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

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ABSTRACT

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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^r) and streptomycin-sensitive (Sm^s), 6% were Cu^sSm^r, 24% were Cu^rSm^r, and 46% were Cu^sSm^s at the concentrations tested. Of 192 strains isolated in 1982 and 1983, 25% were Cu^rSm^s, 7% were Cu^sSm^r, none were Cu^rSm^r, and 68% were Cu^sSm^s. In DNA colony hybridizations with digoxigenin-labeled probes, the *cop*ABCD probe from *P. syringae* pv. *tomato* hybridized with 10% of the Cu^r strains isolated in 1992 and 1993, and the *cop*J probe from *P. syringae* pv. *syringae* hybridized with a different 6% of the Cu^r strains isolated in 1992 and 1993. Neither probe hybridized with any Cu^r strains isolated in 1982 and 1983. A DNA probe encoding the streptomycin resistance determinant *str*A-*str*B 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 *cop*ABCD, *cop*J, and *str*A-*str*B 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 fall 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^r) and streptomycin resistance (Sm^r) 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^r determinants from *P. syringae* pv. *tomato* (8) and *P. syringae* pv. *syringae* (21) have been cloned and characterized. The *cop*ABCD operon from *P. syringae* pv. *tomato* confers resistance through a copper-sequestering system external to the cytoplasm (5). The *cop*J operon from *P. syringae* pv. *syringae* shares some structural similarities with *cop*ABCD, but a different mechanism for Cu^r is suspected (21). To compare the genotypes of Cu^r *P. syringae* strains with previously cloned Cu^r determinants, *cop*ABCD (3) and *cop*J (21) were used in DNA colony hybridizations.

One type of Sm^r, conferred by *str*A-*str*B aminoglycoside phosphotransferase genes, has been identified in several phytopathogenic bacteria including *E. amylovora* (6), *X. campestris* pv. *vesicatoria* (19), and *P. syringae* pvs. *syringae* (25) and *papulans* (11). The ecology and evolution of the *str*A-*str*B 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^r and Sm^r 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% NaHOCl (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 aqueous 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 1×10^8 CFU/ml ($OD_{590} = 0.3$).

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 $CuSO_4 \cdot 5H_20$ (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 Al513, which grows on 0.80 mM $CuSO_4 \cdot 5H_20$, and strain Al487, which does not grow on 0.16 mM $CuSO_4 \cdot 5H_20$, 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 $CuSO_4 \cdot 5H_20$ 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 recorded as streptomycin resistant.

DNA colony hybridizations. The *cop*ABCD probe from *P. syringae* pv. *tomato* consists of a 4.5-kb *Pst*I copper resistance determinant cloned in pUC18 and maintained in *Escherichia coli* DH5α (16). The 6.5-kb *cop*J probe from *P. syringae* pv. *syringae*, also a Cu^r determinant, (21) was restriction-digested from pLAFR3 to produce a 2-kb *Eco*RI fragment, subcloned into pUC18 and transformed into *E. coli* DH5α by the methods of Sambrook et al. (22). The plasmid-borne Sm^r determinant from *P. syringae* pv. *syringae* PSR1, a 3.7-kb *Pst*I fragment, cloned into pBluescript SK and maintained in *E. coli* DH5α (25), was used as a probe for Sm^r. This fragment has high identity to *str*A-*str*B genes from the broad-host-range plasmid RSF1010 (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

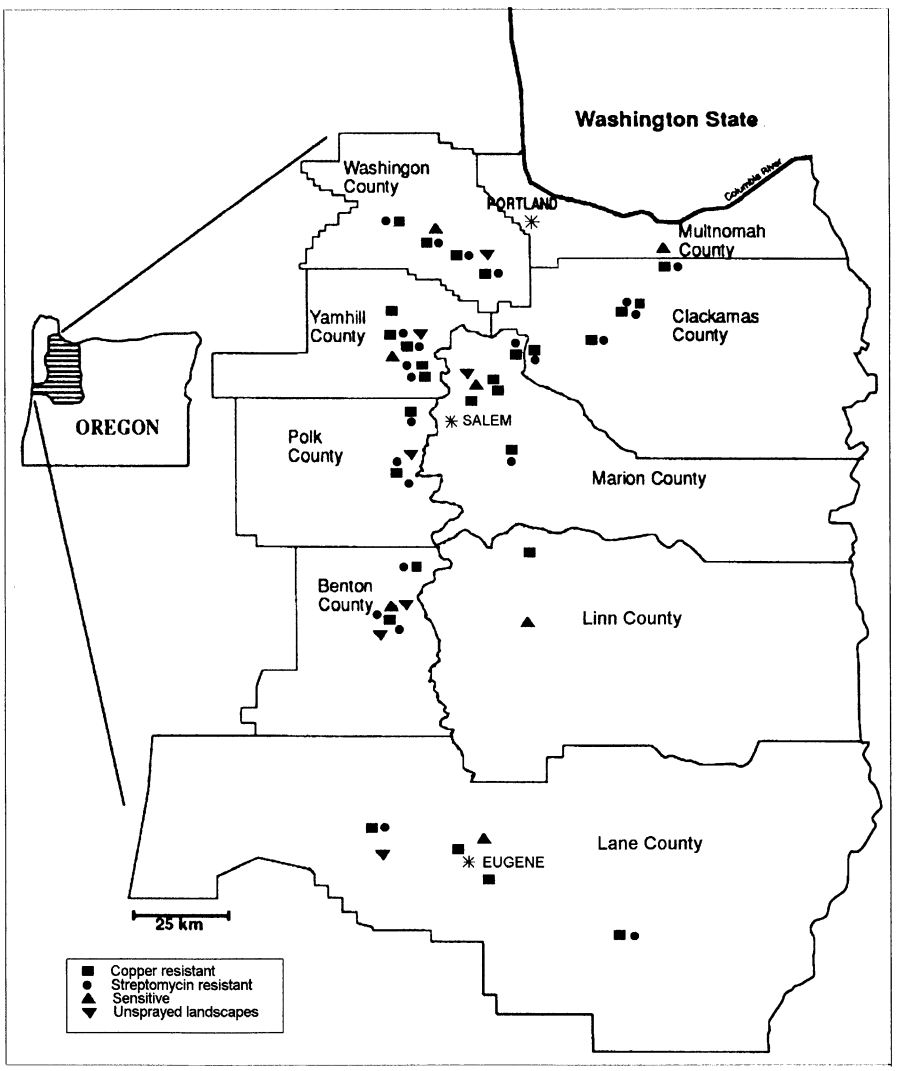

Fig. 1. Commercial nurseries and landscape plantings in the Willamette Valley, Oregon, where strains of *Pseudomonas syringae* were isolated in 1992 and 1993. Symbols show nurseries or landscapes where copper-resistant, streptomycin-resistant, and copper-and-streptomycin-sensitive strains were collected.

copper-resistant mutants were observed from copper-sensitive strain Al487 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 Sm^s strain FF5 occurred at a frequency of approximately 1 in 10^6 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 *cop*ABCD probe for Cu^r determinants hybridized with 10% of the strains isolated in 1992 and 1993, and all of the strains that hybridized were copper resistant. The *cop*J probe for Cu^r 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 *cop*ABCD or *cop*J 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 *cop*ABCD or the *cop*J probe.

The *str*A-*str*B Sm^r 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.

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

Family, genus, and species[a]	Common name	Resistance phenotype (no. of strains)[b]			
		Cu^sSm^s	Cu^rSm^s	Cu^sSm^r	Cu^rSm^r
Aceraceae					
Acer palmatum Thunb.	Japanese maple	34	21	4	21
A. platanoides L.	Norway maple	4	0	1	0
A. rubrum L.	Red maple	7	5	0	1
A. saccharum Marsh.	Sugar maple	0	1	0	0
A. truncatum Bunge	Shantung maple	4	1	0	0
Berberidaceae					
Berberis aquifolium Pursh	Oregongrape	0	2	0	0
Caprifoliaceae					
Viburnum dentatum L.	Arrow-wood	1	5	0	1
Celastraceae					
Euonymus alatus (Thunb.) Siebold	Spindle tree	0	0	0	3
Ericaceae					
Vaccinium corymbosum L.	Highbush blueberry	1	15	0	4
Hamamelidaceae					
Liquidambar styraciflua L.	Sweet gum	2	0	0	2
Hydrangeaceae					
Philadelphus coronarius L.	Mock orange	6	0	0	2
Magnoliaceae					
Magnolia grandiflora L.	Southern magnolia	7	0	0	5
Oleaceae					
Forsythia viridissima Lind.	Golden bells	6	4	0	2
Syringa × *chinensis* Willd.	Chinese lilac	0	3	0	9
S. amurensis Rupr.	Amur lilac	1	2	0	0
S. × *persica* L.	Persian lilac	3	0	0	3
S. vulgaris L.	Common lilac	100	48	23	55
Rosaceae					
Prunus armeniaca L.	Apricot	5	0	0	0
P. avium (L.) L.	Sweet cherry	5	0	0	0
P. laurocerasus L.	Cherry laurel cv. Otto Leuken	11	0	0	2
P. serrulata Lindl.	Japanese flowering cherry	3	2	0	1
Pyrus communis L.	Common pear	3	2	1	0
P. pyrifolia (Burm. f.) Nakai	Asian pear	9	0	0	0
Sorbus aucuparia L.	Mountain ash	0	1	0	0
Tiliaceae					
Tilia cordata Mill.	European linden	3	0	0	0

[a] Latin binomials follow those in *Scientific and Common Names of 7,000 Vascular Plants in the United States.* 1995. L. Brako et al. American Phytopathological Society, St. Paul, MN.

[b] Copper sensitive Cu^s; streptomycin sensitive, Sm^s; copper resistant, Cu^r; streptomycin resistant, Sm^r.

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^r.

The *cop*ABCD and *cop*J probes hybridized with 16% of the copper-resistant *P. syringae* strains isolated in 1992 and 1993. These two Cu^r determinants have some structural similarities, but they apparently have functional and regulatory differences (21). Genetic similarities in Cu^r determinants are recognized in a diverse collection of bacteria from the genera *Pseudomonas* (8), *Xanthomonas* (34), and *Escherichia* (15). The Cu^r mechanism in *P. syringae* pv. *tomato* is sequestration of Cu^{+2} ions by periplasmic and outer membrane proteins with a two-component regulatory system (18). *copJ* confers Cu^r to *P. syringae* pv. *syringae* differently, possibly with an ef-

flux mechanism that prevents copper ions from accumulating inside the cell.

The Willamette Valley collection of copper-resistant strains, some of which hybridized with *cop*ABCD and others with *cop*J, 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^r 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 *str*A-*str*B gene probe. The 2% of strains that grew on KBS but did not hybridize with the probe may have an Sm^r mechanism other than that conferred by the *str*A-*str*B genes. Jones et. al. (11) observed that a small percentage of streptomycin-resistant *P. syringae* pv. *papulans* strains did not hybridize to *str*A-*str*B. The 4% of the strains that hybridized with the probe but did not grow on KBS may have a nonfunctional copy of the *str*A-*str*B genes. The excellent agreement between growth on KBS and hybridization with the *str*A-*str*B 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^r conferred by *str*A-*str*B genes is widespread among commensal and pathogenic bacteria from animals, plants, and humans, which suggests they share a common gene pool (28). The *str*A-*str*B genes from *P. syringae* are located within

Table 2. Plant sources and resistance phenoypes of *Pseudomonas syringae* strains isolated in 1982 and 1983

Family, genus, and species[a]	Common name	Resistance phenotype (no. of strains)[b]			
		Cu^sSm^s	Cu^rSm^s	Cu^sSm^r	Cu^rSm^r
Aceraceae					
Acer palmatum Thunb.	Japanese maple	12	4	1	0
A. rubrum L.	Red maple	6	3	0	0
Anacardiaceae					
Rhus hirta (L.) Sudw.	Staghorn sumac	2	0	0	0
Araliaceae					
Aralia spinosa L.	Devil's walking stick	1	0	0	0
Betulaceae					
Corylus avellana L.	European filbert	5	4	0	0
Celastraceae					
Euonymus alatus (Thunb.) Siebold	Spindle tree	5	3	0	0
Cornaceae					
Cornus florida L.	Flowering dogwood	6	5	1	0
Ericaceae					
Vaccinium corymbosum L.	Highbush blueberry	2	3	2	0
Fabaceae					
Gleditsia triacanthos L.	Honey locust	3	0	0	0
Laburnum anagyroides Medick.	Golden chain tree	0	2	0	0
Fagaceae					
Fagus grandifolia Ehrh.	American beech	1	3	0	0
Magnoliaceae					
Magnolia grandiflora L.	Southern magnolia	5	3	0	0
Magnolia × *soulangiana* Soul.-Bod.	Chinese magnolia	8	1	0	0
Moraceae					
Morus alba L.	White mulberry	1	0	0	0
Oleaceae					
Fraxinus americana L.	White ash	3	0	0	0
Forsythia viridissima Lind.	Golden bells	4	0	0	0
Syringa vulgaris L.	Common lilac	12	4	2	0
Paeoniaceae					
Paeonia suffruticosa Andr.	Tree peony	3	1	0	0
Rosaceae					
Malus sylvestris Mill.	Crabapple	1	0	0	0
Prunus avium (L.) L.	Sweet cherry	8	0	3	0
P. persica Batsch	Peach	1	2	0	0
P. triloba Lindl.	Flowering almond	3	1	1	0
Pyrus pyrifolia (Burm. f.) Nakai	Asian pear	10	2	3	0
Rosa odorata (Andr.) Sweet	Tea rose	4	0	0	0
Sorbus aucuparia L.	Mountain ash	1	0	0	0
Salicaceae					
Populus tremuloides Michx.	Trembling aspen	7	5	0	0
Salix babylonica L.	Weeping willow	2	0	0	0
Thymelaeaceae					
Daphne odora Thunb.	Winter daphne	4	2	0	0
Tiliaceae					
Tilia americana L.	American linden	7	0	0	0
Tilia cordata Mill.	European linden	4	0	0	0

[a] Latin binomials follow those in *Scientific and Common Names of 7,000 Vascular Plants in the United States.* 1995. L. Brako et al. American Phytopathological Society, St. Paul, MN.

[b] Copper sensitive Cu^s; streptomycin sensitive, Sm^s; copper resistant, Cu^r; streptomycin resistant, Sm^r.

Table 3. Number of *Pseudomonas syringae* strains isolated in 1982 and 1983 and 1992 and 1993, resistant to copper (Cu^r) and/or streptomycin (Sm^r)

Resistance phenotype[a]	No. of strains (%)	
	1982/1983	1992/1993
Cu^sSm^s	131 (68)	215 (46)
Cu^rSm^s	48 (25)	112 (24)
Cu^sSm^r	13 (7)	29 (6)
Cu^rSm^r	0 (0)	111 (24)
Total	192 (100)	467 (100)

[a] Cu^s = copper sensitive, strains unable to grow on medium containing 0.32 mM copper sulfate. Cu^r = copper resistant, strains able to grow on medium containing 0.32 mM copper sulfate. Sm^s = streptomycin sensitive, strains unable to grow on medium containing 100 µg of streptomycin sulfate per ml. Sm^r = streptomycin resistant, strains able to grow on medium containing 100 µg of streptomycin sulfate per ml.

Table 4. Number of *Pseudomonas syringae* strains isolated in 1992 and 1993 that hybridized with copper resistance determinants *cop*ABCD and *cop*J

Resistance phenotype[a]	No. of strains (%) (n = 467)	
	*cop*ABCD	*cop*J
Cu^sSm^s	0 (0)	0 (0)
Cu^rSm^s	21 (5)	20 (4)
Cu^sSm^r	0 (0)	0 (0)
Cu^rSm^r	24 (5)	10 (2)
Total	45 (10)	30 (6)

[a] Cu^s = copper sensitive, strains unable to grow on medium containing 0.32 mM copper sulfate. Cu^r = copper resistant, strains able to grow on medium containing 0.32 mM copper sulfate. Sm^s = streptomycin sensitive, strains unable to grow on medium containing 100 µg of streptomycin sulfate per ml. Sm^r = streptomycin resistant, strains able to grow on medium containing 100 µg of streptomycin sulfate per ml.

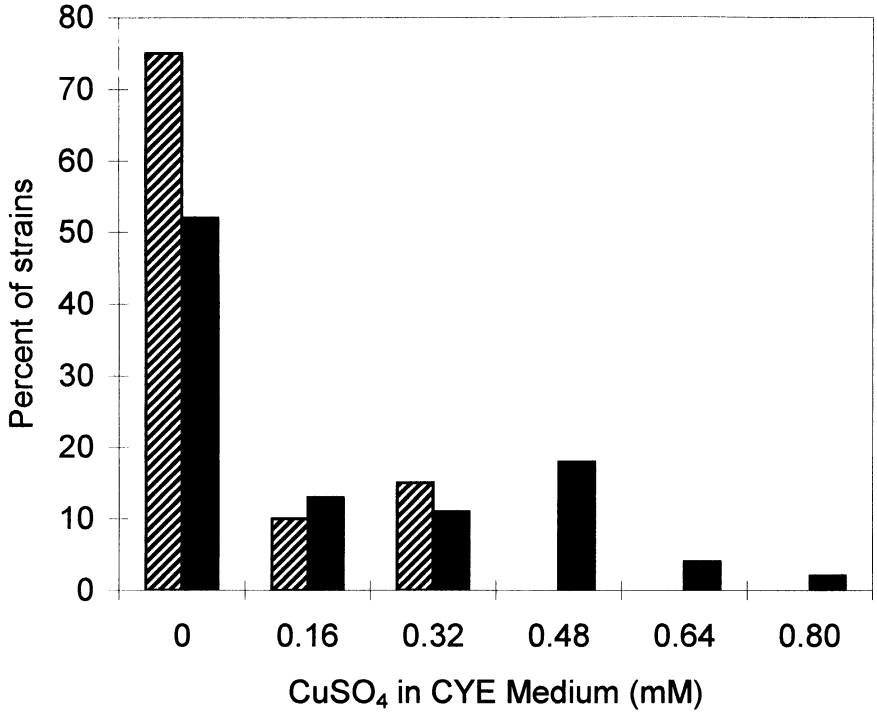

Fig. 2. Frequency distribution of minimum inhibitory concentrations of $CuSO_4 \cdot 5H_2O$ in casitone-yeast extract medium for *Pseudomonas syringae* strains isolated in 1982 and 1983 (hatched bars) and 1992 and 1993 (solid bars).

the transposable element *Tn*5393, which is usually borne on a conjugative plasmid (27). Plasmid transfer has been implicated in the rapid dissemination of *str*A-*str*B genes within populations of plant pathogenic bacteria (29). This study shows that *str*A-*str*B homologues were present in *P. syringae* strains isolated in Pacific Northwest 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 Cu^r and Sm^r genes, the ease of gene transfer 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 application of bactericides had selected for many different *P. syringae* genotypes with transferable resistance determinants.

No Cu^r or Sm^r was detected in any of the strains of *P. syringae* isolated from landscaped-planted lilacs either by growth on amended media or by colony hybridization. These strains were isolated from mature shrubs in public parks and private gardens 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. syringae* with resistance to both copper and streptomycin seriously compromises current nursery chemical control programs. Growers needed to explore alternative methods of disease control, including host resistance, and biological and cultural controls. In many nurseries, growing susceptible 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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