Ecology and Epidemiology

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

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__ABSTRACT__


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 inermis; and 20 g
of semi-decomposed leaves and debris (plant refuse) were collected from under sour cherry trees in each of the three orchards used in a previous study (16). Collection dates were July 10, 18 June, 12 July, 2 October, and 20 November 1977 and March 29, April 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-mI portions were seeded on two petri plates containing King's Medium B (12) (MB) amended with 50μg/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 pseudomonal 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), tyrosine activity (T), and tartrate utilization (Ta). Some isolates also were tested for levan formation; for p-sorbitol, l-erythritol, and L(+)-lactic, citric, malic, and maleic acid 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 earlier (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. florescens_, 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 λ = 620 nm in a Bausch and Lomb spectrophotometer (about 1 x 10⁶ cells per milliliter). Sour cherry leaves and shoots also were inoculated with some isolates at 1 x 10⁷ cells per milliliter.

**Selection of rifampin resistant mutants.** Rifampin resistant mutants (Rif') were selected by screening high populations of _P. syringae_ isolates from weeds and of _P. morsprunorum_ from Montmorency sour cherry leaves on cMB amended with 50μg/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 strains of _P. syringae_ or _P. morsprunorum_ at a concentration of 1 x 10⁵ 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 cm² 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 50μg/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.

**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>
<thead>
<tr>
<th>Source of isolates</th>
<th>Lactase as sole C source</th>
<th>Hydrolysis of arbutin</th>
<th>Production of syringomycin</th>
<th>Growth on sucrose broth</th>
<th>Lysis by phage A7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yt</td>
<td>Yc</td>
<td>We</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Weeds</strong></td>
<td>169/200</td>
<td>111/116</td>
<td>147/228</td>
<td>64.5</td>
<td>16.4</td>
</tr>
<tr>
<td>Broadleaf</td>
<td>41/70</td>
<td>46/49</td>
<td>32/71</td>
<td>13/42</td>
<td>11/44</td>
</tr>
<tr>
<td>Grasses</td>
<td>107/108</td>
<td>55/57</td>
<td>95/131</td>
<td>17/31</td>
<td>11/78</td>
</tr>
<tr>
<td>Plant refuse</td>
<td>21/22</td>
<td>10/10</td>
<td>20/26</td>
<td>4/4</td>
<td>0/12</td>
</tr>
<tr>
<td><strong>Percent</strong></td>
<td>84.5</td>
<td>95.6</td>
<td>64.5</td>
<td>44.2</td>
<td>16.4</td>
</tr>
</tbody>
</table>

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

* Positive isolates over the total number of isolates tested.
<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>Tobacco hypersensitivity Ratio(^1) (%)</th>
<th>Fruit Ratio (%)</th>
<th>Pathogenic to cherry: Leaves Ratio (%)</th>
<th>Shoots Ratio (%)</th>
<th>Peach seedlings Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeds:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broadleaf</td>
<td>73/106</td>
<td>68.9</td>
<td>48/91</td>
<td>52.7</td>
<td>29/29</td>
</tr>
<tr>
<td>Grasses</td>
<td>129/175</td>
<td>73.7</td>
<td>86/130</td>
<td>65.1</td>
<td>22/24</td>
</tr>
<tr>
<td>Plant refuse</td>
<td>29/53</td>
<td>54.7</td>
<td>10/36</td>
<td>15.9</td>
<td>6/7</td>
</tr>
<tr>
<td>Total and %</td>
<td>231/334</td>
<td>69.2</td>
<td>144/257</td>
<td>56.0</td>
<td>57/60</td>
</tr>
</tbody>
</table>

\(^1\) Ratio: Number of positive isolates over the total number of isolates tested.

**DISCUSSION**

_P. syringae_ was common among the oxidase-negative and green fluorescent pseudomonads isolated from grassed and broad-leaf herbaceous plants and from plant refuse collected from under sour cherry trees. _P. mors-prunorum_ was isolated three times from weeds but never from plant refuse. Thus, _P. syringae_, but not _P. mors-prunorum_, 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. mors-prunorum_ were at levels which could not be detected by our methods. Nevertheless, by the same methods, large numbers of _P. mors-prunorum_ 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. mors-prunorum_ 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. mors-prunorum_ 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. mors-prunorum_ 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. mors-prunorum_ 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 (i.e., 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.

**LITERATURE CITED**


