Variations in *Pseudomonas syringae* Isolated from Grass Species Occurring in Woody Plant Nurseries in the Pacific Northwest

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ABSTRACT

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High subpopulations of *Pseudomonas syringae* were detected from naturally occurring grass species on the edges of nursery production areas and from sudangrass and cereal ryegrass used as green manure and cover crops, respectively. Leaf samples obtained from diseased sudangrass and symptomless cereal ryegrass and roadside grass species in November yielded large populations of pathogenic and ice-nucleation-active strains of *P. syringae*. Populations approaching 10^9 cfu/g of fresh tissue were isolated from sudangrass samples, whereas populations of 10^6 cfu/g were obtained from ryegrass and roadside grass samples. Of 81 randomly selected strains from these isolations tested, 58 (72%) were similar to *P. syringae*; of these, 34 (59%) were ice-nucleation-active at −5 C. Thirty-one of the *P. syringae* strains (54%) induced a hypersensitive response in tobacco leaves, and 29 (50%) were pathogenic on green fruit of yellow pear tomato.

Additional key words: hypersensitivity, LOPAT

The nursery industry in Oregon is a major producer of woody ornamental and fruit trees in the United States. During the past few years, the industry has experienced an increase in both incidence and severity of a bacterial infection affecting many deciduous plant species. Strains of *Pseudomonas syringae* were detected on 40 of 44 plant species surveyed, and more than 50% of these strains were pathogenic on green fruit of yellow pear tomato (4). More than 50% of these *P. syringae* were also ice-nucleation-active (INA) at −5 C (3); however, not all INA strains were pathogenic on green tomato fruit. Conversely, not all of the pathogenic strains induced ice formation at this temperature.

One of our concerns was to identify the source of these strains of *P. syringae*. Strains of INA *P. syringae* are widely distributed in nature, occurring on herbaceous as well as deciduous plant species (10,11,14). No information is available, however, regarding where and how *P. syringae* overwinters in nurseries that produce woody plants. In some woody plants, populations of *P. syringae* are often undetectable on dormant tissues during the winter and early spring (D. C. Gross and L. W. Moore, *unpublished*). Burr et al (2), however, reported that low populations of *P. syringae* could be isolated from dormant buds on some cultivars of apple trees but not from other cultivars. Furthermore, high populations of *P. syringae* can develop quickly after budbreak (5).

Populations of *P. syringae* decrease to undetectable levels on seven woody plant species (lilac, linden, magnolia, dogwood, aspen, Japanese pear, and maple), including stem and bud tissue samples, during the winter months in Oregon (S. Baca and L. W. Moore, *unpublished*). Therefore, sources exterior to the woody plants may be involved in the initial spread of inoculum with the onset of a new spring season.

Several studies have demonstrated that *P. syringae* can exist as an epiphyte on weeds, grasses; apparently healthy plants, and several nonhost plants (6,7). Arsenijeric (1) showed that *P. syringae* isolated from sudangrass (*Sorghum sudanense*) could infect woody plants and that strains of *P. syringae* isolated from woody hosts were pathogenic to sudangrass; however, this has not been documented in the United States.

Sudangrass is commonly planted as a green manure crop in many nurseries in the Pacific Northwest and then plowed under the soil in late October. Cereal ryegrass (*Secale cereale*) is subsequently planted in these fields and between rows of nursery stock as a winter cover crop. In some nurseries, strips of sudangrass are left unplowed between rows of nursery stock so machinery can be moved through the production area during the wet winter months.

Because sudangrass and cereal ryegrass have been implicated as potential sources of *P. syringae* inoculum and are currently used in the cultural management schemes of Pacific Northwest nurseries, we investigated the potential of these grass species for harboring *P. syringae* and the distribution of pathogenic and INA strains of *P. syringae* among these grasses.

MATERIALS AND METHODS

Selection and maintenance of bacterial strains. A total of 300 bacteria that fluoresced on King's medium B (KB) were purified by repeated streaking on this medium until colonies from two successive streakings remained pure in colony morphology. Eighty-one strains were selected at random from the 300, and working strains were maintained at 4 C on slants of potato-dextrose agar amended with 0.5% (w/v) calcium carbonate ($CaCO_3$), a buffering agent, and 0.1% chloranthalonil to reduce fungal contamination. Permanent collections of the *P. syringae* strains were prepared immediately to reduce the marked phenotypic variations observed during subculturing. Bacteria for permanent storage were grown on slants of KB medium for 48 hr at 24 C and suspended in 3 ml of sterile glycerol and distilled water solution (30:70, v/v). Duplicate 1-ml aliquots of these suspensions were stored at −70 C.

Sampling procedures. Leaf samples of grasses from four nursery sites within the Willamette Valley of western Oregon were collected during November 1983, placed in plastic bags, and transported to the laboratory on ice. The tissues were held at 4 C until processed. All samples were processed within 24 hr by placing 1 g of fresh plant material in 10 ml of sterile distilled water. Tissue samples were allowed to soak for 30 min, then were vortexed for 15–30 sec and serially diluted. Aliquots (0.1 ml) were spread on duplicate plates of KB and incubated for 48 hr at 24 C before counting colonies that fluoresced under near-ultraviolet light (350 nm). Isolated colonies representative of all colony morphologies were selected at random for further characterization.

Biochemical and pathogenicity tests. Eighty-five strains from sudangrass,

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cereal ryegrass, and roadside grasses along with four known strains of *P. syringae* were characterized by LOPAT (12), levan production (13), oxidase reaction (13), pectate reaction (13), and tobacco hypersensitivity (13). Levan production was determined with a 5% sucrose-augmented minimal medium. Production of a domed colony within 72 hr was considered a positive reaction. Oxidase reaction was determined by streaking a representative colony on filter paper soaked with a 1% solution of *N', N'*-tetramethyl-*p*-phenylenediamine hydrochloride. Production of a dark blue color within 10 sec was considered a positive reaction. Pectate reaction was determined with three replicates of cut potato pieces by dropping 0.1 ml of a 10^9-cfu/ml solution onto each piece. Potatoes were then placed in a humidity chamber and tested at 3 and 7 days for evidence of pectate utilization. Arginine dehydrolase reaction was determined with Thornley's media (13) by stabbing a loopful into the semisoft agar and observing for a color change. Production of a deep red color within 72 hr was considered a positive reaction. Tobacco hypersensitivity was tested in tobacco cultivar White Burley with a 10^9-cfu/ml suspension infused into the abaxial surfaces of tobacco leaves. Tests were replicated twice, with the test plants left out at room temperature (68 F) in natural light and humidity. Strains were scored positive if a white collapsed area was evident within 24 hr. Pathogenicity to green fruit of yellow pear tomato (*Lycopersicon esculentum* Mill.) (3) was also determined using a cell suspension of about 10^6 cfu/ml of each strain, on young shoots of peach, and on greenhouse-grown seedlings of sudangrass and cereal ryegrass (*Secale cereale*). Inoculations to peach (*Prunus persica*) and grass seedlings (*Sorghum sudanense*) by several strains were made by puncturing the plant leaves with a 23-gauge needle, placing a drop of the 10^6-cfu/ml suspension over the injury, and enclosing the plants in a plastic tent with a relative humidity of greater than 80% for 7 days. Tomato fruit were injected with 0.1 ml of a 10^6-cfu/ml at three sites on each fruit, and the inoculated fruits were incubated in a plastic-covered crisper to maintain high relative humidity. Tests were replicated twice with sterile water injected into green fruit to serve as controls for each pathogenicity trial.

INA. All strains were tested for INA at −5 C (8). A suspension of about 10^8 cfu/ml was used to test for INA by the freeze-drop method of Vali as modified by Lindow et al (9). Activity was determined only at −5 C. The test for INA was recorded as positive if one of 10 drops of the bacterial suspension froze within 30 sec.

RESULTS

Distribution of *P. syringae* on grass species in Oregon nurseries. Populations of fluorescent pseudomonads were isolated from all grass samples collected at four nursery sites in western Oregon. Mean populations averaged greater than 10^6 cfu/ml of fresh tissue in both the cereal ryegrass and roadside grass samples and exceeded 10^9 cfu/ml in standing fields of sudangrass (Table 1).

Biochemical and physiological tests. The 81 selected fluorescent pseudomonads varied from one another when characterized. For example, only 59/81 (73%) of these pseudomonads were identified as putative *P. syringae* by their negative oxidase and arginine dihydrolase reactions. Variations also were observed in the percentage of *P. syringae* isolated from individual grass species; 59% of the strains isolated from sudangrass, 81% of the strains from cereal ryegrass, and 95% of the strains from roadside grass were identified as putative *P. syringae* (Table 2). Of the 24 sudangrass strains that resembled *P. syringae* in oxidase and arginine dihydrolase reactions, 92% were levan-positive, 71% induced a hypersensitivity reaction in tobacco leaves, and all were pectate-negative. One strain from sudangrass was identical to the reference strains of *P. syringae*, except it was arginine dihydrolase-positive. Within the 21 strains isolated from cereal ryegrass that resembled *P. syringae* in oxidase and arginine dihydrolase reactions, 71% were levan-positive and 71% induced a hypersensitive response in tobacco leaves. All strains were pectate-negative. In the roadside grass strains, the variations from the control strains were not as prominent. For instance, 95% of the strains resembling *P. syringae* were levan-positive, and all of these strains induced a hypersensitive response in tobacco and were pectate-negative.

INA. Thirty-five of the 59 (59%) fluorescent pseudomonads identified as *P. syringae* were INA at −5 C, but the percentage of *P. syringae* strains from each grass species active as ice nuclei ranged from 42 to 72% (Table 3). For example, 15/24 of the sudangrass strains (63%), 7/17 (42%) of the ryegrass strains, and 13/18 (72%) of the strains from roadside grasses were INA.

Pathogenicity of *P. syringae* strains. All 59 *P. syringae* strains were tested for their ability to induce water-soaking and necrosis in immature green fruits of yellow pear tomato; however, variations were observed among the grass species in percentage of strains with this ability (Table 4). Of the *P. syringae* strains isolated from sudangrass, cereal ryegrass, and roadside grass, 58, 47, and 61%, respectively, produced disease in tomato fruit. Responses were similar to those of the known *P. syringae* strains included as controls.

Preliminary testing of two strains of *P. syringae* from each grass species was conducted to further determine pathogenicity or cross-infectivity to sudangrass, cereal ryegrass, and young leaves of peach trees in the greenhouse. Both strains from sudangrass induced water-soaked symptoms, which progressed to necrosis in both the sudangrass seedlings and the peach leaves. Only one strain from cereal ryegrass was pathogenic to both sudangrass and peach leaves. Both roadside grass strains were pathogenic on sudangrass but not on peach leaves. None of the strains from grasses induced necrotic changes in cereal ryegrass seedlings.

Table 1. Mean[a] population of *Pseudomonas syringae* per gram of fresh tissue from three grass species collected from Oregon nurseries during November 1983

Host	Number of sites	Mean population (cfu/g fresh tissue)	Symptom
Sudangrass	3	$>1.6 \times 10^9$	Leaf spot
Cereal ryegrass	3	5.6×10^6	Asymptomatic
Roadside grass species	3	5.4×10^4	Asymptomatic

[a] Average population from three nursery sites.

Table 2. Characterization of fluorescent pseudomonads isolated from three grass species collected from three Pacific Northwest nurseries during November 1983

Test	Controls (reference strains)[a] (4)[b]	Source of strains: Sudangrass (24)	Cereal ryegrass (17)	Roadside grass species (18)
Levan production (%)	75	92	71	95
Oxidase (negative) (%)	100	100	100	100
Pectate (negative) (%)	100	100	100	100
Arginine dihydrolase (negative) (%)	100	100	96[c]	100
Tobacco hypersensitivity (positive) (%)	100	71	71	95

[a] Two *Pseudomonas syringae* strains obtained from L. Moore and S. Sule.
[b] Numbers of strains tested given in parentheses.
[c] One strain arginine dihydrolase-positive.

Table 3. Ice nucleation activity (INA) of *Pseudomonas syringae* strains recovered from three grass species in Pacific Northwest nurseries during November 1983

Source of *P. syringae* strains	Number of strains tested	INA at −5 C[a] (%)	Total fluorescent pseudomonads[b] (%)
Reference strains[c]	4	100	100
Sudangrass	24	63	37
Cereal ryegrass	17	42	33
Roadside grass species[d]	18	72	68

[a] Calculated by INA/total *P. syringae* × 100.
[b] Calculated by INA/total fluorescent pseudomonads × 100.
[c] Two *P. syringae* strains obtained from L. Moore and S. Sule.
[d] Taxonomic identification of grass species inconclusive.

Table 4. Pathogenicity testing of *Pseudomonas syringae* strains isolated from grass species in Oregon nurseries during November 1983

Pathogenicity tests	Reference strains	Source of strains: Sudangrass[a]	Cereal ryegrass[b]	Roadside grass species[c]
Green tomato fruit	100%	58%	47%	61%
Sudangrass seedlings	+[d]	+	+	+
Cereal ryegrass seedlings	−[d]	−	−	−
Peach leaves	+	+	+	+

[a] Four strains of *P. syringae* tested, two strains positive.
[b] Two strains of *P. syringae* tested, one strain positive.
[c] Two strains of *P. syringae* tested, two strains positive.
[d] + = At least one pathogenic event and − = nonpathogenic.

DISCUSSION

Pathogenic/INA strains of *P. syringae* exist at high population levels on the cultivated grasses and on grasses bordering the nursery sites, but the traits of pathogenicity and INA vary among the strains. Populations of total fluorescent bacteria from diseased sudangrass were 30 times higher than on the other two grasses. Disease symptoms were not evident on either cereal ryegrass or roadside grass species. Although the proportion of fluorescent pseudomonads on sudangrass that was pathogenic/INA was lower than on the other two grasses, more than 50% of the fluorescent pseudomonads from diseased sudangrass samples were of the *P. syringae* phenotype. In contrast, 83 and 93% of the fluorescent pseudomonads isolated from symptomless cereal ryegrass and roadside grasses, respectively, were of the *P. syringae* phenotype.

The use of sudangrass and cereal ryegrass species as alternate green manure and cover crops within the nursery boundaries appears risky in that they may act as reservoirs of inoculum of *P. syringae*. Likewise, the borders of grass around the nursery may contribute to the spread of *P. syringae* throughout the nursery and surrounding fields, and both should be considered in the disease management programs of nurseries producing woody nursery stock.

These findings suggest that *P. syringae* from these grass species could initiate diseases early in the spring. Researchers like Gross, Moore, and others have shown that populations of *P. syringae* are low to undetectable on many woody plant species during the extremes of winter and in some woody hosts during the hot days of summer. The near absence of *P. syringae* from these woody tissues during the winter followed by a rapid increase in the spring may be due to transfer of *P. syringae* from the adjacent grasses to the trees. Moreover, *P. syringae* may exist inside apple buds as demonstrated by Burr and Katz (2); however, this has been shown for relatively few woody plant species. In our sampling of twigs and dormant buds, we cut and soaked intact buds to obtain interior as well as exterior bacterial colonizers; however, we found no indication that *P. syringae* was colonizing the interiors of these buds.

Although selected strains of the *P. syringae* isolated from the three grass species studied incited pathogenic changes in greenhouse-grown sudangrass as well as in young leaves of peach trees, none of the strains produced disease changes in greenhouse-grown cereal ryegrass. This is consistent with the findings of Lindemann et al (8) that ryegrass is able to be colonized by *P. syringae* but is not susceptible to infections by *P. syringae*. Despite the nonsusceptibility of cereal ryegrass, it still harbors epiphytic populations of pathogenic/INA *P. syringae* that could serve as an inoculum source for adjacent woody plants. Of greatest importance to the nursery industry is the demonstration of cross-infectivity of these strains between grasses and woody hosts. Further work is needed to definitively establish the cross-infectivity of these *P. syringae* from grass species as pathogens of other woody hosts.

Our findings agree with the published findings of Arsenijeric (1) that strains of *P. syringae* from herbaceous hosts may infect woody plants and vice versa. We are now testing the hypothesis that *P. syringae* is involved in a cycle of infection and reinfection between certain grass species cultivated in the nursery and adjacent woody plants.

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