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Distribution of Ice Nucleation-Active Bacteria on Plants in Nature

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A replica plating method for rapid quantitation of ice nucleation-active (INA) bacteria was developed. Leaf washings of plant samples from California, Colorado, Florida, Louisiana, and Wisconsin were tested for the presence of INA bacteria. Of the 95 plant species sampled, 74 were found to harbor INA bacteria. Only the conifers were, as a group, unlikely to harbor INA bacteria. All of the INA bacteria isolated resembled either *Pseudomonas syringae* or *Erwinia herbicola*. Sufficient numbers of INA bacteria were present on the samples to account for the ice nuclei associated with leaves that are necessary for freezing injury to occur. Numbers of INA bacteria were large enough to suggest that plant surfaces may constitute a significant source of atmospheric ice nuclei.

Ice nucleation-active (INA) strains of *Pseudomonas syringae* van Hall and *Erwinia herbicola* (Löhnis) Dye have been shown to incite frost damage to corn and other plants (2, 36). Leaf surface populations of these bacterial species limit supercooling in the plant parts on which they reside by initiating damaging ice formation at temperatures of −2 to −4°C (37). Since plants do not have intrinsic ice nuclei active at these relatively warm temperatures, INA bacteria have a primary role in limiting supercooling and, thus, inciting frost damage to plants in nature.

Large epiphytic populations of INA strains of both *P. syringae* and *E. herbicola* have been found on corn under field conditions (37). However, the general involvement of these bacteria in inciting frost damage to plants in nature would require their ubiquitous presence on frost-sensitive plants at the time of frost hazard.

Strains of *P. syringae* (all phytopathogenic, oxidase, and arginine dihydrolase negative, fluorescent pseudomonads grouped under the nomenspecies *P. syringae* by Doudoroff and Palleroni [11]) have been found to be present, without causing disease, on such diverse plants as cherry (6, 7, 9, 17); pear (8, 38); peach (12, 18); olive (15); bean (16, 33); soybean (30–32); and hairy vetch, alfalfa, red clover, and lilac (16). Although *P. syringae* is pathogenic to many plants, it has been found as a resident on a number of apparently healthy plants, including both known hosts and plants not known to be hosts. *Erwinia herbicola* has been found on many plants, such as vegetables (4, 19); a number of grasses, including perennial rye grass, sugarcane, barley, rye, oats, and wheat (10, 14, 20, 22, 23, 28, 29, 46); seeds of rice, wheat, oats, rye, clover, and timothy (13, 24, 39, 45, 46; H. Chcanowska, Rev. Appl. Mycol. **45**:436, 1966); cotton (3); and leaves and flowers of fruit trees (5, 21, 40). However, the importance of bacterial ice nucleation in frost injury has been recognized only recently (2, 36, 37), and the isolates obtained in the earlier studies were not tested for ice nucleation activity. Thus, the distribution of INA bacteria has not been reported previously.

The present study examines the quantitative distribution of INA strains of both *P. syringae* and *E. herbicola* on diverse plants in different geographical locations in the United States to substantiate the possibility of their general involvement in inciting frost damage to plants. A preliminary report has been published (S. Lindow et al., Proc. Am. Phytopathol. Soc. **4**:107, 1977).

MATERIALS AND METHODS

Sampling technique. Samples collected near Madison, Wis., were placed in sterile plastic bags and plated immediately upon return to the laboratory. Samples from distant locations were sent to Madison by air express, by air mail, or, in some cases, hand-carried, with time between collecting and plating being 1 to 3 days in most cases. (The effects of this delay have not been determined, but comparisons of results with those samples collected locally have not suggested any gross differences.) Samples from southern Florida were supplied by R. S. Cox from December 1976 to March 1977, and by D. J. Hagedorn on 30 December 1976. California and Colorado samples were collected by R. C. Schnell in April and June 1976,

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respectively. The Louisiana samples were collected on 4 June 1976. Wisconsin samples were assayed in May and October of 1976 and in April and May of 1977. In the distribution study, single samplings for most plant species locations were made. For seasonal population trends, at least one sample was taken from each control (nontreated) plot in each of several replications.

Several whole leaves made up the sample for small-leaved species; portions of at least two leaves were used for large-leaved species. Samples of ca. 10 to 20 g were cut into pieces ca. 5 cm^2 and placed in 500-ml Erlenmeyer flasks with 200 ml of 0.1 M phosphate buffer (pH 7.0) with 0.1% (wt/vol) peptone (Difco). Flasks were shaken on either a rotary or a reciprocal shaker for ca. 2 h before appropriate dilution plantings were made on nutrient agar (NA) for total population or NA + 2.5% (vol/vol) glycerol (NAG) for INA bacteria. Several other media were used in the INA assays, i.e., Crosse medium (6), King medium B (27), and nutrient dextrose agar with 50 μg of cycloheximide per ml; INA colonies were most consistently detected on NAG, however. Leaf washings were all plated on NA, NAG, and at least one of the other media; there was usually good agreement between total colonies on NA and either NAG or King medium B, and between INA colonies on NAG and either King medium B or Crosse medium. In preliminary assays, homogenization of leaves was compared with leaf washing, and similar results were obtained. Since the leaf washing was more expeditious, it was used for the assays reported here.

Detection of INA colonies by replica freezing. A rapid system for assaying colonies was patterned after the replica plating technique developed by Lederberg and Lederberg (34). A square of sterile velvet cloth stretched across a circular rubber block was first pressed against the surface of the dilution plate with colonies to be assayed and then pressed onto a sheet of paraffin-coated aluminum foil. The replica on the paraffin surface retained substantial numbers of bacteria from the original dilution plate. Six such replicates were made on a sheet (20 by 25 cm) of foil. The edges (ca. 1 cm) of the sheet were then folded upward to form a "boat" which could be floated on the surface of a refrigerated bath held at the desired temperature, usually −5°C. A fine mist of the ice nucleus-free water was sprayed on the surface of the boat. After about 2 min, discrete areas of ice (having a frosty, white appearance) were enumerated visually for each dilution plate. Ambiguous areas were tested physically for solidity with the tip of a sterile toothpick. From the total number of colonies, the number of areas of ice formation and other pertinent values, the total and INA bacteria per gram (fresh weight) of sample were calculated. Pure cultures of INA isolates were obtained by making transfers from the areas of ice formation on the −5°C surface and restreaking to obtain single bacterial colonies.

Since ice nucleation activity of bacteria can be strongly influenced by the medium on which the cells are grown (36), the quantitation of INA bacteria can be strongly influenced by the medium on which the cells are plated. In addition, when only a small fraction (<0.1%) of the total colonies are active, plates bearing very large numbers of colonies must be tested by the replica freezing procedure. Active colonies can be missed under these conditions due to intercolony antagonism on our nonselective media. Thus, the accuracy of the procedure varies from nearly as good as that of dilution plating (when INA bacteria represent a high proportion of the population) to only an order of magnitude estimate (when INA bacteria represent <0.1% of the total), and many estimates of INA bacterial populations are probably low.

RESULTS

Geographic distribution. (i) California samples (6-8 April 1976). Total bacterial populations varied from 6×10^5 to over 10^9 cells per g (fresh wt), with most samples being close to 10^7 cells per g (Table 1). When more than one sample from the same plant species was assayed, total populations were similar in most cases. Populations of INA bacteria varied widely among species, and, in some cases, such as *Prunus domestica*, from sample to sample from the same species. *Juglans regia*, *Prunus dulcis*, and one sample of *P. domestica* had the highest INA bacterial populations. INA bacteria made up widely varying fractions of the total population, ranging from nearly 41% for *J. regia* to <0.001% for tomatoes.

All of the INA bacteria isolated in pure culture from these samples resembled *P. syringae*, i.e., they produced a fluorescent green pigment on King's medium B and gave negative oxidase and arginine dihydrolase reactions. Twelve *P. syringae*-like isolates from six of the plant species also incited frost damage to corn seedlings when applied 24 h before freezing at −5°C.

The leaf-washing liquid from the *P. dulcis* sample was tested directly for ice nucleation activity by using a droplet-freezing procedure previously described (36, 43). As droplet temperature was gradually lowered, ice nucleation was detected at −1.9°C, and all 30 10-μl droplets were frozen at −2.4°C, indicating that the washing liquid contained >340 nuclei/ml that were active above −2.4°C. On the basis of the replica plating of a dilution series, the number of INA bacteria in the leaf wash was ca. 5×10^5 cells per ml.

(ii) Colorado samples (8 June 1976). Very low total populations of bacteria were found, ranging from 10^2 to 2.4×10^6 cells per g (fresh weight). Populations on conifers were especially low (10^2 to $>10^4$ cells per g). Bacteria with ice nucleation activity were detected on only 3 of the 13 species assayed. Species without detectable INA bacteria included both annuals and perennials. All INA bacteria isolated from these plants resembled *P. syringae*.

(iii) Florida samples (15 December 1976 to March 1977). Total bacterial populations varied widely, ranging from 9×10^3 on *Nicotiana*

TABLE 1. *Total and INA bacterial populations in leaf washings from various plant species and locations in the United States*

Genus and species[a]	{Log [cells/g (fresh wt)] – [total/INA]}[b]						
					Wis.		
	Calif.	Colo.	Fla.	La.	May 1976	Oct 1976	Apr–May 1977
Annuals							
Allium cepa L.		5.2/2.5					
Amaranthus sp. L.			6.0/3.1				
Ambrosia trifida L.					5.9/4.6 (2)		
Beta vulgaris L.						4.9/4.3	
Brassica hirta Moench.			7.5/5.4				
B. oleracea L. Gongylodes group						3.2/ND[c]	
B. oleracea L. Capitata group		3.2/ND				4.1/1.5	
B. oleracea L. Botrytis group						4.4/ND (2)	
B. rapa L.						4.4/2.9	
Capsicum frutescens L.			8.4/6.9			5.1/4.6	
Citrullus lanatus (Thunb.) Matsum. & Nakai						5.2/4.9	
Cucumis melo L.						5.2/3.5	
C. sativus L.			7.7/5.1			6.5/4.3 (2)	
Cucurbita maxima Duchesne			7.7/4.2			4.8/2.9	
Glycine max (L.) Merrill			6.1/2.3				
Hordeum vulgare L.		2.4/ND					
Lactuca sativa L.			7.8/5.6				
Lycopersicon esculentum Mill.	8.5/ND		8.5/7.4 (2)			4.5/2.9	
Mimosa L. sp.				6.7/ND			
Nicotiana tabacum L.			3.9/ND				
Phaseolus vulgaris L.			7.4/4.4 (2)			6.4/5.2 (2)	
Pisum sativum L.					5.7/2.8 (2)		
Raphanus sativus L.			8.0/6.3				
Solanum tuberosum L.						5.5/2.9	
Tagetes patula L.						7.1/5.0	
Triticum aestivum L.							4.5/2.9
Zea mays L.	6.4/4.5	3.2/ND	6.1/3.3		6.1/ND	7.3/3.3	
Zinnia elegans Jaeg.						6.7/6.2	
Woody plants							
Acer negundo L.					6.9/6.8		
Amelanchier arborea (Michx.f.) Fern.					5.5/3.6	6.1/ND	
Brassaia actinophylla Endl.			7.4/4.7				
Carissa grandiflora (E. H. Mey.) A. DC.			6.5/ND				
Carya ovata (Mill.) C. Koch					6.3/3.0	6.0/4.0	
Citrus L. sp.			7.1/2.8 (4)				
Codiauem variegatum (L.) Blume.			5.4/ND				
Elaeagnus angustifolia L.		4.5/ND					
Juglans nigra L.					6.5/4.9 (3)	4.9/3.4 (2)	
J. regia L.	7.7/7.3 (2)						
Lagerstroemia indica L.				7.1/5.8 (2)			
Livistona R. Br. sp.				5.3/ND			
Magnolia virginiana L.				6.2/4.1			
Malus sylvestris Mill.					6.9/5.6	6.5/3.3	
Platanus occidentalis L.		2.9/ND					
Populus angustifolia James		4.7/2.6					
P. sargentii Dode		3.3/ND					
P. tremuloides Michx.							3.8/3.2
Prunus americana Marsh.					6.3/4.7 (2)		
P. armeniaca L.					7.6/7.1 (2)	4.7/ND	
P. avium (L.) L.	6.6/4.1				7.3/6.9	5.1/3.4	
P. cerasus L.						5.0/2.3	
P. domestica L.	6.9/6.1 (3)				7.1/7.0 (3)	5.2/3.1	

TABLE 1.—*Continued*

Genus and species[a]	{Log [cells/g (fresh wt)] – [total/INA]}[b]						
	Calif.	Colo.	Fla.	La.	Wis.		
					May 1976	Oct 1976	Apr–May 1977
P. dulcis (Mill.) D. A. Webb	7.5/6.4						
P. persica (L.) Batsch.	7.5/5.6 (2)						
P. serotina J. F. Ehrh.					5.1/3.1		4.9/3.3
P. virginiana L.					5.2/4.1		
Pyrus communis L.	6.5/4.8				5.2/3.6	6.1/ND	
Quercus macrocarpa Michx.						3.8/2.1	6.4/5.9
Q. rubra L.						4.7/ND	3.8/1.9
Q. virginiana Mill.				5.7/ND			
Ribes L. sp.							6.9/6.4
Rubus idaeus L.					4.4/2.7		
Sambucus canadensis L.							6.2/5.5
Sorbus americana Marsh.					4.9/2.5		
Syringa vulgaris L.					4.2/3.7		
Vitis riparia Michx.							2.9/1.8
V. vinifera L.	6.6/4.7					4.6/3.1	
Herbaceous perennials							
Agropyron repens (L.) Beaurois.							4.4/4.1
Asparagus officinalis L.	7.3/4.8				4.1/2.4		
Chrysanthemum x *morifolium* Ramat.			6.5/2.1				
Fragaria chiloensis (L.) Duchesne						4.9/4.1	
Medicago sativa L.		4.5/ND			6.8/4.9 (2)	5.2/4.7	7.3/5.8
Musa L. sp.				6.3/3.7			
Narcissus pseudonarcissus L.					5.2/3.7		
Oenothera biennis L.							7.3/6.5
Pelargonium x *hortorum* L. H. Bailey			7.4/6.0				
Phyllostachys Siebold & Zucc. sp.				5.9/4.5			
Poa pratensis L.		6.4/4.2					
Rheum rhabarbarum L.						3.6/ND	
Taraxacum officinale Wiggers							5.9/4.6
Trifolium pratense L.							4.2/3.0
Conifers							
Abies balsamea (L.) Mill.					3.4/ND	3.3/2.1	
A. concolor (Gord.) Lindl. ex Hildebr.						2.1/ND	
A. fraseri (Purgh) Poir.					5.4/ND		
Juniperus virginiana L.		4.8/ND					3.1/2.6
Picea glauca (Moench) Voss					5.9/ND	3.1/ND	2.2/ND
P. pungens Engelm.						3.8/ND	
Pinus ponderosa Dougl. ex P. Laws. & C. Laws.		2.6/ND (2)					
P. resinosa Ait.					4.8/ND	2.1/ND	2.8/ND
P. strobus L.						5.2/ND	3.1/ND
Pseudotsuga menziesii (Mirb.) Franco		2.3/ND			5.1/2.9	3.1/ND	
Tsuga canadensis (L.) Carriere							2.9/ND

[a] Latin binomials follow those in *Hortus Third: a Concise Dictionary of Plants Cultivated in the United States and Canada*, 1976, MacMillan, New York.

[b] First figure of each pair is log of the total bacterial population; the second is the log of the INA population. Assays were on single samples except where noted with figure in parentheses. Where multiple samples were assayed, the log of the means is given.

[c] ND, INA bacteria were not detected, i.e., <100 cells/g (fresh weight).

tabacum to 10^8 cells per g (fresh weight) on *Lycopersicon esculentum* and *Raphanus sativus*. The INA bacterial populations also covered a wide range, representing <0.01% of the population for Natal plum to 7.1% for tomato. No INA bacteria were detected on 3 of the 18 species

assayed. Relatively low populations of INA bacteria were found on *Citrus* sp., and none was detected on several waxy-leaved ornamentals. Both *E. herbicola* and *P. syringae* were isolated from these samples in approximately equal numbers.

(iv) Louisiana samples (4 June 1976). Colonies of INA bacteria (mostly *E. herbicola* and some *P. syringae*) were obtained from four of the seven plant species sampled.

(v) Wisconsin samples (May 1976 and April and May 1977). Various plant species were assayed. Dormant twigs, leaves, and flowers were used from some woody plants, depending on developmental stage. Total populations ranged from 10^3 to 6×10^7 cells per g (fresh weight). INA bacteria were not detected on six of the eight conifers tested. In young leaves of *Acer negundo* and blossoms and young leaves of *P. domestica*, *P. armenica*, and *P. avium*, very high INA bacterial populations were found—more than 10^7 INA cells per g (fresh weight)—up to 95% of the total bacteria present. In May 1976, all INA bacteria isolated in pure culture for additional characterization resembled *P. syringae*; in the spring of 1977, both *P. syringae*- and *E. herbicola*-like isolates were obtained.

(vi) Wisconsin samples (October 1976). Total bacterial populations were lower than in the May assays, particularly on conifers and deciduous tree leaves. However, several herbaceous annuals, such as *Tagetes*, *Cucumis*, *Phaseolus*, *Zea*, and *Zinnia*, had relatively high total populations (10^6 to 10^7 cells per g [fresh weight]). INA populations on the *Prunus* species that had been very high in May were generally much lower in October, as had been reported by others for *P. syringae* (7, 38). No INA bacteria were detected on six of the seven conifer species tested. Colonies resembling both *P. syringae* and *E. herbicola* made up nearly equal fractions of the cultures isolated in October.

In addition to the species listed in Table 1, INA bacteria have been found on *Ulmus americana*, *Populus alba*, and *Cucurbita pepo* in Wisconsin at various times during the growing season. Seasonal variations of number of INA bacteria on *C. pepo* in Wisconsin will be discussed below.

Summary of plant species and INA bacteria. One or more samples from each of 95 plant species was assayed quantitatively for total and INA bacteria. The geographical distribution and plant type are summarized in Table 2. In all species sampled, except those from Colorado, the majority carried detectable levels of INA bacteria. Overall, about three-quarters of the species carried INA bacteria. For annuals, herbaceous perennials, and woody perennial plants other than conifers, a large majority of the species carried INA bacteria, but for conifers the converse was true. On the conifers, total as well as INA bacterial populations were generally low.

TABLE 2. *Summary of numbers of plant species from which INA bacteria were isolated, compared with those from which INA bacteria were not detected, on the basis of geographical source of sample and type of plant from which samples were obtained*

Source of samples	No. of plant species	
	INA bacteria present	INA bacteria not detected
Location		
Calif.	9	1
Colo.	3	10
Fla.	15	3
La.	4	3
Wis.	54[a]	10
Type of plant		
Annuals	25	5
Herbaceous perennials	13	1
Woody angiosperms	33	7
Conifers	3	8
Total no. of different species[b]	74	21

[a] Three species, *Ulmus americana*, *Populus alba*, and *Cucurbita pepo*, are included in totals, but are not in Table 1.
[b] Duplications of plant species eliminated.

Characteristics of INA bacteria collected. During 1975 and 1976, more than 1,000 isolates of INA bacteria recovered from leaf washings of a large number of plant species were selected at random for further characterization in pure culture. Of these, 24% were yellow pigmented and resembled *E. herbicola* in appearance. All of these isolates grew on NA containing 0.1% 2,3,5-triphenyl 2H-tetrazolium chloride or 5% NaCl, conditions which would inhibit the growth of most *Xanthomonas* species (35). The other 76% of the INA isolates resembled *P. syringae*, i.e., they produced a fluorescent pigment and gave negative oxidase and arginine dihydrolase reactions. The greater number of *P. syringae* isolates may have been due, in part, to the fact that the majority of the isolations were made in the early part of the growing season when *P. syringae* seems to predominate. After July 1, *E. herbicola* was found frequently and predominated on some samples.

The plant samples assayed for total and INA bacteria were essentially normal in appearance, i.e., they were free of any apparent disease symptoms. Although *P. syringae* isolates were obtained from many samples, no obvious pathogenic relationships were involved. The pathogenic potential of the *P. syringae* isolates has not yet been determined.

Seasonal trends of epiphytic bacterial populations. Samples were taken at intervals

of 1 to 3 weeks from replicated plots of several crop plants near Arlington, Wis. during the 1976 season, and total and INA bacterial populations were determined. Seasonal trends of these bacteria on bean, tomato, soybean, and pumpkin leaves are shown in Fig. 1. On snap bean and tomato leaves, there was a gradual increase in both total and INA bacteria throughout the season, with the INA fraction being about 1% on beans, but only 0.3% on tomatoes. This trend is similar to that on corn reported earlier (37), in which total populations reached 10^7 to 10^8 cells per g (fresh weight) late in the season and INA bacteria comprised about 1% of the total population. Although the total bacteria on soybean and pumpkin leaves increased during the season, the estimates of the INA populations were somewhat erratic. For soybeans, the INA populations showed a decrease in late August, but the September readings were again quite high. The pumpkin INA population reached a maximum of 10^5 in early August and later decreased to 3×10^4 cells per g (fresh weight) in September.

In general, these annual plants had relatively low bacterial populations early in the season with rather substantial increases as the season progressed. The INA fraction was approximately proportional to the total population and was typically 1% or less. However, it is apparent that INA bacteria are present throughout the season and are in substantial numbers late in the season. In contrast, some of the woody perennials, e.g., *Prunus* spp., had very high populations of both total and INA bacteria early in the season.

DISCUSSION

INA bacteria are commonly present as epiphytes on a diversity of plant species from widely separated areas of the United States. The distribution of INA bacteria appears to be sufficiently broad that we can conclude that INA bacteria are present on plants in sufficient numbers and with sufficient frequency to adequately explain the lack of supercooling of frost-sensitive

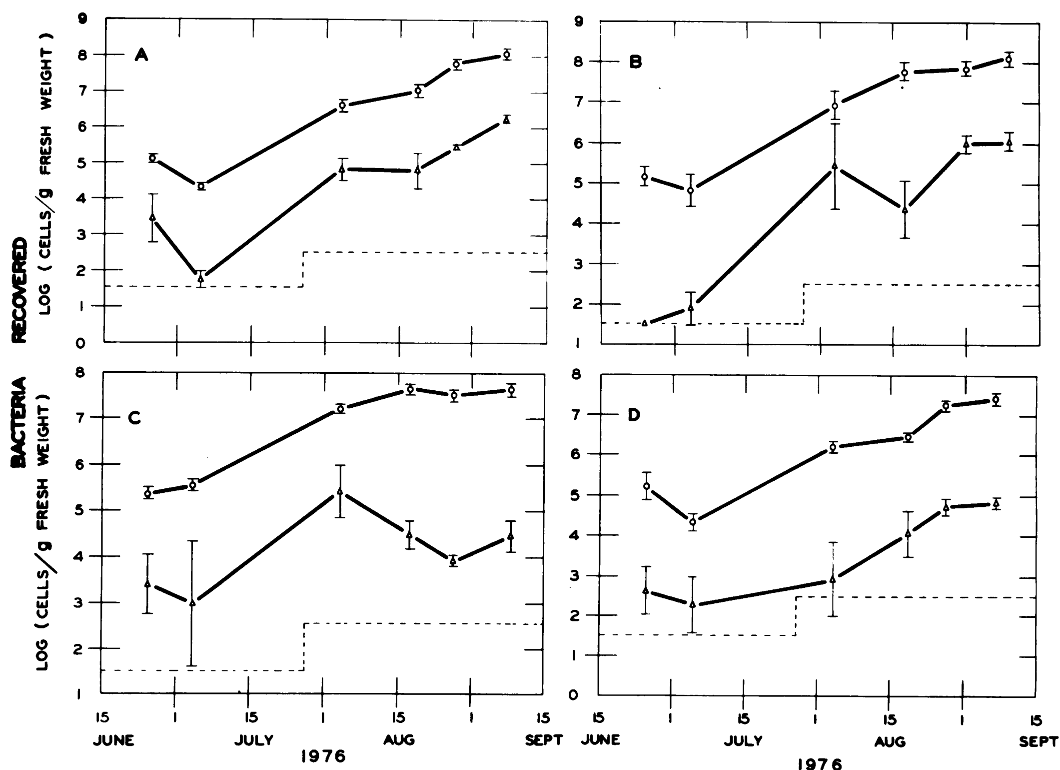

FIG. 1. *Total and INA bacterial populations on normal, nontreated leaves of (A) beans; (B) soybeans; (C) pumpkins; and (D) tomatoes, sampled during the 1976 growing season. Total bacteria (○) were determined on NA, and INA bacteria (△) were determined on NAG by dilution plating of washings of 10 to 20 g of leaves of each species from each of three replications at each date. Vertical bars represent the standard error of the mean of log populations from the three separate determinations of each sampling date. Dashed lines indicate the estimated limits of detection of INA bacteria.*

plants below about −2 to −5°C. INA bacteria were detected on 74 of the 94 plant species sampled. Of the remaining 20 plant species, 8 were conifers and 12 were based on assays of single samples. Because the limit of sensitivity of the replica freezing technique is ca. 100 cells per g or ca. 0.1% of the total population, whichever is larger, INA bacteria may not have been detected on some samples, even though they may have been present. Thus, negative findings based on a single sample should be considered tentative. The limit of sensitivity of the replica freezing technique is high enough that we could have missed INA bacterial populations sufficient to prevent supercooling of the sample leaves. Replicated samples from field trials frequently did not produce positive values for each sample in spite of the fact that the overall population of INA bacteria on the test plants was quite high. Conifers, however, appear to present a special case. Not only are the levels of INA bacteria either very low or below detection on these plants, but total numbers of epiphytic bacteria are also very low.

The overall abundance of INA bacteria on plant surfaces, taken together with the extent of (nonconiferous) terrestrial plant cover, may provide an excellent source of atmospheric ice nuclei active at relatively warm temperatures (41, 44). The content of atmospheric ice nuclei active at or above −15°C increases with movement of air masses over land (1) and may reflect the contributions of nuclei by terrestrial plants.

The seasonal trends of epiphytic bacteria for the four annual crops on which populations were followed through the season are in general agreement with those reported by Dickinson et al. (10) for *Lolium perenne*, for which populations rose from 1×10^5 to 2×10^5 colonies per g (fresh weight) in May to 11×10^6 in September on the older leaves, then back to 2×10^6 in October on newly formed leaves. Stout (42) found populations of 10^7 to 8×10^8 cells per g (wet weight) on the same grass species, with somewhat higher populations in summer and autumn and lower populations in winter and early spring. Kerling (26), in a study on *Beta vulgaris* leaves, reported a progressive increase from ca. 30 to 50 colonies per cm^2 in May and June to 2,000 to 3,000 colonies per cm^2 in July and August and then lower counts in September and October.

Kaku (25), in his freezing-temperature distribution studies with *Buxus* and *Ilex* species, found that mature leaves appeared to have more effective or a higher concentration of ice nucleators than did immature leaves at temperatures between −2 and −6°C, and that before full maturity, size did not affect ice nucleus content. This difference between immature and mature leaves could be explained on the basis of numbers of INA bacteria present, probably being fewer on young leaves and increasing with age, as has been shown in the present work for beans and tomatoes.

Pseudomonas syringae, which is a pathogen on many plants, has been found residing on a number of plants without causing disease symptoms in agreement with earlier reports, e.g., Ercolani et al. (16). It is possible that some isolates from a particular plant would be pathogenic on that plant species under the most favorable conditions. Thus, it is possible that the large populations of *P. syringae* present as epiphytes on leaves of most plants serve as a source of inoculum for infection of either the plant on which they reside or for nearby plants. However, we have not examined the pathogenic host range of the isolates that we obtained on the basis of ice nucleation activity.

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