Occurrence and Nature of Ice Nucleation-Active Strains of *Pseudomonas syringae* on Apple and Peach Trees in Georgia

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ABSTRACT

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Bacteria that nucleate ice crystal formation (INA) were isolated from 60 and 80% of tissue samples collected from each of two apple orchards at approximately monthly intervals from November 1984 until October 1985. Populations varied from 2×10^2 to $>1.0\times 10^4$ cfu/g of green tissue. In a peach orchard sampled similarly, INA bacteria were detected in 20% of the samples at populations of 5.5×10^3 cfu/g and higher. Populations of INA bacteria ranging from 10^2 to $>4.0\times 10^4$ cfu/g of tissue were isolated from 13 of 24 apple flower samples collected in 1985. In 1986, four of 13 samples had populations of INA bacteria ranging from 10^2 to more than 3.9×10^4 cfu/g. Only one of 13 peach flower samples collected in 1985 had detectable levels of INA bacteria, but levels of 1.4×10^5 cfu/g or higher were detected in seven of eight similar samples collected in 1986. Although most of 286 representative INA strains from the apple and peach trees tested were *Pseudomonas syringae* pv. *syringae*, they were diverse in pathogenicity and virulence on green tomato fruit, green bean pods, and peach seedlings. About 61% of 140 INA strains from apple grew on casitone-yeast extract-glycerol (CYE) medium containing 200 μg/ml of streptomycin sulfate, and resistance apparently was related to streptomycin use for fire blight or blister spot control. Less than 2% of 119 strains from peach trees grew when exposed to 200 μg/ml of streptomycin. About 37% of the apple strains—but none of the peach strains—grew on CYE medium with 60 μg/ml of copper.

It is now generally accepted that certain bacteria associated with plants can contribute to frost damage by serving as nuclei for ice formation. Although *Pseudomonas syringae* van Hall, *P. fluorescens* Migula, *P. viridiflava* (Burkholder) Dowson, *Erwinia herbicola* (Lonis) Dye, and *Xanthomonas campestris* pv. *tranlucens* (Jones, Johnson, & Reddy) Dye have been reported to be ice nucleation-active (INA) organisms, certain pathovars of *P. syringae*, particularly *P. s.* pv. *syringae*, appear to be the most common and most active bacteria associated with a wide range of economically important plant species (5,8,15,19,26,29,34). Certain strains of INA *P. syringae* are believed to facilitate their own ingress and pathogenesis by causing freeze injury within host tissue (33,34,41). Despite the considerable amount of work on INA bacteria in recent years, relatively little has been reported on their occurrence, nature, and importance on frost-sensitive plants in the southeastern United States. In South Carolina (7) and West Virginia (2), INA bacteria were isolated infrequently from peach tissues collected at critical temperature periods, and their role in inciting frost damage was disputed. Since apple and peach are important crops that are subject to both spring frost damage and attack by pathovars of *P. syringae*, a study was initiated to determine the occurrence and characteristics of INA bacteria associated with these crops in Georgia.

In our preliminary surveys, streptomycin resistance was common among INA strains from some orchards but not others. Copper tolerance also has been reported among strains of *P. syringae* (1), and both streptomycin sulfate and copper compounds have been suggested as control measures for INA bacteria and frost damage (25,26,28). We determined the extent of resistance to both compounds among INA strains from apple and peach.

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MATERIALS AND METHODS

Association of INA bacteria with apple and peach trees. *Orchards sampled—intensive survey.* Trees in one peach orchard and two apple orchards were sampled 10 times from November 1984 until October 1985. One apple orchard and one peach orchard were located in Wilkes County (Fig. 1). The other apple orchard was located at the University of Georgia Mountain Branch Station in Union County (Fig. 1). At each location, four trees separated by 40–80 m were sampled by removing 10 terminal twigs, 15–45 cm long, from each tree. The twigs were placed in plastic bags and stored on ice until processed the same day in the laboratory. Buds, flowers, young fruit, or leaves, depending on the sampling time, were assayed.

Orchards sampled—extensive survey. In 1985, trees in 13 peach orchards located in five counties and 24 apple orchards in eight counties were sampled once during the spring bloom period (Fig. 1). In 1986, trees in eight peach orchards located in four counties and 13 apple orchards in five counties were sampled similarly (Fig. 1). In each orchard a composite sample consisting of a total of 20–30 twigs was collected from 20 trees selected at random from a 175 × 175 m section. The twigs were transported to the laboratory as described above, and opening buds or flowers were removed for assay.

Laboratory assay procedures. Buds, flowers, and young fruits were macerated with a mortar and pestle; young leaves were cut into 1- to 2-cm pieces with scissors. Tissue samples (10 g) were placed in 500-ml flasks containing 200 ml of sterile 0.1 M phosphate buffer (1:2 [v/v] of KH_2PO_4 [13.6 g/L] and K_2HPO_4 [17.4 g/L]), pH 7.1, amended with 0.1% peptone (w/v). The flasks were shaken for 30 min on a wrist-action shaker at medium speed. Serial dilutions were prepared in sterile distilled water, and selected dilutions predicted to give countable plates were plated, 0.1/plate, on 10 plates of medium B of King et al (KMB) (22) amended with 100 μg/ml of cycloheximide to inhibit fungal growth. Plates were incubated at 25 C, and the numbers of INA bacteria were determined after 48–72 hr using the modified replica-plate technique of Lindow et al (27). Three plates were replicated separately onto paraffin-coated aluminum foil that was floated on a −5 C ethylene glycol-water bath. The surface was sprayed with a fine mist of sterile phosphate buffer from a chromatography sprayer, and frozen drops were counted after 3 min.

Frozen drops were picked separately, suspended in 0.5 ml of sterile distilled water, and streaked on plates of KMB; after 48–72 hr at 25 C, representative colonies were picked for further characterization.

If INA bacteria were not detected by the replica-plate technique, a confirmation test was run by testing 25–45 individual colonies from the unused dilution plates. The colonies were picked, streaked on nutrient glycerol agar (NGA; 3.3 g of peptone, 2.7 g of nutrient broth, 2.0 g of yeast extract, 15 g of agar, 25 ml of glycerol, 1 L of distilled water), and grown for 24 hr at 25 C. A suspension containing 10^8 cfu/ml was prepared by turbidity adjustment in phosphate buffer. Ten 20- to 30-μl drops of the suspension were placed on the surface of 5.7-cm diameter aluminum weighing pans floated on a ethylene glycol-water bath at −5 C. Drops that froze within 3 min were counted as positive for ice nucleation. Strains that consistently froze (all 10 drops) in less than 1 min were kept for characterization.

Maintenance of strains. Working cultures of the strains collected were grown in tubes of nutrient yeast-dextrose agar (NYDA) (21) for 48 hr and stored at 6 C. For extended storage, strains were held in screw-cap tubes of sterile distilled water at 20 C for 2–3 mo until they could be prepared in a dehydrated state on glass beads in sealed ampules for storage at room temperature (J. B. Jones, *personal communication*).

Characterization of INA bacteria from apple and peach trees. Because nearly all INA bacteria recovered from apple and peach trees produced water-soluble fluorescent pigments on KMB and presumptively were pseudomonads, tests known to distinguish saprophytic from pathogenic types and to separate species and pathovars within the group were used (18,24,30,39). A total of 156 strains from apple and 130 strains from peach were characterized. These were selected to be representative of INA populations in the various orchards sampled. Strains that gave a hypersensitive reaction (HR) in tobacco (23) were also tested for pathogenicity on selected hosts.

Biochemical and physiological tests. All strains were tested for their LOPAT reactions (24): For levan production, a cell suspension was streaked on nutrient sucrose agar (nutrient agar + 5% sucrose); the appearance of domed, mucoid colonies after 72 hr of incubation was positive for levan production. For oxidase reaction, a cell mass from a 24-hr culture growing on KMB plates was smeared with an L-shaped sterile glass rod onto moistened Taxo differentiation disks; the appearance of a dark red color within 1 min was considered to be positive. For pectolytic activity, cells from a 24-hr KMB plate culture of each strain were streaked on plates of sodium polypectate media as described by Hildebrand and Schroth (18) except the medium was adjusted to pH 5.0 and 8.0; the plates were observed for pit formation after 3 and 5 days. For arginine dihydrolase activity, tubes of Thornley's 2A medium were stab-inoculated with cells from a 24-hr KMB plate culture of each strain, and the surface of the medium was layered with 5 mm of sterile Vaseline (40); a color change to red after 2 days at 25 C was considered positive. For tobacco HR, a 10^8 suspension of each strain prepared by turbidity adjustment was infiltrated into the interveinal areas of leaves of *Nicotiana tabacum* L. 'Hicks' with a hypodermic needle and syringe; plants were placed on a greenhouse bench at 23–30 C and observed for tissue collapse within 18 hr (23).

Capacity of the strains to utilize the organic substrates erythritol, DL-lactate, D(−)-tartrate, and sucrose for growth was determined using the methods of Misaghi and Grogan (30) as modified by Jones et al (21). A bioassay to determine syringomycin production was performed by the methods of DeVay et al (11) except that Difco potato-dextrose agar (PDA) was used instead of a naturally prepared formula. *Geotrichum candidum* Link ex. Pers. was used as the bioassay organism, and zones of inhibition were measured after 24 hr at 25 C.

Pathogenicity tests. Strains were tested for pathogenicity on mature green tomato (*Lycopersicon esculentum* Mill. 'Chico III') fruit, green bean (*Phaseolus vulgaris* L.) pods, and peach (*Prunus persica* (L.) Batsch 'Elberta') seedlings, all hosts used previously for characterizing fluorescent pseudomonads (6,12,14,32,35). Tomato fruit and green bean pods were inoculated with 268 representative INA strains from apple and peach. Peach seedlings were inoculated with 180 strains. Tomato fruit and green bean pods were surface-sterilized prior to inoculation by immersing them in a 0.5% sodium

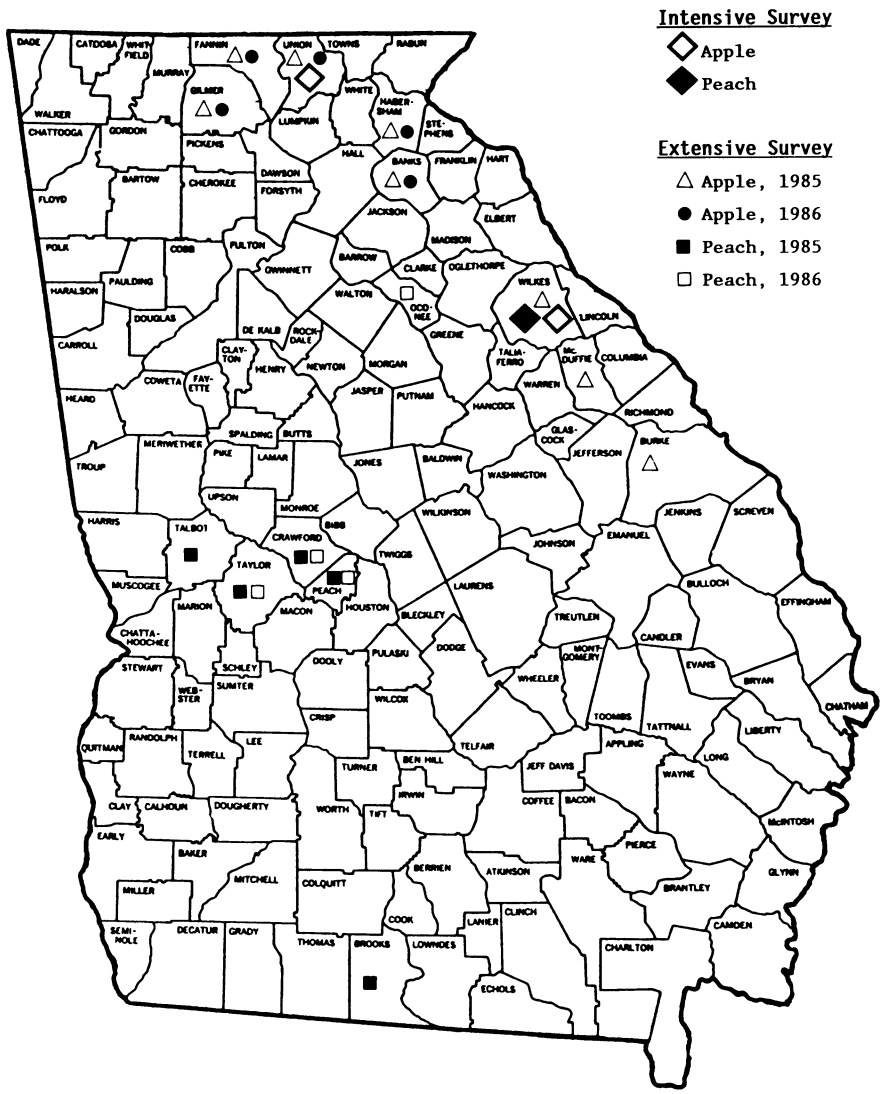

Fig. 1. Locations in Georgia of peach and apple orchards sampled for the presence of ice nucleation-active bacteria.

hypochlorite solution for 2 min, then rinsing them in running tap water for 1 min. Inocula for all hosts were harvested from KMB cultures grown for 24–48 hr at 25 C. Suspensions containing 10^8 cells were prepared by turbidity adjustment in sterile distilled water. A drop of suspension was placed on each mature green tomato fruit, and the fruit were stab-inoculated with a sterile dissecting needle through the drops and 1.0–2.0 mm into the fruit. Green bean pods were inoculated similarly except that three to five inoculations were made along the length of the pod. Inoculated fruit and pods were placed in 9 × 25 cm humidity chambers and incubated on a laboratory bench at 25–27 C. Disease severity was recorded after 5 days using the following scale: − = no necrosis, + = necrosis limited to needle puncture site, ++ = necrosis and water-soaking ≤1.0 cm in diameter around the puncture site, and +++ = necrosis and water-soaking >1 cm in diameter. Three-week-old peach seedlings were inoculated by injecting 0.3 ml of a suspension with a needle and syringe into shoot tips. A known strain (B-48) of *P. s.* pv. *syringae* that was ice nucleation-positive and capable of inciting bacterial canker of peach was included for comparison. Inoculated plants were placed on a greenhouse bench at 20–28 C. Results were recorded after 5 days on a scale where − = no symptoms, ± = dry localized lesion, ++ = moderately severe spreading lesion, and +++ = death of the shoot tip. Each pathogenicity study included three replications of each host-strain combination, and the entire study was repeated once. Controls using sterile distilled water were included in all inoculation experiments.

Streptomycin resistance and copper tolerance among strains. Loopfuls of 10^8 cell suspensions of INA bacteria (163 from apple and 134 from peach) were streaked onto plates of KMB amended with 250 μg/ml of streptomycin sulfate. Growth response was recorded after 48 hr at 25 C. Many of the same strains (140 from nine apple orchards and 119 from eight peach orchards) also were streaked on gradient plates containing streptomycin sulfate or copper (S. Lindow, *personal communication*). A 15-ml volume of casitone-yeast extract-glycerol (CYE) medium (42) amended with 200 μg/ml of Cu^{++} was added to plates. The plates were then slanted so the media solidified to a depth of 1 and 7 mm on opposite sides. The plates were returned to a horizontal position and another 15 ml of unamended CYE medium was added. The plates were allowed to stand for 1 day to permit a streptomycin or copper gradient to develop and were then streaked perpendicular to the gradient with a Puritan cotton-tipped applicator dipped in a slightly turbid suspension of the test strain. When a strain grew on the gradient plate, a mass of cells was removed with a loop from the area of highest antibiotic or Cu^{++} and suspended in water. A cotton-tipped applicator was dipped into the suspension, then streaked onto CYE plates containing 25, 50, 100, or 200 μg/ml of streptomycin sulfate or 7.5, 15, 30, or 60 μg/ml of Cu^{++}. The chemical level at which growth occurred was recorded after 48 hr. All the chemical sensitivity tests were run at 25 C and were replicated three times and repeated once.

RESULTS

Association of INA bacteria with apple and peach trees. *Intensive survey.* Populations of INA varied greatly among orchards and sampling dates when two apple orchards and one peach orchard were sampled 10 times during 1984–1985 (Table 1). INA bacteria were associated more commonly with apple than with peach tissue.

Extensive survey. Populations of INA bacteria also varied greatly with location and year when apple and peach orchards were sampled once during the bloom periods of 1985 and 1986 (Tables 2 and 3). Approximately 54 and 31% of the apple orchards sampled in 1985 and 1986, respectively, yielded INA bacteria (Table 2). Only about 8% of the peach orchards sampled in 1985 yielded INA bacteria, whereas populations were relatively high in about 88% of the orchards sampled in 1986 (Table 3).

Characterization of INA bacteria from apple and peach trees. *Biochemical and physiological tests.* Essentially all bacteria that were INA-positive at −5 C were fluorescent pseudomonads. A few yellow-pigmented INA strains believed to be *E. herbicola* were isolated but were not included in the study because they were active at temperatures lower than −5 C. All 286 fluorescent INA strains characterized were negative for oxidase and arginine dihydrolase, and all except one strain produced an HR in tobacco. Approximately 53% of the strains were positive for levan production. Most of the strains did not show pectolytic activity. Only five and 14 strains caused pitting on polypectate gel at pH 5.0 and 8.0, respectively. More than 95% of the strains utilized erythritol, DL-lactate, and D(−)-tartrate. Although 84% of the strains caused inhibition of *G. candidum*, a presumptive test for syringomycin production, the inhibition zones varied from 1 to 28 mm among strains, suggestive of differences in syringomycin production.

Pathogenicity tests. The INA strains from apple and peach trees varied in pathogenicity and virulence on mature green tomato fruit, green bean pods, and peach seedlings (Table 4). On green tomato fruit the reaction ranged from no evidence of necrosis at the point of inoculation to a water-soaked lesion that reached 2.5 cm in diameter. Most of the strains were weakly to moderately virulent on tomato fruit. Lesion development on green bean pods ranged from a slight rot near the inoculation point to water-soaked lesions that measured 2.5 cm in diameter after 5 days. Most strains were weakly to moderately virulent on bean pods. The most severe reaction on the peach seedlings was the death of the shoot tip beyond the point of inoculation. Approximately 9% of the strains from peach and 4% of the strains from apple caused complete shoot tip death. The known bacterial canker strain of *P. s.* pv. *syringae* used as a positive control caused shoot tip death similar to the most virulent INA strains tested. None of the

Table 1. Populations of ice nucleation-active (INA) bacteria associated with various tissues of apple and peach trees collected at two locations in Georgia during 1984–1985

Sampling date		Bacterial counts[a] (cfu/g green tissue)		
		Wilkes County		Union County
		Peach	Apple	Apple
1984	November	0	2×10^2	0
	December	0	1.8×10^3	2×10^2
1985	January	0	1.7×10^3	0
	February	0	10^2	1.0×10^4
	March	5.3×10^3	0	...[b]
	April	...	...	2×10^2
	May	0	TNTC	TNTC
	July	TNTC[c]	3×10^2	1.2×10^3
	August	0	7×10^2	0
	September	0	TNTC	0
	October	0	0	0

[a] Appropriate dilutions of macerated buds, flowers, leaves, or young fruits were plated on medium B of King et al (22) amended with 100 μg/ml of cycloheximide. INA bacteria counts were determined at −5 C by the replica-plate method of Lindow et al (27). Values are means of samples from four trees.

[b] Samples were not run.

[c] TNTC = too numerous to count. Populations of INA bacteria were so high that the entire plate froze before individual colony counts could be determined. Populations were estimated to be above 10^4 cfu/g.

strains that were highly virulent on peach seedlings were highly virulent on green bean pods or tomato fruit. About 40% of the peach strains and 36% of the apple strains were weakly or moderately virulent on peach, causing lesions 0.3–2.5 cm in length. Many of the INA strains from apple and peach were not pathogenic on peach seedlings; only discoloration occurred at the point of inoculation. The water controls for the tomato, bean, and peach inoculations showed no evidence of disease.

Streptomycin resistance and copper tolerance among strains. Seventy-nine of 85 INA strains of *P. syringae* obtained from the Wilkes County apple orchard from November 1984 to October 1985 grew on KMB amended with 250 μg/ml of streptomycin sulfate. In this orchard, streptomycin had been used three or four times annually for control of fire blight caused by *Erwinia amylovora* (Burr.) Winslow et al. Only one of 23 strains from the apple orchard sampled intensively in Union County grew at the 250-μg/ml level of streptomycin sulfate. This orchard had never received a streptomycin spray. Forty-eight of the 55 strains obtained from seven commercial apple orchards in Fannin, Gilmer, and Union counties in 1985 and 1986 also grew on the streptomycin-amended medium. Although grower spray records were incomplete, it was determined that all seven orchards had received streptomycin for fire blight control. One of the six had received additional sprays for blister spot caused by *P. s.* pv. *papulans* (Rose) Dhanvantari. Only five of 90 strains of INA *P. s.* pv. *syringae* obtained from the peach orchard sampled intensively in Wilkes County grew on the amended medium, and none of the 44 strains collected from eight peach orchards in Crawford, Oconee, Peach, and Talbot counties grew in the presence of streptomycin. None of the peach orchards had received streptomycin sprays. Similar results were obtained when 140 strains from apple and 119 strains from peach were streaked on CYE-streptomycin gradient plates (Table 5). About 78% of the apple strains grew on the gradient plates, and 69% of strains transferred from gradient plates grew on CYE amended with 200 μg/ml of streptomycin sulfate. However, only 6% of the peach strains grew on the streptomycin gradient plates, and less than 2% of the transfers grew on CYE medium containing 200 μg/ml of streptomycin (Table 5). The apple strains also were more tolerant to Cu^{++} than the peach strains (Table 5). Over 87% of the apple strains grew on the CYE-copper gradient plates and 37% grew on CYE amended with 60 μg/ml of Cu^{++}. About 58% of the peach strains grew on the Cu^{++} gradient plates, but none grew at the highest level of Cu^{++}. There were no records to indicate that copper sprays had been used in any of the apple or peach orchards sampled.

Table 2. Populations of ice nucleation-active (INA) bacteria associated with apple flowers collected at various locations in Georgia during 1985 and 1986

Orchard designation	Orchard location (county)	Bacterial counts[a] (cfu/g green tissue)
	1985	
1	Union	9.3×10^3
2	Union	0
3	Union	10^2
4	Banks	9.3×10^3
5	Banks	1.3×10^2
6	Habersham	TNTC[b]
7	Burke	0
8	Burke	0
9	McDuffie	0
10	Wilkes	2.5×10^4
11	Gilmer	TNTC
12	Gilmer	TNTC
13	Gilmer	1.3×10^2
14	Gilmer	1.3×10^2
15	Gilmer	6.7×10^2
16	Gilmer	0
17	Gilmer	7.0×10^2
18	Gilmer	0
19	Gilmer	4.0×10^4
20	Gilmer	0
21	Gilmer	0
22	Gilmer	0
23	Gilmer	0
24	Fannin	0
	1986	
101	Habersham	0
102	Union	0
103	Union	10^2
104	Banks	0
105	Banks	0
106	Banks	0
107	Banks	0
108	Gilmer	0
109	Gilmer	3.9×10^4
110	Gilmer	0
111	Gilmer	0
112	Fannin	1.4×10^4
113	Fannin	TNTC

[a] Appropriate dilutions of macerated flowers were plated on medium B of King et al (22) amended with 100 μg/ml of cycloheximide and counts made after 48–72 hr at 25 C. The sample was a composite of 20–30 twigs from 20 trees in each orchard. INA bacteria counts were determined at −5 C by the replica-plate method of Lindow et al (27).

[b] TNTC = too numerous to count. Populations of INA bacteria were so high that the entire plate froze before individual colony counts could be determined. Populations were estimated to be above 10^4 cfu/g.

DISCUSSION

We concluded that INA bacteria are frequently associated with tissues of apple and peach trees in Georgia, although populations may vary greatly depending on sampling time and location. Seasonal and location fluctuations (15,20) and even differences in populations among leaves on a given plant (20) have been reported in previous studies on INA bacteria. In the Pacific Northwest, Gross et al (15) reported that 30 and 75% of fruit tree orchards sampled in the Yakima Valley of Washington and the Hood River Valley of Oregon, respectively, were positive for INA bacteria. Moisture and temperature are important factors influencing populations of INA bacteria (15,20). Because of the number of locations and long distances involved in our surveys, it was not possible for us to collect precise environmental data. However, we believe that the somewhat drier conditions during sampling in 1985 than in 1986 may explain the striking differences in populations of INA bacteria in the peach extensive survey.

In our studies, INA bacteria were present in some orchards on both apple and peach flowers during the spring when freeze damage would most likely occur. However, our failure to detect them in more than half of the apple and peach orchards sampled during the bloom periods of 1985 and 1986 and the work of others (2,7) raise doubts as to whether

INA bacteria alone play a major role in freeze losses on these crops in the southeastern United States. Bentley and Zehr (7) failed to control frost damage in two peach orchards in South Carolina with antibiotics or ice nucleation inhibitors. Antibiotic or copper sprays applied during the critical bloom period have failed to control frost damage in Georgia on apple and peach (*unpublished*). The systemic invasion of fruit trees by *P. s.* pv. *syringae* would also hinder control efforts (13,37). The presence in the tissue of woody fruit trees of intrinsic nonbacterial ice nuclei that are operative at the same temperatures (−1.5 to −5 C) as INA bacteria may account for major frost damage (2–4,10,17).

Most of the 286 strains of INA bacteria that were isolated from apple and peach trees and characterized in the present study fit the description of *P. s.* pv. *syringae*. They were negative for oxidase and arginine dihydrolase and utilized erythritol, DL-lactate, and sucrose (18). All except three strains gave an HR in tobacco (23) and 84% produced syringomycin (11) or a similar antifungal compound. Although levan production is reportedly useful in identifying fluorescent pseudomonads (24), only about half of our strains gave a definite positive test. The usefulness of levan production for differentiating pseudomonads has been questioned (30). Only 5% of the strains were pectolytic when tested on polypectate media (18). Since strains of *P. s.* pv. *syringae* are characteristically nonpectolytic, a few of our strains were not identified to species. Our results are similar to those reported by Gross et al (16) in the Pacific Northwest. They found that all 82 strains of INA bacteria from deciduous fruit trees tested were *P. syringae*, 96% of the strains produced an HR in tobacco, 82% produced syringomycin, and 78% produced levan.

If there is linkage relationship between tobacco HR and pathogenicity, as generally assumed (23), essentially all the INA strains we isolated from apple and peach trees had pathogenic potential. However, the strains proved to be extremely diverse in pathogenicity and virulence on mature green tomato fruit, green bean pods, and peach seedlings. Some strains from both apple and peach trees were highly virulent on peach seedlings. Some strains from both hosts were not pathogenic on any host tested. Gross et al (16) found that only 50% of the INA strains of *P. syringae* from fruit trees in the Pacific Northwest were pathogenic on immature pear and sweet cherry fruit, although essentially all the grains gave an HR in tobacco. They found no host specificity of pome and stone fruit strains; strains that were highly virulent on cherry were also highly virulent on pear. Roos and Hattingh (36) contend that each fruit variety supports a heterogeneous population of *P. s.* pv. *syringae*, and some of the strains may be more virulent on other hosts. Disease expression may depend on the proper host-pathogen combination, critical environmental conditions, and tree stress (36). In our pathogenicity tests, there was no evidence of host specificity among the peach or apple strains of INA *P. s.* pv. *syringae*. Additional host range studies are needed because strains that were nonpathogenic on the three hosts tested could be pathogenic on other hosts or under other conditions, since they did elicit an HR in tobacco. Baca et al (6) inoculated green tomato fruit and found variation in pathogenicity among INA strains isolated from seven woody plant species in nurseries in the Pacific Northwest.

The occurrence of streptomycin resistance among INA strains of *P. s.* pv. *syringae* from apple was related to the intensive use of the compound for fire blight and, in one orchard, for blister spot control. Recently, Burr et al (9) reported widespread resistance to streptomycin among strains of *P. s.* pv.

Table 3. Populations of ice nucleation-active (INA) bacteria associated with peach flowers collected at various locations in Georgia during 1985 and 1986

Orchard designation	Orchard location (county)	Bacterial counts[a] (cfu/g green tissue)
	1985	
1	Brooks	0
2	Brooks	0
3	Brooks	0
4	Brooks	0
5	Brooks	0
6	Crawford	0
7	Taylor	0
8	Taylor	0
9	Peach	0
10	Peach	0
11	Peach	0
12	Talbot	3×10^2
13	Talbot	0
	1986	
101	Crawford	TNTC[b]
102	Crawford	TNTC
103	Crawford	1.4×10^5
104	Taylor	TNTC
105	Taylor	0
106	Peach	1.7×10^5
107	Peach	TNTC
108	Oconee	TNTC

[a] Appropriate dilutions of macerated flowers were plated on medium B of King et al (22) amended with 100 μg/ml of cycloheximide and counts made after 48–72 hr at 25 C. The sample was a composite of 20–30 twigs from 20 trees in each orchard. INA bacteria counts were determined at −5 C by a replica-plate method of Lindow et al (27).

[b] TNTC = too numerous to count. Populations of INA bacteria were so high that the entire plate froze before individual colony counts could be determined. Populations were estimated to be above 10^4 cfu/g.

Table 4. Pathogenicity of ice nucleation-active strains of fluorescent bacteria obtained from apple and peach trees on mature green tomato fruit, green bean pods, and peach seedlings

Plant or plant part inoculated[a]	Source of strain	Number of strains tested	Number of strains with different levels of virulence[b]			
			−	±	++	+++
Green tomato fruit	Apple	151	16	122	13	0
	Peach	117	8	68	30	11
Green bean pod	Apple	151	3	76	66	6
	Peach	117	3	38	66	10
Peach seedling	Apple	114	30	57	23	4
	Peach	66	31	14	15	6

[a] Green tomato fruit and green bean pods were inoculated by placing drops of inoculum (10^8 cfu/ml) on the fruits and stabbing through the drops with a dissecting needle. Peach seedlings were injected in the shoot tip with 0.3 ml of a suspension (10^8 cfu/ml).

[b] Based on a relative scale where − = no disease, ± = lesion limited to the point of inoculation, ++ = lesion ≤ 1.0 cm on tomato or green bean or moderately severe on peach, and +++ = lesion > 1.0 cm on tomato or green bean or shoot tip death beyond point of inoculation on peach seedlings.

Table 5. Streptomycin and copper resistance among ice nucleation-active strains of *Pseudomonas syringae* obtained from apple and peach trees in Georgia

Host of origin	No. of orchards	No. of strains tested	No. of strains that grew on gradient plates[a]		No. of strains that grew on plates with different levels (μg/ml) of streptomycin sulfate or copper[b]							
					Streptomycin				Copper			
			Strepto-mycin	Copper	25	50	100	200	7.5	15	30	60
Apple	9	140	109	122	109	109	109	96	119	117	61	52
Peach	8	119	7	69	6	6	6	2	66	42	2	0

[a] Gradient plates were prepared by pouring plates with 15 ml of CYE medium (42) amended with 200 μg/ml of streptomycin sulfate or 60 μg/ml CU^{++}, allowing the plates to solidify in a slanted position, and pouring an additional 15 ml of unamended CYE, which provided low to high levels of chemical across the plate. The strains were streaked perpendicular to the chemical gradient with a cotton-tipped applicator dipped in a cell suspension, and growth was recorded after 48 hr at 25 C.

[b] CYE medium was amended with streptomycin sulfate or Cu^{++} and streaked with a cotton-tipped applicator dipped in a cell suspension; growth was recorded after 48 hr at 25 C.

papulans from western New York and showed an association between the number of sprays and the occurrence of resistant strains. Resistance was associated with a conjugative plasmid. We isolated *P. s.* pv. *papulans* from one Mutsu apple orchard during this work but did not test it for streptomycin resistance. Resistant strains of *P. s.* pv. *syringae* occurred rarely in samples from peach orchards where streptomycin had never been used. Although earlier work with *E. amylovora* showed that resistant strains may be found where streptomycin was not used (38), there was evidence that its use provided a selective pressure that favored development of resistant populations (31). Our results provide evidence that streptomycin use results in the development of resistance among nontarget organisms, in this case INA strains of *P. syringae*. The rationale for the presence of copper-tolerant strains on apple and peach trees is not apparent, since copper compounds are not currently used on these crops. The known existence of streptomycin-resistant strains of *P. syringae* plus the presence of copper-tolerant (1) strains in certain orchards may limit the usefulness of these compounds for control of INA bacteria, as has been suggested in earlier reports (25,26,28).

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