Bacterial Brown Spot of Wild Rice

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ABSTRACT

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Bacterial brown spot (BBS) of wild rice (*Zizania aquatica*) is characterized by elliptical to spindle-shaped lesions 2–12 mm long and 1–10 mm wide, which may become irregularly shaped or diffuse with lengths exceeding 100 mm. Three strains from BBS lesions were pathogenic to wild rice but not corn, wheat, or barley. Nine strains from BBS lesions were identified as *Pseudomonas syringae* pv. *syringae* and were distinguished from 11 strains of pv. *zizaniae* from bacterial leaf streak of wild rice by utilization of trigonelline and L(+)lactate and by production of syringomycin but not tyrosinase.

In 1980, a new disease of cultivated wild rice (*Zizania aquatica* L.) was found in Minnesota. The symptoms were very similar to those of brown spot caused by *Bipolaris oryzae* (Breda de Haan) Shoem. and *B. sorokiniana* (Sacc. in Sorok.) Shoem. (1,6). Lesions, however, tended to be more irregularly shaped with diffuse margins and many lesions also had translucent spots or slits in their centers. Isolations from these lesions consistently yielded bacteria rather than either of the two fungal pathogens known to cause brown spot. The relationship of bacterial brown spot (BBS) with the previously reported (2) bacterial leaf streak (BLS) of wild rice was unknown. The purpose of this research was to study the etiology of this new disease and to identify the pathogen.

MATERIALS AND METHODS

Isolation of bacteria. Lesions were cut into 1-mm^2 sections with a sterile scalpel and immediately placed in a drop of sterile distilled water (SDW). After 5–10 min, the drop was diluted 10- to 1,000-fold in SDW and a loopful was streaked onto plates of King's medium B (KMB) (7). Cultures were incubated at 24 C in the dark and examined after 2 and 4 days. Single colonies were streaked once on KMB and strains were subsequently transferred to slants of nutrient agar (Difco, Detroit, MI 48232) and stored at 5 C.

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Characterization of isolates. Nine strains from BBS lesions were compared with 11 strains of *Pseudomonas syringae* pv. nov. *zizaniae* (Bowden and Percich, 1983) from BLS of wild rice. In addition, reference cultures of *P. syringae* pv. *syringae* van Hall from corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), and lilac (*Syringa vulgaris* L.) were obtained from E. L. Stromberg, W. Shane, and the American Type Culture Collection (Rockville, MD 20852), respectively. Biochemical tests were performed as described (2). Syringomycin production was bioassayed with *Geotrichum candidum* Link (4).

Pathogenicity tests. Plants were grown and pathogenicity tests performed as described (2). Three strains from BBS of wild rice were tested for pathogenicity to wild rice (cultivar Netum), corn (cultivar Golden Cross Bantam), wheat (cultivar Angus), and barley (*Hordeum vulgare* L. 'Morex'). In addition, *P. syringae* pv. *syringae* reference strains were tested for pathogenicity to wild rice.

RESULTS

Symptoms. The initial symptom of bacterial brown spot (BBS) of wild rice is a small water-soaked spot or short streak on the leaf. A diffuse greenish brown halo with a dark brown margin rapidly develops around the central water-soaked area. Later, the greenish brown area becomes chestnut or tan (Fig. 1). Small or narrow lesions appear uniformly dark brown. Typical lesions are roughly elliptical or spindle-shaped and 2–12 mm long by 1–10 mm wide. Lesions are often irregularly shaped or diffuse and as long as 100 mm or more. Very rarely, white exudate is present in the center of the lesion. Frequently, the original water-soaked areas can be seen as translucent spots or slits in the centers of lesions. In older lesions, these translucent areas may

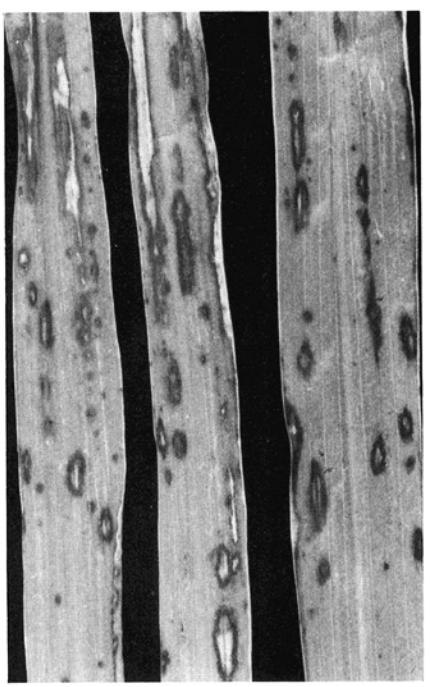

Fig. 1. Bacterial brown spot of wild rice caused by *Pseudomonas syringae* pv. *syringae* (field inoculation).

Fig. 2. Bacterial leaf streak of wild rice caused by *P. syringae* pv. *zizania* (natural infection).

Table 1. Comparison of bacterial brown spot (BBS) and bacterial leaf streak (BLS) strains from wild rice and reference strains of *Pseudomonas syringae* pv. *syringae*

Item	Host and strain of *P. syringae*				
	Wild Rice		Corn	Wheat	Lilac
	BLS	BBS	(Holcus-1)	(CI)	(ATCC 19310)
No. of strains	11	9	1	1	1
Characters					
Fluorescein	11[a]	9	1	1	1
Oxidase	0	0	0	0	0
Arginine dihydrolase	0	0	0	0	0
Potato soft rot	0	0	0	0	0
Levan	11	9	1	1	1
Esculin hydrolysis	10	9	1	1	1
L-tyrosine hydrolysis	11	0	0	0	0
Gelatin hydrolysis	9	9	1	1	1
Syringomycin	0	9	1	1	1
Utilization of:					
D-glucose	11	9	1	1	1
Sucrose	11	9	1	1	1
L(+)arabinose	11	9	1	1	1
D(+)galactose	11	9	1	1	1
Fructose	11	9	1	1	1
D(+)mannose	11	9	1	1	1
D(+)trehalose	0	0	0	1	0
D(+)cellobiose	0	0	0	0	0
Citrate	11	9	1	1	1
L(+)tartrate	0	0	0	0	0
D(−)tartrate	0	2	0	0	0
D(−)quinate	11	9	1	1	0
L(+)lactate	0	9	2	1	1
Anthranilate	0	0	0	0	0
Succinate	11	8	1	1	0
Acetate	11	9	1	1	0
DL-homoserine	0	0	0	0	0
I-erythritol	10	9	1	1	1
D-mannitol	11	9	1	1	1
D-sorbitol	11	9	1	1	1
I-inositol	9	9	1	1	1
Trigonelline	0	8	1	1	1
Betaine	11	9	1	1	1
L-serine	11	9	1	1	1
DL-ornithine	0	0	0	0	0
DL-β-hydroxybutyrate	0	1	0	0	0

[a] Number of strains positive.

fall out, giving leaves a shot-hole appearance.

Symptoms of BLS are typically quite different (Fig. 2). Occasionally, BLS strains cause small brown spots or short streaks that resemble BBS, but BLS lesions have less diffuse margins and are translucent throughout, whereas BBS lesions are opaque or have only a central translucent area.

Isolation and characterization. Bacteria were isolated in large numbers from the translucent areas of lesions. Twenty strains were obtained from seven locations in Aitkin, Clearwater, Polk, and Ramsey counties in Minnesota. All strains produced white, domed, circular, smooth colonies with entire margins on KMB and were fluorescent, Gram-negative, and oxidase-negative. A subset of nine strains was found to be arginine dihydrolase-negative, strictly aerobic, potato soft rot-negative, and produced levan (Table 1). Based on these tests, BBS isolates were identified as *Pseudomonas syringae* van Hall (9).

Strains of *P. syringae* from BBS lesions were similar to reference strains of *P. syringae* pv. *syringae* but differed from BLS strains of *P. syringae* in several respects. First, BBS colonies were typically domed and smooth with entire margins, whereas strains from BLS were typically raised and finely granular with slightly undulate margins. In addition, BBS strains were able to grow on trigonelline (with one exception) and L(+)lactate but were unable to hydrolyze tyrosine, whereas BLS strains had opposite reactions. Finally, BBS strains produced syringomycin but BLS strains did not.

Pathogenicity tests. When infiltrated into wild rice leaves at 10^2 colony-forming units (cfu) per milliliter, strains from BBS lesions produced individual water-soaked lesions with necrotic halos that resembled the field symptom. At greater concentrations, water-soaked lesions coalesced and were accompanied by extensive necrosis extending acropetally and basipetally along the leaf blade. Strains isolated from artificially produced BBS lesions were identical to the originals.

Corn, wheat, and barley were not infected by strains from wild rice; however, *P. syringae* pv. *syringae* strains from corn and wheat, but not lilac, produced typical BBS symptoms on wild rice.

DISCUSSION

Strains of *P. syringae* from BBS differed from strains causing BLS of wild rice in colony morphology, utilization of trigonelline and lactate, and production of tyrosinase and syringomycin. Therefore, two symptomatologically and biochemically distinct pathovars of *P. syringae* attack wild rice.

P. syringae pv. *syringae* is reported to attack a wide range of hosts including lilac, stone fruits, pome fruits, citrus, legumes, tomato, walnut, rose, and a variety of grasses (4). Gross et al (5) suggested that within pv. *syringae*, there may be many ecotypes that are host specific. These strains may deserve status as individual pathovars according to current standards (3), but the convention is to group all strains that produce syringomycin together in pv. *syringae*. By this criterion and the biochemical similarity of BBS strains and reference strains of pv. *syringae* as well as the fact that certain pv. *syringae* strains from cereals produced BBS symptoms on wild rice, BBS strains from wild rice are identified as *P. syringae* pv. *syringae*.

Isolations in 1980 indicated that in some fields, *P. syringae* was the dominant brown spot pathogen and may have affected more than 5% of the leaf area in one field (8). BBS has also been observed in natural stands.

One of the problems in evaluating the importance of BBS is the difficulty in distinguishing it from brown spot caused by *Bipolaris* spp. Careful observation, however, usually reveals the translucent slit typical of BBS. Another difference is that lesions caused by *Bipolaris* spp. often have a chlorotic halo that is never seen with BBS lesions. Finally, BBS lesions are often irregular and have associated areas of diffuse necrosis, whereas lesions caused by *Bipolaris* spp. are typically more uniformly elliptical and have well-defined margins.

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