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Effect of Plant Species and Environmental Conditions on Epiphytic Population Sizes of *Pseudomonas syringae* and other Bacteria

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ABSTRACT

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Selected biological and environmental effects influenced epiphytic colonization of plants by *Pseudomonas syringae, Escherichia coli, Salmonella typhimurium, Aeromonas hydrophila,* and *Rhizobium meliloti* when tested in a growth chamber at 24 C. Epiphytic population size varied with plant host, environmental conditions, and among strains of *P. syringae* tested. Strains of *P. syringae* achieved only slightly larger population sizes than strains from other genera when incubated on inoculated plants for 48 hr, and near 100% relative humidity (RH). However, the strains of *P. syringae* maintained populations at least 25 times higher after a subsequent 72 hr at 40% RH. Epiphytic population sizes of 15 different strains of *P. syringae* varied up to 10-fold on a given plant species, indicating epiphytic diversity within this bacterial species. Relative population sizes of three strains of *P. syringae* on plants under field conditions were predicted by growth chamber populations. Neither epiphytic strains, pathogenic strains, or toxin producing groups were associated with greater epiphytic population sizes. Different plant species varied up to 17-fold in the sizes of bacterial populations supported. Maceration of inoculated plant tissue increased bacterial population size estimates relative to cells removed by sonication, but only after low RH incubations.

Many species of bacteria, including phytopathogens, are found on aerial plant surfaces. Some may only casually occupy leaves, are unable to multiply, and soon disappear unless replenished (10). Others are epiphytic residents, able to grow and survive on plants. The relative importance of healthy plants to the natural life cycle and demographics of most bacterial species or of individual bacterial strains is largely unknown and may vary with such factors as plant species and climate.

Pseudomonas syringae is a diverse species containing phytopathogenic members representing many pathovars and strains that

also occur as plant epiphytes (10). Some strains are pathogenic to specific plant hosts, whereas hosts of others are unknown or possibly nonexistent (17). Many strains are capable of inciting warm temperature frost injury to sensitive plants by producing ice nuclei that catalyze ice crystal formation (20). Ecological diversity within strains of *P. syringae* with respect to epiphytic growth and survival has seldom been investigated quantitatively. Diversity is expected since different pathovars and strains are found at different times of year (4,22) at different spatial locations on a crop (1), on different plant species and at different geographical locations (7,19). An understanding of the factors that most strongly influence epiphytic growth and persistence, and of the variability among different strains in response to these factors, would identify the range of epiphytic adaptability within *P. syringae*.

Epiphytic population size may estimate the degree to which a given strain is ecologically suited to particular niches and has been used to describe various bacteria/plant interactions. High populations of particular bacterial species and pathovars are associated with higher probabilities of plant disease (24) and increased frost injury (16,20). Large epiphytic populations of antagonistic bacteria affect biological control of fireblight (28) and of frost injury (18). Epiphytic bacterial populations may also form significant sources of inoculum for spread, increasing the chance of successful colonization of adjacent plants. Quantitative comparisons of epiphytic population sizes among strains can thus be used to partially predict their epidemiology on plants.

The purpose of this study was to examine selected factors for their influence on bacterial population size in a random sampling of diverse strains of *P. syringae*. Preliminary results of this study have been reported (23).

MATERIALS AND METHODS

Bacterial strains. Nineteen different bacterial strains representing five genera were used in colonization studies. Fifteen strains of *P. syringae* were collected from diverse geographical locations (Table 1). Strains were tested in four groups due to sampling constraints and for comparison purposes. One group included nine strains isolated as epiphytes (Experiment 1). Two groups each included three strains isolated as leaf pathogens (Experiments 2 and 3). The fourth group included four bacterial species from genera not associated with plant leaves; *Rhizobium meliloti, Escherichia coli, Salmonella typhimurium,* and *Aeromonas hydrophila* plus three selected strains of *P. syringae* tested in previous experiments (Experiment 4) (Table 1). Spontaneous mutants resistant to 100 ppm of rifampicin were selected after spreading 10^9 cells on King's medium B (KB) plates

TABLE 1. Characteristics of bacterial strains used in epiphytic colonization experiments

Strain	Bacterial species	Source of strains[a]	Hosts infected	Toxin production[b]	Geographical origin	Supplier[c]
Experiment 1[d]						
4R	*Pseudomonas syringae*	Pear	None	0.0	Lafayette, CA	1
6R	*P. syringae*	Pear	None	0.0	Lafayette, CA	1
9R	*P. syringae*	Strawberry	None	0.0	Salinas, CA	2
468R	*P. syringae*	Almond	None	0.0	Modesto, CA	1
563R	*P. syringae*	Navel orange	Navel orange	9.5	Visalia, CA	1
584R	*P. syringae*	Navel orange	None	5.0	Visalia, CA	1
714R	*P. syringae*	Almond	None	0.0	Fresno, CA	1
1109R	*P. syringae*	Potato	None	10.0	Tulelake, CA	1
1199R	*P. syringae*	Potato	None	0.5	Hillsboro, OR	1
Experiments 2 and 3[e]						
Group 1						
821R	*P. syringae* pv. *syringae*	Tomato	Tomato[f]	0.0	Florida	3
765R	*P. syringae* pv. *lachrymans*	Cucumber	Cucumber	0.0	Ohio	6
407R	*P. syringae* pv. *syringae*	Bean	Bean	11.0	Wisconsin	5
Group 2						
22R	*P. syringae* pv. *pisi*	Pea	Pea	0.0	Oregon	4
230R	*P. syringae* pv. *coronafaciens*	Oat	Oat	0.0	Wisconsin	5
655R	*P. syringae* pv. *syringae*	Corn	Corn	4.5	Wisconsin	5
Experiment 4[g]						
RM1R	*Rhizobium meliloti*	Alfalfa root nodule	NT[h]	NT	unknown	7
EC1R	*Escherichia coli*	Fresh enteric isolate	NT	NT	California	8
ST1R	*Salmonella typhimurium*	Unknown	NT	NT	unknown	8
AH1R	*Aeromonas hydrophila*	Freshwater pond	NT	NT	California	9

[a] Epiphytes were recovered from washings of healthy leaves. Pathogens were recovered from lesions.
[b] Experimental procedure of Gross and DeVay 1977 (8) used for measurement of in vitro production of syringomycin or syringotoxin. Numbers shown are the average radius in millimeters of inhibition to *Geotrichum candidum*. Tests were replicated four times.
[c] Strains were obtained from the following sources: 1) S. E. Lindow, Univ. of California, Berkeley; 2) J. Lindemann, Advanced Genetic Sciences, Inc., Oakland, CA; 3) J. B. Jones, Univ. of Florida; 4) M. L. Powelson, Oregon State Univ.; 5) S. Hirano, Univ. of Wisconsin, Madison; 6) C. Leben, Ohio State Univ., Wooster; 7) Carolyn Napoli, Univ. of California, Berkeley; 8) K. Grant, Univ. of California, Berkeley; and 9) R. Hedrick, Univ. of California, Davis.
[d] Strains isolated as epiphytes.
[e] Strains isolated as pathogens.
[f] Strain 821R was pathogenic after stab inoculation but not by spray inoculation.
[g] Strains not normally associated with plant leaves.
[h] NT = not tested.

containing 100 μg/ml of rifampicin (KBR). All strains were stored in 15% glycerol at −80 C. Strains of *P. syringae* and appropriate control strains were tested for growth on minimal media, ice nucleation activity (19), pathogenicity on a range of hosts, levan production (15), oxidase reaction (26), tobacco hypersensitivity (12), arginine dihydrolase reaction (29), and syringomycin/syringotoxin production (8). For pathogenicity tests, all strains were spray inoculated as well as infiltrated into bean (*Phaseolus vulgaris* L. 'Bountiful'), tomato (*Lycopersicon esculentum* Mill. 'Bonny Best'), oat (*Avena sativa* L. 'Cal Red'), potato (*Solanum tuberosum* L. 'White Rose'), cucumber (*Cucumis sativus* L. 'National Pickling'), pea (*Pisum sativum* L. 'Spring'), and corn (*Zea mays* L. 'P×20') leaves and stems at a concentration of 10^6 cells/ml. All strains were also infiltrated into these plant species at a concentration of 10^8 cells/ml. Pear (*Pyrus communis* L. 'Bartlett') fruit and navel orange (*Citrus sinensis* (L.) Osbeck) seedlings were wounded by stabbing with a sterile toothpick and swabbed with a suspension of 10^6 cells/ml. Watersoaking and/or lesion development on leaves or stems were considered evidence for pathogenicity. Desiccation and necrosis at the inoculation site within 24 hr were considered a hypersensitive reaction.

Plant inoculation. All bacterial strains were grown for 48 hr at 24 C on KBR. Cells were suspended in sterile distilled water, their concentrations determined spectrophotometrically and adjusted to a concentration of 5×10^5 colony-forming units (cfu) per milliliter by dilution. Cell concentration was confirmed by dilution plating aliquots of each inoculation suspension onto KBR. The bacterial suspensions were sprayed to runoff with an atomizer onto 4–6-wk-old greenhouse-grown plants in 20-cm diameter pots. Plants were not overhead watered before inoculation to keep leaf surfaces relatively free of bacteria.

Five separate experiments using a total of seven different plant species were performed. The group of strains of *P. syringae* isolated as epiphytes were sprayed on bean Eagle, tomato Peto 95, oat Cayuse, and potato to form the first experiment. The two groups of strains isolated as plant pathogens were sprayed on bean Eagle; tomato Peto 95; oat Cayuse; corn; cucumber; and pea and formed experiments 2 and 3. Strains 714R, 22R, 821R, representing the highest populations achieved in each of experiments 1, 2, and 3, plus nonfoliar strains were sprayed on both bean Eagle and corn in the fourth experiment. Strains 714R, 9R, and 4R, representative of strains having high, intermediate, or low populations from experiment 1 were sprayed on bean Eagle in a fifth experiment to compare survival of strains in growth chamber and field conditions. Each strain was sprayed on one pot of each plant species in three or four replicates.

Plant incubation conditions. For each experiment, all inoculated plants were sequentially subjected to two different types of environmental conditions: humid with low light levels for 48 hr followed by either a dry, high light incubation of 72 hr or an uncontrolled outdoor environment for 12 days. The wet environment was achieved by enclosing the moistened plants in clear plastic bags at 24 C with a 10-hr light period (240 $\mu E\ m^{-2}\ sec^{-1}$) in a large growth chamber (Conviron, model PGW36). Plants were then unbagged and subjected to drying in the growth chamber that maintained 40% RH and 24 C with a 14-hr daily exposure of 960 $\mu E\ m^{-2}\ sec^{-1}$. The uncontrolled environment was 12 consecutive sunny days having average daytime maximum temperatures of 25 C and nighttime minimum temperatures of about 10 C and no rainfall.

Sampling procedure. Fifteen to 40 individual leaves (15–20 g fresh weight) were collected from each pot (replicate) of plants at each harvest date and tested for bacterial population size. Samples were harvested before inoculation, immediately before inoculation, immediately after inoculation, after wet and dry growth chamber incubation, or periodically during incubation of plants under field conditions. Immediately after inoculation and following high humidity incubation, leaf surfaces were left wet and weights were converted to dry leaf surface weights using conversion factors (range 0.70–0.95). The factors were unique to each plant species and based on measured water retention (data not shown).

Measurement of bacterial populations. Bacteria were removed from leaves by sonication (9) and enumerated by plating suitable 10-fold serial dilutions on appropriate media as described previously (19). After harvest, the leaves were submerged in 100 or 200 ml of sterile washing buffer containing 0.1 M potassium phosphate and 0.1% Bactopeptone, pH 7.0, and sonicated for 7 min in a Bransonic 52 ultrasonic cleaner at 20 C to remove cells from the leaves. Serial dilutions of sonicates from the uninoculated plants were plated on KB containing 50 μg/ml of benomyl and 100 μg/ml of cycloheximide (KBCB) and also SSM media (M. Sasser, J. Lindemann, and S. Hirano, *unpublished*) (12.0 g of sorbitol, 0.8 g of $K_2PO_4 \cdot 3H_2O$, 0.8 g of $K_3PO_4 \cdot 3H_2O$, 0.13 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of L-histidine, 128 mg of Cetrimide, and 15 g of agar in 1 L of H_2O). These media facilitated the determination of both total and pseudomonad background contamination, respectively. The plates were incubated at 24 C and bacterial colonies counted after up to 6 days. Appropriate 10-fold serial dilutions from all other samples were plated on KBCB containing 100 μg/ml of rifampicin (KBRCB). The plates were incubated at 27 C for 2–4 days, counted, and the bacterial population size per gram fresh weight of leaves determined. Distinctive colony morphologies among rifampicin-resistant strains permitted identification of mixtures of recovered bacteria on KBRCB. Mixtures were never found. After samples of leaf sonicates were removed for dilution plating, sonicates and leaves were pooled and ground for 20 sec at low speed in a blender and filtered through one layer of cheesecloth. Appropriate 10-fold serial dilutions of this macerate were plated on KBRCB to estimate total population sizes of *P. syringae*.

Statistical methods. Statistical computations were made using software provided by Statistical Analysis Systems (release 5.16) (SAS Institute Inc., Cary, NC) (27). The SAS GLM procedure was used to perform analysis of variance (ANOVA) on log-transformed population sizes to determine significant biological and environmental effects influencing epiphytic population sizes. Mean comparisons were made with the Ryan-Einot-Gabriel-Welsh (REGW) multiple range test. This test controls the type I experimentwise error rate and has a lower type II experimentwise error rate than many other tests (21). Linear regression analysis was used to test for a direct relationship between some variables.

Experiments 1–3 were split-split plot designs with environment as the whole plot, strain and plant as the subplots, and removal method as the sub-subplot. Experiment 4 was a split plot with environment as the whole plot and strain and plant as the subplots. Experiment 5 was a randomized complete block (RCB) design.

SAS was unable to analyze the entire split-split plot designs of experiments 1–3. Consequently, removal method was analyzed, together with interactions, in an RCB design. This does not affect the significance or mean comparisons of environment, strain, and plant effects but could change the significance of removal method and its interactions slightly. However, since both removal method and environment were at only two levels and were highly significant, these results are probably acceptable.

RESULTS

Biochemical, physiological, and microbiological tests confirmed the prototrophy and identity of the strains of *P. syringae* used (data not shown). Pathogenic strains infected only one host species (Table 1) with only two examples of cross infectivity. Limited watersoaking and small lesions were observed when 10^6 cells/ml of strains 407R and 655R were infiltrated into pea and cucumber, respectively. All strains, including epiphytic isolates, induced a hypersensitive reaction in most nonhost plants after infiltration at 10^8 cells/ml. Occasional hypersensitive responses were also observed after inoculation with 10^6 cells/ml. Although strain 563R was isolated as an epiphyte on healthy navel orange, it was also pathogenic to citrus. One-third of the strains of *P. syringae* were inhibitory to *Geotrichum candidum* in culture, an indication of syringotoxin or syringomycin production (Table 1).

The average of log bacterial population sizes on plants before inoculation was 3.4 and 1.8 cells/g fresh weight of bacteria

culturable on KBCB and SSM media for all experiments and all plant species tested. Plant species did not differ in the background population sizes of bacteria that were supported before inoculation (data not shown).

Although the density of bacteria in all suspensions sprayed onto plants was approximately 5×10^5 cells/ml, the numbers of cells retained on plants varied. Beans, tomatoes, and cucumbers retained equal numbers of bacteria (~4.3 log cells/g fresh weight) but significantly more than oats, peas, and corn (~3.4 log cells/g fresh weight). Strain 765R was recovered in lower numbers from leaf samples taken immediately after inoculation than other bacterial strains (data not shown).

General effect of biological and environmental factors on populations of *P. syringae*. Analysis of variance indicated that strain identity, plant species, physical environment, and removal method significantly affected the estimated size of epiphytic bacterial populations in all of four growth chamber incubations in which wet conditions were followed by dry conditions (Table 2). However, interactions of strain identity with plant species and environment, and interactions of environment with removal method, occurred in one or more experiments. Symptomatic tissue was observed after dry incubation for all pathogen and susceptible host combinations (except tomato and strain 821R), resulting in high population sizes. These data were excluded from the analysis of variance of main effects and interactions.

Variation in bacterial population sizes among strains, plants, physical environments, and removal methods. *Strains.* The relative ability of strains to establish and maintain epiphytic populations was measured by comparing mean population sizes of each strain averaged over all plants and both wet and dry environments (Table 3). Three groups of strains having significantly different population sizes were identified among the nine strains of *P. syringae* isolated as epiphytes. Two different classes were found in each of the two experiments using three strains isolated as pathogens (Table 3). Three different classes were identified when five genera were compared. In this last trial, the strains of *P. syringae* had the highest population sizes (averaged over all plants and wet and dry incubation environments), other genera were somewhat lower, and the strain of *Rhizobium meliloti* was not recoverable by the end of the dry incubation. Strains 714R, 821R, and 22R, which achieved the highest population sizes in three separate previous experiments, had equal population sizes when tested together (Table 3).

Epiphytically isolated strains 714R, 9R, and 4R represented the highest, median, and lowest population sizes, respectively, averaged over all plants and both controlled environments (Table 3). These strains maintained the same relative population sizes under field conditions in a subsequent trial when population sizes from beans over all harvest dates were averaged (Table 4).

Toxin production by strains of *P. syringae* was not associated with strains achieving and maintaining the highest population sizes since many strains producing toxins ranked lower than those that did not (Tables 1, 3, and 4).

Plant species. The relative ability of plant species to support epiphytic colonization was measured by comparing mean bacterial population sizes on each plant species, obtained from averages of all strains and two moisture and light intensity environments (Table 5). Three classes of plants supporting significantly different population sizes of bacteria were identified with epiphytically isolated strains of *P. syringae* as well as with both groups of pathogenic strains. However, particular plant species were not always associated with high or low populations. Beans supported significantly higher populations of bacteria than corn when strains from five different genera were compared.

Humidity and light environments. The effect of light intensity and humidity was measured by comparing mean bacterial population sizes after exposure to two incubation conditions for all plants and strains (Table 6). Populations increased after inoculation until the end of the wet incubation by 100- to 1000-fold. *Salmonella, Escherichia,* and *Aeromonas* strains, which are not normally found as epiphytes on leaves, multiplied on both beans and corn, although *Rhizobium* did not (Table 3). Population sizes of these bacterial genera recovered after dry, high light incubation averaged slightly less than 10% that of populations after wet incubation, except for *Rhizobium,* which was not recoverable after dry incubation (Tables 3, 5, and 6). From immediately after wet incubation to 12 days under field conditions, losses of all viable cells of *P. syringae* averaged 80%, although survival rates varied

TABLE 2. Summary analysis of variance (ANOVA) of biological and environmental effects influencing population sizes of *Pseudomonas syringae* on plants

	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	Strains isolated as epiphytes		Strains isolated as pathogens (group 1)[a]		Strains isolated as pathogens (group 2)[a]		Strains 714R, 22R, 821R and strains not isolated from from plant leaves	
Source of variation	df[b]	SS[c]	df	SS	df	SS	df	SS
Whole plot	7	...	7	...	7	...	5	...
Environment (E)	1	195.41***[d]	1	115.13***	1	136.88***	1	97.16***
Block (B)	3	13.52***	3	2.07*	3	3.46**	2	0.05
Error A	3	2.12	3	0.50	3	0.75	2	0.046
Subplots	70	...	31	...	31	...	26	...
Strain (S)	8	52.05***	2	11.67***	2	6.83***	6	82.23***
Plant (P)	3	19.59***	5	48.22***	5	40.14***	1	13.21***
S × P	24	15.69*	10	5.78**	10	10.16***	6	4.92***
E × S	8	20.14***	2	3.06***	2	0.14	6	7.93***
E × P	3	8.25***	5	7.95***	5	7.22***	1	1.51***
E × S × P	24	11.90	7	4.62**	7	1.08	6	0.79
Error B	423	154.69	225	47.75	223	50.37	48	5.66
Total	500	497.39	263	281.10	261	275.66	77	181.40
Removal (R)[e]	1	12.23***	1	11.15***	1	10.72***	...	...
E	1	201.49***	1	144.24***	1	149.08***	...	...
R × E	1	4.75**	1	3.38**	1	2.07*	...	...
B	3	14.16***	3	2.05	3	3.43	...	...
Error	494	266.70	257	121.27	255	111.21	...	...

[a] Data from susceptible host and pathogen combinations after dry incubation was deleted for ANOVA. Note df for E × S × P = 7.
[b] df = degrees of freedom.
[c] SS = sums of squares.
[d] Asterisks indicate: ***significance at $P = 0.001$; **significance at $P = 0.01$; *significance at $P = 0.05$.
[e] SAS was unable to handle the entire split-split plot so removal method was analyzed separately as a randomized complete block. Interactions not shown were not significant.

TABLE 3. Population sizes of bacterial strains averaged among nine plants under controlled conditions

Strain tested	Bacteria recovered (log cells/g fr wt) Wet incubation environment	Dry incubation environment	Mean of both environments
Experiment 1[u]			
584R	7.0 a[v]	5.8 a	6.3
468R	7.0 a	5.6 ab	6.3
563R	6.9 a	5.5 ab	6.2
714R	6.8 a	5.9 a	6.4
9R	6.7 ab	5.1 b	5.9
1109R	6.4 bc	5.1 b	5.8
6R	6.4 bc	5.1 b	5.8
4R	6.3 c	4.3 c	5.4
1199R	6.2 dc	5.6 ab	5.9
Mean of all strains	6.6	5.3	
Experiment 2[w]			
821R	6.9 a	5.0 a	6.0
407R	6.2 b	4.9 a	5.6
765R	6.2 b	4.8 a	5.6
Mean of all strains	6.4	4.9	
Experiment 3[x]			
22R	6.7 a	5.2 a	6.1
230R	6.4 b	5.0 b	5.8
655R	6.3 b	4.8 b	5.7
Mean of all strains	6.5	5.0	
Experiment 4[y]			
22R	6.9 a	5.0 a	6.0 a
821R	6.9 ab	5.1 a	6.0 a
714R	6.6 abc	5.2 a	5.9 a
AH1R	6.6 abc	3.6 b	5.0 b
EC1R	6.5 bc	3.5 b	5.0 b
ST1R	6.4 c	3.6 b	5.0 b
RM1R	3.0 d	0.0[z]	3.0 c

[u] Strains of *Pseudomonas syringae* isolated as epiphytes. Means reported are the average of four replicates of four different plant species for each strain from dilution plates of leaf macerates.

[v] For each column, means followed by the same lower case letter do not differ according to the Ryan-Einot-Gabriel-Welsh multiple range test ($P = 0.05$). Analysis of variance of these data appears in Table 2.

[w] Strains of *P. syringae* pathogenic to tomato, cucumber, and beans. Means reported are the average of four replicates of six different plant species for each strain from dilution plates of leaf macerates.

[x] Strains of *P. syringae* pathogenic to pea, oat, and corn. Means reported are the average of four replicates of six different plant species for each strain from dilution plates of leaf macerates.

[y] Strains of *P. syringae* 714R, 821R, and 22R and strains not isolated from plant leaves. Means reported are the average of three replicates of two different plant species for each strain from dilution plates of leaf sonicates.

[z] < 100 cells/g fr. wt.

greatly among strains (Table 4).

Recovery method. The method used to dislodge bacteria from leaves significantly affected the number of viable cells recovered when averaged over all strains, plants, and humidity/light environments tested (Table 2). Population estimates from dilution plating of leaf macerates equalled or were higher than from leaf sonicates, especially on tissue that had been subjected to drying (Table 6).

Interactions among removal method, physical environment, strain, and plant. A strong interaction between removal methods used to dislodge bacteria from leaves and the humidity and light conditions that prevailed immediately before harvest (Table 2) allowed comparison of the importance of each removal method for each type of environment (Table 6). After dry incubation, significantly lower estimates of population size were obtained from dilution plates of leaf sonicates than from leaf macerates in all experiments. Maceration increased the total bacterial population size estimated after dry incubation by approximately 70% compared with sonication only. However, the differences between recovery after sonication or maceration were smaller (40% with strains isolated as pathogens) or not significant (strains isolated as epiphytes) after wet incubations (Table 6). Regression analysis of all data indicated that the percentage change in population size upon drying estimated by sonication and maceration removal methods was independent of population size. This was true for both wet ($r = 0.14$) and dry ($r = 0.22$) environments.

Analysis of variance tests indicated a significant probability that light intensity and humidity determined the relative population sizes of individual strains in three of four experiments (Table 2). For example, strain 1199R attained a relatively low population size during wet incubation but sustained relatively lower losses during dry incubation (Table 3). Most strains exhibited only a slight relative survival advantage in one environment or the other. Regression analysis of population sizes of *P. syringae* revealed that the percent decrease in population size after change from wet to dry conditions was not directly related to the population size after wet conditions ($r^2 = 0.05$). However, the strains of *Aeromonas, Escherichia,* and *Salmonella* that achieved similar population sizes to representative strains of *P. syringae* after wet incubation had population sizes much smaller than the strains of *P. syringae* after dry incubation (Table 3). Light and humidity conditions also affected the population sizes of bacteria recovered from each plant species in all experiments (Table 2). However, mean comparisons failed to identify large differences in the relative bacterial population size on any plant species upon change from wet to dry incubation (Table 5).

Plant species differentially influenced the population sizes of all strains of *P. syringae* (Table 2). No strain had clearly higher populations on certain plants and clearly lower populations on other plants relative to other strains (epiphytically isolated strains not shown, Table 7). Instead, mean comparisons identified groups of plants where strain population sizes were significantly different and other groups of plants where these same strains had

TABLE 4. Comparison of field and growth chamber conditions on population size of *Pseudomonas syringae*

Strain tested	Bacteria recovered[v] (log cells/g fr wt)[x] Incubation conditions: Wet growth chamber	Field conditions: 3 days	5 days	12 days	Mean	Bacteria recovered[w] (log cells/g fr wt)[y] Incubation conditions: Growth chamber Wet	Dry
714R	7.5 a A[z]	6.8 a B	6.9 a B	6.9 a B	7.0 a	7.3 a	6.1 a
9R	7.3 a A	6.2 b B	6.0 b B	5.6 b C	6.3 b	7.0 ab	5.3 b
4R	7.3 a A	5.7 b B	5.2 c B	4.8 c C	5.8 c	6.8 b	4.6 c

[v] Experiment 5: Inoculated plants were grown for 48 hr at near 100% RH and 240 $\mu E/m^2$ in the growth chamber and then incubated for the time indicated under field conditions of sunny days and no rainfall.

[w] Experiment 1: Inoculated plants were grown for 48 hr at near 100% RH and 240 $\mu E/m^2$ followed by 72 hr at 40% RH and 960 $\mu E/m^2$ in a growth chamber.

[x] Means reported are average of three replicates for each strain at each sample time from dilution plates of leaf sonicates.

[y] Means reported are the average of four replicates for each strain at each sample time from dilution plates of leaf sonicates.

[z] For each column, means followed by the same lower case letter do not differ according to the Ryan-Einot-Gabriel-Welsh multiple range test ($P = 0.05$). For each row, means followed by the same upper case letter do not differ according to this test. Analysis of variance of these data appears in Table 2.

populations of equal sizes. Both groups of phytopathogenic strains showed no epiphytic preference for their compatible hosts after wet incubation (Table 7). After dry incubation, epiphytic populations could no longer be distinguished from bacteria growing pathogenically, preventing statistical comparisons.

DISCUSSION

Population sizes of bacteria on leaves were determined by biological and environmental factors including strain phenotype, plant species, and physical environmental conditions. The inclusion of 19 bacterial strains, seven plant species, and two contrasting physical environments allowed a portion of the natural biological and physical variables influencing this unique ecological habitat to be evaluated. The range of responses of bacteria to diverse plant species and moisture/light intensities allows inferences about epiphytic populations under natural conditions. conditions.

The 15 strains of *P. syringae* varied significantly in their ability to establish and maintain epiphytic populations. Because growth dynamics of all the strains on leaves were not determined, differences in population sizes estimated after 48 hr of incubation on leaves among strains could have been due to variable growth rates instead of differences in maximum population sizes achieved. However, preliminary studies and previous work (21) revealed that 48 hr was sufficient time for strains of *P. syringae* to reach maximum possible population sizes on leaves when inoculated with the cell concentrations used here, even when less favorable conditions prevailed. Up to a 10-fold difference in population size attained over all plants and both humidity and light intensities suggests that strains group within *P. syringae* are diverse ecologically. The superiority of some strains to multiply and persist may convey some ecological advantage over others. Because competition among strains for leaf sites depends at least partially on epiphytic population size (18), rapidly multiplying strains or strains that achieve higher absolute cell numbers should exclude slower growing ones if both reach leaf sites at the same time. Increased disease risk is also related to higher epiphytic population size (24). Colonization at high numbers and survival ability were not mutually exclusive in this study. For example, strain 584R in experiment 1 reached very high numbers during the humid, low light incubation and maintained a relatively high population during the dry, high light incubation.

In this study, the relative population sizes of three strains of *P. syringae* from growth chamber experiments (Table 3) predicted the relative population sizes of these strains under field conditions (Table 4). Prescreening of strains in a growth chamber may be useful for identification of strains having superior fitness for use in competitive exclusion of organisms causing plant disease or frost injury.

Strains of *P. syringae* showed a greater ability to establish and maintain epiphytic populations than any of the strains from four genera not isolated from leaves. The difference was pronounced in dry, high light intensity incubation conditions, after which the

TABLE 5. Population size estimates of bacteria inoculated on plants under different light intensities and humidities

Plant tested	Bacteria recovered (log cells/g fr wt)		
	Wet/bright incubation environment	Dry/dim incubation environment	Mean of both environments
Experiment 1[v]			
Bean	6.9 a[w]	5.6 a	6.2
Tomato	6.6 b	5.4 a	6.0
Oat	6.6 b	5.3 ab	5.9
Potato	6.2 c	5.0 b	5.8
Mean of all plants	6.5	5.3	
Experiment 2[x]			
Bean	7.0 a	5.7 a	6.3
Cucumber	7.0 a	5.8 a	6.3
Tomato	6.9 a	5.4 a	6.2
Pea	5.9 b	5.1 a	5.5
Corn	6.0 b	4.5 b	5.2
Oat	5.7 b	4.5 b	5.1
Mean of all plants	6.4	5.2	
Experiment 3[y]			
Bean	7.0 a	5.3 a	6.2
Tomato	7.0 a	5.3 a	6.2
Cucumber	7.1 a	5.0 ab	6.1
Corn	6.1 b	5.0 ab	5.7
Oat	6.1 b	5.1 bc	5.6
Pea	5.7 c	4.9 c	5.2
Mean of all plants	6.5	5.1	
Experiment 4[z]			
Bean	6.7 a	4.7 a	5.8
Corn	5.6 b	4.0 b	4.9
Mean of both plants	6.2	4.4	

[v] Strains of *Pseudomonas syringae* isolated as epiphytes. Means reported are the average of four replicates of nine different strains for each plant from dilution plates of leaf macerates.

[w] For each column, means followed by the same lower case letter do not differ according to the Ryan-Einot-Gabriel-Welsh multiple range test ($P = 0.05$). Analysis of variance of this data appears in Table 2.

[x] Strains of *P. syringae* pathogenic to tomato, cucumber, and bean. Means reported are the average of four replicates of three different strains for each plant from dilution plates of leaf macerates.

[y] Strains of *P. syringae* pathogenic to pea, oat, and corn. Means reported are the average of four replicates of three different strains for each plant from dilution plates of leaf macerates.

[z] Strains not isolated from plant leaves and strains of *P. syringae* 714R, 821R, and 22R. Means reported are the average of three replicates of seven different strains for each plant from dilution plates of leaf sonicates.

TABLE 6. Bacterial population sizes on plants under different light intensities and humidities estimated by maceration and sonication of leaves

Incubation environment	Bacteria recovered (log cells/g fr wt)	
	Macerate	Sonicate
Experiment 1[v]		
Wet, low light	6.6 a A[w]	6.5 a A
Dry, high light	5.6 b A	5.1 b B
Mean	6.1	5.7
Experiment 2[x]		
Wet, low light	6.5 a A	6.3 a B
Dry, high light	5.2 b A	4.6 b B
Mean	5.9	5.5
Experiment 3[y]		
Wet, low light	6.6 a A	6.4 a B
Dry, high light	5.3 b A	4.7 b B
Mean	6.0	5.6
Experiment 4[z]		
Wet, low light	Not tested	6.1 a
Dry, high light	Not tested	4.3 b

[v] Strains of *Pseudomonas syringae* isolated as epiphytes. Means reported are the average of four replicates of four different plant species and nine strains for each environment.

[w] For each column means followed by the same lower case letter do not differ according to the Ryan-Einot-Gabriel-Welsh multiple range test ($P = 0.05$). For each row, means followed by the same upper case letter do not differ according to this test. Analysis of variance of these data appears in Table 2.

[x] Strains of *P. syringae* pathogenic to tomato, cucumber, and bean. Means reported are the average of four replicates of six different plant species and three strains for each environment.

[y] Strains of *P. syringae* pathogenic to pea, oat, and corn. Means reported are the average of four replicates of six different plant species and three strains for each environment.

[z] Strains not isolated from plant leaves and strains of *P. syringae* 714R, 821R, and 22R. Means reported are the average of three replicates of two different plant species and seven strains for each environment.

populations of *P. syringae* were at least 25 times those of the most numerous nonpseudomonads. However, during continuously wet and low light intensity incubation conditions, the ability of *E. coli, A. hydrophila,* and *S. typhimurium* to multiply on plants was nearly equal to all strains of *P. syringae* tested. Except for *R. meliloti,* which did not multiply, wet incubation was not sufficient to separate *P. syringae* from the other bacterial species in epiphytic competence. *P. syringae* is commonly isolated from leaves (5,9), while the other species are not (13). Thus, survival, and not ability to grow on leaves, may be a major factor determining specific bacterial population sizes on plants in the absence of competition. Dry incubation conditions may be a useful way to distinguish strains that are normally epiphytes from those that are normally nonresidents, provided bacteria behave similarly in natural plant canopies.

Production of syringomycin (or syringotoxin), or pathogenic potential on a given host, was not associated with establishment and maintenance of epiphytic populations in this study. Syringomycin and syringotoxin can damage plant cells and might increase nutrient leakage (8), which could lead to higher epiphytic population sizes. The hypothesized role of toxins in epiphytic colonization assumes that nutrients are limiting and that in vitro toxin production mirrors production on plants. However, data from these experiments do not support a major role for these toxins in phylloplane colonization.

An environmental interaction with strain identity indicates that light intensity and humidity conditions will modify the relative ability of strains to become established and persist on plants. This might partially explain reported population changes in bacterial strains and species over a growing season (22). Bacterial population sizes before drying were not related to the fractional death of cells upon change from wet to dry incubation conditions, suggesting that strains that multiply to high levels under wet conditions do not necessarily also persist and vice versa.

The plant species that bacteria colonize influence their epiphytic population size. Populations of *P. syringae* varied as much as 17-fold from plant to plant. However, the relative ranking of the seven plant species as epiphytic hosts of *P. syringae* in this study depended somewhat on which group of strains was inoculated. This result further indicates that epiphytic population sizes are determined by interactions of strains, plants, and their physical environment. A factor contributing to the lack of consistent rankings of plant hosts was the absence of one or more plant species in each experiment, thus decreasing the strength and number of comparisons possible. Generally, waxy cuticled plants such as corn, oat, and pea tended to have lower bacterial populations than rough, trichomatic leaved plants such as bean, tomato, and cucumber. Some of this population difference between different plants could have been due to the difficulty in achieving equal inoculum retention between the two leaf types. However, the lengthy incubation period should have allowed sufficient time for complete colonization of leaves even from unequal amounts of inoculum (21).

Some studies have indicated that certain plants and cultivars tend to support greater epiphytic bacterial populations than others (16,19). Quantitative studies have been limited in scope, however, involving only one bacterial strain and three cultivars of the same plant species (2,5). Thus, the general receptivity of a given cultivar to colonization by a range of strains has not previously been determined. This study compared differences in colonization of plants by many strains quantitatively under controlled conditions for the first time and suggests that plant canopies of different crops under natural conditions will likely support different population sizes of *P. syringae* and thus have different disease and frost risks. An environment interaction with plant species indicates that humidity and light intensity will change the relative sizes of

TABLE 7. Interaction of plant species, strain identity, and environmental conditions on population estimates of bacteria inoculated and incubated on plants under controlled conditions

Strain tested	Bacteria recovered (log cells/g fr wt)					
	Bean	Corn	Cucumber	Tomato	Pea	Oat
Experiment 2[v]						
Wet incubation						
821R	7.3 a A[w]	6.3 a BC	7.5 a A	7.3 a A	6.7 a B	6.2 a C
765R	6.7 ab A	5.7 b B	6.7 b A	6.5 c A	5.7 b B	5.6 b B
407R	7.0 b A	5.9 b B	6.7 b A	6.9 b A	5.2 b C	5.4 b C
Dry incubation						
821R	5.2 b AB	4.4 a C	5.1 b AB	5.5 a AB	5.5 a A	5.0 a B
765R	5.3 b B	4.5 a C	7.3*a A	5.4 a B	4.5 b C	4.5 a C
407R	6.7*a A[x]	4.5 a CD	5.1 b BC	5.3 a B	5.3 a B	4.1 b D
Experiment 3[y]						
Wet incubation						
22R	7.1 a A	6.4 a B	7.4 a A	7.1 a A	6.2 a B	6.3 a B
230R	6.9 a A	5.8 b C	6.8 b AB	6.9 a A	5.8 ab C	6.4 a BC
655R	7.0 a A	6.1 ab B	7.0 b A	7.0 a A	5.2 b C	5.8 b BC
Dry incubation						
22R	5.1 a B	5.0 a B	5.4 a B	5.6 a B	6.2*a A	5.0 b B
230R	5.4 a AB	4.9 a BC	4.6 b C	5.3 a AB	4.8 b BC	5.8*a A
655R	5.5 a A	5.1*a AB	5.0 b AB	5.1 a AB	3.9 c C	4.4 c BC
Experiment 4[z]						
22R	6.3 a A	5.6 a B				
821R	6.4 a A	5.5 a B				
714R	6.7 a A	5.1 ab B				
AH1R	5.6 b A	4.6 b B				
EC1R	5.4 b A	4.5 b B				
ST1R	5.4 b A	4.6 b B				
RM1R	3.2 c A	2.9 c A				

[v] Strains of *Pseudomonas syringae* pathogenic to tomato, cucumber, and bean. Means reported are the average of four replicates of two different environments for each strain from dilution plates of leaf macerates.

[w] For each column, means followed by the same lower case letter do not differ according to the Ryan-Einot-Gabriel-Welsh multiple range test ($P = 0.05$). For each row, means followed by the same upper case letter do not differ according to this test. Analysis of variance of these data appears in Table 2.

[x] * indicates disease symptoms from pathogenic strain and susceptible host combination.

[y] Strains of *P. syringae* pathogenic to pea, oat, and corn. Means reported are the average of four replicates of two different environments for each strain from dilution plates of leaf sonicates.

[z] Strains 22R, 821R, and 714R and strains not isolated from plant leaves, mean of wet and dry incubations.

bacterial populations on different plant species. For example, bacteria appear to survive desiccation stress relatively better on plants such as bean and potato than on corn and oat. The lack of dramatic differences in bacterial population sizes among plant species upon shift from wet to dry environments indicates that such differences may be small. However, if plants are subjected to numerous fluctuations in their environment, these bacterial population differences upon environmental shifts may be cumulative. In some regions, wetting and drying occur daily.

A plant × strain interaction indicated that individual strains became established and/or persisted more readily on certain plants compared with other strains. However, from plant to plant there were no large changes in relative population sizes of individual strains. For example, a strain's population size on bean was similar to that on pea relative to other strains. Susceptible host plants did not necessarily support higher epiphytic populations of a compatible pathogenic strain than of nonpathogens after 48 hr of incubation.

Pathogenic and nonpathogenic strains of *P. syringae* achieved and maintained equal populations on plants when tested together (Table 3). This suggests that population sizes of known pathogenic strains on a given plant are not higher overall than strains for which hosts are unknown or nonexistent and vice versa. Apparently, pathogenic and nonpathogenic strains cannot be separated by their epiphytic establishment and persistence capabilities.

Several reports of epiphytic populations of pathogens on susceptible hosts have appeared. Ercolani (4) demonstrated that a strain of *P. syringae* pv. *morsprunorum* pathogenic to cherry and a strain of *P. s. syringae* pathogenic to pear only persisted epiphytically on their respective host plants. Higher population sizes of *P. s. glycinea* have been found on susceptible cultivars than on resistant cultivars of soybean (22), as well as pathovar *phaseolicola* on bean (25) and pathovar *syringae* on bean (2,3). In these five studies, populations from unnoticed or symptomless infections may have been counted as epiphytes. In contrast, equal populations of *P. s. syringae* were found on susceptible and resistant wheat cultivars (6). Unfortunately, no information on average epiphytic population sizes for a range of different nonpathogenic strains was presented in these studies. Susceptible cultivars may have simply supported higher epiphytic populations for all or most bacterial species, and observed differences among cultivars may have been unrelated to specificity. The number of reports of pathogenic host preferences by epiphytic bacteria previously published suggests that the conditions examined in this study were not equivalent, or that the link is less common than previous studies would indicate.

In general, higher cell numbers were recovered when leaves were macerated than when they were sonicated. The lack of strain or plant effects on removal indicates that both sonication and maceration may be very efficient at removing cells from plants and differences in the extent of adhesion or localization that would influence removal are overcome by both removal methods. However, recovery by sonication was less efficient than by maceration after dry incubation, indicating that the use of sonication for removal may cause some populations to be underestimated when bacterial populations recovered from plants grown under different climatic conditions are compared. The reasons for this environmental effect were not investigated. Bacteria may adhere to leaf surfaces more tightly after dry conditions, or bacteria remaining viable on dry plants may be located in leaf sites that prevent easy dislocation by sonication. Bacterial population size (estimated by maceration) was not related to the efficiency of cell removal by sonication, suggesting that the location and/or adhesion of bacterial cells at high or low population sizes was similar. Susceptible hosts and pathogenic strain combinations incubated in either environment were not different from other strain and plant combinations with regard to efficiency of cell removal by sonication. This supports a report in which several species of bacteria were washed from the leaves of different host and nonhost plants with equal efficiency (14) but disagrees with another report in which susceptible host and pathogen combinations had a lower rate of removal than a nonhost/nonpathogen combination (11). Differences in techniques and precision may be responsible for these opposing reports. However, if differential adherence rates with hosts and nonhosts exist, they are apparently subtle and probably may be safely ignored in epidemiological studies when using the removal methods employed here.

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