* and *V. dahliae* (Table 5). In 1989, but not 1988, there was a significant positive correlation between Verticillium wilt and pink-eye in potatoes inoculated with *V. dahliae.* In contrast, there was a lack of correlation between Verticillium wilt symptoms and the incidence of pink-eye in the noninoculated control. Such a correlation is not surprising, because Verticillium wilt symptoms observed were mild or absent in the noninoculated controls.

Although the presence of Verticillium wilt was not necessary for the development or expression of pink-eye, there was a positive association between the two diseases. This positive association suggests that enhancing resistance to Verticillium wilt may suppress the development of pink-eye.

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