Suppression of Ice Nucleation-Active *Pseudomonas syringae* by Antagonistic Bacteria in Fruit Tree Orchards and Evaluations of Frost Control

Y. S. Cody, D. C. Gross, E. L. Proebsting, Jr., and R. A. Spotts

First and second authors, Department of Plant Pathology, Washington State University, Pullman 99164-6430; third author, Department of Horticulture, Irrigated Agricultural Research and Extension Center, Washington State University, Prosser 99350; and fourth author, Department of Botany and Plant Pathology, Mid-Columbia Agricultural Research and Extension Center, Oregon State University, Hood River 97031.

Scientific Paper 7466, College of Agriculture and Home Economics, Research Center, Washington State University, Pullman. This report is based, in part, on research conducted and supported as a part of SAES Western Regional Research Project W-130 and by grants from the Washington Tree Fruit Research Commission and Microlife Technics.

We thank S. E. Lindow for providing cultures of antagonistic bacteria and H. MacCrindle-Zimmerman for help in the statistical analysis.

Accepted for publication 9 January 1987 (submitted for electronic processing).

ABSTRACT

Cody, Y. S., Gross, D. C., Proebsting, E. L., Jr., and Spotts, R. A. 1987. Suppression of ice nucleation-active *Pseudomonas syringae* by antagonistic bacteria in fruit tree orchards and evaluations of frost control. Phytopathology 77:1036-1044.

Bacteria composing a major component of fruit tree flower surfaces were isolated and screened for in vitro inhibition of ice nucleation-active (INA) *Pseudomonas syringae* pv. *syringae* and control of frost injury in greenhouse and orchard trials. Nearly all of the inhibitory strains isolated were identified as either *P. fluorescens* or *P. putida*. Greenhouse evaluations on young corn plants showed that some strains of these bacteria significantly suppressed (by up to 98%) the INA *P. s.* pv. *syringae* population resulting in fewer ice nuclei active at −4.5 C. INA$^-$ strain W4N1613$^-$ of *P. s.* pv. *syringae* was comparable to selected strains of *P. fluorescens* and *P. putida* in effectiveness, whereas strain M232A of *Erwinia herbicola* was inferior to these strains. Applications of the most promising strains to pear trees at Hood River, OR, and sweet cherry and apple trees at Prosser, WA, at the tight cluster stage of bud development resulted in antagonist populations ranging from 10^4 to over 10^6 colony-forming units (cfu)/g throughout bloom. Substantial decreases in populations did not occur until late May or June when the weather became warmer and drier. A 10- to 100-fold suppression of INA *P. syringae* was observed for the most suppressive bacterium, which was INA$^-$ strain W4N1613$^-$ of *P. s.* pv. *syringae*. This strain reached populations of over 10^6 cfu/g to compose virtually 100% of the total bacterial flora. Populations for all *P. fluorescens* and *P. putida* strains on pear, sweet cherry, and apple were surprisingly similar; little suppression of INA *P. syringae* was observed because their populations sometimes exceeded that of the antagonists. None of the antagonists, however, controlled frost injury to pear following radiation frosts ranging from −4 to −4.5 C (air temperatures) in 1982 and 1983, respectively. In addition, incidence of fruit russet and Pseudomonas blossom blast were indistinguishable for treated and untreated trees. These results are discussed in relation to the presence of an intrinsic, wood-associated ice nucleus, active at about −2 C, that impedes control of frost injury to fruit trees through suppression of INA bacteria.

Additional key words: biological control, *Erwinia herbicola*.

Frost injury is one of the primary factors limiting deciduous fruit tree production in the Pacific Northwest (PNW). Flowers and fruitlets are susceptible to killing injury during radiation frosts when temperatures are −2 C or colder (31). The presence of ice nuclei and the temperatures at which they are active modulate the extent to which water within fruit tree tissues will supercool. A few species of plant bacteria have been identified as significant sources of biogenic ice nuclei that are active at temperatures above −5 C (18,21,26). Ice nucleation-active (INA) strains of *Pseudomonas syringae* Van Hall and *Erwinia herbicola* (Lönis) Dye are commonly found on plant surfaces, including those of fruit trees (8,20). Previous studies (5,12,21) demonstrated that herbaceous plants, such as corn and bean, lack intrinsic ice nuclei and, in the absence of INA bacteria, are able to supercool to temperatures of −5 C. A log-linear relationship was observed between the size of the INA bacterial population and the degree of frost injury (19,25). Although lower populations of INA bacteria on herbaceous plants as a rule translates into less frost injury at temperatures above −5 C, this same relationship is not observed on fruit trees because they contain an indigenous, nonbacterial ice nucleus within woody stem tissue that is active at temperatures of approximately −2 C and below (1−3,10,11,30). Consequently, lower INA bacterial populations may not result in a correspondingly low level of frost injury because the wood-associated ice nucleus appears to govern frost susceptibility in fruit tree systems.

Field studies by Lindow (15,17) on pear and by Lindow and Connell (24) on almond showed less frost injury following treatments with either bactericides (i. e., streptomycin, oxytetracycline), ice nucleation inhibitors (e. g., phosphoric acid), or bacterial antagonists that outcompeted native INA bacteria for nutrients on foliage and/or inhibited their growth. The use of antagonistic bacteria to control INA bacteria is particularly attractive because they are capable of persisting throughout the growing season after one or two applications. Lindow (15) reported that the fluorescent *Pseudomonas* strain A510, when applied at 20% bloom, rapidly colonized developing pear flowers. Frost injury was subsequently less by about 80% for immature fruitlets after a radiation frost of −3 C. This level of frost control was equal to that observed for various bactericides and ice nucleation inhibitors. Furthermore, several other strains suppressed populations of INA bacteria over 100-fold, significantly improving frost hardiness of pear fruitlets (15,17). In a report (24) of simulated frost analyses of detached almond spurs, those spurs supporting a population of a bacterium (strain T7-3) antagonistic to INA *P. syringae* showed approximately 50% less frost susceptibility than untreated spurs at −3 C. Thus, these results, in contrast to results from studies (1−3,10,11,30) of inherent supercooling of floral buds and associated injury levels, suggest that frost protection can be achieved through control of INA bacteria.

Detailed studies (22) of the population dynamics and suppressiveness of a non-INA strain (M232A originally from corn) of *E. herbicola*, antagonistic to INA *E. herbicola* and *P. syringae* on corn, showed a significant linear correlation between the logarithm of the INA bacterial population and frost injury; injury levels were inversely related to the population of the antagonist on leaves and, correspondingly, the ice nuclei active at −5 C were

reduced. Strain M232A of *E. herbicola* was more effective in suppressing INA *E. herbicola* than *P. syringae* because frost damage was 57–77% and 11–17% less, respectively, for plants sprayed with these two species of INA bacteria. In field studies (23) during a natural frost between −1.5 and −2.5 C, injury was about 50% less on corn leaves with established populations of strain M232A as compared with leaves without the antagonist. Thus, it was concluded that antagonists that suppress the size of native INA bacterial populations on annual crops such as corn will significantly lessen frost injury, frequently at levels approaching those obtained with bactericides and ice nucleation inhibitors.

If antagonists of INA bacteria are to protect PNW fruit trees from frost injury they must be able to suppress *P. syringae*, because this INA bacterium is the only one found associated with fruit tree floral buds during the period when frosts are likely to occur (8). However, the distribution of INA *P. syringae* is variable and profoundly affected by the specific environmental conditions. For example, INA bacteria were isolated from 30% of the orchards surveyed in the Yakima Valley (average annual precipitation below 20 cm) in contrast to almost 75% of the orchards surveyed in the cooler Hood River Valley (average annual precipitation above 75 cm) (8). The population of INA bacteria ranged up to over 10^6 colony-forming units (cfu)/g of flowers, and in some instances composed over 90% of the total bacterial population in Hood River Valley orchards. INA bacterial populations fluctuated in a cyclic manner. Numbers were highest during the cool, wet spring when buds were developing and low to undetectable during the warm, dry summer months. Populations gradually increased in the autumn and subsequently decreased during dormancy.

Most of the INA *P. syringae* strains isolated from PNW orchards were identified as *P. syringae* pv. *syringae* Van Hall based on the ability to produce the phytotoxin, syringomycin, and infect both immature sweet cherry and pear fruit (9). Frost injury has long been associated with Pseudomonas blossom blast of pear, whereby wounding and cellular leakage provide a means for entry and growth of the bacterium as well as a hospitable environment for further invasion of tissues (28,29). Consequently, the greatest benefit of control of INA *P. s.* pv. *syringae* may be lower incidences of blossom blast of pear and bacterial canker of stone fruit trees.

This study summarizes greenhouse and field evaluations of bacteria isolated from fruit trees as antagonists of INA *P. syringae*. In addition to strains native to PNW orchards, strains (i. e., A510 and M232A) previously shown (15,17,22,23) to be effective in biocontrol of INA *P. syringae* and in reducing frost injury were also evaluated. The population dynamics of the major epiphytic bacteria on fruit trees (*P. s.* pv. *syringae, P. putida* (Trevisan) Migula, *P. fluorescens* (Trevisan) Migula, and *E. herbicola*) were followed in two ecologically distinct environments and related to populations of INA *P. syringae*, severity of frost injury, and disease incidence.

MATERIALS AND METHODS

Bacterial strains. *P. s.* pv. *syringae* strain B301D (synonym = strain #55) was isolated in 1959 from diseased Comice pear flowers in England by C. M. E. Garrett (East Malling Research Station, Maidstone, Kent, England). It expresses a frequency of ice nucleation of 3×10^2 cells/−5 C ice nucleus and is pathogenic to both pear and sweet cherry (9). A mutant (strain W4N1613$^-$) of strain B301D was selected for lack of ice nucleation activity at −5 C after exposure to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Cody and Gross, *unpublished*); strain W4N1613$^-$ is fully virulent. *P. fluorescens* (strains W720 and W722), *P. putida* (strains W700, W713, and W718), and a fluorescent pseudomonad (strain W719), which shared characteristics of both of these species, were isolated from deciduous fruit tree orchards in the PNW as described below. Fluorescent pseudomonad strain A510 (15,17) and *E. herbicola* strain M232A (22) were provided by S. E. Lindow (University of California, Berkeley). Spontaneous rifampicin-resistant (50 μg/ml) derivatives of the above strains were used in greenhouse and field evaluations.

Bacterial strains were maintained on nutrient broth-yeast extract (NBY) (34) agar or as frozen (−20 C) suspensions in a glycerol-mineral salts buffer (8). Lyophilized strains served as reference stocks.

Selection of antagonists. Commercial orchards of pear (*Pyrus communis* L.), apple (*Malus domestica* Borkh.), sweet cherry (*Prunus avium* L.), peach (*Prunus persica* L.), and apricot (*Prunus armeniaca* L.) in the Yakima Valley and Wenatchee areas of Washington and the Hood River and Rogue River valleys of Oregon were sampled in 1980 and 1981 (8). Trees from 50 orchards were sampled during the period of peak frost hazard when buds were in the first bloom to late bloom stages. Sampling procedures for flowers and leaves and enumeration of surface bacteria by dilution plating were performed as previously described (8). Bacterial colonies composing at least 10%, according to size and morphology, of the total bacterial population (determined as colony-forming units) were selected, grown on NBY agar, and purified to colony uniformity. All bacterial strains were grown on King's medium B (KB) (13) agar at 20 C for 3 days before screening for inhibition of *P. s.* pv. *syringae* strain B301D. Bacterial suspensions (approximately 5×10^7 cfu/ml), prepared in phosphate buffer (12.5 mM, pH 7.2), were spotted (four strains per plate, 5-μl droplets) onto duplicate KB agar plates and incubated for 48 hr at 25 C. Plates were then lightly oversprayed with INA *P. s.* pv. *syringae* strain B301D (approximately 5×10^7 cfu/ml) and incubated an additional 48 hr at 25 C. The diameters of inhibition zones were recorded.

Greenhouse tests. The procedure of Lindow et al (19) was used to test bacteria that were inhibitory to INA strain B301D in vitro for ability to suppress cell numbers of INA *P. s.* pv. *syringae* on corn plants (N.K. 199; Northrup King, Minneapolis, MN) and lessen the number of −5 C ice nuclei. Corn seeds were planted (five seeds per pot) in sterilized vermiculite, watered daily with Hoagland's nutrient solution, and grown in the greenhouse (25 C day, 20 C night) with supplemental lighting (18 hr). Bacteria were grown for 48 hr at 20 C on KB agar, suspended (5×10^8 cfu/ml) in sterile water and sprayed onto the corn plants (three-leaf stage) until the point of runoff (five pots per strain). Plants were maintained in a mist chamber for 48 hr before being sprayed to the point of runoff with a suspension (5×10^4 cfu/ml) of INA *P. s.* pv. *syringae* strain B301D. Strain B301D was grown on KB agar for 48 hr at 20 C before suspension in sterile water. Plants were maintained in the mist for an additional 48 hr before evaluations of bacterial populations and frost susceptibility. Each treatment was replicated using six pots, five plants per pot.

To evaluate antagonism, corn plants were cut just above the soil line and transported to the laboratory in plastic bags. Bacteria were washed from corn surfaces and the populations of INA strain B301D and the particular challenge strain were determined by dilution plating on KB agar with and without rifampicin (50 μg/ml) (8). The number of −5 C ice nuclei per gram of corn tissue was determined using a modified (21) method of Vali (33). The proportion of corn plants harboring bacterial ice nuclei sufficient to induce ice formation at temperatures at or above −4.5 C was determined by immersing individual plants into test tubes containing 24 ml of deionized water and subjecting them to −4.5 C in a 50% (v/v) water/ethanol refrigerated bath (11). Once equilibrated at −4.5 C, the tubes were incubated for 20 min and the number of frozen tubes recorded. A temperature of −4.5 C was used because approximately 10% of the tubes with untreated leaves were frozen versus approximately 90% of the tubes with leaves treated only with the INA strain B301D (Table 1). Each trial consisted of 40 plants, three trials per treatment. An EX-400 water bath connected to an EN-850 refrigeration unit was used in these analyses; the accuracy of this unit is estimated by the manufacturer (Neslab Instruments, Inc., Portsmouth, NH) to be ± 0.007 C.

Preparation of antagonistic bacteria and *P. s.* pv. *syringae* for field tests. Bacterial antagonists were prepared for field trials by growing them on KB agar for 48 hr at 20 C and then suspending them in sterile phosphate buffer (12.5 mM, pH 7.2). The suspensions were transported immediately to the field and further diluted with well water (one plate per 12 L of water) to yield approximately 5×10^7 cfu/ml. Cell suspensions of *P. s.* pv. *syringae*

strain B301D were prepared the same way except they were diluted to 1–5 × 10^6 cfu/ml for inoculation of orchards at Prosser, WA.

Field tests. Each year, antagonists were sprayed to runoff on deciduous fruit trees in the tight cluster and first bloom stages of development.

In 1982, antagonists W700, W718, W719, W720, W722, W4N1613$^-$, and M232A were applied to Red and Golden Delicious apple and Bing sweet cherry orchards at the Irrigated Agriculture and Extension Center at Prosser, WA. To supplement the native INA bacterial population at Prosser, *P. s.* pv. *syringae* strain B301D was applied to both orchards at a moderate inoculum concentration (5 × 10^6 cfu/ml). Application dates were 5 and 20 April for the antagonists, and 5 and 13 April for the INA *P. s.* pv. *syringae*. At the Mid-Columbia Experiment Station at Hood River, OR, Anjou pear trees were treated with strains W700, W718, W719, W720, W722, W4N1613$^-$, and M232A. All strains were applied 25 March and 16 April to the orchards at Hood River.

In 1983, *P. putida* strain W718, INA$^-$ *P. s.* pv. *syringae* strain W4N1613$^-$, and *E. herbicola* strain M232A were applied to Red and Golden Delicious apple and Bing sweet cherry orchards at Prosser and to a commercial Anjou pear orchard near Hood River. Strain B301D was spray inoculated (1–2 × 10^6 cfu/ml) as in 1982 at Prosser to supplement the native INA bacterial population. Application dates for antagonists were 25 March and 6 April on sweet cherry and 6 and 21 April on apple at Prosser. INA bacteria were applied 30 March on sweet cherry and 12 April on apple at Prosser. At Hood River, all strains were applied on 17 March and 5 April.

In 1984, *P. putida* strain W718, *P. s.* pv. *syringae* strain W4N1613$^-$, and *Pseudomonas* sp. strain A510 were applied to trees in a commercial Anjou pear orchard near Hood River. Procedures for the preparation and application of bacteria were as in 1983. Application dates were 5 and 19 April 1984.

Within each orchard, trees sprayed with bacteria were compared with unsprayed trees and trees sprayed with a tank mix of streptomycin sulfate (120 ppm; Agristrep 17, Pfizer Chemical Co.) and oxytetracycline (80 ppm; Mycoshield, Pfizer Chemical Co.) approximately every 2 wk from tight cluster to young fruit stages for a total of three applications. All treatments at Prosser and Hood River were applied with a conventional orchard sprayer. Buffer trees were positioned between treated trees whenever possible to minimize contamination with bacteria by drift. All orchards were maintained under normal commercial cultural practices.

Treatments in the Delicious apple and Bing sweet cherry orchards at Prosser were established in a randomized complete block design consisting of two (apple) or three (sweet cherry) replications per treatment with four (apple) or two (sweet cherry) trees per replication. The experiments in the two Anjou pear orchards at Hood River were similarly designed, each having three replications per treatment and two trees per replication. Twenty buds (or flowers or fruitlets, depending on the stage of development) were randomly selected from each replication at 2-wk intervals throughout the frost season, beginning with tight cluster and ending with the young fruit stages. Buds, flowers, or fruitlets were collected, transported on ice to the laboratory, and processed (11) within 24 hr. An additional 40 buds, flowers, or fruitlets were collected from selected treatments for use in determination of the mean ice nucleation temperature (MNT) (described below).

Evaluation of antagonists in field tests. All treatments were monitored for effects on INA bacterial populations and on the number of bacterial ice nuclei at −5 C. In addition, frost injury was determined and blossom blast was monitored on pear trees at Hood River. Populations of INA bacteria, antagonistic bacteria, and total bacteria were determined for each replication in all treatments as described earlier (8). Bacterial numbers were related to fresh weights of floral and fruitlet tissues; the minimum detection levels were 10 cfu/g. The number of −5 C ice nuclei per gram (fresh weight) of tissue was determined for each replication as described above. A MNT was determined by placing individual flower clusters or individual fruit into test tubes containing 24 ml of deionized water (11); 40 tubes were prepared for each treatment. Test tubes were placed in a refrigerated water bath, and the temperature was successively lowered from −1.5 C by 1-degree increments. Tubes were incubated for 20 min at each temperature, and the number of tubes frozen at that temperature was recorded. The MNT was calculated from the average temperature at which the 40 tubes froze. Frost injury within a treatment was determined for the Anjou pears at Hood River using 10 flower clusters per tree (six trees per treatment). All flowers in each cluster were sectioned and evaluated for killing injury by noting discoloration to the ovary and/or pistil. Frost injury to surviving fruit was evaluated approximately 1 mo after a frost as the frequency of external fruit russeting using 25 fruit per tree. The severity of Pseudomonas blossom blast per treatment was noted as the total number of infected clusters per tree (six trees per treatment).

RESULTS

Selection of antagonists. Bacteria were isolated from fruit trees during the period of peak frost hazard when buds were in the first bloom and full bloom stages of development. Over 50 bacterial strains, present as at least 10% of the total indigenous bacterial population, were initially tested for in vitro inhibition of INA *P. s.* pv. *syringae* strain B301D on KB agar. Nearly all of the bacteria inhibitory to INA *P. s.* pv. *syringae* were identified as either *P. fluorescens* or *P. putida*. Approximately 20% of the strains produced inhibition zones on KB agar, a low iron medium that is conducive to the production of pyoverdin siderophores by fluorescent pseudomonads (7,35). Not surprisingly, most of the inhibitory compounds appeared to be bacteriostatic.

TABLE 1. Comparisons of bacterial antagonists for suppression of ice nucleation-active (INA) *Pseudomonas syringae* pv. *syringae* in greenhouse assays on corn

Strain	Antagonist population[a] as % of total bacterial population	INA bacteria/g[b] (no.)	INA bacteria as % of INA bacterial check	Percentage of tubes frozen at −4.5 C[c]	Log no. of −5 C ice nuclei/g
Check	NA[d]	NA	NA	7.5 (11.9)*	0.1*
INA bacterium[e]	NA	1.5 × 10^6 (5.95)	100.0	91.7 (76.5)	2.3
W4N1613$^-$[e,f]	98.2	1.9 × 10^5 (5.07)*	13.2	65.8 (55.3)	1.4*
W718[e,f]	96.7	4.1 × 10^4 (4.32)*	2.3	65.8 (55.1)	1.1*
W713[e,f]	93.1	8.7 × 10^4 (4.73)*	6.0	74.2 (61.2)	0.7*
M232A[e,f]	99.9	5.8 × 10^6 (5.52)	37.2	84.2 (67.0)	0.6*
LSD 0.05[g]	···	··· (0.74)	···	··· (23.8)	0.9

[a] Populations determined using six replications per treatment, five plants per pot, 2 days after challenging with the ice nucleator. Populations were approximately 10^6–10^7 cfu/g.

[b] INA bacterial populations for each replication were converted to log values for statistical analysis; the means of the logs are shown in parentheses.

[c] Average of three trials. Original values were transformed to arcsin prior to statistical analysis; arcsin values are shown in parentheses.

[d] None applied.

[e] INA *P. s.* pv. *syringae* strain B301D applied to just runoff at approximately 5 × 10^4 cfu/ml 2 days after application of antagonists.

[f] Antagonists applied to just runoff at approximately 5 × 10^8 cfu/ml and kept in a mist chamber for 2 days before challenging with INA bacteria.

[g] The protected least significant differences (LSD) from plants treated solely with the INA bacterium are shown at $P = 0.05$; * denotes significant values.

Greenhouse evaluations of bacteria for antagonism towards INA *P. s.* pv. *syringae*. PNW strains of *P. fluorescens* and *P. putida* that were inhibitory to INA *P. s.* pv. *syringae* in vitro were then screened in greenhouse trials (Table 1). These potential antagonists were compared with an INA derivative of *P. s.* pv. *syringae* (W4N1613$^-$) and an *E. herbicola* (M232A) strain. All of the fruit tree bacteria grew approximately 10-fold from an initial population of approximately 5×10^6 cfu/g; this represented over 90% of the total number of bacteria. Initial populations of INA *P. s.* pv. *syringae* were approximately 5×10^3 cfu/g. After 48 hr the INA bacterium grew to approximately 10^6 cfu/g in the absence of applied antagonists (Table 1).

All bacterial antagonists except *E. herbicola* strain M232A significantly ($P = 0.05$) suppressed the log number of INA bacteria on corn leaves as compared with plants treated solely with the INA bacterium. *P. putida* strain W718 was the most effective antagonist in greenhouse trials, resulting in 98% fewer INA *P. s.* pv. *syringae* cells on foliar surfaces (Table 1). The number of ice nuclei active at −4.5 C was also lower on plants treated with the bacterial antagonists. The number of tubes frozen at −4.5 C in treatments with either *P. putida* strain W718 or *P. s.* pv. *syringae* strain W4N1613$^-$ was 26% less than in the INA bacterial check; *E. herbicola* strain M232A was the least effective, resulting in only 7.5% fewer frozen tubes. The number of −5 C ice nuclei per gram (fresh weight) of corn tissue was suppressed by 87–98% as compared with the INA bacterial check, depending on the strain. *E. herbicola* strain M232A was comparable to the pseudomonads, significantly ($P = 0.05$) suppressing the number of −5 C ice nuclei.

Colonization of fruit trees by antagonists at Hood River. Bacterial strains that suppressed the growth of INA *P. syringae* on corn leaf surfaces in greenhouse trials generally maintained high populations on floral bud surfaces in orchards. For example, strains of *P. putida* (W718), *P. s.* pv. *syringae* (INA$^-$ W4N1613$^-$), and *E. herbicola* (M232A) applied to Anjou pear trees at Hood River at the tight cluster stage of bud development colonized new vegetative growth at subsequent stages of development (Fig. 1). Populations did not substantially decrease until late May or June when the weather became warmer and drier.

The population dynamics of *P. putida* strain W718 resembles that of the other *P. putida* and *P. fluorescens* strains that were tested over a 4-yr period on pears at Hood River. This includes strain A510, a fluorescent pseudomonad reported by Lindow (15,17) to inhibit frost injury to pears. In the case of strain W718 (Fig. 1A), the population rarely increased much above 5×10^4 cfu/g and usually composed less than 10% of the total bacterial population at full bloom. Accordingly, there was little or no suppression of natural INA bacterial growth on floral bud surfaces because INA *P. syringae* populations eventually exceeded (approximately 10^5 cfu/g) those of the *P. putida* and *P. fluorescens* strains, as observed for strain W718 (Fig. 2A). A precipitous drop in cell numbers occurred for strain W718 in early May, and populations were undetectable by mid-June (Fig. 1A). In contrast, populations of INA *P. syringae* generally exceeded 10^3 cfu/g during this period.

INA strain W4N1613$^-$ of *P. s.* pv. *syringae* (Fig. 1B) was a more aggressive colonist of pear floral surfaces than any strain of *P. putida* or *P. fluorescens*, as well as strain M232A of *E. herbicola* (Fig. 1C). From an initial population of approximately 10^4 cfu/g, strain W4N1613$^-$ multiplied to over 10^6 cfu/g at full bloom and composed more than 95% of the total bacterial population. Populations of indigenous INA *P. syringae* were only 1–10% that of the check trees (Fig. 2B). This pattern of growth for strain W4N1613$^-$ and suppression of indigenous INA *P. syringae* was observed in Hood River trials in 1982, 1983, and 1984.

The population of strain M232A of *E. herbicola* was approximately 5×10^4 cfu/g from green cluster to postbloom stages of development and declined to undetectable levels by early June (Fig. 1C). No suppression of INA *P. syringae* was observed relative to the check trees during this sample period (Fig. 2C). Similar population curves were observed for strain M232A on Hood River pear trees in 1981, 1982, and 1983.

Colonization of fruit trees by antagonists at Prosser. Biological control experiments at Prosser were complicated by the likelihood that, because of the dry climate, only low numbers of INA bacteria would be present in experimental orchards (8). Consequently, INA strain B301D of *P. s.* pv. *syringae* was applied to experimental trees at $1–5 \times 10^6$ cfu/ml after establishment of the various antagonists on floral bud surfaces for a few days. The population of INA strain B301D increased from $2–5 \times 10^4$ cfu/g to approximately 10^6 cfu/g at full bloom on check trees (Fig. 3E); thereafter, there was a steady decline in its population regardless of the treatment. Because the climate at Prosser is drier and warmer than that at Hood River, INA *P. syringae* populations, as well as the populations of most antagonists, were commonly undetectable by late June (Fig. 3).

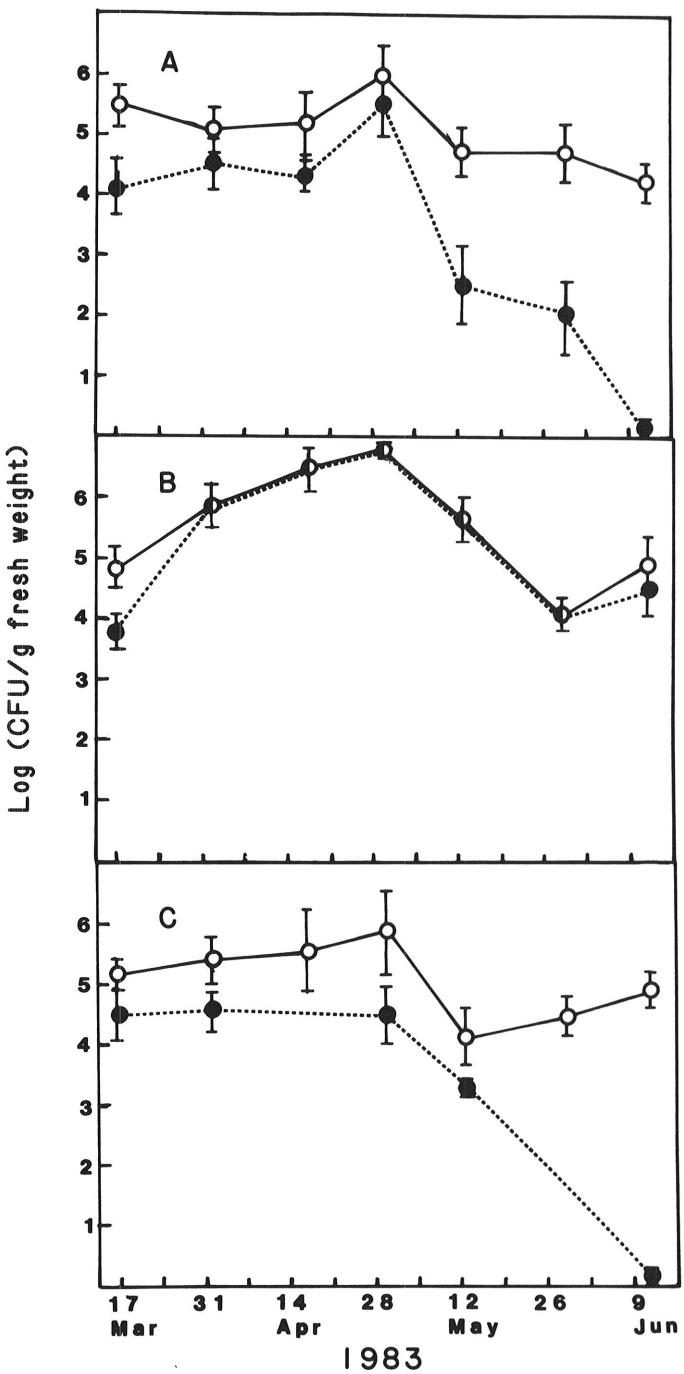

Fig. 1. Populations of total (o), and antagonistic (•) bacteria on flower buds, flowers, and fruitlets of Anjou pear trees at Hood River, OR, in 1983. Antagonistic strains W718 (*Pseudomonas putida*), **A**, W4N1613$^-$ (ice nucleation-active-minus *P. syringae* pv. *syringae*), **B**, and M232A (*Erwinia herbicola*), **C**, were applied at the tight cluster (17 March) and first bloom (2 April) stages of development. Vertical bars show the standard error of the mean of log populations.

All bacterial antagonists established populations exceeding 10^6 cfu/g on sweet cherry and apple trees, and these populations were maintained through bloom. For example, on sweet cherry trees in 1983 strain W718 of *P. putida,* INA$^-$ strain W4N1613$^-$ of *P. s.* pv. *syringae*, and strain M232A of *E. herbicola* (Fig. 3A, B, and C, respectively) all composed at least 50% of the total bacteria through April, by which time young fruitlets had formed. The population of strain M232A of *E. herbicola* increased to a higher level at Prosser on sweet cherry (Fig. 3C) and apple trees than on pear trees at Hood River (Fig. 1C). Despite the presence of high populations of antagonists, INA bacteria were not greatly suppressed because trees treated with any given strain of antagonist developed INA bacterial populations of similar size as check trees (Fig. 3E). Only the bactericide treatment of streptomycin and oxytetracycline lowered populations of INA bacteria to low (10^1–10^2 cfu/g) or undetectable levels (Fig. 3D). These results were observed over a 3-yr period (1981–1983) on sweet cherry and apple trees.

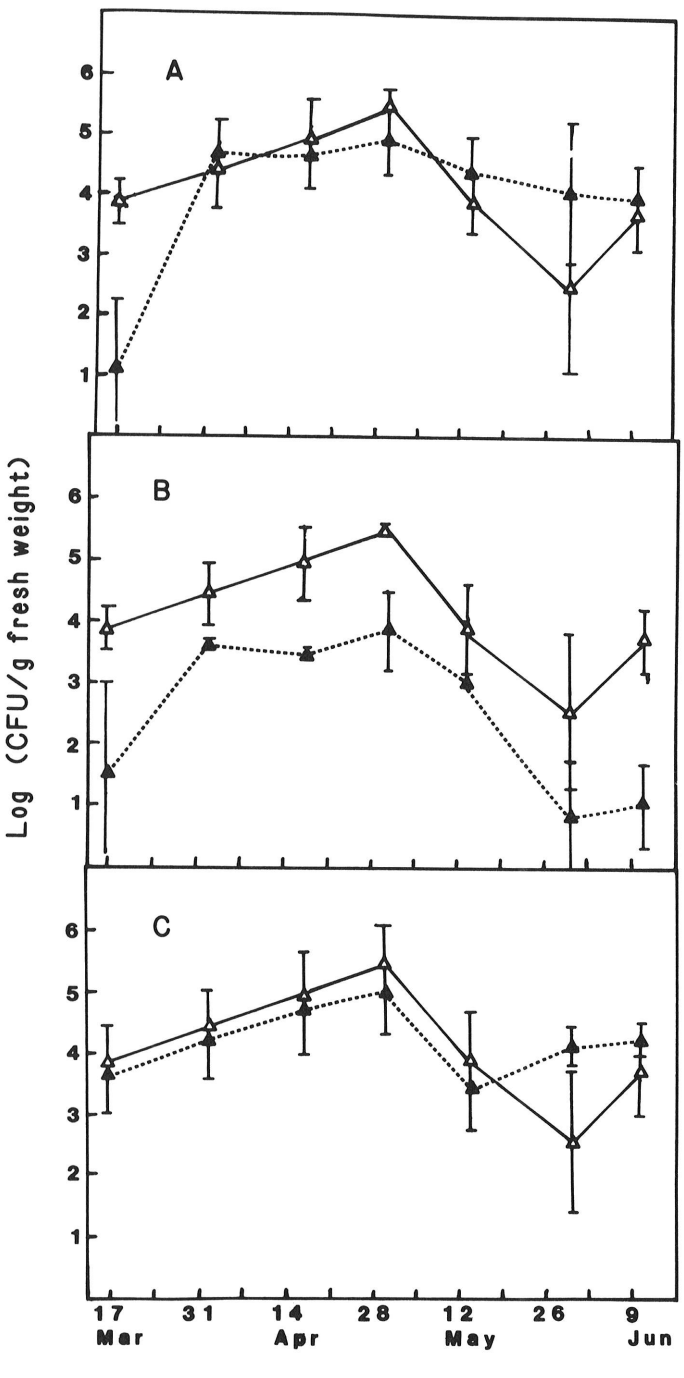

Fig. 2. Populations of ice nucleation-active (INA) bacteria on flower buds, flowers, and fruitlets of Anjou pear trees at Hood River, OR, in 1983 sprayed with antagonists (▲) as compared with those of the unsprayed check (△). Antagonistic strains W718 (*Pseudomonas putida*), **A,** W4N1613$^-$ (INA$^-$ *P. syringae* pv. *syringae*), **B,** and M232A (*Erwinia herbicola*), **C,** were applied at the tight cluster (17 March) and first bloom (2 April) stages of development. Vertical bars show the standard error of the mean of log populations.

Field evaluations of frost injury and disease. Antagonists were evaluated for their effectiveness in reducing frost injury and Pseudomonas blossom blast after a natural frost (air temperature approximately −4 C, first bloom stage) on 19 April 1982 in an Anjou pear orchard at Hood River (Table 2). Despite good colonization of developing flowers by all strains of antagonists (approximately 10^5 cfu/g), frost injury occurred in all treatments. Treatment with strain W718 of *P. putida* resulted in 62% less frost injury as compared with the check; however, this difference was not significant ($P = 0.05$). Subsequent evaluations of external fruit russet corroborated flower injury data. Strain W718 of *P. putida* was best showing 11% less fruit russeting, albeit not significant ($P = 0.05$).

Pseudomonas blossom blast was detected in frosted trees at a low level by late April (Table 2). The trees treated with antibiotic sprays had the lowest incidence of blossom blast (3.3 infected clusters per tree) as compared with the check (6.7 infected clusters per tree); however, the bactericides and the antagonists had no significant effect on the incidence of blossom infection.

Frost injury in an Anjou pear orchard at Hood River in 1983 occurred during prebloom stages of development (Table 3). Although the frost on 9 April at the first white stage of flower development was relatively intense (−4.5 C air temperature), the second frost on 15 April at first bloom was mild (−2 C air temperature). Despite the presence of high populations of strain W718 of *P. putida*, INA$^-$ strain W4N1613$^-$ of *P. s.* pv. *syringae*, and strain M232A of *E. herbicola* at the times of these frosts (Fig. 1), injury levels were about the same as that of the check (Table 3). Even the bactericides, which significantly ($P = 0.05$) suppressed populations of INA bacteria to low levels (approximately 10^2 cfu/g) at this time, did not lessen frost injury. Evaluations of external fruit russeting showed no difference between treatments. However, the 10- to 100-fold suppression of INA bacterial populations by treatment with either bactericides or strain W4N1613$^-$ (Fig. 2B) was manifested as lower MNTs for excised pear flowers and fruitlets (−4.4 and −4.0 C, respectively) than that of the check (−3.5 C) (Table 3). Furthermore, −5 C ice nuclei were not detected in samples collected from treatments of bactericides or strain W4N1613$^-$, whereas the check averaged about two −5 C ice nuclei per gram (fresh weight). In contrast, the MNTs for flowers treated with strains W718 of *P. putida* and M232A of *E. herbicola* were about the same as that of the check throughout the bloom period. Samples from these treatments averaged about 1.5, −5 C ice nuclei per gram (fresh weight).

The incidence of blossom blast was low in 1983, showing no discernible separation of treatments (Table 3).

Frost injury analysis of treatments on sweet cherry and apple trees at Prosser resembled results at Hood River; no significant differences in frost injury levels were observed for trees treated with antagonists. Frosts during this period varied in intensity, air temperatures ranging from −2 to −5 C during bloom and postbloom stages.

DISCUSSION

Lindow (15,17) and Lindow and Connell (24) have reported that certain plant bacteria can greatly suppress the growth of native INA bacterial populations on fruit tree flower surfaces, resulting in significantly less frost injury. The results from our orchard studies also show that bacteria can be easily applied to fruit trees before bloom and that populations ranging from 10^4 to over 10^6 cfu/g (depending on the strain) can be maintained throughout bloom, a developmental stage especially prone to frost injury at temperatures beginning at approximately −1.5 C (1,11).

Suppression of indigenous INA *P. syringae* on pear trees was evident for some antagonistic strains at Hood River where INA populations were 10- to 100-fold less than on untreated trees. Nevertheless, significant INA *P. syringae* populations were established in the presence of these antagonists. For example, INA *P. syringae* populations of over 10^3 cfu/g were achieved in the presence of strain W4N1613$^-$ of *P. s.* pv. *syringae*, which was noted to be the most effective antagonist (Fig. 1B). Because only 10^3–10^4 cells are needed for expression of one ice nucleus at −5 C by most strains of *P. syringae* (8,12,25), at least one bacterial ice nucleus per gram of flower tissue could be expected to occur in the presence of the most suppressive bacterial strain. However, this level of suppression may be sufficient to reduce the MNT of detached flowers. Pear flowers (excised from all stem tissue) colonized by strain W4N1613$^-$, for example, consistently had a lower MNT (−4.0 C) as compared with the check (−3.5 C) (Table 3). Such a low MNT for strain W4N1613$^-$ is particularly noteworthy because it approached the MNT recorded for pear flowers treated with bactericides (−4.4 C).

Although antagonists generally composed a major component of the total bacterial flora resulting in suppression of INA *P. syringae* populations in some instances, appreciably less frost injury was not observed following frosts of approximately −4 C (Tables 2 and 3). This was not surprising because it was discovered that a nonbacterial source of ice nucleation is intrinsically associated with stem tissues which, like INA *P. syringae*, are active at warm freezing temperatures from −1.5 to −5 C (1,2,11,30). Flowers or fruitlets attached to or excised from a 5-cm stem segment were found to exhibit MNTs of approximately −3 and −5 C, respectively, in the absence of detectable INA bacterial populations. Simulated frost analyses showed no differences in killing injury to attached peach and sweet cherry flowers, supporting either low or high INA *P. syringae* populations, after exposure to temperatures ranging between −3 and −4 C (11). Only when the flowers were excised from the stem was killing injury found to be significantly less for those that were essentially free of INA bacteria. This showed that ice formation was initiated in the woody stem, and from there the ice spread to the vegetative floral tissues. Similar studies by Ashworth et al (3) of peach corroborate these findings; they also failed to observe a relationship between

TABLE 2. Field evaluation on Anjou pear of potential antagonists of ice nucleation-active (INA) *Pseudomonas syringae* at Hood River, OR, in 1982

Treatment	INA bacteria/g[a] (no.)	Frost injury[b] (% injured flowers/tree)	External fruit russet[c] (% fruit/tree)	Blossom blast[d] (no. infected clusters/tree)
Check	1.1×10^5 (4.55)	27.7	74.0	6.7
Bactericides[e]	5.1×10^4 (4.20)	20.3	71.3	3.3
W722	3.9×10^4 (3.54)	27.0	81.3	7.7
W720	3.0×10^3 (2.72)	31.8	70.7	4.7
W719	4.2×10^4 (3.15)	25.3	80.0	13.8
W718	2.5×10^4 (2.29)	17.3	63.3	5.3
W700	3.0×10^4 (3.31)	18.8	72.7	5.8
LSD 0.05[f]	··· (2.92)	19.4	16.4	10.5

[a] Populations from samples collected at full bloom on 24 April. INA bacterial populations for each replication were converted to log values for statistical analysis; the means of the logs are shown in parentheses.
[b] Frost injury occurred 19 April in the first bloom stage of development after exposure to a minimum air temperature of −4 C. Evaluations of internal injury to ovaries and/or pistils were made (22 April) on all flowers contained within a cluster, 10 clusters per tree.
[c] Evaluations of 25 fruit per tree on 20 May.
[d] Counts of blossom blast were completed 20 May.
[e] Streptomycin (120 ppm) and oxytetracycline (80 ppm).
[f] The protected least significant difference (LSD) between the means for the check and bacterial or bactericide treatments are shown at $P = 0.05$.

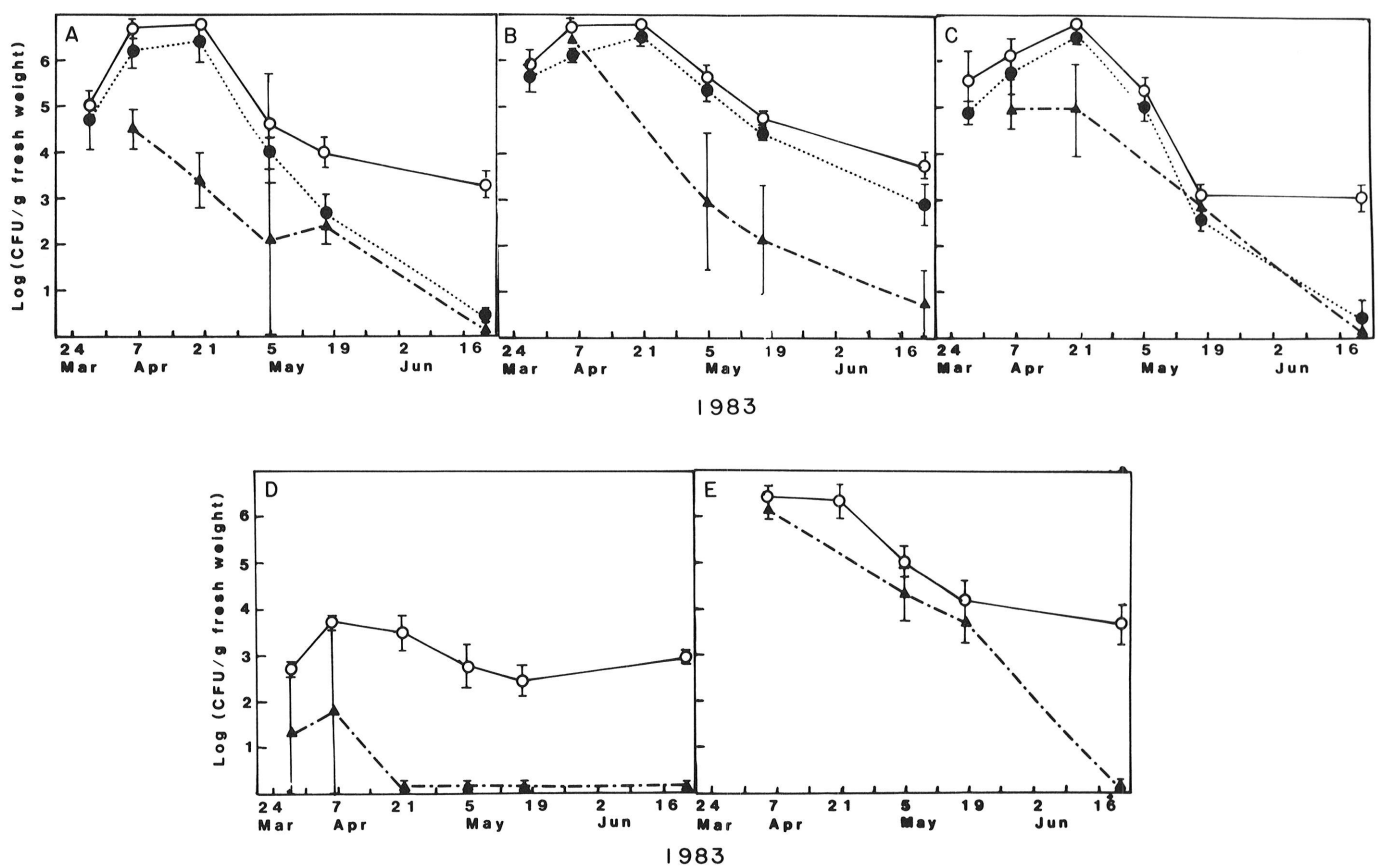

Fig. 3. Populations of total (o), ice nucleation-active (INA) (▲), and antagonistic (•) bacteria on flower buds, flowers and fruitlets of Bing sweet cherry trees at Prosser, WA, in 1983. Antagonistic bacteria W718 (*Pseudomonas putida*), **A**, W4N1613$^-$ (INA$^-$ *P. syringae* pv. *syringae*), **B**, and M232A (*Erwinia herbicola*), **C**, were applied at the tight cluster (25 March) and first bloom (6 April) stages of development. Bactericides (120 ppm streptomycin; 80 ppm oxytetracycline), **D**, were applied every 2 wk. Unsprayed, **E**, trees served as a check treatment. INA *P.s.* pv. *syringae* strain B301D was applied on 30 March. Vertical bars show the standard error of the mean of log populations.

the population of INA bacteria and the temperature at which ice formed in peach shoots. A stochastic mechanism of ice nucleation was ascribed (3,5) to peach because the probability of ice formation depended on sample volume and time of exposure; as the temperature decreased, ice nucleation increased exponentially. Recently, the wood-associated ice nucleus was concluded to be nonbacterial since prolonged soaking of stems selectively inactivated the wood-associated ice nucleus and not the bacterial ice nucleus (Gross and Proebsting, Jr., *unpublished*). Suppression of the populations of INA *P. syringae* by antagonistic bacteria therefore would result in correspondingly lower MNTs for flowers only if they were either excised from stems or attached to stems that were soaked in water. A further complication, observed in laboratory tests, is that higher MNTs induced by INA bacteria reduced frost injury in prebloom flower buds of peach and sweet cherry (11).

The use of antagonistic bacteria to suppress INA *P. syringae* may be more successful in controlling fruit tree diseases than frost injury. Indeed, it was shown that 50% of the INA *P. syringae* isolated as epiphytes from pome and stone fruit orchards in the PNW were pathogenic to immature pear and sweet cherry fruit (9). Although we observed a 10- to 100-fold suppression of the INA *P. syringae* population on pear trees with some antagonistic strains such as W4N1613$^-$ (Fig. 2B), suppression was not reflected in lower incidences of blossom blast (Tables 2 and 3). It should be cautioned, however, that very low levels of disease developed in these trees, making such comparisons tenuous. Despite several reports (28,29) that frost injury can be important in predisposing pear flowers to *Pseudomonas* infection, the environmental conditions accompanying the frosts at Hood River did not substantially favor flower infections. A preliminary report of Lindow (14) noted that fruit tree bacteria initially selected for antagonism of INA bacteria also lessened the incidence of fire blight, caused by *E. amylovora* (Burr.) Winslow et al, up to 64%; this level of control was comparable to that obtained using a mixture of streptomycin and oxytetracycline. Beer et al (6) have also reported the successful control (up to 86%) of fire blight on apple with antagonistic strains of *E. herbicola*. These examples illustrate the profound impact of certain fruit tree bacteria on pathogenic components of the microflora. The obvious goal is to improve the effectiveness of biological control through selection of more suppressive strains that work in a diversity of environments where fruit trees are grown.

Antagonists in this study were selected first for ability to develop high populations on fruit tree foliar surfaces and second for inhibition of INA *P. syringae* growth. It is important to note that all antagonistic bacteria were isolated from fruit tree flower and leaf surfaces in the PNW and composed at least 10% of the total bacterial flora. Subsequent screenings for antagonism of INA *P. syringae* on KB agar identified several inhibitory strains. Antibiosis on KB agar has been widely used for selecting potential biocontrol agents of plant pathogens (35). Inhibition of INA *P. syringae* probably resulted from the production of siderophores, which either could not be taken up by cells of *P. syringae* or had higher affinities for iron than the siderophore produced by *P. syringae*; antibiotics are rarely produced on media such as KB agar (7,35).

Further screening by the method of Lindow et al (19,22) for in vivo suppression of INA *P. syringae* on corn leaf surfaces identified several strains that significantly inhibited the growth of INA *P. syringae* and significantly suppressed the number of −5 C ice nuclei per gram (fresh weight) of leaf tissue (Table 1). In our trials, *E. herbicola* strain M232A was not as effective as the *P. putida* and *P. fluorescens* strains in suppressing the population of INA *P. syringae*. This was not surprising because Lindow et al (22) reported that strain M232A more effectively suppressed INA *E. herbicola*. Consequently, subsequent efforts were aimed at selecting fluorescent pseudomonads antagonistic to INA *P. syringae*, the only source of bacterial ice nuclei detected in PNW orchards during bloom (8).

Colonization of fruit trees in the field by antagonistic strains was characterized by discrete patterns for the major fruit tree species of bacterial epiphytes. INA$^-$ strain W4N1613$^-$ of *P. s.* pv. *syringae* was the most aggressive colonist, composing virtually 100% of the total bacterial population on pear at Hood River [1983 (Fig. 1B) and 1984] and sweet cherry and apple at Prosser [1983 (Fig. 3B)]. Populations of *P. s.* pv. *syringae* strain W4N1613$^-$ at Hood River during bloom were consistently 10- to 100-fold higher than the populations of strain M232A of *E. herbicola* or any strain of *P. putida* or *P. fluorescens*. The ability of *P. s.* pv. *syringae* to attain populations between 10^6 and 10^7 cfu/g, which appears to be near the epiphytic carrying capacity of bacteria on flower surfaces (8,9,15,24), may reflect its ability to suppress native INA *P. syringae* through competition for nutrients (17,18). INA$^-$ *P. s.* pv. *syringae* would be most apt to inhabit the same niches as indigenous INA *P. syringae*; therefore, a spray application of this bacterium to trees after budbreak, but before bloom, would ensure rapid buildup to high populations in advance of rampant growth of native INA *P. syringae*. In corn leaf assays, Lindow (16) first recognized the value of INA$^-$ *P. syringae* strains in suppressing INA bacteria. Construction of *P. syringae* strains carrying a deletion of all or part of the *ice* gene is one promising approach of developing strains highly suppressive to INA bacteria (27). Our results tend to support such concerted efforts to use INA$^-$ *P. syringae* strains as biological control agents.

In contrast to the INA$^-$ strain of *P. s.* pv. *syringae*, all of the *P. fluorescens* and *P. putida* strains maintained lower populations (10^4–10^5 cfu/g) through bloom on pears at Hood River, which was followed by a noticeable drop-off in numbers beginning in early May. Surprisingly similar population curves developed for all

TABLE 3. Field evaluation on Anjou pear of potential antagonists of ice nucleation-active (INA) *Pseudomonas syringae* at Hood River, OR, in 1983

Treatment	INA bacteria/g[a] (no.)	Frost injury[b] (% injured flowers/tree)	External fruit russet[c] (% fruit/tree)	Blossom blast[d] (no. infected clusters/tree)	Mean ice nucleation temperature[e] (C)
Check	2.4×10^5 (5.04)	12.5	40.7	7.2	−3.5
Bactericides[f]	6.5×10^2 (1.11)*	9.4	50.7	3.3	−4.4
W718	2.9×10^5 (4.84)	13.9	50.0	3.7	−3.5
W4N1613$^-$	4.1×10^3 (3.61)	13.3	55.3	6.3	−4.0
M232A	1.2×10^6 (4.88)	15.4	42.7	7.2	−3.6
LSD 0.05[g]	··· (2.33)	9.1	17.4	7.7	1.1

[a] Populations from samples collected 17 April (Fig. 2). INA bacterial populations for each replication were converted to log values for statistical analysis; the means of the logs are shown in parentheses.

[b] Frost injury occurred 9 April (−4.5 C minimum air temperature) and 15 April (−2 C minimum air temperature) at the first white and first bloom stages of development, respectively. Evaluations done at full bloom (18 April) on 10 flower clusters per tree. All flowers in each cluster were sectioned and evaluated for internal injury to ovaries and/or pistils.

[c] Evaluations on 24 May of 25 fruit per tree.

[d] Counts of blossom blast were completed 6 May.

[e] Mean ice nucleation temperatures shown as the average of six determinations during the blossom season.

[f] Streptomycin (120 ppm) and oxytetracycline (80 ppm).

[g] The protected least significant difference (LSD) between the means for the check and bacterial or bactericide treatments are shown at $P = 0.05$ (*).

strains of *P. putida* and *P. fluorescens*; these curves also resembled those for fluorescent pseudomonads on California pear trees in the spring (15,17). Somewhat higher populations were established for these two species by bloom on sweet cherry at Prosser (Fig. 3A). It was also observed that sweet cherry flowers were colonized more heavily than apple and pear flowers. By late June these bacterial strains were generally undetectable, reflecting the hot, dry summer conditions characteristic of both Prosser and Hood River. Populations also were not observed to increase selectively in the fall when environmental conditions were more suitable for pseudomonad growth. Consequently, reapplications would be necessary to reestablish significant epiphytic populations. Fluorescent pseudomonad strain A510, which was reported by Lindow (15) to be the most effective strain in controlling frost injury (by 85%) to immature Bartlett pear fruit after a radiation frost of −3 C, was not appreciably different from our *P. putida* or *P. fluorescens* strains in aggressiveness of colonization, persistence, or ability to suppress INA *P. syringae* in a pear orchard at Hood River.

Strain M232A of *E. herbicola* was decidedly inferior to INA$^-$ strain W4N1613$^-$ of *P. s.* pv. *syringae* in colonizing floral tissues to high population densities. Populations of strain M232A generally ranged from 10^4–10^5 cfu/g during bloom periods, which is considerably below the peak populations of 10^6–10^7 cfu/g reported for this strain on corn leaves in Wisconsin (23). Strain M232A, nevertheless, averaged up to 10-fold higher populations at Prosser than at Hood River, perhaps reflecting the slightly warmer and, consequently, more favorable climate at Prosser during the spring. During the dry summer months, however, populations of strain M232A were usually undetectable (detection level = 10 cfu/g). In light of these observations it is important to note that *E. herbicola* is not a common epiphytic bacterium on fruit trees in the PNW. This is in sharp contrast to the prevalence of *E. herbicola* on fruit trees in other parts of the United States (6). For instance, INA *E. herbicola* has never been found on trees during the spring despite extensive surveys for such strains (8). Thus, it was concluded that *E. herbicola* is not as ecologically fit for survival on PNW fruit trees as the fluorescent pseudomonads. Because the pseudomonads are also more suppressive of INA *P. syringae* (22), these bacteria probably are more suitable for use in biological control.

A full explanation is not apparent for the dichotomy of results obtained by Lindow (15,17) and Lindow and Connell (24) relative to those reported here concerning the effectiveness of antagonistic bacteria in lessening frost injury in fruit trees. The effect of an intrinsic wood-associated ice nucleus active at about −2 C will certainly have a governing role in ice formation during most radiation frosts in the PNW, making any form of INA bacterial control ineffective (1–3,10,11). However, differential temperatures between the flowers or fruitlets and the woody stem tissue may occasionally occur during mild radiation frosts, wherein the floral fruit tissues are preferentially susceptible to heat loss due to exposure to nighttime radiation to the sky (2,11). Ashworth et al (4) measured the temperatures of ice formation in peach trees under field conditions and noted transient differences of 1 to 2 C between blossom and shoot temperatures. Nevertheless, these temperatures were not sustained, and differences of less than 0.5 C predominated. Temperature differences may become, however, more pronounced as fruitlet size increases and as foliage shelters the woody tissue. Superficial frost injury to the epidermis of pears, resulting in frost rings and russet, is associated with temperatures that are insufficient to kill the fruit. Suppression of INA bacteria by antagonists may be adequate to reduce surface ice nuclei, resulting in significantly less frost injury when subjected to minor differential temperatures between fruit and wood. The frost injury data of Lindow (15,17) for pear and Lindow and Connell (24) for almond, which showed less injury using certain antagonists, appeared to result from mild frosts (−3 C minimum air temperature) of this type. In contrast, russet of pear fruit was not controlled by antagonists in trials at Hood River, although frosts were more intense in these orchards (−4 to −4.5 C minimum air temperature; Tables 2 and 3).

The concept that antagonistic bacteria can suppress the population size of INA *P. syringae* on fruit trees is supported by our results. However, over a 4-yr period we were frustrated in our repeated efforts to obtain any significant benefit from control of INA *P. syringae*. Both natural and simulated frosts resulted in killing injury levels to flowers and fruitlets that were indistinguishable despite large contrasts in the population sizes of INA *P. syringae* on tissue surfaces (10,11). Such data probably reflect the effect of the intrinsic, wood-associated ice nucleus, complicating the implementation of INA bacterial control practices for frost protection. Nevertheless, suppression of INA *P. syringae* populations may be beneficial in controlling disease, which can be severe following frost injury (9,28,29). The use of antagonistic bacteria for biocontrol of *P. syringae* will require greater suppression than observed in our orchard studies to give effective and predictable control for orchardists. Further selection and development of INA$^-$ strains of *P. s.* pv. *syringae* appear to have the greatest potential. Thus far, strains have been primarily selected for ability to suppress growth of the target bacterium by occupying common foliar sites and using nutrients necessary for growth. The importance of antibiosis in antagonism of INA bacteria has been questioned by Lindow (16); additional studies are needed to judge the value of screening for antagonists on KB agar. Selection for antagonism as a result of bacteriocin production may enhance their capacity to suppress indigenous strains of *P. syringae*. Indeed, Smidt and Vidaver (32) have detected production of syringacin W-1 by *P. s.* pv. *syringae* at significant levels in bean that were inhibitory to a sensitive strain of *P. s.* pv. *syringae*, but not its bacteriocin-resistant derivative. Thus, biocontrol continues to be an attractive alternative for control of *P. syringae*. If methods can be developed for temporary or permanent inactivation of the wood-associated ice nucleus, then control of INA bacteria in orchards will become an urgent priority.

LITERATURE CITED

1. Andrews, P. K., Proebsting, E. L., Jr., and Gross, D. C. 1983. Differential thermal analysis and freezing injury of deacclimating peach and sweet cherry reproductive organs. J. Am. Soc. Hortic. Sci. 108:755-759.
2. Andrews, P. K., Proebsting, E. L., Jr., and Gross, D. C. 1986. Ice nucleation and supercooling in freeze-sensitive peach and sweet cherry tissues. J. Am. Soc. Hortic. Sci. 111:232-236.
3. Ashworth, E. N., Anderson, J. A., and Davis, G. A. 1985. Properties of ice nuclei associated with peach trees. J. Am. Soc. Hortic. Sci. 110:287-291.
4. Ashworth, E. N., Anderson, J. A., Davis, G. A., and Lightner, G. W. 1985. Ice formation in *Prunus persica* under field conditions. J. Am. Soc. Hortic. Sci. 110:322-324.
5. Ashworth, E. N., Davis, G. A., and Anderson, J. A. 1985. Factors affecting ice nucleation in plant tissues. Plant Physiol. 79:1033-1037.
6. Beer, S. V., Rundle, J. R., and Norelli, J. L. 1984. Recent progress in the development of biological control for fire blight—A review. Acta Hortic. 151:195-201.
7. Gross, D. C. 1985. Regulation of syringomycin synthesis in *Pseudomonas syringae* pv. *syringae* and defined conditions for its production. J. Appl. Bacteriol. 58:167-174.
8. 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.
9. Gross, D. C., Cody, Y. S., Proebsting, E. L., Jr., Radamaker, G. K., and Spotts, R. A. 1984. Ecotypes and pathogenicity of ice nucleation-active *Pseudomonas syringae* isolated from deciduous fruit tree orchards. Phytopathology 74:241-248.
10. Gross, D. C., and Proebsting, E. L., Jr. 1984. The role of ice nucleation-active *Pseudomonas syringae* in frost injury to floral buds of deciduous fruit trees. Pages 34-36 in: Proc. 2nd Working Group on *Pseudomonas syringae* Pathovars. Hellenic Phytopathological Society, Athens, Greece. 117 pp.
11. Gross, D. C., Proebsting, E. L., Jr., and Andrews, P. K. 1984. The effects of ice nucleation-active bacteria on temperatures of ice nucleation and freeze injury of *Prunus* flower buds at various stages of development. J. Am. Soc. Hortic. Sci. 109:375-380.
12. Hirano, S. S., Baker, L. S., and Upper, C. D. 1985. Ice nucleation temperature of individual leaves in relation to population sizes of ice

nucleation active bacteria and frost injury. Plant Physiol. 77:259-265.
13. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
14. Lindow, S. E. 1982. Integrated control of frost injury and fire blight of pear with antagonistic epiphytic bacteria. (Abstr.) Phytopathology 72:946.
15. Lindow, S. E. 1982. Population dynamics of epiphytic ice nucleation active bacteria on frost sensitive plants and frost control by means of antagonistic bacteria. Pages 395-416 in: Plant Cold Hardiness and Freezing Stress: Mechanisms and Crop Implications, Vol. 2. P. H. Li and A. Sakai, eds. Academic Press, New York.
16. Lindow, S. E. 1982. Role of antibiosis in antagonism against ice nucleation active bacteria by epiphytic bacteria. (Abstr.) Phytopathology 72:986.
17. Lindow, S. E. 1983. Methods of preventing frost injury caused by epiphytic ice-nucleation-active bacteria. Plant Dis. 67:327-333.
18. Lindow, S. E. 1983. The role of bacterial ice nucleation in frost injury to plants. Annu. Rev. Phytopathol. 21:363-384.
19. Lindow, S. E., Arny, D. C., and Upper, C. D. 1978. *Erwinia herbicola*: A bacterial ice nucleus active in increasing frost injury to corn. Phytopathology 68:523-527.
20. Lindow, S. E., Arny, D. C., and Upper, C. D. 1978. Distribution of ice nucleation-active bacteria on plants in nature. Appl. Environ. Microbiol. 36:831-838.
21. Lindow, S. E., Arny, D. C., and Upper, C. D. 1982. Bacterial ice nucleation: A factor in frost injury to plants. Plant Physiol. 70:1084-1089.
22. Lindow, S. E., Arny, D. C., and Upper, C. D. 1983. Biological control of frost injury: An isolate of *Erwinia herbicola* antagonistic to ice nucleation active bacteria. Phytopathology 73:1097-1102.
23. Lindow, S. E., Arny, D. C., and Upper, C. D. 1983. Biological control of frost injury: Establishment and effects of an isolate of *Erwinia herbicola* antagonistic to ice nucleation active bacteria on corn in the field. Phytopathology 73:1102-1106.
24. Lindow, S. E., and Connell, J. H. 1984. Reduction of frost injury to almond by control of ice nucleation active bacteria. J. Am. Soc. Hortic. Sci. 109:48-53.
25. Lindow, S. E., Hirano, S. S., Barchet, W. R., Arny, D. C., and Upper, C. D. 1982. Relationship between ice nucleation frequency of bacteria and frost injury. Plant Physiol. 70:1090-1093.
26. Maki, L. R., Galyan, E. L., Chang-Chien, M., and Caldwell, D. R. 1974. Ice nucleation induced by *Pseudomonas syringae*. Appl. Microbiol. 28:456-459.
27. Orser, C., Staskawicz, B. J., Panopoulos, N. J., Dahlbeck, D., and Lindow, S. E. 1985. Cloning and expression of bacterial ice nucleation genes in *Escherichia coli*. J. Bacteriol. 164:359-366.
28. Panagopoulos, C. G., and Crosse, J. E. 1964. Frost injury as a predisposing factor in blossom blight of pear caused by *Pseudomonas syringae* van Hall. Nature (London) 202:1352.
29. Paulin, J.-P., and Luisetti, J. 1978. Ice nucleation activity among phytopathogenic bacteria. Pages 725-731 in: Proc. 4th Int. Conf. Plant Pathogenic Bact. Station de Pathologie Vegetale et Phytobacteriologie, ed. Gibert-Clarey, France. 978 pp.
30. Proebsting, E. L., Jr., Andrews, P. K., and Gross, D. C. 1982. Supercooling young developing fruit and floral buds in deciduous orchards. HortScience 17:67-68.
31. Proebsting, E. L., Jr., and Mills, H. H. 1978. Low temperature resistance of developing flower buds of six deciduous fruit species. J. Am. Soc. Hortic. Sci. 103:192-198.
32. Smidt, M. L., and Vidaver, A. K. 1982. Bacteriocin production by *Pseudomonas syringae* PsW-1 in plant tissue. Can. J. Microbiol. 28:600-604.
33. Vali, G. 1971. Quantitative evaluation of experimental results on the heterogeneous freezing nucleation of supercooled liquids. J. Atmos. Sci. 28:402-409.
34. Vidaver, A. K. 1967. Synthetic and complex media for the rapid detection of fluorescence of phytopathogenic pseudomonads: Effect of the carbon source. Appl. Microbiol. 15:1523-1524.
35. Xu, G.-W., and Gross, D. C. 1986. Selection of fluorescent pseudomonads antagonistic to *Erwinia carotovora* and suppressive of potato seed piece decay. Phytopathology 76:414-422.