Evaluation of Weeds and Plant Refuse as Potential Sources of Inoculum of *Pseudomonas syringae* in Bacterial Canker of Cherry

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ABSTRACT

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Pseudomonas syringae, which causes bacterial canker of sour cherry, was recovered from weeds and plant refuse collected under Montmorency sour cherry trees in three locations in Michigan. Isolates of *P. syringae* were recovered periodically from 7 May 1977 to 18 May 1978, while *P. morsprunorum* was recovered from only three of 54 weed samples and not from plant refuse. In greenhouse experiments, isolates of *P. syringae* from weeds and plant refuse were pathogenic to sour cherry leaves and shoots, peach seedlings, and sweet cherry fruit, and under wet conditions colonized and infected uninjured Montmorency sour cherry leaves. The potential of weeds and plant refuse as sources of inoculum for bacterial canker of cherry is discussed.

Additional key words: bacterial blast.

Pseudomonas syringae and *P. Morsprunorum* (species concept *sensu* Bergey's Manual 7th edition [1], are the causal agents of bacterial canker of sweet and sour cherry (*Prunus avium* L. and *P. cerasus* L., respectively) in Michigan (11,16). These organisms are related etiologically and epidemiologically, and are distinguishable by several determinative tests (4,11,16).

The sources of inoculum for bacterial canker of sweet and sour cherries have not been well defined under Michigan conditions. Resident populations (*sensu* Leben [17]) of *P. morsprunorum* and of *P. syringae* were suggested as sources of inoculum for bacterial canker of sweet cherry in England (2,3) of Montmorency sour cherry in Michigan (16), of almonds and peaches in California (7), and for blast of pear and citrus trees in several countries (4,18), respectively. In addition, weeds were suggested as a source of *P. syringae* for bacterial canker of stone fruit trees (7), blast of pears (18), and bacterial brown spot of beans (8).

The objectives of this research were to determine: if *P. morsprunorum* and *P. syringae* existed in weeds and plant refuse in cherry orchards and if the pseudomonads from those sources were pathogenic to Montmorency sour cherry.

MATERIALS AND METHODS

Isolation from weeds and plant refuse. Samples consisting of 25 g fresh wt of broadleaf herbaceous plants composed mainly of *Taraxacum officinale, Stellaria media, Trifolium* spp., and *Rumex* sp.; 25 g of grasses composed mainly of *Bromus inermes;* and 20 g

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of semi-decomposed leaves and debris (plant refuse) were collected from under sour cherry trees in each of the same three orchards used in a previous study (16). Collection dates were 7 and 16 May, 8 June, 12 July, 2 October, and 20 November 1977 and 30 March, 29 April, and 18 May 1978. Samples were carried to the laboratory in a cold ice chest and maintained at 5 C until processed, always within 24 hr.

Each sample was shaken for 30–60 sec in 500 ml of sterile distilled water. The wash water was diluted in a tenfold series and 0.1-ml portions were seeded on two petri plates containing King's Medium B (12) (MB) amended with 50 μg/ml cycloheximide (cMB). Plates were incubated at 20 C for 4–5 days. It was determined in preliminary studies that 20 C improved the selectivity of the isolation procedure and delayed the coalescence of the colonies. Plates with individual colonies were examined under the dissecting microscope and up to 20 suspect pseudomonad colonies per sample were selected at random for study. Selection was based on colony appearance (16).

Physiological and biochemical properties. The 20 suspect colonies were streaked on MB and checked for colony appearance (16), cytochrome oxidase activity (14), and production of green-fluorescent pigmentation (12). Up to 10 oxidase-negative and fluorescent colonies were selected from each group of 20 colonies and were maintained as separate isolates. Each isolate was characterized in the following determinative tests we previously designated as GATTa (16): gelatin liquefaction (G), aesculin hydrolysis (A), tyrosinase activity (T), and tartrate utilization (Ta). Some isolates also were tested for levan formation; for D-sorbitol, *i*-erythitol, and L (+) lactic, citric, malic, and maleic acids utilization; for β-glucosidase activity on arbutin medium; for growth characteristics in 5% sucrose-nutrient broth; for syringomycin production on potato dextrose agar (PDA, Difco) (10); and for resistance to bacteriophage A7 (5,6,16).

Hypersensitivity and pathogenicity. Bacterial isolates were tested for ability to induce a hypersensitive reaction on leaves of tobacco cultivar White Burley (13) and for pathogenicity to immature sweet cherry fruits, Montmorency sour cherry leaves and shoots, and Halford peach seedlings by the methods described previously (16). Five sweet cherry fruits were inoculated per isolate and positive results were recorded if one or more fruits developed symptoms in a moist chamber at 20 C within 48–72 hr. Known isolates of *P. syringae* and *P. morsprunorum* used in a previous study (16), an isolate of *P. fluorescens*, and sterile distilled water were used as controls. Sour cherry and peach were inoculated by using a hypodermic syringe to inject leaves or green shoots. Plants were held under greenhouse conditions until symptoms developed.

Inoculations were performed with 24- to 48-hr-old bacterial suspensions prepared from cultures growing on MB and adjusted to 70–80% transmittance at $\lambda = 620$ nm in a Bausch and Lomb spectrophotometer (about 1×10^7 cells per milliliter). Sour cherry leaves and shoots also were inoculated with some isolates at 1×10^5 cells per milliliter.

Selection of rifampin resistant mutants. Rifampin resistant mutants (Rif^r) were selected by screening high populations of *P. syringae* isolates from weeds and of *P. morsprunorum* from Montmorency sour cherry leaves on cMB ammended with 50 μg/ml rifampin (Calbiochem, San Diego, CA 92112). Several resistant strains were detected and isolated, but only strains with in vitro growth rates similar to the parent isolate, that induced a hypersensitive reaction on tobacco leaves, and that were pathogenic to cherry fruit were used in pathogenicity and population dynamics studies on Montmorency sour cherry.

Population trends on Montmorency sour cherry. Two-year-old Montmorency sour cherry trees on Mahaleb rootstock growing in pots (capacity 1 L) were sprayed to run-off with suspensions of Rif^r strains of *P. syringae* or *P. morsprunorum* at a concentration of 1×10^7 cells per milliliter. Rifampin resistant strains allowed us to differentiate weed isolates from resident populations that may have existed on the sour cherry trees. Inoculated trees were held in a growth chamber at 20 C under different humidity conditions: low (≤ 80%), high RH (≃ 100%), and high RH with supplemental moisture from a humidifier to keep the leaves wet. Samples of about 200 cm^2 of leaf tissue were taken after 24 hr and every 48 hr thereafter for 9 days. Only the four or five newest leaves present at the time of inoculation were sampled. Leaves were shaken in 0.5 ml distilled water per square centimeter of leaf area, the wash water was diluted in a tenfold series, and 0.1-ml portions were seeded on each of four plates containing cMB amended with 50 μg/ml rifampin. The plates were incubated at 20–22 C for 5 days and the colonies were counted. Because ~95% of the bacteria applied to leaves died in the first 24 hr, populations were measured beginning 24 hr after inoculation.

TABLE 1. Characterization of green-fluorescent and oxidase-negative pseudomonad bacteria recovered from weeds and plant refuse collected from under sour cherry trees in Michigan

Source of isolates	Number of isolates	$GATTa^+$[a] (no.)	$GATTa^+$[a] (%)	$GATTa^-$ (no.)	$GATTa^-$ (%)	$GATTa^\pm$ (no.)	$GATTa^\pm$ (%)
Weeds							
Broadleaf	106	83	78.3[b]	1	0.9	22	20.8
Grasses	175	137	78.3	3	1.7	35	20
Plant refuse	53	32	60.4	0	0.0	21	39.6
Total	334	252	75.4[b]	4	1.2	78	23.4

[a]The $GATTa^+$ isolates are positive for gelatin liquefaction and aesculin hydrolysis, but negative for tyrosinase activity and tartrate dissimilation. The $GATTa^-$ isolates are negative for the first two tests and positive for the last two. The $GATTa^\pm$ isolates are heterogenous for one or more of the four tests.

[b]Percentages of the number of isolates per column relative to the total number tested.

RESULTS

Isolation. Twenty-six of 28 (92.8%) grass samples, 9 of 22 (40.9%) broadleaf weed samples, and 13 of 20 (65%) plant refuse samples yielded oxidase-negative and green-fluorescent bacterial

TABLE 2. Further characterization of $GATTa^+$ (gelatin-liquifying) bacterial isolates recovered from weeds and plant refuse collected from under sour cherry trees in Michigan

Source of isolates	Lactate as sole C source	Hydrolysis of arbutin	Production of syringomycin	Growth on sucrose broth[a] Yt	Yc	Wc	Lysis by phage A7
Weeds							
Broadleaf	41/70[b]	46/49	32/71	13/42	29/42	0/42	11/44
Grasses	107/108	55/57	95/131	17/31	14/31	0/31	11/78
Plant refuse	21/22	10/10	20/26	4/4	0/4	0/4	0/12
Total	169/200	111/116	147/228	34/77	43/77	0/77	22/134
Percent	84.5	95.6	64.5	44.2	55.8	0	16.4

[a]Determined in 50 ml nutrient broth containing 5% (w/v) sucrose, Yt = yellow and translucent supernatant; Yc = yellow and cloudy supernatant; and Wc = white and cloudy supernatant.

[b]Positive isolates over the total number of isolates tested.

TABLE 3. Pathogenicity of oxidase-negative and green fluorescent bacterial isolates recovered from weeds and plant refuse collected from under sour cherry trees in Michigan in 1977–1978

Source of isolates	Tobacco hypersensitivity		Pathogenic to cherry: Fruit		Leaves		Shoots		Peach seedlings	
	Ratio[a]	(%)	Ratio	(%)	Ratio	(%)	Ratio	(%)	Ratio	(%)
Weeds:										
Broadleaf	73/106	68.9	48/91	52.7	29/29	100	28/39	71.8	4/4	100
Grasses	129/175	73.7	86/130	65.1	22/24	91.6	21/45	46.6	11/13	84.6
Plant refuse	29/53	54.7	10/36	15.9	6/7	85.7	10/10	100	5/5	100
Total and %	231/334	69.2	144/257	56.0	57/60	95.0	59/94	62.8	20/22	90.9

[a]Ratio: Number of positive isolates over the total number of isolates tested.

colonies. They were isolated as early as 30 March 1978, when the trees were dormant and snow partially covered the ground, and as late as 20 November 1977, when the trees were dormant. The detection of oxidase-negative and green-fluorescent pseudomonads from plant refuse was more difficult than from weeds due to interference from large numbers of miscellaneous bacteria in the plant refuse.

Physiological and biochemical properties. A total of 334 oxidase-negative and green-fluorescent pseudomonad isolates were characterized. Four isolates (1.2%) were GATTa$^-$, 252 (75.4%) were GATTa$^+$, and 78 (23.4%) were GATTa$^\pm$ (Table 1). The latter isolates differed from GATTa$^+$ and GATTa$^-$ isolates in one or more of the four tests included in GATTa. The GATTa$^-$ isolates were subsequently identified as *P. morsprunorum* and the GATTa$^+$ isolates as *P. syringae*. The four GATTa$^-$ isolates were recovered from weeds in three of 54 isolation attempts.

About 85% of the GATTa$^+$ isolates (Table 2) utilized L (+) lactic acid as a sole carbon source, 95% had β-glucosidase activity on arbutin agar medium, 64.5% were positive for syringomicin production, and all yielded a yellow supernatant fluid with a clear or translucent appearance in 5% sucrose nutrient broth. Of 134 GATTa$^+$ isolates, 16.4% were lyzed by phage A7. Most GATTa$^+$ isolates utilized citric and malic acids, D-sorbitol, and *i*-erythritol, but not maleic acid, as sole carbon sources. They produced large, dome, and mucoid colonies on sucrose medium, characteristics which are typical of levan-forming bacteria, although a few isolates produced flat, ruff colonies.

Hypersensitivity and pathogenicity studies. Of 334 pseudomonad isolates from weeds and plant refuse, 69.2% induced a hypersensitive reaction in 24 hr and 56% were pathogenic to sweet cherry fruits (Table 3). On Montmorency sour cherry leaves, pathogenic isolates induced a water-soaking reaction which later became dark brown, necrotic, and often was surrounded by a chlorotic halo. Of 94 isolates tested, 59 produced lesions when inoculated at 1×10^7 cells per milliliter into green shoots of Montmorency sour cherry. When a few of the pathogenic isolates were inoculated into shoots at 1×10^5 cells per milliliter, all produced lesions. Pathogenic isolates induced water-soaking on Halford peach seedlings that became brown and necrotic 10 days after inoculation. Some strains completely girdled the shoots in 15 days.

Population trends on Montmorency sour cherry. The population of four Rif^r strains of *P. syringae* and of one Rif^r strain of *P. morsprunorum* increased and produced a leaf spotting and blasting of the youngest leaves 4–5 days after inoculation when the plants were maintained under high RH and with free water on leaf-surfaces. Bacterial populations 9 and 11 days after inoculation were 100% to >300% of those after 24 hr. For example, populations of a Rif^r strain of *P. syringae* (isolate 6T-9-8) were 6.9×10^4 cells per milliliter of wash water 24 hr after inoculation, 3.1×10^4 cells per milliliter after 9 days, and 1.9×10^5 cells per milliliter after 11 days. When the trees were maintained at low RH, or at high RH without free moisture on the leaves, bacterial populations rapidly declined during the first 8 hr to about 5% of the population 1 hr after inoculation. Populations continued to decline with time until bacteria could no longer be recovered from the leaves. Symptoms never were observed on plants maintained without free water on leaf-surfaces.

DISCUSSION

P. syringae was common among the oxidase-negative and green fluorescent pseudomonads isolated from grasses and broad-leaf herbaceous plants and from plant refuse collected from under sour cherry trees. *P. morsprunorum* was isolated three times from weeds but never from plant refuse. Thus, *P. syringae*, but not *P. morsprunorum*, appears to be a normal component of the weed and plant refuse microflora found in Michigan sour cherry orchards in 1977–1978, or the bacterial populations of *P. morsprunorum* were at levels which could not be detected by our methods. Nevertheless, by the same methods, large numbers of *P. morsprunorum* were detected on cherry tissues (16). Since *P. syringae* was present on weeds in November 1977, and was recovered from healthy green leaves exposed as snow melted away in March 1978, it is possible that the bacteria may overwinter on weeds in Michigan.

The apparent ubiquity of *P. syringae* in sour cherry orchards is similar to that reported in peach and almond orchards by English and Davis (7). However, *P. morsprunorum* is much less widespread, occurring only on the cherry tree itself and on prunes (19). Thus, the disease phase of its life cycle appears only casual and not essential for the survival of *P. syringae* in cherry orchards while that of *P. morsprunorum* is important for its survival.

Free water appears to be a critical factor for establishing resident populations and for penetration of Montmorency sour cherry leaves. Populations of *P. syringae* and *P. morsprunorum* rapidly declined and symptoms never developed when the bacteria were sprayed onto sour cherry leaves maintained at low or high RH, but without visible moisture on the leaves. Similar relationships have been found for *P. morsprunorum* in England (9).

Because isolates of *P. syringae* from weeds and plant refuse infected uninjured Montmorency sour cherry leaves under growth chamber conditions, we conclude that weeds and plant refuse are possible sources of primary inoculum for bacterial canker incited by *P. syringae*. The relative importance of these sources of bacteria, compared to the other sources of primary inoculum (ie, the cherry tree itself), in the development of epidemics of bacterial canker is not yet clear. It is desirable to know to what extent the bacteria are disseminated by splashing rain from contaminated weeds and plant debris to cherry. Now that we have isolates of *P. syringae* with suitable markers for identification, studies of the cross-contamination of cherry and weed hosts by *P. syringae* can be undertaken.

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