Copper and Streptomycin Resistance in Strains of Pseudomonas syringae from Pacific Northwest Nurseries

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ABSTRACT

Growers' reports of disease-control failures following the application of copper and streptomycin bactericides led to collecting and testing strains of Pseudomonas syringae for resistance to copper and streptomycin. A comparison of strains isolated from 25 species of diseased woody plants in the Willamette Valley, Oregon, in 1992 and 1993 was made with strains collected in 1982 and 1983 from 30 diseased woody plant species in Oregon and Washington. On differential media supplemented with copper sulfate or streptomycin sulfate, the growth of 467 isolates recovered in 1992 and 1993 was determined. Twenty-four percent of the isolates were found to be copper-resistant (Cu') and streptomycin-sensitive (Sm'), 6% were Cu'Sm', 24% were Cu'Sm', and 46% were Cu'Sm' at the concentrations tested. Of 192 strains isolated in 1982 and 1983, 25% were Cu'Sm', 7% were Cu'Sm', none were Cu'Sm', and 68% were Cu'Sm'. In DNA colony hybridizations with digoxigenin-labeled probes, the copABCD probe from P. syringae pv. tomato hybridized with 10% of the Cu' strains isolated in 1992 and 1993, and the copI probe from P. syringae pv. syringae hybridized with a different 6% of the Cu' strains isolated in 1992 and 1993. Neither probe hybridized with any Cu' strains isolated in 1982 and 1983. A DNA probe encoding the streptomycin resistance determinant strA-strB from P. syringae pv. syringae hybridized with 98% of the strains that grew on King's medium B with 100 μg of streptomycin sulfate per ml (KBS) and 4% of the strains that did not. This is the first report of copABCD, copI, and strA-strB homologues in strains of P. syringae from Pacific Northwest nurseries. The emergence of strains resistant to both copper and streptomycin shows that growers need to explore new methods to control Pseudomonas diseases.

Pseudomonas syringae causes tip dieback, bud and flower blast, canker, and leaf spot on a wide variety of deciduous woody plants in Pacific Northwest nurseries (4, 13). The nursery industry was Oregon's most valuable agricultural sector in 1994 with gross sales of $385 million (33). During the early 1990s, the severity and frequency of Pseudomonas diseases have increased, and annual losses have been estimated at $8 million for ornamentals alone. Recommendations for control of P. syringae on many of these crops include a full application of fixed copper (13). Nurseries commonly apply multiple sprays of copper-containing bactericides, streptomycin sulfate, or both, beginning in the dormant season and continuing through the end of flowering or until leaves are fully unfurled. Even under these intensive regimens, disease management has often been ineffective. The reasons for poor control are unknown and pathogen populations may have been selected for resistance to copper and streptomycin. Plasmid-encoded copper resistance (Cu') and streptomycin resistance (Sm') are becoming increasingly widespread in several genera of phytopathogenic bacteria including Erwinia, Xanthomonas, and Pseudomonas (7). Strains of P. syringae pv. syringae resistant to high concentrations of copper (1) and to both copper and streptomycin (24) have been isolated in western U.S. fruit orchards. In Oklahoma, strains resistant to both copper and streptomycin have been isolated in commercial woody plant nurseries (25).

The Cu' determinants from P. syringae pv. tomato (8) and P. syringae pv. syringae (21) have been cloned and characterized. The copABCD operon from P. syringae pv. tomato confers resistance through a copper-sequestering system external to the cytoplasm (5). The copI operon from P. syringae pv. syringae shares some structural similarities with copABCD, but a different mechanism for Cu' is suspected (21). To compare the genotypes of Cu' P. syringae strains with previously cloned Cu' determinants, copABCD (3) and copI (21) were used in DNA colony hybridizations.

One type of Sm', conferred by strA-strB aminoglycoside phosphotransferase genes, has been identified in several phytopathogenic bacteria including E. amylovora (6), X. campestris pv. vesicatoria (19), and P. syringae pv. syringae (25) and papulans (11). The ecology and evolution of the strA-strB genes in plant pathogenic bacteria has been reviewed (29).

The purpose of this research was to evaluate copper and streptomycin resistance in P. syringae from Pacific Northwest nurseries, compare the resistance of strains isolated in 1992 and 1993 with strains isolated in 1982 and 1983, compare unsprayed landscape plants with those in commercial nurseries, and test previously cloned Cu' and Sm' determinants as probes in colony hybridizations. A preliminary report of the survey has been published (23).

MATERIALS AND METHODS
Isolation of Pseudomonas syringae. Diseased woody plants were collected from 44 commercial nurseries specializing in woody ornamentals and seven landscape plantings of lilac in the Willamette Valley, Oregon, March through May of 1992 and 1993 (Fig. 1). Plant samples with tip dieback, bud and blossom blast, canker, or leaf spot were surface-disinfested for 60 s in 0.525% NaOCl (10% Clorox bleach) followed by two 60-s rinses in sterile distilled water (sdw). A 1-g sample from the margin between diseased and healthy tissue was macerated, aseptically transferred to 10 ml of sdw, and allowed to stand for 1 h at room temperature. Loopfuls of the resultant aeous suspension were streaked onto King's medium B (KB) (12) and incubated for 48 h at 28°C. Characteristic colonies were re-streaked to ensure purity. Bacteria were preserved at ~80°C in 1.6 ml of sterile Luria-Bertani broth (17) and 0.4 ml of sterile glycerol (J. T. Baker, Phillipsburg, NJ). The isolation of P. syringae strains in 1982 and 1983 has been described (4).

Characterization of strains. Strains isolated in 1992 and 1993 were identified as P. syringae based on fluorescence on KB under UV-light at 350 nm and negative test results for both cytochrome oxidase (14) and arginine dihydrolase activity (32). The strains came from a diverse group of woody plants and pathogenicity tests on

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the original hosts were not feasible. Because of the diversity of ecotypes of *P. syringae* (2,9,10,20,35), and the possibility of plasmid transfer between genotypes (30, 31), no classifications were made below species. Strains isolated in 1982 and 1983 were characterized by fluorescence on KB and cytochrome oxidase and arginine dihydrolase activity by Canfield et al. (4).

**Preparation of inoculum for media screening.** *Pseudomonas syringae* strains were recovered from frozen storage, streaked on KB, and incubated at 28°C for 48 h. Individual colonies were suspended in SDW to a concentration of approximately 1 x 10⁶ CFU/ml (OD₆₅₀ = 0.3).

**Copper resistance.** Casitone-yeast extract (CYE), a low-complexing mineral salts medium similar to that described by Zevenhuizen et al. (36) and modified by Anderson et al. (1), was used to evaluate strains for resistance to free copper ions. Bacterial suspensions in 10-μl aliquots were spotted on CYE medium containing CuSO₄·5H₂O (Anderson Labs, Fort Worth, TX) at concentrations of 0, 0.16, 0.32, 0.48, 0.64, 0.80, or 0.96 mM. *P. syringae* strain AI513, which grows on 0.80 mM CuSO₄·5H₂O, and strain AI467, which does not grow on 0.16 mM CuSO₄·5H₂O, were included as controls (1). Cultures were incubated at 28°C for 72 h, and the minimum concentration that prevented colony growth (minimum inhibitory concentration, MIC) was recorded. Strains able to grow on 0.32 mM CuSO₄·5H₂O or greater were considered copper resistant. Each test was done three times.

**Streptomycin resistance.** Resistance to streptomycin was determined by spotting 10-μl aliquots of bacterial suspension onto plates of KB amended with filter-sterilized (0.2 μm) aqueous streptomycin sulfate (Sigma Co., St. Louis, MO) made to a final concentration of 100 μg/ml (KBS). *P. syringae* strains G1 and FF5 with resistance and sensitivity, respectively, to 100 μg of streptomycin sulfate/ml (25) were included as controls. Cultures were incubated at 28°C for 48 h. Those with growth equivalent to that of strain G1 were considered streptomycin resistant.

**DNA colony hybridizations.** The copA/BCD probe from *P. syringae* pv. *tomato* consists of a 4.5-kb PsfI copper resistance determinant cloned in pUC18 and maintained in *Escherichia coli* DH5α (16). The 6.5-kb cop1 probe from *P. syringae* pv. *syringae*, also a Cu²⁺ determinant, (21) was restriction-digested from pAFR3 to produce a 2-kb EcoRI fragment, subcloned into pUC18 and transformed into *E. coli* DH5α by the methods of Sambrook et al. (22). The plasmid-born Sm² determinant from *P. syringae* pv. *syringae* PSR1, a 3.7-kb PsiI fragment, cloned into pBluescript SK and maintained in *E. coli* DH5α (25), was used as a probe for Sm². This fragment has high identity to strA-strB genes from the broad-host-range plasmid RSFI010 (25).

DNA fragments were labeled with digoxigenin-11-dUTP (Genius kit; Boehringer-Mannheim Biochemicals, Indianapolis, IN) as described by the manufacturer. Pre-hybridizations were a minimum of 1 h at 68°C. Post-hybridization washes were two 5-min washes at 22°C in 2× SSC (1× SSC = 0.15 M NaCl + 0.015 M sodium citrate) plus 0.1% sodium dodecyl sulfate (SDS) and two 30-min washes at 68°C in 0.1× SSC plus 0.1% SDS. Colony hybridizations were done twice.

**RESULTS**

**Isolation and characterization of *P. syringae* strains.** Isolations were made from 25 plant species with tip dieback, bud and blossom blast, canker, or leaf spot in 1992 and 1993 (Table 1). Nursery managers provided samples from plant genera with the highest incidence and severity of *Pseudomonas* diseases, hence the large number of samples from lilac (*Syringa vulgaris* L.) and Japanese maple (*Acer palmatum* Thunb.). A total of 467 strains, 435 from commercial nurseries and 32 from landscape-planted lilacs, were isolated and characterized as *P. syringae*. Strains resistant to copper, streptomycin, or both, were obtained from 38 of the 44 nurseries; no resistant strains were obtained from the seven landscape plantings (Fig. 1). The collections in 1982 and 1983 yielded 192 strains of *P. syringae* from 30 species of woody plants (Table 2) in 32 nurseries in western Oregon and Washington (4).

**Copper and streptomycin resistance.** Twenty-four percent of the strains isolated in 1992 and 1993 and 25% of the strains isolated in 1982 and 1983 were resistant to copper. The highest MIC of copper sulfate in CYE for strains isolated in 1992 and 1993 was 0.80 mM; the highest MIC of copper sulfate in CYE for strains isolated in 1982 and 1983 was 0.32 mM (Fig. 2). None of the 32 strains collected in 1992 and 1993 from landscape-planted lilacs were resistant to copper. No spontaneous

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**Fig. 1.** Commercial nurseries and landscape plantings in the Willamette Valley, Oregon, where strains of *Pseudomonas syringae* were isolated in 1992 and 1993. Symbols show nurseries or landscapes where copper-resistant, streptomycin-resistant, and copper-and-streptomycin-sensitive strains were collected.
copper-resistant mutants were observed from copper-sensitive strain AI487 grown on 0.16 mM copper sulfate.

On KBS, 6% of the strains isolated in 1992 and 1993 and 7% of the strains isolated in 1982 and 1983 were streptomycin resistant (Table 3). None of the strains from landscape-planted lilacs were resistant to streptomycin. Spontaneous streptomycin-resistant mutants from Sm³ strain FFS occurred at a frequency of approximately 1 in 10⁶ cells and produced small, nonconfluent colonies on KBS.

Of the strains isolated in 1992 and 1993, 24% were resistant to both copper and streptomycin (Table 3). None of the 192 strains collected in 1982 and 1983 were resistant to both copper and streptomycin.

DNA-DNA colony hybridizations. The copABCD probe for Cu⁺ determinants hybridized with 10% of the strains isolated in 1992 and 1993, and all of the strains that hybridized were copper resistant. The copI probe for Cu⁺ determinants hybridized with a different 6% of the strains from 1992 and 1993, and all strains that hybridized were again copper resistant. The strains that hybridized with either copABCD or copI were equally split between the streptomycin-sensitive and the streptomycin-resistant phenotype (Table 4). Although neither of the probes hybridized with any copper-sensitive strain, 66% of the copper-resistant strains were not detected. None of the copper-resistant strains isolated in 1982 and 1983 hybridized with either the copABCD or the copI probe.

The strA-strB Sm³ determinant provided 94% agreement between growth on KBS and hybridization with the probe for strains isolated in 1992 and 1993 and 1982 and 1983. Two percent of the strains grew on KBS but did not hybridize with the probe, and 4% hybridized with the probe but did not grow on KBS.

**DISCUSSION**

Copper and streptomycin resistance was widespread in strains of *P. syringae* isolated from commercial woody plant nurseries in the Pacific Northwest. In comparing strains isolated in 1992 and 1993 with strains isolated in 1982 and 1983, the number of copper-resistant/streptomycin-sensitive and copper-sensitive/streptomycin-resistant strains has not increased. However, while no strains isolated in 1982 and 1983 were resistant to both copper and streptomycin, this phenotype constituted 24% of the strains isolated in 1992 and 1993. The percentage of copper-sensitive/streptomycin-sensitive strains was reduced from 68% in 1982 and 1983 to 46% in 1992 and 1993. In addition, the MIC of copper sulfate increased fourfold, from a high of 0.32 mM in the strains isolated in 1982 and 1983 to a high of 0.80 mM in the strains from 1992 and 1993. The increase in both percentage of strains resistant to both copper and streptomycin, and the concentration of copper to which they are resistant, may help explain why chemical applications no longer provide adequate control of *Pseudomonas syringae* diseases.

The MIC of copper sulfate that prevented growth of strains of *P. syringae* from the Willamette Valley was higher than that measured in strains of *P. syringae* pv. *tomato* from Southern California (3) but lower than that in strains of *P. syringae* pv. *syringae* from Northern California (21) or from Hood River, OR (24). These differences could reflect ecotype variation (10), or different selection pressures from sprays on nursery crops versus vegetable or fruit crops. Alternately, Willamette Valley strains may have different genetic determinants that confer Cu⁺.

The copABCD and copI probes hybridized with 16% of the copper-resistant *P. syringae* strains isolated in 1992 and 1993. These two Cu⁺ determinants have some structural similarities, but they apparently have functional and regulatory differences (21). Genetic similarities in Cu⁺ determinants are recognized in a diverse collection of bacteria from the genera *Pseudomonas* (8), *Xanthomonas* (34), and *Escherichia* (15). The Cu⁺ mechanism in *P. syringae* pv. *tomato* is sequestration of Cu²⁺ ions by periplasmic and outer membrane proteins with a two-component regulatory system (18). *copJ* confers Cu⁺ to *P. syringae* pv. *syringae* differently, possibly with an ef-

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**Table 1.** Plant sources and resistance phenotypes of *Pseudomonas syringae* strains isolated in 1992 and 1993

<table>
<thead>
<tr>
<th>Family, genus, and species</th>
<th>Common name</th>
<th>Cu/Sm²</th>
<th>Cu/Sm³</th>
<th>Cu/Sm²</th>
<th>Cu/Sm³</th>
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<tr>
<td>Aceraceae</td>
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<td><em>Acer palmatum</em> Thunb.</td>
<td>Japanese maple</td>
<td>34</td>
<td>21</td>
<td>4</td>
<td>21</td>
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<td><em>A. platanoides</em> L.</td>
<td>Norwegian maple</td>
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<td>0</td>
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<td><em>A. rubrum</em> L.</td>
<td>Red maple</td>
<td>7</td>
<td>5</td>
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<td><em>A. saccharum</em> Marsh.</td>
<td>Sugar maple</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>A. truncatum</em> Bunge</td>
<td>Shantung maple</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Berberidaceae</td>
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<tr>
<td><em>Berberis aquifolium</em> Pursh</td>
<td>Oregongrape</td>
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<td>2</td>
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<td>0</td>
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<td>Caprifoliaceae</td>
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<tr>
<td><em>Viburnum dentatum</em> L.</td>
<td>Arrow-wood</td>
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<td>5</td>
<td>0</td>
<td>1</td>
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<tr>
<td><em>Euonymus alatus</em> (Thunb.) Siebold</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>Ericaceae</td>
<td></td>
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<tr>
<td><em>Vaccinium corymbosum</em> L.</td>
<td>Highbush blueberry</td>
<td>1</td>
<td>15</td>
<td>0</td>
<td>4</td>
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<tr>
<td>Hamamelidaceae</td>
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<td></td>
<td></td>
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<tr>
<td><em>Liquidambar styraciflua</em> L.</td>
<td>Sweet gum</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
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<td>Hydrangeaceae</td>
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<tr>
<td><em>Philadelphus coronarius</em> L.</td>
<td>Mock orange</td>
<td>6</td>
<td>0</td>
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<tr>
<td>Magnoliaceae</td>
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<tr>
<td><em>Magnolia grandiflora</em> L.</td>
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<tr>
<td><em>Forsythia viridissima</em> Lind.</td>
<td>Golden bells</td>
<td>6</td>
<td>4</td>
<td>0</td>
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<tr>
<td><em>Syringa x chinesis</em> Wildl.</td>
<td>Chinese lilac</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>9</td>
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<tr>
<td><em>S. amurensis</em> Rupr.</td>
<td>Amur lilac</td>
<td>1</td>
<td>2</td>
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<td>0</td>
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<tr>
<td><em>S. x persica</em> L.</td>
<td>Persian lilac</td>
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<td>0</td>
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<td><em>S. vulgaris</em> L.</td>
<td>Common lilac</td>
<td>100</td>
<td>48</td>
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<td><em>Pruus armeniacus</em> L.</td>
<td>Apricot</td>
<td>5</td>
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<tr>
<td><em>P. avium</em> (L.) L.</td>
<td>Sweet cherry</td>
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<td>0</td>
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<tr>
<td><em>P. laurocerasus</em> L.</td>
<td>Cherry laurel cv. Otto Leuken</td>
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<td>0</td>
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<tr>
<td><em>P. serralata</em> Lindl.</td>
<td>Japanese flowering cherry</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pyrus communis L.</td>
<td>Common pear</td>
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<td>2</td>
<td>1</td>
<td>0</td>
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<tr>
<td><em>Pyrus pyrifolia</em> (Burm. f.) Nakai</td>
<td>Asian pear</td>
<td>9</td>
<td>0</td>
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<tr>
<td><em>Sorbus aucuparia</em> L.</td>
<td>Mountain ash</td>
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<td>1</td>
<td>0</td>
<td>0</td>
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<td>Tiliaceae</td>
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<tr>
<td><em>Tilia cordata</em> Mill.</td>
<td>European linden</td>
<td>3</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>


* Copper sensitive Cu⁺; streptomycin sensitive, Sm³; copper resistant, Cu⁺; streptomycin resistant, Sm³.
flux mechanism that prevents copper ions from accumulating inside the cell.

The Willamette Valley collection of copper-resistant strains, some of which hybridized with copABCD and others with cop1, but most with neither, may be due to the movement of resistant strains across geographical areas or may represent a continuum of resistance mechanisms with varying degrees of relatedness to one another. The probes failed to detect 68% of the copper-resistant strains of *P. syringae* from Northwest nurseries. Apparently, there is a different mechanism of Cu' functioning in these strains.

Ninety-eight percent of the streptomycin-resistant *P. syringae* strains, both copper-sensitive and copper-resistant, collected in 1992 and 1993 and in 1982 and 1983 hybridized with the *strA-strB* gene probe. The 2% of strains that grew on KBS but did not hybridize with the probe may have an Sm' mechanism other than that conferred by the *strA-strB* genes. Jones et al. (11) observed that a small percentage of streptomycin-resistant *P. syringae pv. pappulans* strains did not hybridize to *strA-strB*. The 4% of the strains that hybridized with the probe but did not grow on KBS may have a nonfunctional copy of the *strA-strB* genes. The excellent agreement between growth on KBS and hybridization with the *strA-strB* genes allows colony hybridization to increase the efficiency and accuracy of detection of streptomycin-resistant strains while avoiding spontaneous streptomycin-resistant mutants on KBS test medium.

Sm' conferred by *strA-strB* genes is widespread among commensal and pathogenic bacteria from animals, plants, and humans, which suggests they share a common gene pool (28). The *strA-strB* genes from *P. syringae* are located within

<table>
<thead>
<tr>
<th>Table 2. Plant sources and resistance phenotypes of <em>Pseudomonas syringae</em> strains isolated in 1982 and 1983</th>
</tr>
</thead>
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<tr>
<td><strong>Family, genus, and species</strong></td>
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<tr>
<td><strong>Aceraceae</strong></td>
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<td><strong>Anacardiaceae</strong></td>
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<td><strong>Araliaceae</strong></td>
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<td><strong>Tiliaceae</strong></td>
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*b* Copper sensitive Cu'; streptomycin sensitive, Sm'; copper resistant, Cu'; streptomycin resistant, Sm'.

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<table>
<thead>
<tr>
<th>Table 3. Number of <em>Pseudomonas syringae</em> strains isolated in 1982 and 1983, resistant to copper (Cu') and/or streptomycin (Sm')</th>
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<tbody>
<tr>
<td><strong>Resistance phenotype</strong></td>
</tr>
<tr>
<td>Cu'Sm'</td>
</tr>
<tr>
<td>Cu'Sm'</td>
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<tr>
<td>Cu'Sm'</td>
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<tr>
<td>Cu'Sm'</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

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*a* Cu' = copper sensitive, strains unable to grow on medium containing 0.32 mM copper sulfate. Cu' = copper resistant, strains able to grow on medium containing 0.32 mM copper sulfate. Sm' = streptomycin sensitive, strains unable to grow on medium containing 100 µg of streptomycin sulfate per ml. Sm' = streptomycin resistant, strains able to grow on medium containing 100 µg of streptomycin sulfate per ml.

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<thead>
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<th>Table 4. Number of <em>Pseudomonas syringae</em> strains isolated in 1992 and 1993 that hybridized with copper resistance determinants <em>copABCD</em> and <em>copJ</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resistance phenotype</strong></td>
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<td></td>
</tr>
<tr>
<td>Cu'Sm'</td>
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<td>Cu'Sm'</td>
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<td>Cu'Sm'</td>
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<tr>
<td>Cu'Sm'</td>
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<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

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*a* Cu' = copper sensitive, strains unable to grow on medium containing 0.32 mM copper sulfate. Cu' = copper resistant, strains able to grow on medium containing 0.32 mM copper sulfate. Sm' = streptomycin sensitive, strains unable to grow on medium containing 100 µg of streptomycin sulfate per ml. Sm' = streptomycin resistant, strains able to grow on medium containing 100 µg of streptomycin sulfate per ml.
Fig. 2. Frequency distribution of minimum inhibitory concentrations of CuSO₄·5H₂O in casitone-
yeast extract medium for Pseudomonas syringae strains isolated in 1982 and 1983 (hatched bars) and
1992 and 1993 (solid bars).

the transposable element Tn5393, which is
usually borne on a conjugative plasmid
(27). Plasmid transfer has been implicated in
the rapid dissemination of strA-strB genes
within populations of plant pathogenic
bacteria (29). This study shows that
strA-strB homologues were present in P.
syringae strains isolated in Pacific North-
west nurseries more than a decade ago.
There has been no appreciable change in
the number of copper-resistant/streptomycin-
sensitive strains: 7% in 1982 and 1983
versus 6% in 1992 and 1993. However,
24% strains isolated in 1992 and 1993 are
copper- and streptomycin-resistant while
this phenotype was not present in the
strains isolated in 1982 and 1983. This
may reflect a recent compatibility between
Cu²⁺ and Sm³⁺ genes, the ease of gene trans-
fer within populations, or a response to
the selection pressure of increased applications of
bactericides over the past decade.

The copper- and streptomycin-resistant
phenotype has been previously reported in
P. syringae pv. syringae with resistance
being stable over many generations in vitro
(25,26). Genetic analysis of P. syringae pv.
syringae from nurseries in Oklahoma
grouped 12 plasmid types based on their
size and resistance phenotype. Sundin et al.
(25) concluded that the repeated applica-
tion of bactericides had selected for many
different P. syringae genotypes with trans-
ferable resistance determinants.

No Cu²⁺ or Sm³⁺ was detected in any of
the strains of P. syringae isolated from
landscape-planted lilacs either by growth
on amended media or by colony hybridiza-
tion. These strains were isolated from ma-
ture shrubs in public parks and private gar-
dens within similar geographic areas of the
Willamette Valley, and presumably have
not been sprayed with either copper or
streptomycin for many years. If there were
copper- or streptomycin-resistant strains of
P. syringae associated with these plants
while they were in nurseries, these strains
have not persisted in the landscape setting.

The presence of populations of P. syrin-
gaee with resistance to both copper and
streptomycin seriously compromises cur-
rent nursery chemical control programs.
Growers need to explore alternative
methods of disease control, including host
resistance, and biological and cultural
controls. In many nurseries, growing sus-
ceptible plants such as lilacs under plastic
shelters during the winter and early spring,
protecting them from rain and frost, has
improved disease control.

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