Population Dynamics and Diversity of *Pseudomonas syringae* on Maple and Pear Trees and Associated Grasses

Dean K. Malvick and Larry W. Moore

Former graduate research assistant and associate professor, respectively, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331-2902.

Submitted as technical paper no. 8407 from the Oregon State University Agricultural Experiment Station.

This research was supported in part by funding from the Oregon Nursery Advisory Council.

The authors thank M. L. Powelson, H. M. El-Nashaar, C. L. Bender, and J. Fletcher for reviewing this manuscript, and J. D. Burd for assistance with graphics.

Accepted for publication 1 June 1988 (submitted for electronic processing).

ABSTRACT

Malvick, D. K., and Moore, L. W. 1988. Population dynamics and diversity of *Pseudomonas syringae* on maple and pear trees and associated grasses. Phytopathology 78:1366-1370.

The number of epiphytic *Pseudomonas syringae* isolated from maple twigs and leaves between July 1985 and September 1986 was erratic (undetectable to 10^5 cfu/g), whereas the number isolated from pear was more stable and often higher (10^3 to 10^6 cfu/g). *P. syringae* was isolated consistently (about 10^4–10^7 cfu/g) from perennial rye, orchard, red fescue, annual rye, and brome grasses growing among trees in the maple nursery and from perennial rye grass in the pear orchard. In greenhouse pathogenicity tests, 87% of the *P. syringae* isolates from maple trees was pathogenic in maple seedlings, whereas 15% of the isolates from pear trees was pathogenic in young pear trees. Of the isolates tested from grasses, 55% from the maple nursery was pathogenic in maple seedlings, and 29% from grass in the pear orchard was pathogenic in young pear trees. These data indicate that grasses and trees support reservoirs of inoculum of pathogenic *P. syringae*. Indigenous isolates from a maple nursery were variable relative to pathogenicity and DNA restriction-fragment analysis, indicating that epiphytic populations of *P. syringae* from the grasses and trees were heterogenous.

Additional keywords: epiphytic bacteria, inoculum sources, restriction endonuclease fingerprinting.

Blast and tip dieback of pear (*Pyrus* spp.) and leaf spot of red maple (*Acer rubrum* L.) (1,4,5) are common diseases caused by *Pseudomonas syringae* van Hall in the Pacific Northwest of the U.S. Although these diseases are economically important in Oregon nurseries and orchards (4,5), chemical control methods are often unsuccessful. An important, unanswered question related to control concerns the sources of inoculum of *P. syringae* for maple and pear trees. Two potential inoculum sources have been implicated. One source is the host trees themselves. Isolates of *P. syringae* that were pathogenic to fruit trees have been obtained from symptomless bark, twigs, and leaves (7,16). A second potential inoculum source is the grass grown as a ground cover between rows of woody plants in orchards and nurseries. Grasses and weeds in orchards have yielded isolates of *P. syringae* that were pathogenic to fruit trees (9,17,21,22); however, epiphytic populations on grass have not been monitored to determine population levels over different seasons, nor has dispersal of this bacterium from grasses to trees been shown. In the studies cited, the relative quantities of inoculum available on grasses and trees were not determined.

In addition to studying population dynamics of *P. syringae* on grasses and trees, we wanted to know if the isolates of *P. syringae* from these two environments were similar. If they were the same, it would indicate that they had a common origin. Pathogenicity studies were used to identify one component of similarity. A second method of analysis was desirable to provide a more specific measure of similarity among isolates of *P. syringae* from trees and grasses. For this purpose, restriction endonuclease analysis of total cellular DNA was applied. This method was reported to distinguish accurately among different strains of the same bacterial species (3), including *Erwinia carotovora* subsp. *carotovora* (8), *Xanthomonas campestris* pv. *citri* (11), and *Rhizobium* spp. (19). This report describes the occurrence and diversity of indigenous *P. syringae* on maple trees, pear trees, and grasses over a 1-yr period in western Oregon.

MATERIALS AND METHODS

Experimental sites. Epiphytic populations of *P. syringae* were

© 1988 The American Phytopathological Society

monitored at two experimental sites near Corvallis, OR. One site was a plot of red maple trees (cv. Red Sunset) interplanted with different grass species. The plot contained three rows of 3- to 4-m tall maple trees that were planted in 1982. Populations of *P. syringae* were monitored on eight maple trees. Six species of grass that are planted as cover crops in commercial Oregon nurseries and orchards were grown among the rows of trees. Each grass species was planted in two plots, 10–15 m^2, in August 1985. The grass species were perennial rye (*Lolium perenne* L. 'Manhattan II'), field brome (*Bromus arvensis* L.), annual rye (*Lolium multiflorum* Lam.), orchard (*Dactylis glomerata* L. 'Potomac'), sudan (*Sorghum sudanense* (Piper) Stapf.), and red fescue (*Festuca rubra* L.). The second site was an orchard containing mixed cultivars of pear trees (*Pyrus communis* L.) planted in 1980, with perennial ryegrass planted among the rows of trees. Epiphytic populations of *P. syringae* were monitored on one tree of each of the following pear cultivars: Eldorado, Honeysweet, and Winter Nelis. Grasses at both sites were mowed as needed.

Sampling, isolation, and characterization of *P. syringae*. Trees at both sites were sampled at 2- to 3-wk intervals between July 1985 and September 1986. Samples from the trees consisted of 2–4 g of twigs from November 1985 to April 1986, and a combination of 1–2 g of leaves (about five leaves cut into 2-cm^2 pieces) and 1–2 g of twigs (about four twigs 2.5 cm long) during the rest of the sampling period. For individual trees, twigs or leaves and twigs were removed from the tip, middle, and basal part of two or three branches and combined into one sample. Grasses were sampled at different intervals from October 1985 to September 1986. Perennial rye was sampled most frequently (2- to 4-wk intervals), because it is planted in many nurseries and orchards more commonly than the other grass species. Sudan was sampled for only 1 mo, because this grass died with the onset of cold weather in November. Other species were sampled at varied intervals (usually 3–7 wk). Grasses were sampled by breaking whole grass plants off about 1 cm above soil level; 2- to 4-g samples were collected separately from each of the two plots for each species. Samples were placed into plastic bags and most were processed within 2 hr, or stored in darkness at 6 C and processed within 5 hr.

Samples were weighed and then washed for 60–75 min on a rotary shaker (140 rpm) in 125-ml Erlenmeyer flasks containing 40 ml of sterile deionized water. Serial, 10-fold dilutions were prepared from the wash water, and 0.1 or 0.4 ml of each dilution was spread in duplicate on King's medium B (KBM) (14) amended with 100 μg/ml chlorothalonil fungicide; the quantity to be plated was determined by the populations isolated on previous sampling dates.

Bacterial colonies that fluoresced bluish-green (FL+) under ultraviolet light (wavelength 350 nm) were counted after 48–54 hr of incubation at 26 C. All FL+ colonies on individual plates were tested for cytochrome oxidase (CO) activity (15) when the number was 10 or less. Otherwise, 10 randomly picked FL+ colonies were tested for CO activity. The percent CO− colonies in this subsample of 10 was used to estimate the total number of FL+, CO− colonies (putative *P. syringae*) in the sample. Data were transformed to $\log_{10}$ to account for log-normal distributions of epiphytic bacterial populations (12). Colonies of FL+, CO− pseudomonads isolated from tree and grass tissues were selected at random and stored in sterile deionized water (13) at 6 C for pathogenicity and DNA-profile studies.

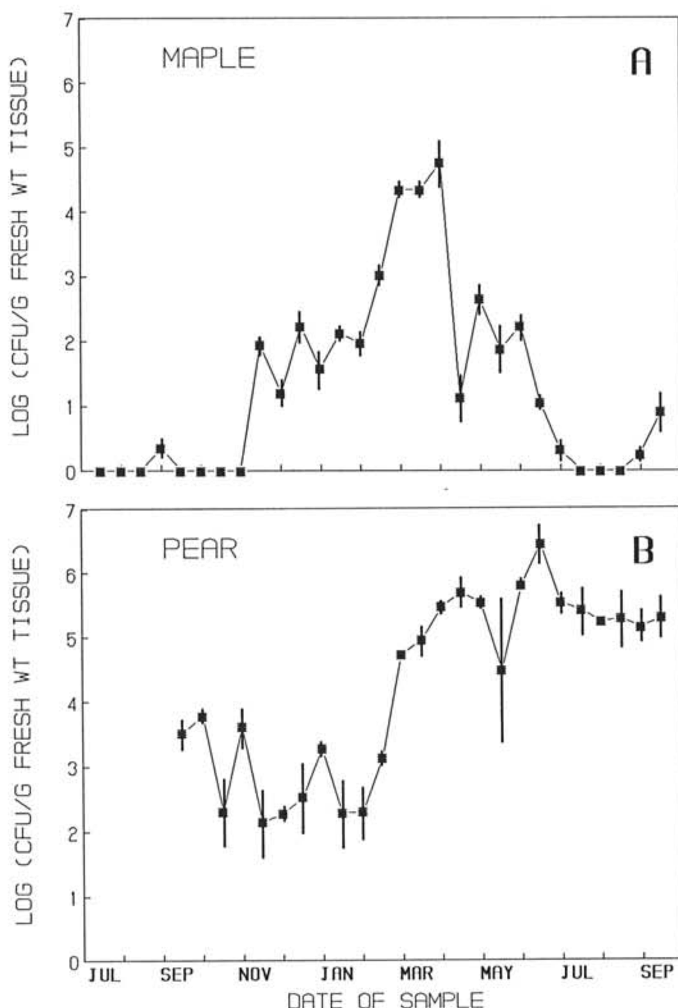

Fig. 1. Mean number of epiphytic *Pseudomonas syringae* isolated from leaves and/or twigs of: **A**, maple and **B**, pear from July 1985 through September 1986. Vertical lines represent standard errors.

Pathogenicity tests. Pure cultures of 142 isolates of *P. syringae* were obtained by repeated subculturing of single colonies to homogeneity on KBM. Inoculum was prepared by growing each isolate for 35–40 hr on KBM at 26 C before suspending it in sterile deionized water to 10^7–10^8 colony-forming units (cfu) per milliliter (A_{600} = 0.04–0.05 in a Spectronic 20 colorimeter; Bausch and Lomb, Rochester, NY).

Six-month-old red maple trees (cv. Red Sunset) were inoculated in the greenhouse with the isolates from trees and grasses in the maple nursery, whereas new growth on pruned pear tree whips (cv. Magnus, with cv. Old Home × Farmingdale rootstocks) were inoculated with isolates from trees and grasses in the pear orchard. Inoculations were made during October 1986 when mean ambient greenhouse temperatures were 23 C (day) and 21 C (night). A 10-μl suspension of each isolate was inoculated to one leaf scar on three different maple or pear trees immediately after leaves were detached. A sterile scalpel tip was inserted through the drop of inoculum 2–4 mm into the leaf scar, and the inoculation site was wrapped with Parafilm (American Can Company, Greenwich, CT). This procedure introduced about 10^4–10^5 cfu at each site. A known pathogenic strain of *P. syringae* (Pss2-3, isolated in our laboratory from grass) and sterile deionized water were applied to leaf scars on each inoculated tree as positive and negative inoculation controls. Incidence and size of lesions were recorded 21 and 44 days after inoculations to maple and pear, respectively.

DNA restriction-fragment profile analysis. Forty isolates of *P. syringae* and four FL+, CO+ pseudomonad isolates from grasses and maple trees in the nursery plot were used for DNA-profile analysis. Pure cultures were grown in yeast-dextrose-peptone broth medium (20) and incubated on a rotary shaker (140 rpm) at 27–29 C for 24 hr. About 0.9 ml of bacterial suspension was spun 2 min at 16,000 *g* in an Eppendorf microcentrifuge (Brinkman Instruments Co., Westbury, NY) at 6 C. The resultant pellet was suspended in 0.25 ml TEG buffer (10 mM Tris-HCl, pH = 8.0; 50 mM ethylenediaminetetraacetic acid [EDTA], pH = 8.0; 1% glucose), 10 μl of 10% sodium dodecyl sulfate was added, and the mixture was heated at 68 C for 10 min. Nucleic acids were extracted with phenol/chloroform, precipitated and washed with ethanol, and resuspended in 30 μl of TE buffer as described by Maniatis et al (18). Approximately 0.9 μg of each DNA preparation was digested with 15 units of *Eco*RI (Bethesda Research Laboratories, Gaithersburg, MD; and United States Biochemical Corporation,

Cleveland, OH) at 37 C for 3 hr. Ten micrograms of RNase A (Sigma Chemical Co., St. Louis, MO) was added 10 min before the end of the incubation period, and digestion was terminated by adding 0.15 M EDTA (pH = 7.5). The digested DNA samples were electrophoresed, stained, and photographed as described by Maniatis et al (18). Electrophoresis was done at 4 V/cm for 0.25 hr, then 2 V/cm for 4.75 hr in 2-mm-thick gels prepared with 0.7% agarose. Digested DNA from a reference strain of *P. syringae* (Pss2-3RNH, isolated in our laboratory from grass) was loaded into each gel as a DNA profile standard. Banding patterns in the DNA profiles were compared visually.

RESULTS

Population trends on maple and pear trees. The number of *P. syringae* isolated from maple trees was variable from July 1985 to September 1986 (Fig. 1A). *P. syringae* was isolated only once from one of the eight maple trees during the first 4 mo of sampling (500 cfu/g on 6 Sept. 1985). Two weeks after leaf fall (7 Nov. 1985), however, *P. syringae* was isolated from seven of the eight maple trees (mean number isolated was 228 cfu/g of twig tissue). The number of *P. syringae* isolated thereafter remained at that level or increased until 5 June 1986, when populations fell below detectable levels. Populations remained at undetectable levels until 28 August 1986 and then increased up to the last sampling on 18 September 1986.

In contrast to the maple trees, *P. syringae* was recovered from pear trees more frequently and in higher numbers (Table 1, Fig. 1B). From September 1985 to September 1986, the mean number of epiphytic *P. syringae* isolated from three pear trees was consistently greater than 1.5×10^2 cfu/g. Populations of *P. syringae* on the pear trees never dropped to undetectable levels as they did on maple trees. There was no significant difference in the number of *P. syringae* isolated from the three cultivars of pear trees ($P = 0.02$).

Disease symptoms were not observed on the maple trees at any time over the sampling period. Symptoms characteristic of bacterial blight caused by *P. syringae*, e.g., necrosis on leaves and branch tips, were observed in April on one of the three pear trees that was sampled (cv. Eldorado).

Population trends on grasses. The brome, red fescue, perennial rye, and orchard grasses in the maple nursery usually yielded populations of *P. syringae* of 3×10^3 to 10^7 cfu/g from October 1985 to September 1986 (Fig. 2). Similar results were obtained from annual ryegrass (data not shown). Populations of *P. syringae* on all five species of grass were similar and followed the same trends. Perennial rye grass in the pear orchard consistently yielded populations of *P. syringae* of 10^4–10^7 cfu/g (data not shown).

Pathogenicity tests. A higher percentage of isolates of *P. syringae* from maple was pathogenic (Table 1), and they induced larger sunken necrotic lesions on young maple trees in the greenhouse than the isolates of *P. syringae* from grasses in the same maple nursery. In contrast, fewer isolates of *P. syringae* from pear trees or grasses in the pear orchard infected pear trees, and lesion size was indistinguishable among isolates. No symptoms occurred on uninoculated areas or where water was applied as a control treatment on the maple or pear trees.

DNA restriction-fragment profiles. Twenty-one different DNA

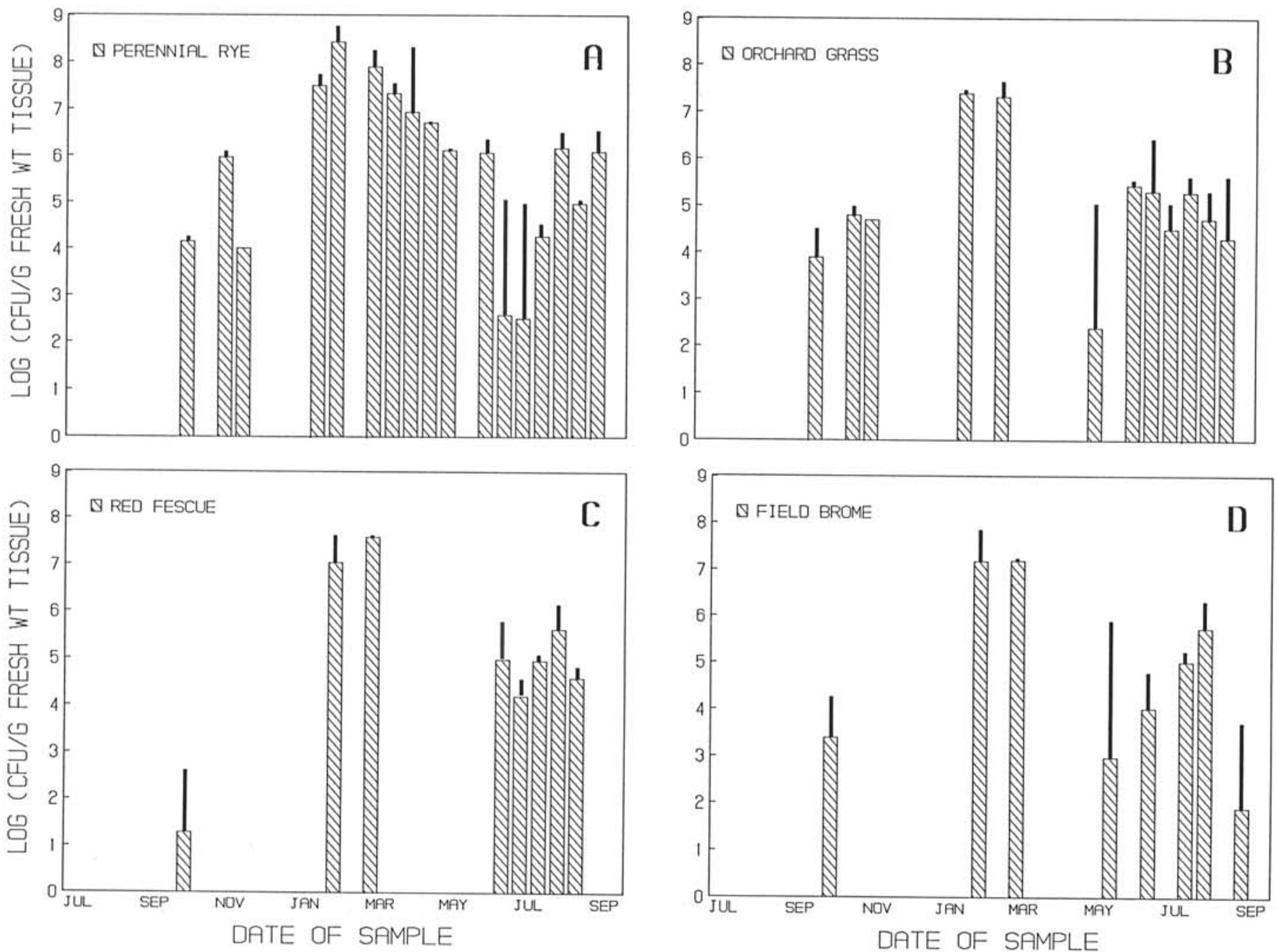

Fig. 2. Mean number of epiphytic *Pseudomonas syringae* isolated from: **A**, perennial rye; **B**, orchard; **C**, red fescue; and **D**, field brome grasses from October 1985 through September 1986. The absence of a bar on any date indicates in most cases that no sample was collected (5% of the grass samples did not yield *P. syringae*). Vertical lines represent standard errors.

profiles were identified on the basis of restriction-fragment banding patterns (Table 2, Fig. 3). Eleven profiles were each found to be unique to one of the 40 isolates (Table 2, DNA patterns K–U), whereas 10 profiles were found in more than one isolate (Table 2, DNA patterns A–J). Two unique profiles were found among four CO+ pseudomonad isolates from the maple nursery; these were different than all the profiles from isolates of *P. syringae*.

DISCUSSION

This study indicated that both trees and grasses may be significant reservoirs of inoculum of *P. syringae* in Oregon nurseries and orchards, and those reservoirs may vary in relative quantitative importance during different seasons of the year. Epiphytic populations on trees, when present, are a likely source of inoculum due to the close proximity of those populations and potential infection sites. The importance of grasses as an inoculum reservoir is not as apparent but is implicated in this study by three results from the maple nursery: in the summer, high populations of *P. syringae* were isolated from grass and none was detected on maple; the number of *P. syringae* isolated from grasses was consistently higher and more stable than the number isolated from maple trees over the 11-mo period when both were sampled simultaneously; and populations of *P. syringae* peaked on grasses before populations peaked on trees.

DNA-profile analyses were designed to provide further evidence for inoculum sources. We hypothesized that if grasses were a source of inoculum, then isolates with identical DNA profiles would be obtained from grasses and trees. The results, however, neither strongly support nor contradict this hypothesis; only two of the 21 DNA profiles reported were from both grasses and trees. It remains unknown, therefore, what quantity of inoculum the grasses and trees contribute to infections on trees.

Grasses are potentially less important as a reservoir of inoculum in the pear orchard than in the maple nursery. Epiphytic populations of *P. syringae* were maintained at fairly high levels on pear, whereas *P. syringae* was sometimes undetectable on maple, indicating that an external source of inoculum such as grass may be more important for maple. Further, in contrast to the low populations of *P. syringae* isolated from maple trees and the high numbers isolated from grasses in the nursery, the populations isolated from pear trees and grasses in the orchard were always at similarly high levels. These results suggest that pear trees and grasses provide a better habitat for survival and multiplication of *P. syringae* than maple trees over much of the year, or that *P. syringae* is more readily washed from grass and pear tissues. In addition, the roles of tree and grass inoculum reservoirs may vary in different environments (e.g., in maple nurseries and pear orchards).

Epiphytic populations of *P. syringae* apparently were diverse in the maple nursery. The fact that more of the isolates from trees (87%) were pathogenic in maple than those from grasses (55%) is one indication that the populations on these different plant types were heterogenous. This result also suggests that the isolates from maple trees may be better adapted to pathogenesis and survival on trees than the isolates from grasses. The variety of DNA profiles found among the isolates of *P. syringae* analyzed is another indication that diverse populations of *P. syringae* were distributed throughout the maple nursery. With the exception of annual ryegrass, all plant species, i.e., maple trees and five species of grass,

TABLE 1. Isolation frequency and pathogenicity of epiphytic isolates of *Pseudomonas syringae* from maple trees, pear trees, and grasses

Source of isolates/location	Isolation frequency: Number of samples	Isolation frequency: Samples with *P. syringae* (%)	Pathogenicity: Number of isolates	Pathogenicity: Isolates pathogenic (%)[a]
Maple/nursery	262	38	47	87.2
Grass/nursery[b]	173	96	42	54.8
Pear/orchard	90	95	39	15.4
Grass/orchard	60	100	14	28.6

[a] Pathogenicity of isolates from the maple nursery and pear orchard in stems of young maple and pear trees, respectively.
[b] Grasses included five species planted in the maple nursery.

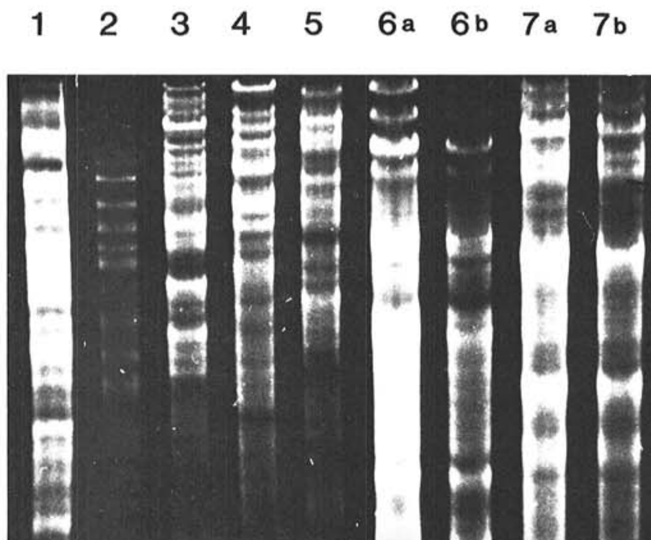

Fig. 3. Restriction-fragment banding patterns of cellular DNA from seven different isolates of *Pseudomonas* digested with *Eco*RI. Lanes 1, 2, 5, 6, and 7 show DNA profile types H, C, I, F, and A, respectively (see Table 2), from isolates obtained from a red maple nursery. The a and b lanes for 6 and 7 show the result when different quantities of DNA were loaded; lane a contains twice as much DNA as lane b. The DNA profile in lane 3 is from a cytochrome oxidase-positive fluorescent isolate of *Pseudomonas* spp. The DNA profile in lane 4 is from *P. syringae* strain Pss2-3 used as a DNA profile standard.

TABLE 2. Grouping of epiphytic isolates of *Pseudomonas syringae* from maple trees and grasses in a maple nursery based on DNA restriction-fragment banding patterns

DNA pattern[a]	Plant origin[b]	Number of isolates within profile	Pathogenicity[c]
A	PR	2	P,P
	F	1	P
	Or	2	P,P
B	AR	2	P,N
C	T	3	P,P,P
D	F	4	X
E	B	3	P,N,N
F	B	2	P,P
G	T	2	P,P
H	PR	2	X
	T	1	P
I	T	1	P
	PR	1	X
J	S	3	X
K	T	1	X
L	T	1	X
M	Or	1	X
N	S	1	X
O	T	1	X
P	T	1	P
Q	Or	1	P
R	PR	1	X
S	Or	1	X
T	Or	1	P
U	B	1	P

[a] Each DNA pattern represents a different DNA restriction-fragment banding profile from cellular DNA digested with *Eco*RI. (See Fig. 3.)
[b] T = red maple trees (from six different trees), PR = perennial ryegrass, F = red fescue, Or = orchard grass, AR = annual ryegrass, B = brome grass, S = sudan grass.
[c] Pathogenicity of isolates in stems of young maple trees. P = pathogenic, N = nonpathogenic, X = pathogenicity not tested.

yielded at least two to eight isolates of *P. syringae* with different DNA profiles. This variation among DNA profiles agrees with the heterogeneity among isolates of *P. syringae* previously found in nurseries (i.e., with respect to hypersensitivity in tobacco, ice nucleation activity, and pathogenicity) (2). Whereas DNA restriction-fragment profile analysis is only one measure of diversity within the bacterial population, it appears to be a useful tool to compare the identity of individual isolates of *P. syringae* that cannot be distinguished morphologically or by traditional biochemical methods.

It is unknown why disease symptoms were not observed on maple trees, because pathogenic isolates of *P. syringae* were present on the trees over much of the year. Environmental conditions seemed to favor infection by *P. syringae* during the time covered by this study; this is supported by nursery operators who reported infections caused by *P. syringae* on maple in areas near the experimental site. Further, inoculation of a pathogenic strain of *P. syringae* to wounds on several maple trees in the nursery resulted in infection (D. K. Malvick and L. W. Moore, unpublished). Perhaps *P. syringae* is a facultative pathogen on these tissues and infects them only during periods of unique host susceptibility. Another possible reason for the lack of natural infection may be that minimum levels of *P. syringae* required for disease occurrence were not attained. Although threshold populations of epiphytic *P. syringae* required for infection on woody plants have not been well defined, evidence has been reported to support the theory that threshold numbers of *P. syringae* are required for infection of trees. Crosse (6) found that the mean concentration of inoculum of *P. s.* pv. *morsprunorum* required to achieve the same level of disease incidence in two cherry cultivars was about 30 times greater for the resistant cultivar. To our knowledge, a correlation between epiphytic populations of *P. syringae* and disease incidence on maple or pear trees has not been reported.

The seasonal fluctuation of populations on maple and pear trees is similar to that reported for *P. syringae* on stonefruit trees in Washington state (10) and Victoria, Australia (23). Wimalajeewa and Flett (23) suggested that population levels of epiphytic bacteria on trees are influenced most by the availability of substrates for growth, whereas rainfall and temperature have secondary effects. The trees in both experimental sites were exposed to similar rainfall and temperature conditions. Therefore, it seems likely that substrate differences, as influenced by different quality and quantity of exudates, may have influenced population sizes of *P. syringae*. In Victoria, low summer populations of epiphytic *P. syringae* were attributed to low humidity, high temperatures, and high UV irradiation (23). It is probable that the same environmental factors in Oregon affect the epiphytic populations of *P. syringae* on trees in the summer.

The primary source of inoculum is not well defined for tree diseases caused by *P. syringae*. Population levels on trees during this study suggest that trees may be an immediate source of inoculum during much of the year. Indirect evidence was noted in that the highest incidence of infection by *P. syringae* occurs in Oregon on woody nursery plants in the spring, which corresponds to the time in our study when population levels of *P. syringae* were highest on maple and pear trees. If epiphytic populations of *P. syringae* on trees are the immediate source of inoculum, then populations on grasses near the trees would be of little concern in disease-control schemes. The role of epiphytic populations on trees in infection has not been clearly established, however, leaving the contribution of inoculum from grasses a possibility. The questions of inoculum source and threshold populations required for infection remain important relative to the epidemiology of diseases caused by *P. syringae* in orchards and nurseries.

LITERATURE CITED

1. Ark, P. A. 1939. Bacterial leaf spot of maple. Phytopathology 29:968-970.
2. Baca, S., and Moore, L. W. 1987. Variations in *Pseudomonas syringae* isolated from grass species occurring in woody plant nurseries in the Pacific Northwest. Plant Dis. 71:724-726.
3. Bradbury, W. C., Pearson, A. D., Marko, M. A., Congi, R. V., and Penner, J. L. 1984. Investigation of a *Campylobacter jejuni* outbreak by serotyping and chromosomal restriction endonuclease analysis. J. Clin. Microbiol. 19:342-346.
4. Cameron, H. R. 1962. Diseases of deciduous fruit trees incited by *Pseudomonas syringae* van Hall. Oregon St. Univ. Agric. Exp. Stn. Tech. Bull. 66. 64 pp.
5. Canfield, M. L., Baca, S., and Moore, L. W. 1986. Isolation of *Pseudomonas syringae* from 40 cultivars of diseased woody plants with tip dieback in Pacific Northwest nurseries. Plant Dis. 70:647-650.
6. Crosse, J. E. 1957. Bacterial canker of stone fruits. III. Inoculum concentration and time of inoculation in relation to leaf-scar infection of cherry. Ann. Appl. Biol. 45:19-35.
7. Dowler, W. M., and Weaver, D. J. 1975. Isolation and characterization of fluorescent pseudomonads from apparently healthy peach trees. Phytopathology 65:233-236.
8. Drahos, D., Brackin, J., and Barry, G. 1985. Bacterial strain identification by comparative analysis of chromosomal DNA restriction patterns. (Abstr.) Phytopathology 75:1381.
9. English, H., and Davis, J. R. 1960. The source of inoculum for bacterial canker and blast of stone fruit trees. (Abstr.) Phytopathology 50:634.
10. Gross, D. C., Cody, Y. S., Proebsting, E. L., Jr., Radamaker, G. K., and Spotts, R. A. 1983. Distribution, population dynamics, and characteristics of ice nucleation-active bacteria in deciduous fruit tree orchards. Appl. Environ. Microbiol. 46:1370-1379.
11. Hartung, J. S., and Civerolo, E. L. 1987. Genomic fingerprints of *Xanthomonas campestris* pv. *citri* strains from Asia, South America, and Florida. Phytopathology 77:282-285.
12. Hirano, S. S., Nordheim, E. V., Arny, D. C., and Upper, C. D. 1982. Lognormal distribution of epiphytic bacterial populations on leaf surfaces. Appl. Environ. Microbiol. 44:695-700.
13. Iacobellis, N. S., and DeVay, J. E. 1986. Long-term storage of plant-pathogenic bacteria in sterile distilled water. Appl. Environ. Microbiol. 52:388-389.
14. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. and Clin. Med. 44:301-307.
15. Kovacs, N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature 178:703.
16. Latorre, B. A., González, J. A., Cox, J. E., and Vial, F. 1985. Isolation of *Pseudomonas syringae* pv. *syringae* from cankers and effect of free moisture on its epiphytic populations on sweet cherry trees. Plant Dis. 69:409-412.
17. Latorre, B. A., and Jones, A. L. 1979. Evaluation of weeds and plant refuse as potential sources of inoculum of *Pseudomonas syringae* in bacterial canker of cherry. Phytopathology 69:1122-1125.
18. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 545 pp.
19. Mielenz, J. R., Jackson, L. E., O'Gara, F., and Shanmugam, K. T. 1979. Fingerprinting bacterial chromosomal DNA with restriction endonuclease *Eco*RI: Comparison of *Rhizobium* spp. and identification of mutants. Can. J. Microbiol. 25:803-807.
20. Moore, L. W., and Carlson, R. V. 1975. Liquid nitrogen storage of phytopathogenic bacteria. Phytopathology 65:246-250.
21. Roos, I. M. M., and Hattingh, M. J. 1986. Weeds in orchards as potential source of inoculum for bacterial canker of stone fruit. Phytophylactica 18:5-6.
22. Waissbluth, M. E., and Latorre, B. A. 1978. Source and seasonal development of inoculum for pear blast in Chile. Plant Dis. Rep. 62:651-655.
23. Wimalajeewa, D. L. S., and Flett, J. D. 1985. A study of populations of *Pseudomonas syringae* pv. *syringae* on stonefruits in Victoria. Plant Pathol. 34:248-254.