Bacterial Blight of Mock Orange (*Philadelphus* spp.) Caused by *Pseudomonas syringae*

B. W. KENNEDY, Professor, Department of Plant Pathology, University of Minnesota, St. Paul 55108; J. FROYD, Research Scientist, Eli Lilly & Company, Greenfield, IN 46140; and R. BOWDEN, Former Graduate Research Assistant, University of Minnesota

ABSTRACT

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A bacterial leaf and shoot blight occurs regularly on mock orange, a popular woody ornamental shrub in Minnesota. The disease is caused by two variants of *Pseudomonas syringae* that persist in field-grown plants and in planting stock stored from one season to the next. Considerable difference in susceptibility exists among eight cultivars of *Philadelphus* commonly grown in Minnesota. Species of *Viburnum* and *Hydrangea*, ornamental plants in the same subfamily, were resistant.

Mock orange is a widely grown ornamental plant in Minnesota. During the last 15 yr, nurserymen and homeowners have brought specimens to us periodically for diagnosis of a disease characterized by torn and necrotic leaves. Occasionally, distorted growth was apparent on shoots brought in early in the season. Reports of the disease diminish with onset of hot weather, but later in the summer and autumn, after rain, youngest leaves again become diseased, and by early October, a terminal shoot blight becomes apparent. Symptoms are usually first noted on leaves as angular black lesions. A period of water-soaking briefly precedes necrosis. Chlorotic halos 1–7 mm wide commonly surround lesions. Repeated infections blacken large portions of leaves after rain; leaves either die or become shredded by wind. Twigs are killed, turn black and curl at the tips (forming classic "shepherds crook" symptoms), and the entire plant is often stunted.

Our objectives were to ascertain and describe the causal agent and elucidate important factors related to epiphytotics.

MATERIALS AND METHODS

Suspensions of bacteria were obtained from young leaf lesions placed in sterile water, and eight single-colony isolates on nutrient agar were obtained by dilution. Plants were inoculated in most cases by forcing bacterial suspensions into young leaf mesophyll with a 5-ml syringe fitted with a rubber tip. Inoculations were made both on greenhouse- and field-grown *Philadelphus* by introducing 24-hr-old nutrient agar cultures adjusted turbidometrically in distilled water to contain $1–2 \times 10^5$ cfu per milliliter. Inoculated leaves of resistant Lemoine and susceptible Virginal mock orange were allowed to incubate for 0, 24, 48, 72, and 145 hr before being killed and fixed for paraffin sectioning according to methods described by Sass (7). Pathogenicity tests were also made on *Hydrangea* and *Viburnum*.

To study survival of the pathogen, resistant and susceptible plants were obtained from field and nursery storage and assayed by sampling 2-cm sections of stems. These were randomly divided into groups of 10, surface-sterilized in 0.1% sodium hypochlorite, rinsed twice in sterilized water, homogenized for 30 sec in a blender, and filtered. The filtrate was used to inoculate leaves of the cultivar from which the stem originated. Buds from full-grown plants that had overwintered in the field were assayed in a similar manner.

The causal bacterium was identified on the basis of selected physiological tests made on eight isolates from mock orange and five known (control) species and on comparisons with 10 pathovars of *Pseudomonas syringae* van Hall. Tests for potato soft rot, oxidase, levan, liquefaction of gelatin, tobacco hypersensitivity, and tryosinase were performed according to methods of Lelliott et al (6). Schaad's method was used for several tests, including esculin hydrolysis and arginine dihydrolase (8). Carbon source utilization was tested at 0.1% (w/v) concentrations using the methods of Doudoroff and Palleroni (2) and the replica plating technique of Lederberg and Lederberg (5). Production of syringomycin was bioassayed with *Geotrichum candidum* Link ex Fr. according to the methods of Gross and DeVay (3). Oxygen requirements were determined in deep tubes of Difco phenol red dextrose agar (6).

RESULTS

When inoculated by the method described or by various other methods, young leaves of susceptible cultivars developed water-soaked angular lesions within 3–4 days, necrosis occurred by the fifth day, and lesions expanded for an additional 2–5 days. Shoot blight was easily induced in the greenhouse by injecting the pathogen into shoots 6–8 cm from the tip. Of eight cultivars commonly grown in a local nursery, five were susceptible, two were moderately susceptible, and one was resistant (Table 1). In the field, new infections always appeared on the youngest leaves and healthy older leaves remained free of disease even under conditions ideal for disease development. Both leaf and shoot blight occurred on cultivars Coronarius, Enchantment, Golden, and Minnesota Snowflake. Shoot blight was never observed on cultivars Frosty Morn or Sylvia; neither shoot blight nor leaf spots were observed on cultivar Lemoine. Abundance of bacteria, collapse of tissue, and buildup of bacteria in pockets were

Table 1. Natural occurrence of bacterial blight on eight field-grown cultivars of mock orange (*Philadelphus* spp.) during three growing seasons in Minnesota

		Disease	
Species	**Cultivar**	**Leaf spot**	**Shoot blight**
P. coronarius L.	Coronarius	+	+
	Golden	+	+
P. × *lemoinei* Hort.	Lemoine	0	0
P. × *virginalis* Rehd.	Enchantment	+	+
	Frosty Morn	+	0
	Minnesota Snowflake	+	+
	Sylvia	+	0
	Virginal (eastern strain)	+	+

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Table 2. Comparison of two groups of strains of *Pseudomonas syringae* from mock orange with strains of selected pathovars of *P. syringae*

	Strain[b]																	
	Mock orange group								*P. syringae* pathovar									
	1				2				*syringae*				*phaseolicola*	*glycinea*	*pisi*	*tagetis*	*zizaniae*	*striafaciens*
Character[a]	**B**	**C**	**D**	**F**	**A**	**E**	**G**	**H**	**ATCC 19310**	**Holcus -1**	**PSS-B**	**C-1**	**PSPH**	**PSGL**	**PSPI**	**PSTA**	**PSZ-2**	**1514**
Syringomycin	−	−	−	−	+	+	+	−	+	+	+	+	−	−	−	−	−	−
Levan	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Esculin	−	−	−	−	+	+	−	+	+	+	+	+	−	−	−	+	+	+
Tyrosinase	+	+	+	+	−	−	−	−	−	−	−	−	−	+	−	+	+	+
Gelatinase	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	−	N
L(+)Tartrate	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
D(−)Tartrate	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	
D(−)Quinate	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
L(+)Lactate	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−
DL-Homoserine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
I-Erythritol	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+
D-Mannitol	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+
D-Sorbitol	−	−	−	−	+	+	+	+	+	+	+	+	−	−	+	−	+	+
I-Inositol	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Trigonelline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Betaine	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+

[a] + = Positive for character, − = negative for character, and N = not done.
[b] Mock orange strains isolated by authors. For sources of other strains, see Table 1 on page 641 of Bowden and Percich, *Phytopathology* 73.

evident in susceptible cultivars. When any of the eight isolates were introduced at 1 × 10^6 cfu per milliliter into either greenhouse- or field-grown resistant Lemoine, cells became dark at the site of bacterial entry within 24–48 hr and comparatively few bacteria were present after further incubation. Dark-staining cells preceded limited secondary invasion of bacteria into tissues. No symptoms occurred after inoculation with 1 × 10^5 cfu.

Hydrangea arborescens L. 'Grandiflora' and 'Snowhill,' *H. paniculata* Siebold 'Grandiflora' (*Peegee hydrangea*), *Viburnum lantana* L. (wayfaring bush), *V. opulus* L. (European cranberry bush), and *V. trilobum* Marsh. (American highbush cranberry), all members of the same subfamily of Saxifragaceae as *Philadelphus*, showed no symptoms in the field and did not become infected when inoculated.

The pathogen survived in buds and stems of nursery stock either left in the field or dug during November and stored at 2–5 C for 4 mo. All isolates were capable of causing symptoms on healthy young leaves of the cultivar from which they were isolated. The pathogen was isolated from all susceptible cultivars but not from the resistant cultivar Lemoine.

Symptoms were never induced on greenhouse-grown plants by inoculating leaves with aqueous extracts from nursery soil samples collected beneath mock orange foliage at various times throughout the year.

Identification of the pathogen. Results of physiological and biochemical tests are given in Table 2. Isolates from field-grown mock orange cultivars were characterized on nutrient agar by white, circular, convex, smooth colonies with edges entire to irregular. On sterile potato slices, colony color was first creamy white and later became brownish. Fluorescent pigment was produced on King's medium B (4). All isolates grew in the basal salts medium of Ayres, Rupp, and Johnson containing 1% glucose (1). Cells of cultures growing on nutrient agar for 3 days measured 0.4–1.0 × 0.8–3.2 μm and had three or four polar flagella. All produced a hypersensitive reaction on tobacco. None produced indole or H_2S; nitrates were not reduced to nitrites. All isolates from mock orange and reference strains of *P. syringae* were oxidase-negative, arginine dihydrolase-negative, and Gram-negative. None used anthranilate or grew at 37 C.

All eight isolates of the bacterium were pathogenic to one or more cultivars of mock orange, and physiological tests indicate that all isolates are consistent with the general description of *P. syringae* (Table 2). There are two distinct groups, however, and we conclude that isolates A, E, G, and H are consistent with descriptions of *P. syringae* pv. *syringae* Young et al, mainly on the basis of production of syringomycin and utilization of L(+)lactate as well as tests for utilization of tyrosine, esculin, D-tartrate, erythritol, and sorbitol. Isolates B, C, D, and F differ in these characteristics and are not readily identifiable with any pathovar descriptions for *P. syringae* in the *Laboratory Guide for Identification of Plant Pathogenic Bacteria* (8).

DISCUSSION

Bacterial blight of mock orange consistently appears in Minnesota as leaf spot, shoot blight, and stunting. During 3 yr (1968–1970), mock orange buds were activated when daily temperatures averaged 4–10 C. Full leaf growth required 2–5 wk, depending on temperatures. During that period, any measurable amount of rain was adequate for infection to occur. Disease was first observed on 10 May the first year, 14 June the second year, and 31 May the third year. Disease invariably subsided with the onset of warm, dry weather in July and August but was expressed as shoot blight in early autumn. It does not appear to be markedly destructive, however, and our observations indicate that susceptible stock regularly survives. Although such chemical sprays as antibiotics and copper offer promise of reduced severity (B. W. Kennedy, *unpublished*), spraying is not recommended as a routine practice. The causal bacterium is of interest because two distinct variants apparently make up the natural population. Although all are characteristic of *P. syringae*, one group of isolates fits well into the classic descriptions of *P. syringae* pv. *syringae*, whereas the other group does not conform to any pathovar of *P. syringae* described in the recent manual (8). These two variants differ in at least seven characters that are considered important in distinguishing pathovars of the species.

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