Isolation of *Pseudomonas syringae* from 40 Cultivars of Diseased Woody Plants with Tip Dieback in Pacific Northwest Nurseries

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ABSTRACT

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Bacteria were isolated from 40 cultivars of woody plants with tip dieback during 1981 and 1982. Plants most severely affected were maple (*Acer* spp.), dogwood (*Cornus florida*), filbert (*Corylus avellana*), blueberry (*Vaccinium corymbosum*), magnolia (*Magnolia* spp.), lilac (*Syringa* sp.), oriental pear (*Pyrus pyrifolia*), aspen (*Populus tremuloides*), and linden (*Tilia americana*). Of the 556 bacterial strains tested, 466 (84%) were fluorescent, and 303 (65%) of the fluorescent strains were identified as *Pseudomonas syringae*.

Since 1981, nursery workers in the Pacific Northwest have reported increasing losses of woody plants with tip dieback. In many instances, tip dieback was followed by death of the entire tree. *Acer rubrum* L. 'Red Sunset' and 'October Glory' were the first trees reported affected. Another species affected in 1981 was *Pyrus calleryana* Dcne., especially the cultivar Bradford, which had necrotic lesions around leaf scars in addition to tip dieback. One grower reported that thousands of these trees were affected and that 70% were discarded.

Tip dieback occurred most frequently after late spring frosts, suggesting that bacteria active in ice nucleation may have been associated with symptoms. Strains of *Pseudomonas syringae* van Hall with ice-nucleation activity are known to occur in high numbers on many woody plants in the spring (14). Damage to woody plants from the combination of *P. syringae* and frost has been reported (5,23). To confirm the suspicion that *P. syringae* might be involved in tip dieback, we isolated bacteria from the red maples Red Sunset and October Glory in June 1981. This resulted in the acquisition of 20 strains that were fluorescent on King's medium B (10), negative for cytochrome oxidase activity, and active in ice nucleation. On the basis of these results, a more extensive study seemed justified.

This study was designed as a survey to determine the number and variety of woody hosts with tip dieback and the association of *P. syringae* with diseased plant tissues. A separate study made on the bacteria that were active in ice nucleation will be reported later.

MATERIALS AND METHODS

Collection of samples. Samples collected by nursery inspectors and growers were mailed to our laboratory for analysis. We also visited many of the collection sites to observe diseased trees and collect additional specimens. Plant material was obtained from 32 nurseries in Oregon and Washington from December 1981 through July 1982.

Isolation and purification of strains. Plant tissue from the margins of necrotic areas was cut into small (1-g) pieces and soaked in 2 ml of sterile distilled water for 1 hr. Loopfuls of the resulting aqueous suspension were streaked onto King's medium B and three selective media (2; M. Sasser, *personal communication*). After 2 days of incubation at 24 C, the King's medium B plates were examined under ultraviolet irradiation (350 nm) for fluorescent colonies. Bacteria from fluorescent colonies and selective media

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were transferred to 2 ml of sterile distilled water, mixed, and streaked on King's medium B. This procedure was repeated at least once to ensure purity. Single colonies chosen from the initial plates often yielded two colony types, one of which may have been nonfluorescent. The nonfluorescent strains were included in subsequent tests for comparison with the initial parent colony in the event that they might also be pathogenic. Cultures were stored on potato-dextrose agar slants with 0.5% calcium carbonate at 4 C.

Characterization of bacteria. The three characteristics, fluorescence on King's medium B, and cytochrome oxidase-negative and arginine dihydrolase-negative responses, were used to identify strains preliminarily as *P. syringae*. Although these characteristics are shared by *P. viridiflava*, tests were not done to distinguish between the two because of the reported variability of responses (21).

Pathogenicity. Because it was not feasible to inoculate all 40 of the hosts of origin during the study, three tests were used to detect pathogenicity of the strains that were isolated: induction of hypersensitivity in tobacco, induction of necrosis in tomato fruit, and induction of necrosis in peach and cherry leaves. Hypersensitivity of *Nicotiana tabacum* L. 'Samsun NN' (9) to the bacterial strains was tested by inoculating 0.1 ml of a bacterial suspension at 10^8 cfu/ml into leaves and recording the presence of tissue collapse 24 hr later (11,12). Green fruit of Yellow Pear tomato (4) was inoculated with bacteria from 48-hr-old colonies on King's medium B.

Each green tomato fruit was injected at three sites with 0.1 ml of a 10^6-cfu/ml suspension, then placed in a plastic container lined with moist paper towels and incubated for 7 days. Tissue collapse, necrosis, or bacterial ooze was recorded as a positive response (4). The peach and cherry trees were tested by inoculating newly emerging leaves of 2-yr-old potted trees (16). Leaves were pricked with a hypodermic needle, then droplets of 50–100 µl of a 10^6-cfu/ml suspension of the bacteria were added to the wounds. The trees were placed in a humid chamber in the greenhouse at 26 C during daylight and at 20 C at night. After 3 days, the plants were removed from the chamber and left on the greenhouse bench at the same temperatures. Leaves were examined for necrosis after 7 days.

Table 1. Plant sources of bacterial isolates, dates of sampling, and proportion of *Pseudomonas syringae* among strains isolated from each host

Family, genus, and species[a]	Common name	No. of cultivars	Dates of sampling	*P. syringae*[b]
Aceraceae				
Acer ginnala Maxim.	Amur maple	1	Apr. 1982	2/4
A. japonicum Thunb.	Japanese maple	1	Jan. 1982	10/10
A. platanoides L.	Norway maple	1	May & Jul. 1982	15/20
A. rubrum L.	Red maple	5	Dec. 1981, Feb. 1982	17/43
A. saccharum Marsh.	Sugar maple	1[c]	May 1982	0/3
Betulaceae				
Corylus avellana L.	European filbert	1	Jun. & Jul. 1982	50/95
Celastraceae				
Euonymus alata (Thunb.) Siebold	Spindle tree	1	May & Jun. 1982	8/14
Cornaceae				
Cornus florida L.	Flowering dogwood	1	May & Jun. 1982	18/18
Ericaceae				
Vaccinium corymbosum L.	Highbush blueberry	7	May & Jun. 1982	19/57
Rhododendron sp.	Rhododendron	1	Jul. 1982	0/20
Leguminoseae				
Laburnum anagyroides Medic.	Golden chain	1	Jun. 1982	0/6
Magnoliaceae				
Magnolia spp.	Magnolia	3	May 1982	14/20
Myrtaceae				
Eucalyptus sp.	Eucalyptus	1	Jul. 1982	2/41
Oleaceae				
Forsythia sp.	Golden-bells	1	May 1982	9/9
Syringa sp.	Lilac	1	May 1982	14/15
Fraxinus sp.	Ash	1	May 1982	3/4
Paeoniaceae				
Paeonia suffruticosa Andr.	Tree peony	1	May 1982	6/6
Rosaceae				
Malus spp.	Apple	2[c]	Jun. 1982	1/6
Prunus avium (L.) L.	Sweet cherry	1	May & Jun. 1982	8/43
P. triloba Lindl.	Flowering almond	1	May 1982	5/5
Pyrus calleryana Dcne.	Callery pear	1	Jun. 1982	3/10
P. communis L.	Common pear	1	Jun. 1982	1/1
P. pyrifolia (Burm. f.) Nakai	Oriental pear	4	Feb. & Jun. 1982	47/56
Rosa sp.	Rose	1	Sept. 1982	0/3
Salicaceae				
Populus tremuloides Michx.	Quaking aspen	1	Jun. & Jul. 1982	21/27
Salix sp.	Willow	1	May 1982	3/3
Tiliaceae				
Tilia americana L.	Linden	2	Apr. & May 1982	17/17

[a] Latin binomials follow those in *Hortus Third: A Concise Directory of Plants Cultivated in the United States and Canada*. MacMillan, New York. 1976.
[b] Ratios indicate number of strains per total strains isolated that produced a fluorescent, diffusable pigment on King's medium B and were negative for both cytochrome oxidase activity and arginine dihydrolase activity.
[c] Seedlings, not named cultivars.

RESULTS

Strains of *P. syringae* were isolated from plant samples from all 32 nurseries providing material. The woody plants from which bacteria were isolated represented 13 families that included 44 cultivars (Table 1). *P. syringae* has been isolated by previous workers from all of the species of hosts sampled except *Cornus florida, Euonymus alatus, Paeonia suffruticosa,* and *Tilia americana* (6,14,17,22). *Pseudomonas* infections of *Corylus avellana* have not been reported in the United States, although one of the authors (L. W. Moore, *unpublished*) has isolated an oxidase-negative fluorescent bacterium from diseased filberts. The symptoms observed on filbert in the present study were similar to those of filbert blight caused by *Xanthomonas campestris* pv. *corylina*, i.e., death of new buds, stem necrosis, and tip dieback (15). However, more than 50% of the strains recovered from filbert in our survey were *P. syringae*, and no xanthomonad strains were isolated. Psallidas and Panagopolous (18) reported severe infection of filberts by *P. syringae* in Greece.

Differences were observed in symptoms on different cultivars of the same species. In fields where various cultivars of blueberry were grown, all plants of Blue Ray had tip dieback, stem necrosis, dead leaf buds, and floral necrosis, whereas all plants of Blue Crop were symptomless. Among Japanese maple cultivars, all young trees of Sango showed tip dieback, whereas all other cultivars were symptomless. Such differences have been reported previously in apple cultivars (2).

Symptoms in this study included stem and floral necrosis, leaf spots, canker, gummosis, dead buds, and floral wilt in addition to tip dieback (Fig. 1). Whereas tip dieback was seen on all diseased plants, the other symptoms occurred less frequently. *Acer* spp., *Corylus avellana, Vaccinium corymbosum, Cornus florida, Forsythia* sp., *Syringa* sp., *T. americana, Magnolia* spp., and *Pyrus* spp. showed stem necrosis. *Acer* spp., *Magnolia* spp., and *Paeonia suffruticosa* showed leaf spots with chlorotic halos. *V. corymbosum, Syringa* sp., *Magnolia* spp., and *T. americana* showed leaf necrosis. *P. suffruticosa, V. corymbosum, Magnolia* spp., *Pyrus* spp., and *Syringa* sp. showed floral necrosis or wilt. *Cornus florida* showed canker, *Prunus avium* showed

gummosis, and *Corylus avellana* and *V. corymbosum* showed dead buds.

Selective media used in early isolations were discontinued for separate reasons. The medium of Burr and Katz did not support growth of known *P. syringae* strains, and some of the colonies picked from Sasser's media produced no fluorescent pigment when grown on King's medium B. When colonies that did produce a fluorescent pigment were tested for their cytochrome oxidase activity and compared with colonies isolated on King's medium B, only 56% of the fluorescent colonies from Sasser's medium were negative, whereas 80% of those from King's medium B were negative.

In this survey, of 556 strains isolated from diseased plants, 466 (84%) produced fluorescent pigment on King's medium B. Of the fluorescent strains, 303 (65%) were negative in tests for cytochrome oxidase and arginine dihydrolase, major characteristics of *P. syringae* (21). Two hundred fifty-two strains (85%) of those considered to be *P. syringae* induced a hypersensitive response on tobacco.

Thirty-one of the 256 non-*P. syringae* strains (12%) caused hypersensitivity in tobacco. Characteristics of the 31 strains are shown in Table 2. Eleven of these strains were nonfluorescent, and 13 of the fluorescent strains had similar characteristics. Rhododendron, sugar maple, golden chain, and rose were the only plants sampled that yielded no *P. syringae*.

Of the 252 strains that induced a hypersensitive response on tobacco, 173 also induced symptoms on tomato fruit. Twelve strains induced symptoms on tomato fruit but no hypersensitive response in tobacco. A higher percentage of strains induced a hypersensitive response on tobacco than a pathogenic response on tomato (Table 3).

Twenty-eight of 77 *P. syringae* strains inoculated to peach and cherry leaves produced necrosis within 7 days that subsequently spread beyond the site of inoculation.

DISCUSSION

It appears on the basis of this survey that strains of *P. syringae* were associated with tip dieback and other symptoms in a large number of woody plant species in Pacific Northwest nurseries and orchards. Further tests are needed to confirm the pathogenicity of *P. syringae* on *Cornus florida, E. alatus, Paeonia suffruticosa,* and *T. americana* because there are no previous reports of isolation of *P. syringae* from these plants (8). Why the infections occurred so suddenly over such a large geographic region and on such a broad range of woody plant species is unknown.

Symptoms have been shown to differ in their severity from year to year in plants such as *Prunus avium* and other woody hosts infected with *P. syringae* (3). Environmental conditions favorable to symptom development have not been investigated thoroughly, but many growers report that late spring frost following early spring growth and development of succulent tissues result in more diseased trees.

Isolating and testing bacteria from such a large number of hosts in one growing season presents several problems, one being the lack of a good selective medium. Another problem is determining which strains are pathogenic. The hypersensitive response in tobacco has been used to differentiate saprophytic pseudomonads from pathogenic strains (11,12). This response has been especially useful in testing strains isolated from woody plants, because appropriate homologous hosts are often not available. In addition, verification of pathogenicity in woody plants often takes weeks or months. Latorre and Jones (13) reported that 83% of their isolates from cherry that induced hypersensitivity on tobacco were pathogenic when inoculated to cherry trees. Roos and Hattingh (19) tested 403 fluorescent oxidase-negative strains isolated from plum, apricot, peach, and nectarine and found that 79% induced a

Table 2. Characteristics of 31 non-*Pseudomonas syringae* strains that induced a hypersensitive response in tobacco

Number of strains	Fluorescence on King's medium B	Cytochrome oxidase activity	Arginine dihydrolase activity
1	+	+	−
6	+	−	+
13	+	+	+
1	−	+	+
1	−	+	−
2	−	−	+
7	−	−	−

Table 3. Induction of hypersensitive response in tobacco and pathogenicity in tomato fruit by strains of *Pseudomonas syringae* isolated from 14 woody hosts[a]

Host of origin	Hypersensitive response[b]		Pathogenicity[c]	
	Ratio	%	Ratio	%
Acer japonicum	8/10	80	7/10	70
A. platanoides	9/15	60	10/15	67
A. rubrum	13/17	76	7/17	41
Cornus florida	17/18	94	11/18	61
Corylus sp.	44/50	88	31/50	62
Eucalyptus sp.	1/2	50	0/2	0
Euonymus alatus	8/8	100	8/8	100
Magnolia sp.	12/14	91	6/14	43
Populus tremuloides	20/21	95	16/21	76
Prunus avium	5/8	63	4/8	50
Pyrus pyrifolia	24/47	51	23/47	49
Syringa sp.	12/14	86	11/14	79
Tilia americana	14/17	82	12/17	75
Vaccinium corymbosum	23/24	96	19/24	79
Totals	210/265	79	165/265	62

[a] Only those hosts from which more than 10 fluorescent strains were tested are included in this table.
[b] Ratios indicate number of *P. syringae* strains that induced a hypersensitive response on *Nicotiana tabacum* L. 'Samsun NN' relative to total number tested.
[c] Ratios indicate number of *P. syringae* strains that induced tissue collapse, necrosis, or bacterial ooze on green fruit of Yellow Pear tomato relative to total tested.

Fig. 1. Symptoms on representative plants from which *Pseudomonas syringae* was isolated. **(A)** Tip dieback on red maple; **(B)** leaf, stem, and floral necrosis on magnolia; and **(C)** stem necrosis and dead leaf and floral buds on blueberry.

hypersensitive response on tobacco. These strains also induced symptoms on plum leaves and shoots and on woody stems of plums and apricots.

If hypersensitivity in tobacco can be used to predict the pathogenicity of *P. syringae* strains, then 271 strains from this survey could be considered potential pathogens. Most of these (240, or 89%) were presumptively identified as *P. syringae* and the remaining 31 resembled *P. fluorescens* (Table 2) because they were fluorescent, oxidase-positive, and arginine dihydrolase-positive. *P. fluorescens* strains are usually considered saprophytes, but they have been identified as pathogens of garlic and cactus (1,20).

Fifty-one strains characterized as *P. syringae* (10%) in our study induced neither a hypersensitive response in tobacco nor symptoms in tomato. However, these strains should not be excluded from consideration as pathogens until they have been inoculated back to the host from which they were isolated. Latorre and Jones (13) found that 12 of 225 pathogenic strains of *P. morsprunorum* did not induce a hypersensitive response in tobacco but did cause disease when inoculated to sour cherry.

A bioassay that has a high correlation for pathogens that infect woody hosts would be very useful. Green fruit of Yellow Pear tomatoes was used by Cameron (4) for *P. syringae* strains isolated from cherry, but this bioassay needs further testing with known pathogens isolated from other woody hosts.

Recently, Endert and Ritchie (7) inoculated leaves of potted peach trees in a greenhouse with 10^7 cfu/ml of *P. syringae* pv. *syringae* strains from various woody hosts and compared the results with inoculations made on 1- to 3-day-old etiolated hypocotyls of apple, pear, and peach seedlings. They found a high correlation between number of lesions per peach leaf and necrosis on pear ($r = 0.92$) and apple ($r = 0.84$) hypocotyls. Therefore, inoculation of young pear and apple hypocotyls should be useful as a bioassay for pathogenicity. A comparison between necrosis of Yellow Pear tomato fruit, the hypocotyl response, and infection of the host of origin after inoculations of selected strains from this survey might determine which is most useful as a bioassay.

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