Ecotypes and Pathogenicity of Ice-Nucleation-Active *Pseudomonas syringae* Isolated from Deciduous Fruit Tree Orchards

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ABSTRACT

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Ice-nucleation-active (INA) strains of *Pseudomonas syringae* were isolated as epiphytes from pome and stone fruit orchards in the Pacific Northwest (PNW). Fifty percent of the 82 strains were pathogenic in immature pear and sweet cherry fruit. Pathogenic strains isolated from trees in either pome or stone fruit orchards had a corresponding degree of virulence in the two hosts. Nearly all INA strains, however, induced hypersensitivity in tobacco and produced syringomycin. An INA strain of *P. syringae* pv. *syringae* from pear colonized inoculated apricot trees, attaining 10^8 to 10^9 colony-forming units per gram (fresh weight) of flowers at full bloom, and expressed an in vivo frequency of ice nucleation at 6×10^3 cells per −5 C ice nucleus. These high populations were detected after flower infection which was mediated by damage from several mild frosts (ie, −1.3 to −4.7 C). Bacteriocin and phage typing demonstrated no appreciable differences between pome and stone fruit INA *P. syringae*. All INA strains produced at least one bacteriocin and were subdivided into 11 producer groups; groups 6C, 8B, 8F, and 13 contained 88% of the INA strains. Nine phage sensitivity groups were identified, and 73% of the strains were classified in either phage groups 1 or 2. Phages (12B, S3, and Ø17), which had been reported to specifically lyse pear strains of *P. syringae* pv. *syringae* were either weakly virulent or avirulent on INA strains isolated from trees in either pome or stone fruit orchards in the PNW. Phage typing differentiated PNW INA strains from most strains from England whereas bacteriocin typing differentiated them from most California strains. Therefore, at least three major ecotypes of INA *P. syringae* were discerned.

Ice-nucleation-active (INA) bacteria are either the only or major source of biogenic ice nuclei on the surfaces of most plant species at relatively high freezing temperatures. They, therefore, are important in limiting supercooling of plant tissues to between −2 to −5 C to effect frost injury (20,22). For plants devoid of intrinsic ice nuclei, frost injury is directly related to the number of bacterial ice nuclei present at a given temperature, which is contingent upon both the nucleation frequency and the number of INA bacteria associated with the plant (23). Two common epiphytic bacteria, *Pseudomonas syringae* and *Erwinia herbicola*, are the primary sources of bacterial ice nuclei on plant surfaces (20,21). The few INA strains of *Pseudomonas fluorescens* biotype G are soil and water residents (24); their occasional retrieval from plant surfaces

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may merely reflect a casual plant association following deposition of windblown soil particles. Thus, it appears that specific types of INA bacteria have become adapted to the plant surfaces which they inhabit. Their ability to generate ice nuclei may provide a selective advantage for their continued survival and prominence.

Deciduous fruit trees are prone to frost injury at temperatures of −2 to −5 C, particularly during bloom stage when developing flowers have little inherent tolerance to ice formation (29). However, it cannot be assumed a priori that INA bacteria are responsible for induction of frost injury to fruit trees. First, their occurrence during the blooming stage is not uniform in all orchards. For example, only about 30% of the orchards in the Yakima Valley of Washington had detectable INA bacteria, while approximately 75% of the orchards in the Hood River Valley of Oregon contained INA bacteria (10). Secondly, not every cell of an INA bacterium forms an active ice nucleus at a given time or temperature (23). Finally, the woody stem tissue of deciduous fruit trees has an intrinsic source of ice nucleation material of apparently nonbacterial origin, that by itself promotes ice formation at temperatures between −2 and −5 C (1,28). Nevertheless, INA bacteria can induce frost injury of detached flowers at these temperatures. Although the contribution of INA bacteria to frost injury in most Pacific Northwest (PNW) orchards appears limited, their presence suggests that ice nucleation contributes to their survival.

Pseudomonas syringae was the only INA bacterium isolated from pome and stone fruit trees in the PNW, and a diversity of ice nucleation frequencies was recorded for these strains at −5 C (10). These ice nucleation frequencies largely appeared to be normally distributed among the strains that were isolated, the median activity being 10^4 cells per −5 C ice nucleus. Surveys of pathogenic *P. syringae* pv. *syringae* isolated from fruit trees also indicate that most are INA (87% in one study [27]) and that fruit tree strains are among the most active ice-nucleating bacteria (14). Several investigators (26,27) have observed that frost injury predisposes pear blossoms to bacterial blossom blast caused by *P. syringae* pv. *syringae*. Although it is unknown whether the majority of INA *P. syringae* are pathogenic, it is known that their populations are highest during the frost-susceptible stages of fruit tree development (10). The possible induction of frost injury by these bacteria would be advantageous to their growth and survival because wounds and cellular leakage would promote infection.

Fruit trees are perennial and, consequently, the associated INA *P. syringae* may be specifically adapted to and stabilized within this habitat. The fact that fruit tree strains of *P. syringae* pv. *syringae* are nonpathogenic to maize, and presumably other grasses, supports this hypothesis (11). Furthermore, bacteriophage tests have been used to distinguish the host of origin for some strains of *P. syringae* pv. *syringae* that are very similar in physiological characteristics (3). By phage typing, Billing (2) reported that phage 12B specifically lysed 80% of the pear strains isolated in England; all strains isolated from lilac were insensitive, and very weak reactions were recorded for 21% of the stone fruit isolates. Crosse and Garrett (5) reported that *P. syringae* pv. *syringae* isolated from pear were distinguishable from all other isolates of this pathogen by greater sensitivity to phage S3. Garrett et al (9) further showed that pear strains of *P. syringae* pv. *syringae* are specifically adapted to pear and are distinguishable from citrus and lilac strains by sensitivities to phages and bacteriocins. Vidaver and Buckner (36) reported that most of the stone fruit strains from California that were typable by bacteriocin production were in group 14, whereas most pome fruit strains were in group 8F. The above results collectively raise questions about the ability of strains of *P. syringae* pv. *syringae* from pome and stone fruit trees to colonize and infect the heterologous host. Host specificity of pome and stone fruit strains of *P. syringae* pv. *syringae* may also influence the spread and establishment of ice nucleators between pome and stone fruit orchards. Although inoculations of sour cherry leaves with a few pear strains resulted in infection (31), no systematic comparison of pathogenicity on both pome and stone fruits has been made for fruit tree isolates of *P. syringae* pv. *syringae*.

In this investigation, INA *P. syringae* isolated as epiphytes from pome and stone fruit trees in the PNW were evaluated for pathogenicity in immature pear and sweet cherry fruit. The relationship of frost injury to colonization and infection of apricot trees by a pear strain of *P. syringae* was also determined along with the expression of ice nucleation activity of the bacterium in nature. The INA fruit tree strains were further differentiated by typing for bacteriocin production and phage sensitivity.

MATERIALS AND METHODS

Bacterial strains and phages. Eighty-two strains of INA *P. syringae* were isolated from pear (*Pyrus communis* L), apple (*Malus domestica* Borkh.), sweet cherry (*Prunus avium* L.), peach (*Prunus persica* L.), and apricot (*Prunus armeniaca* L.). Orchards were located in the Yakima Valley and Wenatchee area of Washington and the Hood River and Rogue River Valleys of Oregon. Strains were isolated from epiphytic bacterial populations of fruit trees from 1980 to 1982; diseased tissue was avoided in sampling. In general, only one INA strain was retained from an orchard on a given date. All strains were purified by subculturing isolated colonies three times. A list of these INA strains together with the source, determinative tests, syringomycin production, ice nucleation frequencies, bacteriocin and phage groupings, and pathogenicity on immature pear and sweet cherry fruit is available upon request.

The strains of *Pseudomonas* (PS281, PS6, PSC1-B, PS14, PG1-

TABLE 1. Characteristics of representative strains of *Pseudomonas syringae* pv. *syringae* and *P. syringae* pv. *morsprunorum* isolated from areas outside the Pacific Northwest

Species and strain	*Geographic source*	Host plant of origin	Ice-nucleation-active[a]	Bacteriocin producer group[b]	Phage sensitivity group	Pathogenicity to fruit of:	
						pear	sweet cherry
P. syringae pv. *syringae*							
B301D	*England*	pear	+	8F	7	+	+
S8	*England*	pear	+	8F	7	+	+
W50	*England*	pear	+	8F	4	+	+
B15+	*California*	almond	+	14	1	+	+
B3A	*California*	peach	+	14	1	+	+
5D4198	*California*	plum	+	14	1	+	+
5D447	*California*	sweet cherry	+	6C	2	+	+
464	*South Dakota*	maize	+	6C	4	+	+
12D4	*Unknown*	soybean	+	6C	4	+	+
P. syringae pv. *morsprunorum*							
C28(A2)	*England*	sweet cherry	−	8F	10	−	+
C46 RF	*England*	sweet cherry	−	8F	10	−	+

[a] Ice-nucleation-active (+) or inactive (−) at both −5 and −9 C.

[b] Scheme of Vidaver and Buckner (36).

T, PS-Col, PS17, HB6, and GN-2) used to detect bacteriocins were received from A. K. Vidaver (University of Nebraska, Lincoln) and were previously described by Vidaver et al (37). Authentic strains of *P. syringae* pv. *syringae* and *P. syringae* pv. *morsprunorum*, listed in Table 1, were previously described (6,8,9,11,36). Strains S8, W50, C28(A2), and C46 RF and phages A9, A26, S3, and Ø17 were provided by C. M. E. Garrett (East Malling Research Station, Maidstone, Kent, England). Phage 12B was received from E. Billing (East Malling Research Station, Maidstone, Kent, England).

Cultivation and preservation of bacteria. Bacteria were routinely cultivated on nutrient broth-yeast extract (NBY) agar medium (35), and strains were preserved at −20 C in a glycerol-mineral salts buffer (10). Lyophilized cultures served as reference stocks.

Propagation and maintenance of phages. The procedure of Crosse and Garrett (5) was used for the propagation of high titer phage stocks, which were then stored at 4 C in 5 ml of NBY broth over 0.5 ml of chloroform. Phage 12B was propagated in strain B301D; A9 in strain C28(A2); A26 in strain C46 RF; S3 in strain S8; and Ø17 in strain W50.

Characterization of INA bacteria. The 82 PNW INA strains were tested for levan production (19), oxidase reaction (32), pectolytic activity on Hildebrand's A, B, and C pectate media (13), arginine dihydrolase (33), and tobacco hypersensitivity (16) (LOPAT [19]). Inocula for the tests were prepared by growing strains at 25 C in NBY broth to late exponential growth phase; cultures were then centrifuged for 15 min at 11,700 *g*, and the pellet was suspended in potassium phosphate buffer (12.5 mM, pH 7.2) to give a final approximate cell concentration of 10^8 colony-forming units (cfu) per milliliter. Leaves of *Nicotiana tabacum* 'White Burley' were used to test hypersensitivity.

Production of syringomcyin on potato-dextrose agar supplemented with 0.4% (w/v) casamino acids (Difco) was bioassayed using *Geotrichum candidum* (11).

Pathogenicity tests. Immature d'Anjou pear fruit (picked approximately 6 wk after set) and Bing cherry fruit (picked 6–8 wk after set), used for inoculations with INA *P. syringae*, were stored at 4 C. Prior to inoculation, they were surface sterilized for 20 min in 0.25% (v/v) sodium hypochlorite solution containing 0.01% (v/v) of mild detergent, rinsed twice in running water, and soaked in deionized water for 20 min before drying at room temperature.

Inocula were prepared for pathogenicity tests as described above except that final inocula concentrations were 10^4 and 10^6 cfu/ml. Cell suspensions were kept on ice prior to inoculation of fruit.

Inoculations were made with a syringe fitted with a 0.51-mm-diameter (25-gauge) needle. Each strain was inoculated into four pear and seven sweet cherry fruits. Each pear was injected five times (~0.1 ml per injection of 10^6 cfu/ml) and each sweet cherry three times (~0.05 ml per injection of 10^4 cfu/ml). Control fruits were injected with sterile potassium phosphate buffer. The fruits were then suspended in disinfested test tube racks and incubated for 3 days at 25 C inside a closed plastic box lined with moist paper towels. They were then observed for internal and external disease symptoms.

Procedures for monitoring epiphytic bacterial populations. *P. syringae* pv. *syringae* strain B301D, an INA strain from pear, was sprayed to run-off onto three mature apricot trees at a cell concentration of 5×10^8 cfu/ml on 17 March 1982. Trees located in the experimental orchard at Prosser, WA, were at the red calyx developmental stage (stage 4) (29). Inoculum had been grown on King's medium B (15) agar plates and suspended in potassium phosphate buffer prior to the final dilution in water. Following application of the INA bacteria to trees, a subsample of inoculum was diluted and plated onto King's medium B agar to verify viability. Three unsprayed trees served as controls.

Populations of total and INA bacteria were determined on the day of inoculation and at weekly intervals thereafter until early May after which trees were sampled once in June and July. Samples consisted of 15 buds, flowers, or leaves collected from each treated tree. Buds were collected 17 and 23 March, flowers or young fruit from 1 April through 5 May, and leaves thereafter. Samples from treated and untreated trees were separately bulked, placed in plastic bags, and chilled during transport to the laboratory. The following day, two samples of 20 buds, flowers, or leaves from each treatment were weighed and vigorously washed for 2 hr on a rotary shaker (250 rpm) with sterile potassium phosphate buffer containing 0.1% (w/v) peptone (Difco) (20). Between 10 and 15 ml of wash solution was added for each gram (fresh weight) of tissue. The decanted supernatant was centrifuged at 11,700 *g* for 15 min and the pellet was resuspended in 1 ml of sterile potassium phosphate buffer. Petri plates of King's medium B agar supplemented with cycloheximide (40 μg/ml) were inoculated with appropriate dilutions and then incubated for 2–3 days at room temperature (22–24 C). INA bacteria were detected by testing representative colonies in 0.5 ml of sterile potassium phosphate buffer for ice formation following equilibration to −5 and/or −9 C in a refrigerated water bath (Lauda K-4/RD; Brinkmann, Westbury, NY 11590). The replica-plating procedure of Lindow et al (20) was used when INA bacteria were present in low populations and plates exhibited confluent bacterial growth.

Determination of numbers of bacterial ice nuclei. Numbers of bacterial ice nuclei in washed samples collected from the apricot orchard were calculated as described by Vali (34) and modified by Lindow et al (22). Diluted or concentrated samples were spotted (40 10-μl drops) on paraffin-coated aluminum foil boats and floated on a refrigerated bath (−5 C) containing an equal mixture of ethanol and water (20). The number of drops that froze within 2 min was recorded for those dilutions or concentrations that yielded between five and 35 frozen drops. The number of ice nuclei per gram (fresh weight) was then calculated (22).

Bacteriocin production and detection. The induction, production, and storage of bacteriocins was according to the method of Vidaver and Buckner (36). Strains were induced to produce bacteriocin(s) on two separate occasions, and preparations were not stored longer than 1 mo prior to typing. All preparations were spot-tested in 10-μl aliquants on duplicate NBY plates by using the same procedures and indicator strains as previously described (36). Bacteriocin reactions were noted after 16–24 hr of incubation at ambient temperature (22–24 C). Strains of *P. syringae* known to produce characteristic bacteriocins were used to monitor the reproducibility of results on the indicator strains.

Phage typing. Strains were grown to exponential growth phase in 5 ml of NBY broth and adjusted to approximately 10^8 cfu/ml. NBY agar plates were then overlaid with 2.5 ml of NBY soft agar (0.7% [w/v] agar) containing 0.1 ml of bacterial suspension. Duplicate plates were spot-tested (10 μl) with phages adjusted to routine test dilution (RTD), the highest dilution that gave confluent lysis of the propagating strain (5). The phage titers at RTD were 10^3 plaque-forming units (PFU) per milliliter for 12B and S3; 10^4 for A9; 10^5 for Ø17; and 10^6 for A26. Plates were incubated at ambient temperature (22–24 C) for 16–24 hr and phage sensitivities were recorded. The original propagating strains for phages were used to monitor the activity of phage preparations.

RESULTS

Phenotypic characteristics of fruit tree INA bacteria. All 82 INA bacteria isolated from fruit tree orchards in the PNW were identified as *P. syringae*. Most strains conformed to *P. syringae* according to the determinative LOPAT tests. The exceptions were four strains that were pectolytic, 18 strains that did not produce levan, and three strains that did not induce a hypersensitive reaction in tobacco. Syringomycin was produced on PDA plates by 82% of the strains as indicated by the characteristic zones of inhibition to *G. candidum* (Table 2). The reference pathogenic strains of *P. syringae* pv. *syringae*, listed in Table 1, conformed to *P. syringae* in all LOPAT tests and all produced syringomycin.

Pathogenicity of fruit tree INA bacteria. Pathogenic strains of INA bacteria injected into immature pear and sweet cherry fruit caused brown and sunken lesions that spread from the areas of injection (Fig. 1). Lesions produced by highly virulent strains engulfed nearly the whole fruit following incubation for 3 days at 25 C. Injections with potassium phosphate buffer alone or

nonpathogenic INA strains caused no internal or external discoloration of tissue. Of the 82 strains tested for pathogenicity on pear and sweet cherry fruits, 41 were pathogenic. Furthermore, these 41 strains were pathogenic in both fruits, with virulence of individual strains generally correlated between both hosts (Fig. 1).

Pathogenicity of INA strains isolated from pome and stone fruit trees was associated with the geographic location of the orchards (Table 2). Roughly 25% of the INA strains isolated from pome fruit trees in the Yakima Valley were pathogenic to pear and sweet cherry fruits versus 75% of those from the Hood River Valley. In contrast, less than 10% of the INA strains isolated from stone fruit trees in the Yakima Valley were pathogenic.

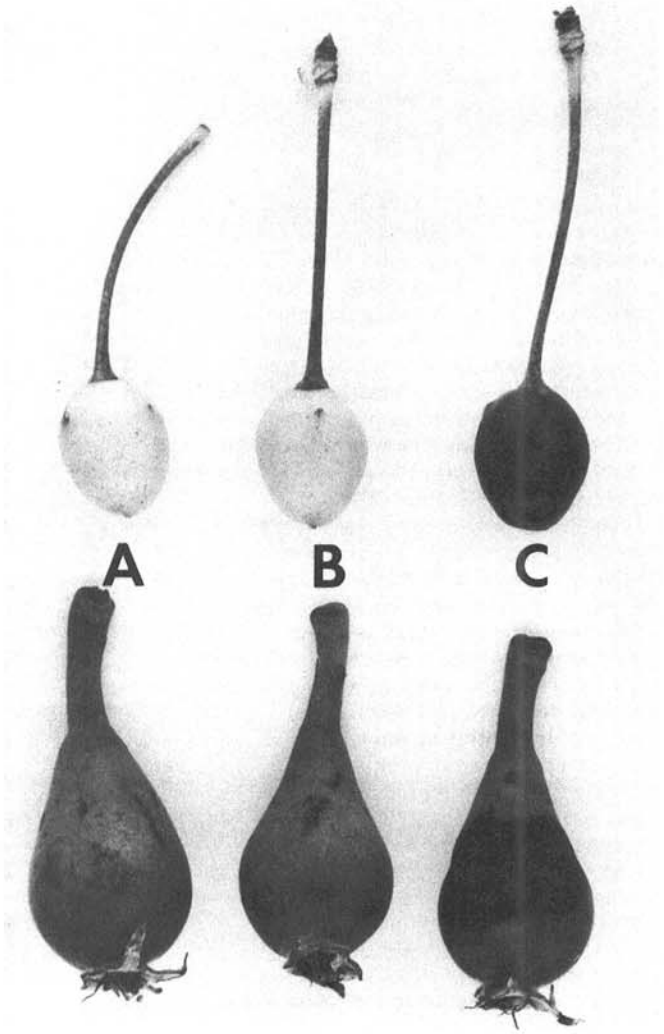

Fig. 1. Pathogenicity of INA strains of *Pseudomonas syringae* isolated as epiphytes from fruit trees in the PNW. Immature Bing cherry and d'Anjou pear fruit were inoculated with potassium phosphate buffer (row A), nonpathogenic pear strain W4N10 (row B), and pathogenic pear strain W4N52 (row C). Fruit were incubated for 3 days at 25 C.

Pathogenic strains of *P. syringae* pv. *syringae* characteristically are nonpectolytic bacteria that cause a hypersensitive reaction in tobacco and produce levan and the phytotoxin, syringomycin (11,19). All but three of the 82 INA strains incited a hypersensitive reaction in tobacco, including all 45 INA strains isolated in the Hood River Valley (Table 2). Furthermore, the three INA strains of *P. syringae* that did not induce hypersensitivity in tobacco were nonpathogenic to pear and sweet cherry. Only one of the four pectolytic INA strains and none of the 18 levan-negative INA strains were pathogenic. Syringomycin was produced on potato-dextrose agar by all except four of the strains pathogenic to immature fruit. However, the quantity of syringomycin produced by the INA strains was not related to virulence. Most nonpathogenic strains, including two (W4N45 and W4N105) that did not cause a hypersensitive reaction in tobacco, also appeared to produce syringomycin (Table 2).

Pear strains (B301D, S8, and W50) from England and four stone fruit strains (+B15, B3A, 5D4198, and 5D447) from California were highly virulent on both pear and sweet cherry fruit (Table 1). In addition, INA strains 464 and 12D4, which cause holcus spot of maize (11), were also pathogenic in these fruits.

INA bacterial colonization of apricot trees. The INA *P. syringae* pear strain, B301D, rapidly colonized buds and flowers of apricot following application in mid-March (Fig. 2). At full bloom (around 1 April), a peak population of 10^8 to 10^9 cfu/g (fresh weight) of blossoms was reached. During this period and thereafter, 11 natural frosts occurred (30 March; 1, 7, 8, 9, 16, 18, 19, 20, and 29 April; and 4 May) with minimum temperatures ranging between −1.3 and −4.7 C. Following a −4.7 C frost on 1 April, substantial petal injury occurred and about 50% of the flowers were killed. Subsequently, the INA bacterial populations of inoculated trees increased 10-fold, largely due to the infection of the injured flowers, particularly the petals. Infections frequently spread from the flowers to stem tissue, resulting in the formation of small cankers and sometimes the exudation of sap. The INA bacteria comprised essentially the total bacterial population until May when warmer and drier conditions prevailed. By July, no INA bacteria were detected, and disease development had ceased.

INA bacteria were not detected on buds and immature flowers of uninoculated trees. Following the frosts at full bloom, the INA bacterium, B301D, spread to uninoculated trees; populations by mid-April generally exceeded 10^7 cfu/g (fresh weight). These trees sustained losses in flower viability similar to those of inoculated trees (*unpublished*).

Bacterial ice nuclei active at −5 C were detected between 10^2 to 10^4/g (fresh weight) throughout most of the period when natural

TABLE 2. Pathogenic properties of ice-nucleation-active bacteria isolated as epiphytes from pome and stone fruit trees in the Yakima and Hood River Valleys[a]

Origin of strains: Location and type of fruit tree	Pathogenicity in fruit of: Pear		Pathogenicity in fruit of: Sweet cherry		Syringomycin production		Hypersensitive reaction	
	Positive[b]	%	Positive[b]	%	Positive[b]	%	Positive[b]	%
Yakima Valley[c]								
Pome fruit	7/26	27	7/26	27	23/26	88	25/26	96
Stone fruit	1/11	9	1/11	9	9/11	82	9/11	82
Hood River Valley[d]								
Pome fruit	33/44	75	33/44	75	34/44	77	44/44	100
Stone fruit	0/1	0	0/1	0	1/1	100	1/1	100

[a] Pome fruit tree strains were isolated from either pear or apple orchards; stone fruit tree strains were isolated from either sweet cherry, apricot, or peach orchards.
[b] Ratio of the number of positive strains to the total number of strains tested.
[c] Includes three strains isolated from pome fruit orchards in the Wenatchee area of Washington.
[d] Includes four strains isolated from pome fruit orchards in the Rogue River Valley of Oregon.

frosts occurred (Fig. 2). Once the populations of INA bacteria dropped to 10^4 cfu/g (fresh weight) or lower, −5 C ice nuclei were not detected. *Pseudomonas syringae* strain B301D grown in vitro will form one −5 C ice nucleus per 3×10^2 cells (12). On 17 March, following application of the INA strain of *P. syringae* to levels of approximately 10^5 cfu/g (fresh weight), there was one −5 C ice nucleus recovered per 10^4 cells. Although the total B301D population in the orchard increased with bud development, the frequency of bacterial ice nucleation remained relatively stable. On April 1, an average flower carried 2.2×10^6 viable INA bacterial cells. Since one −5 C ice nucleus was measured per 6×10^3 cells, 367 −5 C ice nuclei were associated with each flower.

Bacteriocin and phage typing of fruit tree INA bacteria. Each of the 82 fruit tree INA strains produced at least one bacteriocin and they were subdivided into 11 bacteriocin producer groups (Table 3). Groups 6C, 8B, 8F, and 13 contained 88% of the INA strains, while only one to three strains were classified in any remaining groups. Bacteriocin groups 17 and 18 were erected to accommodate one strain each. A few strains appeared to produce two different bacteriocins; these were distinguished by the formation of a large, diffusable zone of inhibition in one case and a zone restricted to the area of application in the second case. Temperate phages were induced from several strains, but their plaques were distinguishable from bacteriocin-induced lysis.

Strains of *P. syringae*, previously classified in representative bacteriocin producer groups, gave the expected results with the exception of strain B15+. Strain B15+ belonged in group 14 rather than in group 4, which was reported earlier (36).

The INA bacteria isolated from PNW orchards formed nine phage sensitivity groups with 73% of the strains classified in either phage groups 1 or 2 (Table 4). All but three strains were sensitive to at least one of the five phages with 90% of the strains sensitive to phage 12B. However, only one strain was sensitive to phage A9 and three to S3. In general, the phages formed turbid, lytic spots that in some instances were barely visible. In contrast, intensely clear zones of lysis were formed on the three pear INA strains from England (Table 1); only strains B301D and S8 were sensitive to all five phages. The two strains of *P. syringae* pv. *morsprunorum*

TABLE 3. Bacteriocin groups of ice-nucleation-active *Pseudomonas syringae* isolated from fruit trees in the Pacific Northwest[a]

Indicator strain	Bacteriocin producer group[b]										
	1	6C	6D	6E	8B	8F	12	13	14	17	18
PS281	+	−	+	−	+	+	−	−	+	+	+
PS6	−	−	−	−	+	+	−	−	+	−	+
PSC1-B	−	−	−	−	+	+	−	−	+	−	+
PS14	−	+	+	−	−	−	+	+	+	−	+
PG1-T	−	+	+	+	−	−	−	+	−	−	−
PS-Col	−	−	−	−	−	−	−	−	−	−	−
PS17	−	−	−	−	+	+	−	−	+	−	+
HB6	−	−	−	−	+	−	−	−	−	−	−
GN-2	−	−	−	−	−	−	+	+	−	+	+
No. of strains[c]	3	15	1	1	27	16	1	14	2	1	1

[a] All strains were induced on at least two different occasions and the bacteriocin group identified using the established *P. syringae* indicator strains and typing scheme of Vidaver and Buckner (36).
[b] Clear or turbid zone = +; resistant reaction = −.
[c] A total of 82 strains from fruit tree orchards in the Pacific Northwest.

TABLE 4. Phage typing patterns of ice-nucleation-active *Pseudomonas syringae* isolated from fruit trees in the Pacific Northwest

Phage	Phage sensitivity group[a]										
	1	2	3	4	5	6	7	8	9	10	11
12B	+	+	+	+	+	+	+	+	−	−	−
A9	−	−	−	−	−	−	+	+	−	+	−
A26	+	+	−	+	−	−	+	+	+	+	−
S3	−	−	−	+	−	+	+	−	−	−	−
Ø17	−	+	−	+	+	+	+	+	−	−	−
No. of strains[b]	33	27	8	2	2	1	0	1	5	0	3

[a] Sensitive = +; resistant = −. Phages were spotted at routine test dilution.
[b] A total of 82 strains from fruit tree orchards in the Pacific Northwest.

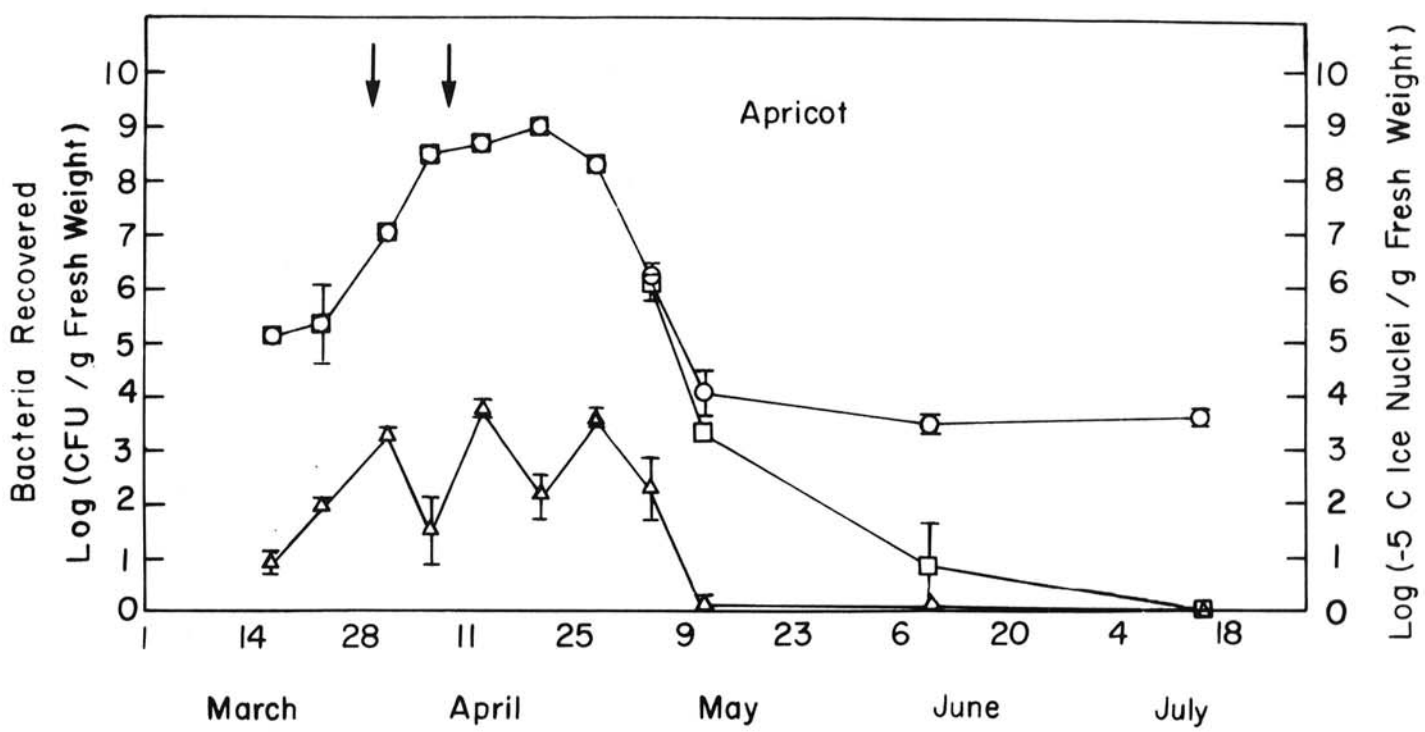

Fig. 2. Colonization of apricot trees by *Pseudomonas syringae* pv. *syringae* strain B301D, an INA pear isolate. Trees in an orchard located at Prosser, WA, were spray inoculated on 17 March with 5×10^8 cfu/ml. Total bacteria per gram (o——o); INA bacteria per gram (□——□) and −5 C ice nuclei per gram (△——△) were measured on a fresh weight basis from 17 March to 15 July 1982. Arrows indicate the time period for bloom. Vertical bars show standard error.

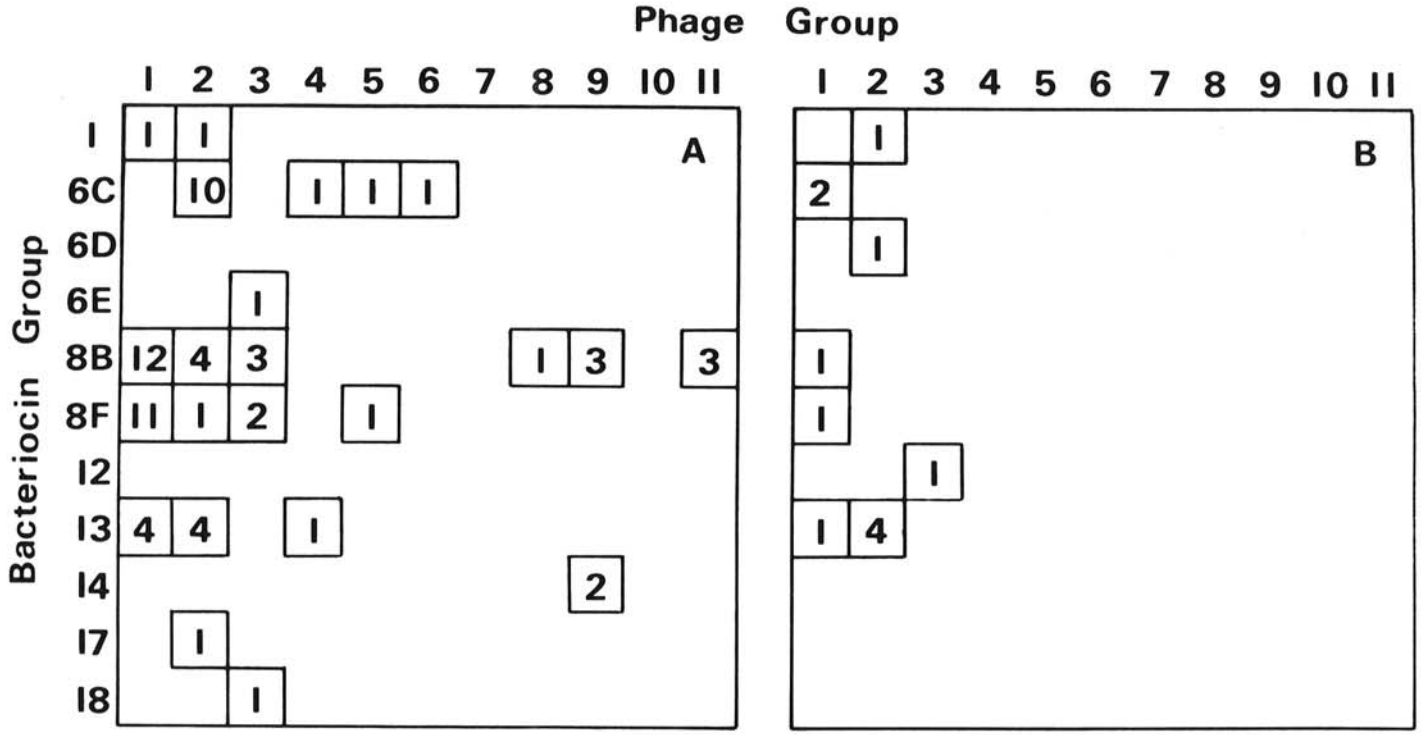

Fig. 3. Diversity of 70 pome (Fig. 3A) and 12 stone (Fig. 3B) fruit strains of INA *Pseudomonas syringae* as related to bacteriocin production group versus phage sensitivity group. The total number of strains that exhibit a particular combination of bacteriocin and phage groups is identified within the square. All strains were isolated from orchards in the PNW.

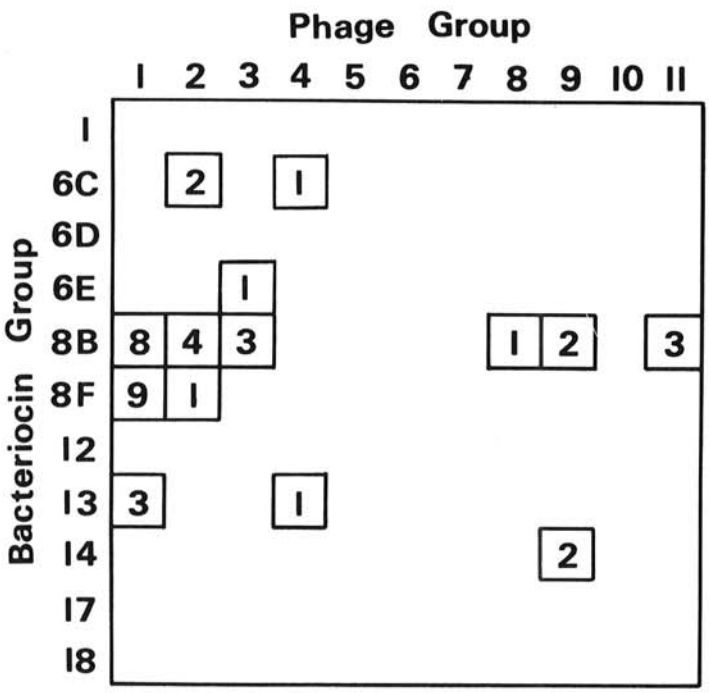

Fig. 4. Diversity of 41 INA strains of *Pseudomonas syringae* pathogenic in both pear and sweet cherry fruit as related to bacteriocin production group versus phage sensitivity group. The total number of strains that exhibit a particular combination of bacteriocin and phage groups is identified within the square. All strains were isolated from orchards in the PNW.

(C28(A2) and C46 RF) were the only members of group 10 in this study, and they exhibited sensitivity to phage A9, a phage largely specific for strains of *P. syringae* pv. *morsprunorum* from England (8). The fruit tree strains from California had a group 1 or 2 phage sensitivity pattern characteristic of most PNW INA strains from fruit trees.

The diversity of the pome fruit strains was compared to that of stone fruit strains by plotting the bacteriocin groups versus the phage groups (Fig. 3). The pome fruit strains exhibited great diversity since 23 different combinations of bacteriocin and phage groups were observed (Fig. 3A). The most common combination was between bacteriocin group 8B and phage group 1. Eight pome fruit strains were insensitive to phage 12B (groups 9 and 11) and only three strains were sensitive to phage S3 (groups 4 and 6); both phages have been reported to be highly specific for pear isolates of *P. syringae* (2,5). Although the stone fruit strains were limited to the first three phage groups, the strains were distributed in seven bacteriocin groups (Fig. 3B). Considerable overlap in bacteriocin and phage groups occurred for the pome and stone fruit INA strains. Strains isolated from the Hood River and Yakima Valleys could not be distinguished from one another by bacteriocin and/or phage typing.

Bacteriocin producer group versus phage sensitivity group of the 41 INA strains that were pathogenic in both pear and sweet cherry fruits was plotted (Fig. 4). With the exception of the two strains in bacteriocin group 14 and one in group 6E, these strains were restricted to the four major bacteriocin producer groups (ie, 6C, 8B, 8F, and 13). Moreover, 51% of the strains pathogenic to both pome and stone fruits were in bacteriocin group 8B; this includes at least three-fourths of the strains placed in this bacteriocin group. Seven phage groups were represented by these pathogenic INA strains.

DISCUSSION

Although INA strains of *P. syringe* in PNW orchards are diverse, most strains are distributed in only a few phage or bacteriocin groups, and the strains appear to be adapted to fruit trees. Deposition of INA *P. syringae* from rainfall (24) or spread from natural vegetation is unlikely to be an important source of the bacterium. *P. syringae* survives as an epiphyte in association with its host even during dormancy (4,7,17). Increasing populations of INA bacteria coincide seasonally with fruit tree development (10), and population levels probably reflect nutritional and environmental aspects of this habitat.

Because 50% of INA isolates of *P. syringae* were pathogenic to

fruit trees, a high degree of host specificity seemed to occur. Furthermore, frost injury, INA bacterial growth, and infection were observed to be interrelated in heavily colonized apricot trees. Thus, INA *P. syringae* can incite frost injury in close proximity to plant cells which in turn provides avenues for ingress of the pathogen to susceptible host tissues (26,27,38,39). However, host specificity of INA *P. syringae* for pome fruit trees versus stone fruit trees was not observed, because all pathogenic strains were pathogenic to both hosts and had corresponding degrees of virulence in these hosts. This was further borne out in the field by blossom infection and canker formation by a pear strain in apricot trees; similar infections also occurred in sweet cherry and peach orchards following spray applications of this same strain (*unpublished*). Seemuller and Arnold (31) also showed that pathogenic pear strains of *P. syringae* pv. *syringae* could infect sour cherry leaves. These comparative studies of pome and stone fruit strains isolated in the PNW or elsewhere show them to be essentially indistinguishable in pathogenic potential. Thus, either pome or stone fruit orchards could qualitatively serve as inoculum sources of pathogenic INA *P. syringae* for other orchards in the vicinity.

Even though 50% of the INA *P. syringae* were pathogenic to fruit trees, tobacco hypersensitivity reactions indicated that virtually all of these strains were potential plant pathogens. The INA *P. syringae* that were nonpathogenic to fruit trees could be pathogens of other plant hosts but still effectively utilize the essential nutrients and environment provided by fruit trees. Furthermore, grasses, tobacco, and, apparently, many other plants are not normally infected by fruit tree strains of *P. syringae* pv. *syringae* following inoculation (11,30). Nevertheless, the host range and specificity of strains of *P. syringae* pv. *syringae* isolated from infected plants remain unresolved. Saad and Hagedorn (30) observed bean isolates of *P. syringae* pv. *syringae* to be highly virulent in their host of origin whereas several fruit tree strains were not pathogenic to bean plants. Conversely, Seemuller and Arnold (31) found many fruit tree strains to be infectious in bean. Pome and stone fruit strains of *P. syringae* pv. *syringae* were reported (11) to be nonpathogenic to maize, although a few holcus spot strains in this study were pathogenic to immature pear and sweet cherry fruits (Table 1). Latorre and Jones (18) correspondingly found that *P. syringae* pv. *syringae* isolated from weeds and plant refuse in cherry orchards were pathogenic to sweet cherry fruit. Thus, *P. syringae* pv. *syringae* appears to be an assemblage of groups of strains that are characterized by a spectrum of host specificity. Several reports (2,3,5,9,25,36) suggest that the strains best suited for long term survival in association with a host are certain specific phage, bacteriocin, or serological types of *P. syringae* pv. *syringae*. Whether these strains are also highly specialized as to host preference remains to be determined.

Bacteriocin production has been a useful tool in differentiating strains of *P. syringae* pv. *syringae*. Vidaver and Buckner (36) found that nearly half of the typable strains isolated from various *Prunus* species in California were contained in bacteriocin producer group 14. In comparison, only two of 82 total PNW INA strains were of group 14. Most of these fruit tree strains were typed in the major bacteriocin producer group 8 which is in accordance with an earlier study of different strains (37). The INA *P. syringae* from fruit trees exhibited relatively specific bacteriocin patterns with just four groups (ie, 6C, 8B, 8F, and 13) containing nearly 90% of all the strains. Together with the two strains in group 14, all of the strains pathogenic to both immature pear and sweet cherry fruits were placed in these groups. However, the two PNW strains in group 14 differed from the California group 14 strains in that they produced diffusable bacteriocins inhibitory to indicator strain PG1-T. The predominance of typical bacteriocin group 14 strains in California orchards and the apparent absence of similar strains in the PNW, therefore, suggests that two different ecotypes of INA *P. syringae* exist in these two different geographic locations. In addition, bacteriocin groups 2 through 5, which are characteristic for strains of *P. syringae* pv. *phaseolicola* and *P. syringae* pv. *glycinea* (37), were notably absent in the fruit tree INA bacteria. Thus, the INA bacteria from PNW orchards are relatively homogeneous in regard to bacteriocin production and fall principally into previously characterized groups of *P. syringae* pv. *syringae*.

Phage sensitivity patterns were used to distinguish PNW orchard strains of INA *P. syringae* from the pear strains that predominate in England. Four phages had been reported (2,5,8,9) to be highly specific for fruit tree strains of *P. syringae* pv. *syringae* or *P. syringae* pv. *morsprunorum* that predominantly occur in England. The phage 12B was highly specific for pear strains in England (2), but most of the INA bacteria isolated either from pome or stone fruit orchards in the PNW were weakly sensitive and none were highly sensitive. Differences in lysis appearance enabled us to readily distinguish the English strains from the PNW INA *P. syringae*. The occurrence of geographically distinct strain ecotypes was further supported by the sensitivity patterns to phage S3 at RTD. Only three of the 82 PNW INA strains were sensitive to phage S3 in contrast to the high sensitivity of the English pear strains (5). Phage Ø17, also relatively specific for pear strains of *P. syringae* (9), was reported to be useful in distinguishing them from strains isolated from citrus and lilac. However, less than half of the PNW INA strains were sensitive, and no specificity for pear INA isolates was observed. In contrast, phage A9 was reported (8) to be specific for cherry strains of *P. syringae* pv. *morsprunorum* (although two of the English pear strains of pv. *syringae* were observed to be sensitive to this phage in this study [Table 1]). The insensitivity of PNW INA strains to phage A9, therefore, suggests that *P. syringae* pv. *morsprunorum* was not present in these orchards even though some strains of this pathovar are known to be INA (14,17). Furthermore, over 80% of the PNW INA *P. syringae* produced syringomycin, which is not produced by *P. syringae* pv. *morsprunorum* (11,17,31). Thus, the two English cherry strains of *P. syringae* pv. *morsprunorum* were placed in a unique phage group, 10, relative to the PNW INA strains. The phage, A26, was observed to give turbid lysis to most PNW INA strains which confirms the sensitivity of strains from several hosts of *P. syringae*, including both the *morsprunorum* and *syringae* pathovars (9). Phage typing was a useful tool for identifying strain groups that are ecologically distinct but physiologically similar. While the pathogenicity of the English pear strains B301D, S8, and W50 on both pear and sweet cherry fruit (Table 1) further exemplifies the lack of host specificity of pome and stone fruit strains, it does not diminish the strong evidence for ecotypes of *P. syringae* for fruit trees.

In conclusion, at least half of the INA pseudomonads associated with fruit trees in the PNW are pathogenic and identified as *P. syringae* pv. *syringae*. The potential ability to induce frost injury to flowers may facilitate infection and enhance the multiplication of the INA pseudomonad component of the bacterial microflora on fruit tree surfaces. Because pome and stone fruit INA strains from the PNW could not be distinguished from one another by phage and bacteriocin typing or by pathogenicity and virulence, following inoculation in immature pear and sweet cherry fruit, the two groups of strains are not qualitatively different. In addition, the pear strain B301D colonized both pome and stone fruit trees to high levels, sometimes exceeding 10^8 per gram of blossoms (fresh weight), and caused infection of stone fruit trees following natural frosts. Thus, there appeared to be no barriers to spread of INA bacteria between pome and stone fruit orchards and subsequent colonization of floral and leaf surfaces of fruit trees. Moreover, the INA strains of *P. syringae* pv. *syringae* isolated from fruit trees in the PNW appear to be relatively diverse in several properties: LOPAT reactions, toxigenicity, ice nucleation frequency (10), and pathogenicity. Phage and bacteriocin typing, however, showed the presence of a distinct ecological group of strains relative to the predominant types occurring in England and California. This may reflect the continual cyclical association of these bacteria with fruit trees and the selection of those INA bacteria best adapted to particular habitats. Although the exact role of INA bacteria in frost injury to fruit trees remains to be elucidated, the ability of most phytopathogenic strains of *P. syringae* pv. *syringae* from fruit trees to be INA suggests that ice nucelation activity is an important adaptive feature which promotes their survival in fruit tree orchards.

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