Pathogenicity of an Isolate of *Verticillium dahliae* from Barley

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ABSTRACT

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A fungus isolated from leaves of barley was determined to be *Verticillium dahliae*. Its pathogenicity was compared with that of known isolates of this fungus from tomatoes, cotton, and potatoes, and with members of various vegetative compatibility groups. Among small grain cereals, oats and barley were susceptible. Wheat was infected but did not develop severe symptoms. Wounding of roots facilitated infection, but was not required. Symptoms on infected oats and barley included chlorosis of leaves, usually in stripes along the leaf margin, followed by necrosis. The isolate originating from barley was similar in pathogenicity to isolates from potatoes or potato soil. Even *V. albo-atrum* from alfalfa was capable of infecting oats and causing slight symptoms. Within barley, there were differences in susceptibility between the various cultivars tested, ranging from highly susceptible to resistant.

Historically, *Verticillium dahliae* Kleb. has been thought to be a pathogen of dicotyledonous plants, with members of the Gramineae considered to be immune or nonhosts (9,11). The only true vascular pathogen of cereals, such as wheat and barley, has been *Cephalosporium gramineum* Nisikado & Ikata (1). In 1985 during a field trip to the Aberdeen Experiment Station in Idaho, I noticed spring barley plants (*Hordeum vulgare* L.) showing symptoms typical of those caused by *C. gramineum*. This seemed unusual because this pathogen primarily attacks fall-sown crops whose roots experience wounding during the winter. Isolations from these barley plants, however, consistently produced pure cultures of a fungus that microscopically appeared to be a *Verticillium* spp.

Control recommendations for Verticillium wilt have often included crop rotation with cereals. While there is evidence that occasionally *V. dahliae* will colonize cereal root surfaces (2,6,7), only the report by Krikun and Bernier (5) has suggested that this pathogen can penetrate into and colonize the vascular system of members of the Gramineae. Isaac and Levy (4) noted that when penetration of barley roots occurs, "microsclerotial formation occurs immediately so that no xylem colonization takes place."

The purpose of this work was to determine the species of *Verticillium* infecting barley, its pathogenic capabilities (compared with isolates of *V. dahliae* of known virulence) towards members of the Gramineae plus dicots commonly attacked by *V. dahliae*, and the factors that might affect its pathogenicity. A portion of this work was published previously (8).

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MATERIALS AND METHODS

Isolates and cultivation. The original isolate of *Verticillium* was obtained from a barley plant (cultivar Hazen) growing at the University of Idaho Experiment Station at Aberdeen. Isolations from the symptomatic plant were made on Czapek's agar after first surface-sterilizing the leaves in 0.5% NaOCl. Other isolates were obtained from various researchers in the United States and included isolates from potatoes, cotton, tomato (race 1 and race 2), and from unknown hosts that represented vegetative compatibility groups 2, 3, and 4 (10). Conidial inoculum of all isolates was produced by growing the cultures in shaken Czapek's broth. In one test, some of the isolates were grown on autoclaved oat kernels for 1 mo, air-dried for 1 wk, briefly comminuted in a Waring Blendor, and used to infest soil.

Plants for pathogenicity studies were grown in either a commercial horticultural mix (Sunshine Mix); a 1:1 mixture of Bozeman silt loam soil with river sand; or a 1:1:1 mix of Bozeman silt loam, sand, and peat moss (= PGC mix). The soil mixtures were pastuerized with aerated steam before use. Plants were fertilized with Peter's solution (20:20:20) as needed to ensure good growth.

RESULTS AND DISCUSSION

Based on production of microsclerotia typical of those produced by known isolates of *V. dahliae*, the isolate from barley was determined to be *V. dahliae* (3). An initial test was conducted to determine if this isolate was pathogenic to "traditional" hosts of this pathogen. An isolate of *V. dahliae* originally from cotton (ATCC 18702) was used for a comparison. For root-dip inoculations, plants were grown in Sunshine Mix for 4–6 wk, uprooted, and their roots either left intact or severed with scissors about 2 cm below the crown. The plants were placed in a conidial suspension (5×10^6/ml) for 5 min and then transplanted back into Sunshine Mix. A similar test was established using infested oat kernels mixed with Sunshine Mix (3 g of inoculum/100 g of mix). In this test, no root wounding occurred.

With conidial inoculum, the only plants to show symptoms were potatoes, but positive isolations of both the cotton

Table 1. Pathogenicity of two isolates of *Verticillium dahliae* to monocots and dicots

	Inoculation technique					
	Root dip[v]				Soil infestation[y]	
	Wounded		Nonwounded		Nonwounded	
Host and cultivar	478[w]	495[x]	478	495	478	495
Tomato						
Marglobe	I[z]	I	I	I	I-S	0
Ace	0	0	0	0	0	0
Potato						
Russet Burbank	I-S	I-S	0	I-S	0	0
Barley						
Klages	I	I	I	I	I-S	0
Wheat						
Pioneer 2369	I	I	I	0	0	0
Oats						
Monida	I	I	I	I	0	I-S

[v] Root dip was 5 min in 1×10^6 conidia/ml.
[w] Isolate 478 = ATCC 18702 from cotton.
[x] Isolate 495 from barley.
[y] Soil infestation was 3 g of infested oat kernels/100 g of soil mix.
[z] I = positive isolation from host tissue, I-S = positive isolation from host tissue plus symptoms of leaf chlorosis, 0 = not isolated.

and barley isolates were made from leaves of potatoes, barley, wheat, and oats (Table 1). With soil infestation, isolate 495 from barley produced symptoms on oats, while the cotton isolate (ATCC 18702) produced symptoms on Marglobe tomatoes and Klages barley.

To further compare the pathogenicity and virulence of barley isolate 495 with other "known" isolates of *V. dahliae*, Earlipak and Pakmore tomatoes with differential resistance to race 1 and race 2 of *V. dahliae* were used along with Russet Burbank potatoes, Pioneer 2369 wheat, Monida oats, and Larker and Klages barley. The tomatoes were grown in Sunshine Mix while the other hosts were grown in the soil:sand mix. Plants to be root-dip inoculated were uprooted, placed in a suspension of conidia (1×10^6/ml) for 15 min, and then transplanted back to the original soil mix. In some cases, the roots were severed before dipping in the inoculum. For soil infestation, 100 ml of conidial inoculum (1×10^6/ml) was poured over the surface of the soil mix and allowed to infiltrate the mix. In some cases, the roots were severed by driving a knife into the soil and moving it back and forth before addition of the inoculum. Three replicates of three pots each were used for each treatment. The plants were grown in a greenhouse with supplemental metal halide lighting for 7 hr per day with a day temperature of 18–21 C and a night temperature of 10–13 C.

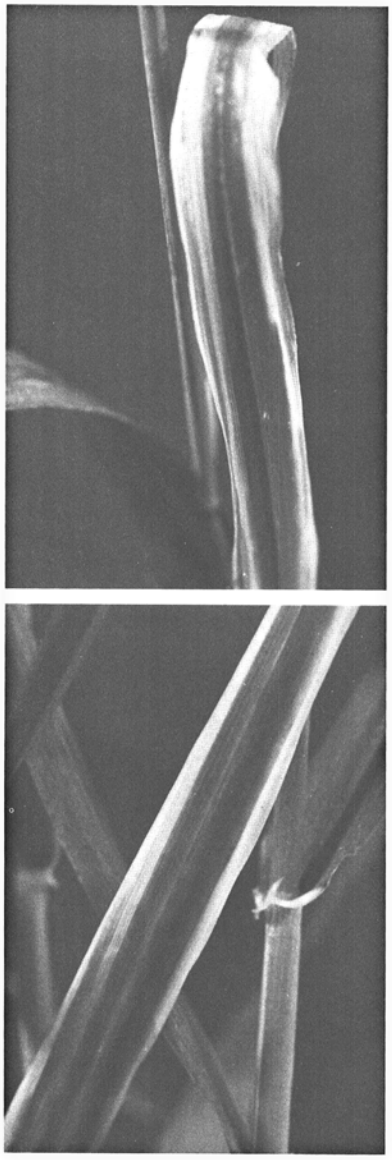

Fig. 1. Symptoms in barley leaves infected with *Verticillium dahliae* showing marginal leaf chlorosis.

With tomatoes, symptoms were read after 6 wk and isolations were made from all plants 7 wk after inoculation. With the other plants, symptoms were read after 8 wk and isolations were made 9 wk after inoculation. In barley, several of the isolates caused yellow stripes to develop in the leaves, often along one side of the leaf (Fig. 1). Eventually the entire leaf would turn chlorotic, then necrotic. The infected plants were somewhat stunted as compared with the noninoculated controls. Except near the base of the plant, there was little evidence of veinal necrosis such as usually occurs in Cephalosporium stripe of wheat and barley (1). In a few instances, the leaf yellowing would occur in a localized area of the leaf without the entire length of the leaf being involved. Yellowed leaves were often quite flaccid.

In oats, there were usually leaf scorching symptoms leading to necrosis, but not always in stripes. Occasionally there would be scattered lesions in the leaves. Wheat developed only a few symptoms involving some marginal leaf scorch, but usually only the lowest leaves were involved. Based on symptom severity, oats were the most susceptible, followed by barley, with wheat showing the fewest symptoms.

Isolates of *V. dahliae*, other than 495 from barley, were also capable of infecting and causing symptoms in the three cereals, as well as in the more traditional hosts, tomatoes and potatoes (Table 2). Again, oats and barley were the most susceptible of the three cereals. The two races of *V. dahliae* from tomato performed as expected on the two differential tomato cultivars. The isolate from cotton was similar to race 1 from tomato. The other isolates, including the one from barley, were only slightly pathogenic to these two tomato cultivars but were pathogenic to potatoes, from which most of the isolates originated. Wounding usually, but not always, resulted in more severe symptoms. Based on this study, it appears that the isolate from barley is most like the isolates from potato, which is not surprising because potatoes had been grown on the land before the barley crop that became infected.

To determine if various cultivars of wheat, oats, and barley differ in their reaction to *V. dahliae*, plants were grown in the PGC soil mix until the fourth leaf stage. They were then uprooted and the roots were severed approximately 5 cm below the crown. They were placed in a conidial suspension (1×10^6/ml) for 10 min and transplanted back into the soil mix. Symptoms were read at heading and isolations were made from symptomatic plants. Four replications of each host were used, with three plants per replication. Race 1 of *V. dahliae* from tomato was also used as a comparison to isolate 495 from barley.

Based on severity of symptoms, race 1 was more pathogenic than isolate 495,

Table 2. Pathogenicity of various isolates of *Verticillium dahliae* on various monocots and dicots when the roots were severed and dipped into a conidial suspension (10^6/ml) for 10 min

		Symptom severity[v]						
		Potato	Tomato[w]		Oats	Barley		Wheat
Isolate	Original host	Russet Burbank	Earlipak	Pakmore	Monida	Klages	Larker	Pioneer 2369
495	Barley	2	2	1	3	3	2	1
499	Tomato, race 1	4	4	1	4	4	4	1
500	Tomato, race 2	2	3	3	...[x]	...	...	...
501	Potato, ID[y]	2	2	1	2	2	1	1
502	Potato, ID	4	2	1	4	2	2	1
505	Potato, WA	4	2	1	4	4	4	2
506	Cotton, CA	3	4	1	4	4	4	1
513	Unknown, VG2[z]	1	3	1	4	3	3	1
514	Unknown, VG3	1	1	1	3	1	4	1
515	Unknown, VG4	1	2	1	3	3	4	1
516	Potato soil, OH	2	1	1	4	4	4	1

[v] Symptom severity score: 0 = no symptoms, 1 = slight lower leaf yellowing, to 4 = severe chlorosis/necrosis of most leaves of plant.
[w] Earlipak susceptible to both race 1 and race 2; Pakmore susceptible to race 2, but resistant to race 1.
[x] Not tested.
[y] Isolates obtained from various researchers in the United States: ID = Idaho, WA = Washington, CA = California, and OH = Ohio.
[z] VG = vegetative group (10).

but both isolates were capable of infecting and causing severe symptoms in many of the cultivars tested (Table 3). There did seem to be differences between cultivars in their susceptibility. Within the 10 cultivars of barley tested, there was a range from very susceptible to resistant. Wheat was the least susceptible of the three cereals tested.

To determine the effect of inoculum density on severity of reaction, Monida oats and Klages barley were uprooted at the four-leaf stage, their roots were clipped 5 cm below the crown, and then they were dipped into conidial suspensions varying from 10^3 to 10^7 per ml for 10 min. They were then transplanted back into the original soil mix. In addition to isolate 495 from barley, race 1 from tomato, isolate 516 from potato soil, and an isolate of *V. albo-atrum* Reinke & Berth. from alfalfa were tested. Symptoms were read at heading and isolations were made on Czapek's agar from symptomatic plants.

While symptoms were observed at levels as low as 10^3 conidia/ml, the most severe symptoms usually developed with 10^6 conidia/ml or higher (Table 4). Even *V. albo-atrum* caused a few mild symptoms on oats at the higher inoculum levels and was recovered in isolations from leaves. Isolate 516 from potato soil was quite similar in pathogenicity to isolate 495 from barley.

To determine the effect of time of exposure to inoculum on infection, plants of Monida oats and Klages barley were uprooted at the four-leaf stage, their roots were clipped 5 cm below the crown, and then they were dipped in a conidial suspension (1×10^6/ml) of isolate 495 for times varying from 1 sec to 10 min. They were then transplanted back into the original soil mix. While the most severe symptoms developed in barley with the 10-min dip, in oats there was little difference between the 1-sec dip and the 10-min dip.

While *V. dahliae* has normally been considered a pathogen of dicots, this study suggests that this pathogen, and its close relative *V. albo-atrum*, can colonize the internal tissues of cereal plants, move within the vascular system to the top of the plant, and cause symptoms that are typical of other vascular pathogens of cereals. Krikun and Bernier (5) also have reported that they could isolate *V. dahliae* from leaf tissues of wheat, oats, and barley infected with isolates from potatoes or peas, but they failed to observe any symptoms on infected plants. They also found microsclerotia in the roots of infected cereals. While root wounding did greatly aid infection, some infection did occur in the absence of wounding. The fact that this disease was first discovered in a spring-sown barley plant suggests that infection probably occurred in this instance without the benefit of root wounding. Because the symptoms are not highly distinctive, they may have been overlooked in the past and confused with nutrient deficiencies that cause leaf yellowing and necrosis or firing.

While it is unlikely that Verticillium wilt will become a serious disease of small grain cereals, there does seem to be resistance available within existing cultivars that breeders could use as sources of resistance if the need arose.

Table 3. Response of various cultivars of wheat, oats, and barley to *Verticillium dahliae* from barley and race 1 from tomato when root-dip inoculated with 1×10^6 conidia/ml for 10 min

Host and cultivar	Check[x]	Symptom severity[y] (*V. dahliae* isolate)	
		Isolate 495 from barley	Race 1 from tomato
Oats			
Monida	0.0	0.5	2.8
Coyote	0.0	1.0	2.0
Park	0.0	1.2	2.8
Cayuse	0.0	0.0	2.2
Otana	0.0	0.5	2.5
Barley			
Betzes	0.0	0.2 a[z]	3.0
Bonanza	0.0	2.8 ab	3.0
Compana	0.0	3.2 bcd	3.8
Klages	0.0	2.8 ab	3.8
Piroline	0.0	4.2 d	4.8
Glen	0.0	2.8 ab	4.8
Larker	0.0	1.2 a	2.8
Melvin	0.0	0.8 a	3.8
Steptoe	0.0	0.2 a	3.0
Unitan	0.0	3.8 cd	4.5
Wheat			
Triticum aestivum			
Pondera	0.0	0.0	0.0
Sheridan	0.0	0.2	1.2
Manitou	0.0	0.2	0.5
T. durum			
Wells	0.0	0.8	0.8
Ward	0.0	0.5	1.2

[x] All figures not significant.
[y] Symptom severity score: 0 = no symptoms, 1 = lowest leaves chlorotic or necrotic, 2 = symptoms on leaves one to two from bottom of plant, 3 = symptoms on leaves one to three from bottom, 4 = symptoms on leaf just below flag leaf, 5 = symptoms on flag leaf.
[z] Means in a column followed by the same letter are not significantly different ($P = 0.05$) using the Student-Newman-Keuls multiple comparison test. Means not followed by a letter are not significant.

Table 4. Effect of inoculum density of *Verticillium dahliae* and *V. albo-atrum* on infection of barley and oats when root-dip inoculated with a conidial suspension

Conidial concentration (no./ml)	Symptom severity[z]			
	V. dahliae isolate			*V. albo-atrum* isolate
	495	499	516	433
Monida oats				
0	0.0	0.0	0.0	0.0
10^3	0.7	1.0	0.0	0.0
10^4	0.3	1.7	1.3	0.7
10^5	1.0	3.0	1.7	0.0
10^6	1.3	4.0	2.0	2.0
10^7	3.0	3.7	1.3	1.3
Klages barley				
0	0.0	0.0	0.0	0.0
10^3	0.0	0.0	0.3	0.0
10^4	0.3	0.0	0.7	0.3
10^5	1.0	1.0	2.0	0.3
10^6	3.3	3.0	2.7	0.3
10^7	2.7	4.0	3.7	0.3

[z] Symptom severity score: 0 = no symptoms, 1 = lowest leaves chlorotic or necrotic, 2 = symptoms on leaves one to two from bottom of plant, 3 = symptoms on leaves one to three from bottom, 4 = symptoms on leaf just below flag leaf, 5 = symptoms on flag leaf.

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