A Bacterial Kernel Spot of Barley Caused by *Pseudomonas syringae* pv. *syringae*

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ABSTRACT

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Necrotic spots on developing barley kernels, observed only rarely before 1977, were common on sprinkler-irrigated barley cultivar Klages in southern Idaho in 1977. Necrotic lesions on barley leaves and leaf sheaths were observed in 1980. Affected kernels had tan to dark brown necrotic spots with distinct margins. A Gram-negative, oxidase-negative, fluorescent bacterium identified as *Pseudomonas syringae* pv. *syringae* was the causal organism. *P. syringae* strains isolated from blighted seed and necrotic leaves were identical in pathogenicity on several hosts and were indistinguishable in physiological tests. Infection of barley lemmas occurred most often in kernels inoculated before the lemma became attached to the seed.

In 1977, a kernel spot of unknown etiology was common on sprinkler-irrigated Klages barley (*Hordeum vulgare* L.) grown in southern Idaho (17). Before 1977, the necrotic spots were observed only rarely and the disease was not severe in 1978–1980. Symptoms appeared as tan to dark brown necrotic spots about 2 mm in diameter with distinct margins (Fig. 1). Lesions were predominantly on the lemma and did not extend into the seed coat. The water-soaked appearance of developing lesions suggested a bacterium was the causal organism. In 1980, tan to dark brown necrotic lesions occurred on leaves and leaf sheaths. These lesions coalesced into elongated areas that expanded laterally. Leaf symptoms were similar to those of leaf necrosis of wheat (8) incited by *Pseudomonas syringae* pv. *syringae* van Hall (1), except the lesions on barley remained dark.

Others have noted an association between *P. syringae* and barley. Galachian (2) reported infection of wheat seedlings under laboratory conditions with *P. syringae* pv. *atrofaciens* (McCulloch) Young, Dye, & Wilkie (1). The pathogen caused basal glume rot on wheat and barley. Sands et al (12) recovered wheat leaf-spotting pseudomonads from barley and wheat seed samples collected in Montana. Koroleva and Sidovenko (6) reported *P. syringae* pv. *atrofaciens* as the causal agent of bacteriosis of barley ears in the USSR.

The objectives of this study were to investigate the etiology of barley kernel blight, specifically to determine if seeds are more susceptible at a particular time during their development and to determine if there was variability among barley cultivars in reaction toward kernel blight. Other objectives were to associate seedling reaction with kernel blight and to determine the susceptibility of other cereal hosts to the pathogen. A preliminary report has been published (10).

MATERIALS AND METHODS

Isolation. Seed lots of blighted Klages barley grown commercially in southern Idaho were obtained from the 1977–1980 crop years. Blighted seed were surface-

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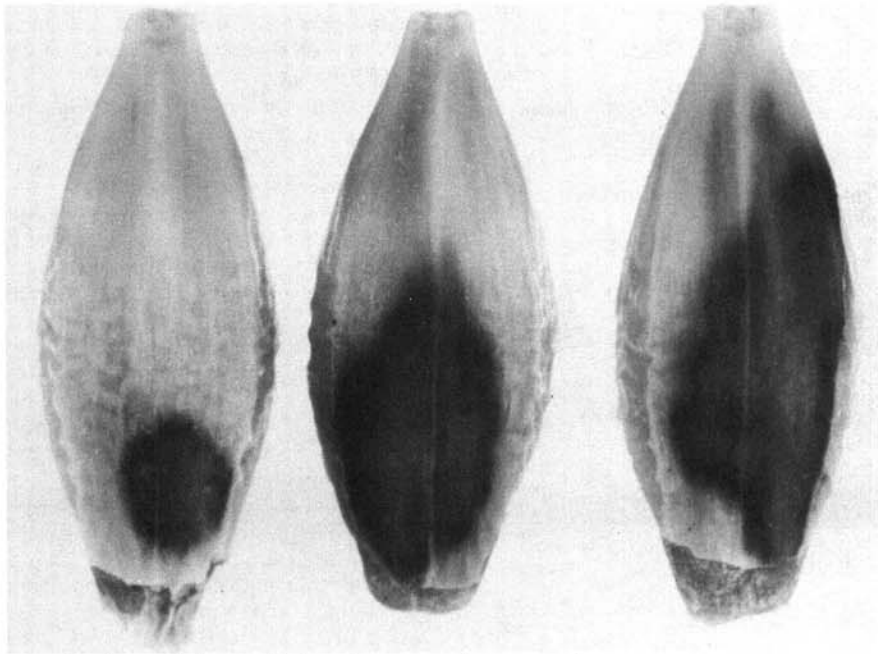

Fig. 1. Barley seed infected with *Pseudomonas syringae* pv. *syringae*.

sterilized in 2% sodium hypochlorite for 60 sec, rinsed in 1,000 ppm sodium thiosulfate, then rinsed in sterile distilled water. Surface-sterilized seed were soaked in sterile distilled water for 24 hr and infected portions of the lemmas and surrounding tissue were then removed and plated on artificial media. Media used for isolation from seed were nutrient agar plus 0.5% sucrose (NA), King's medium B (5) (KB), and Sands' (13) BCBRVB medium.

Leaves were washed in running tap water for 10 min, then a small piece of tissue from the margin of the infected area was removed, rinsed in sterile distilled water, and plated on KB and NA media.

After incubation for 24, 48, or 72 hr at 23 ± 1 C, *Pseudomonas*-like colonies were detected visually by pigment production and/or fluorescence under a model UVL-22 BLAK-RAY (Ultraviolet Products, Inc., San Gabriel, CA) ultraviolet lamp. Bacterial colonies were streaked on KB medium to obtain culture purity.

Pathogenicity. Pathogenicity of bacterial colonies isolated from blighted barley kernels and necrotic leaves was confirmed on Klages barley. Seedlings in the two- to three-leaf stage were inoculated by partial vacuum-infiltration or by hypodermic needle intromission with bacterial cell suspensions of about 1×10^6 colony-forming units per milliliter of liquid. Bacteria were taken from 48-hr cultures on KB medium and suspended in sterile distilled water. Inoculated plants were incubated in a mist chamber for 72 hr at 23 ± 2 C. After incubation, plants were maintained in a greenhouse at 23 ± 2 C with supplemental fluorescent lighting to give an 18-hr day for symptom expression. Bacteria were reisolated from developing lesions by plating on KB medium.

Bacterial strains pathogenic on barley seedlings were tested for pathogenicity on wheat, oats, rye, sorghum, corn, and bean and for their ability to induce hypersensitivity reactions in tobacco leaves. Inoculation and incubation were as described for barley seedlings.

Bacteria reisolated from developing lesions in barley were identified as *P. syringae*. Identification was based on the following tests: Gram stain, oxidase reaction, fluorescence on KB, arginine dihydrolase production, levan production, tobacco hypersensitivity, and ability to utilize various compounds as carbon sources (13).

Antibiotic (rifampin) resistant pathogenic strains (Rf) of *P. syringae* were selected from colonies isolated from blighted barley kernels according to the methods used by Weller and Saettler (16).

Ten plants each of Klages, Piroline, and Vanguard barley were grown in the greenhouse. Each plant had at least three heads. Heads with kernels in the watery ripe to milky stage were spray-inoculated with a 1×10^6 cell suspension of an Rf strain of *P. syringae* with an artist's airbrush. Two uninoculated plants of each cultivar served as controls. Inoculated plants were incubated as described for seedlings. Blighted and unblighted kernels were removed in the early yellowing stage of development. Isolations were made from the kernels by plating portions of the lemmas containing lesions on KB and on KB containing 50 μg/ml rifampin.

Relationship of kernel development and kernel blight. Barley plants of 10 cultivars were grown singly in 15-cm pots in the greenhouse. Five pots of each cultivar were planted three times at 2-wk intervals. Each planting was considered a replicate. When the plants headed, stages of kernel development in each head were rated on a 1–6 scale where 1 = anthesis complete with little or no kernel filling, 2 = caryopsis elongation and filling but without lemma attachment to the caryopsis, 3 = watery ripe, 4 = milk formation, 5 = dough formation, and 6 = slight yellowing of the barley head.

Heads at the various stages of kernel development were simultaneously spray-inoculated with a *P. syringae* strain isolated from blighted seed. The numbers of blighted and unblighted seeds that developed were recorded.

Physiological tests. All *P. syringae* cultures were tested at least once using standard biochemical tests with the API 20E System (Analytab Products, Plainview, NY 11803). Biochemical tests were made on bacteria from isolated colonies on NA in petri plates. Tests were made for the presence of β-galactosidase, arginine dihydrolase, lysine decarboxylase, tryptophane diaminase, and urease; production of hydrogen sulfide, indole, and acetoin; and reduction of nitrates to nitrites or nitrogen gas. Bacteria were also tested for ability to liquefy gelatin and to utilize the following compounds as a carbon source: citrate, glucose, sucrose, rhamnose, arabinose, melibiose, inositol, sorbitol, mannitol, and amygdalin. Results were recorded after a 48-hr incubation period at 25 C.

The presence of oxidase was tested by smearing a loopful of bacteria from a plate on an oxidase test disk (Difco Laboratories, Detroit, MI 48232). Development of pink to purple in 5–30 sec was recorded as a positive test and no color as a negative test.

Strains were streaked on NA containing 5% (w/v) sucrose to test for the formation of levan. The presence of convex mucoid colonies after 3–5 days of incubation was recorded as a positive test.

Results of physiological tests using American Type Culture Collection (ATCC) strains #9004 and #19607 of *P. syringae* were compared with those obtained from blighted seed and leaves.

Table 1. The reactions of eight hosts inoculated with *Pseudomonas syringae* isolated from blighted barley seed and leaves

Host	*P. syringae* source	
	Seed	Leaves
Barley	LN[a]	LN
Wheat	LN	LN
Oats	0	0
Rye	0	0
Corn	0	0
Sorghum	HLS	HLS
Beans	0	0
Tobacco	HR	HR

[a] LN = leaf necrosis, HLS = holcus leaf spot, HR = hypersensitive reaction, and 0 = no reaction.

Table 2. Percent kernel infection of 10 cultivars spray-inoculated with *Pseudomonas syringae*

Cultivar	Percent infection
Moravian III	7.56[a]
Piroline	5.30
Morex	5.27
Vanguard	3.93
Klages	3.83
AB 4302	2.20
Steptoe	1.83
Hector	1.70
Beacon	1.57
Larker	0
LDS, $P = 0.05$	3.36

[a] Numbers represent mean of three replicates with five plants per replicate.

RESULTS

Isolation. *P. syringae* was isolated from 61% of the blighted barley kernels obtained from commercial fields when BCBRVB medium was used and from 21% of the blighted kernels when KB was used. A bacterium identified as *Xanthomonas campestris* pv. *translucens* (Jones, Johnson, & Reddy) Dye (1) was isolated on NA from 7% of the blighted seed.

Pathogenicity. *P. syringae* strains isolated from blighted seed and necrotic leaves were identical in pathogenicity. Results of pathogenicity tests on several hosts are given in Table 1. *P. syringae* strains caused leaf necrosis in barley and wheat seedlings, holcus leaf spot on sorghum, and a hypersensitive reaction on tobacco. No evidence of pathogenicity was observed on inoculated seedlings of oats, rye, corn, or bean.

Tests for pathogenicity on various hosts and standard identification techniques (13) indicated that the bacterium that caused blighted kernels on barley were *P. syringae* pv. *syringae* (1). Inoculation of barley heads with an Rf strain of *P. syringae* resulted in 7, 4.2, and 3.1% of the kernels of Piroline, Klages, and Vanguard barley, respectively. *P. syringae* Rf strains were always reisolated from blighted kernels on KB medium containing 50 μg/ml rifampin and were isolated from 81% of blighted kernels on

KB. *P. syringae* Rf strains were isolated from 45% of inoculated unblighted kernels on KB and 45% of inoculated unblighted kernels when KB medium with 50 μg/ml rifampin was used. Blighted kernels did not develop in the uninoculated controls, and neither the wild nor the rifampin-resistant forms of *P. syringae* were isolated from uninoculated kernels.

Relationship of kernel development and kernel blight. The range of *P. syringae* kernel blight infections among 10 barley cultivars was from 0 to 7.56% (Table 2). No kernel blight infection occurred in Larker barley, an intermediate level of 3.83% occurred in Klages barley, and 7.56% occurred in Moravian III barley. Kernel infection by *P. syringae* was most frequent on plants inoculated before kernel development reached the milky dough stage (Fig. 2), ie, at completion of caryopsis elongation and before the lemma became attached to the caryopsis.

Physiological tests. Strains of *P. syringae* isolated from blighted barley seed and leaves were indistinguishable in physiological tests (Table 3). Characteristics were similar to those of *P. syringae* pv. *atrofaciens* (ATCC 9004) and *P. syringae* pv. *coronafaciens* (Elliott) Young, Dye, & Wilkie (ATCC 19607) (Table 3). *P. syringae* strains from barley gave a negative response when tested for the presence of oxidase, arginine dihydrolase, and urease. All strains gave a positive response for the production of levan and presence of catalase. They did not reduce nitrates to nitrites or nitrogen gas; neither did they generate hydrogen sulfide or indole. Ability to form acetoin (Voges-Prokauer) and liquefy gelatin varied among strains. The strains utilized citrate and produced acids from utilization of glucose, sucrose, arabinose, mannitol, inositol, and sorbitol. Acids were not produced from rhamnose or amygdalin. Acid formation from melibiose was a variable characteristic.

DISCUSSION

Bacterial kernel blight, typified by tan to dark brown necrotic spots on barley kernels, was caused by *P. syringae* pv. *syringae*. This agrees with Koroleva and Sidovenko (6), who reported that this species caused bacteriosis of barley kernels in the Ukraine. The bacterium also causes necrotic lesions on barley leaves and leaf sheaths.

Koch's postulates were successfully completed with *P. syringae* strains from southern Idaho. The bacterium was frequently isolated from blighted seed; barley heads were inoculated, symptoms typical of kernel blight developed, and the bacterium was reisolated from the blighted seed. When antibiotic resistant mutants were used for inoculations, the mutant bacteria were always isolated from blighted seed.

Infection of barley lemmas with subsequent kernel blight development occurred in a higher percentage of kernels when they were inoculated before the lemma became attached to the caryopsis. Lemma attachment occurs shortly after the caryopsis reaches its maximum length (during the watery ripe stage) (3). Jones et al (4) and Shekhawat and Patel (15) have shown that *X. c.* pv.*translucens* enters the leaf and lemma through stomata and that bacterial invasion is confined to the thin-walled parenchyma with intercellular spaces. Only the inner epidermis of the barley lemma contains stomata; the outer epidermal cells have simple pits (7,9,11). We have shown that *P. syringae* pv. *syringae* is less able to infect kernels after the watery ripe developmental stage. We suggest that the bacteria enter the lemma

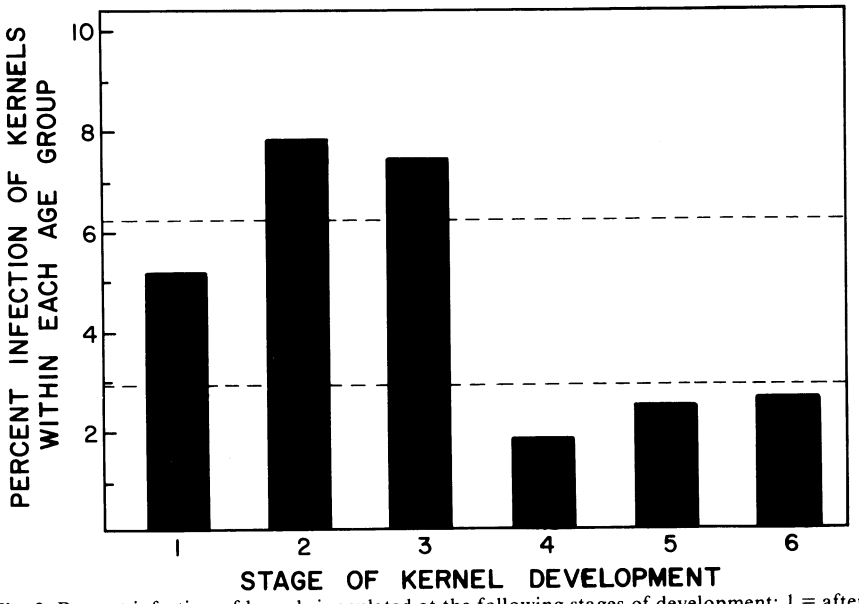

Fig. 2. Percent infection of kernels inoculated at the following stages of development: 1 = after anthesis, 2 = caryopsis elongation complete, 3 = watery ripe, 4 = milk, 5 = dough, and 6 = yellowing. Area between broken lines equals LSD 0.05.

Table 3. Comparison of biochemical and physiological characteristics of two *Pseudomonas syringae* pathovars and oxidase-negative pseudomonads isolated from blighted barley kernels and leaves

Test	Pseudomonads from barley		*P. syringae* pv.	
	Seed[u]	Leaves[v]	*atrofaciens*[w]	*coronafaciens*[x]
Oxidase	–[y]	–	–	–
Hypersensitivity	+	+	+	+
Arginine dihydrolase	–	–	–	–
Motility	+	+	+	+
Gelantin hydrolysis	d[z]	d	+	+
Nitrate reduction	–	–	–	–
Catalase	+	+	+	+
Urease	–	–	–	–
Indole	–	–	–	–
Voges-Proskauer	d	d	–	–
Levan	+	+	+	+
H_2S	–	–	–	–
Carbon substrate				
Citrate	+	+	–	–
Glucose	+	+	+	+
Sucrose	+	+	+	+
Rhamnose	–	–	+	–
Melibiose	d	–	+	–
Arabinose	±	+	+	+
Mannitol	+	+	+	+
Inositol	+	+	+	+
Sorbitol	+	+	+	+
Amygdalin	–	–	–	–

[u] Based on 15 strains from diseased barley seed.
[v] Based on 13 strains from diseased barley leaves.
[w] American Type Culture Collection 9004.
[x] American Type Culture Collection 19607.
[y] – = Negative; + = positive.
[z] d = Between 21 and 79% of the strains were positive for this test.

primarily through the stomata before lemma-caryopsis attachment. Once the lemma becomes attached to the caryopsis, infection may be caused by bacterial entry through pits or wounds in the external epidermis.

Cultivars vary in susceptibility to *P. syringae* pv. *syringae* under greenhouse conditions. Cultivars need to be examined for bacterial kernel and leaf blight susceptibility to the pathogen in the field. Otta (8) and Sellam and Wilcoxson (14) indicated that some wheat cultivars are resistant to leaf necrosis. One might also expect variation in susceptibility among the barley cultivars. Why kernel blight has been observed as a problem only on Klages cannot be explained at this time.

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Figure 1 courtesy of V. D. Pederson.

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