APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1994, p. 3128–3137
0099-2240/94/$04.00+0

Vol. 60, No. 9

Ecological Similarity and Coexistence of Epiphytic Ice-Nucleating (Ice^+) *Pseudomonas syringae* Strains and a Non-Ice-Nucleating (Ice^-) Biological Control Agent

MARK WILSON* AND STEVEN E. LINDOW

Department of Environmental Science, Policy and Management, Division of Entomology, and Plant and Soil Microbiology, University of California, Berkeley, California 94720

Received 4 March 1994/Accepted 16 June 1994

De Wit replacement series were used to study competitive interactions between epiphytic Ice^+ *Pseudomonas syringae* strains and the biological frost control agents Ice^- *P. syringae* TLP2del1 and *Pseudomonas fluorescens* A506. Mixtures containing two strains in different proportions but at a constant total population size were inoculated onto potato leaves. The population sizes of each strain and the total population size were determined when the community had reached equilibrium. A near-isogenic *P. syringae* strain pair exhibited an interaction similar to that expected for strains competing equally for limiting environmental resources. Replacement series with nonisogenic Ice^+ and Ice^- *P. syringae* strain pairs suggested that these strains competed for limiting resources according to their relative competitive abilities. There was no evidence of any niche differentiation between the Ice^+ *P. syringae* strains and the Ice^- *P. syringae* strain. The growth responses of epiphytes following addition of nutrients to the phyllosphere indicated that the epiphytic *P. syringae* populations were nutrient limited and that, under growth chamber conditions, the populations were more limited by the availability of carbon than by the availability of nitrogen. Determination of in vitro carbon source utilization profiles provided further evidence for the lack of niche differentiation between the Ice^+ and the Ice^- *P. syringae* strains. Niche overlap indices calculated for the Ice^+ *P. syringae* strains with respect to Ice^- *P. syringae* TLP2del1 were uniformly high, indicating ecological similarity, and were consistent with the observed low level of coexistence. The biological frost control agent *P. fluorescens* A506 replaced *P. syringae*. This was correlated with a high degree of niche overlap between these species.

In addition to yeasts and filamentous fungi, leaf surfaces support a diverse epiphytic bacterial flora, including both saprophytic and phytopathogenic species. Ice nucleation-active (Ice^+) *Pseudomonas syringae* strains are frequent components of the epiphytic microflora on plants that act as incitants of frost injury during freezing temperatures (16, 21, 31). Naturally occurring non-ice-nucleating (Ice^-) strains of *P. syringae* and *Erwinia herbicola* applied preemptively to plants reduced colonization by Ice^+ *P. syringae* strains and reduced the severity of frost injury (32, 44, 45). The majority of these antagonistic strains exhibited no in vitro antibiosis against Ice^+ *P. syringae*, and antibiosis-deficient mutants of antibiotic-producing strains were no less effective at inhibiting Ice^+ *P. syringae* strains (33, 37). These results suggested that competition for limiting environmental resources, not antibiosis, was the primary mechanism of antagonism between naturally occurring Ice^- bacteria and Ice^+ *P. syringae* strains (37). It was hypothesized that genotypically and, hence, ecologically similar strains would exhibit more effective competitive exclusion than dissimilar strains (34). Near-isogenic Ice^- mutants of Ice^+ *P. syringae* strains were selected following mutagenesis with ethyl methanesulfonate (33). In preemptive exclusion studies, near-isogenic Ice^- mutants significantly reduced the populations of parental Ice^+ *P. syringae* strains (33, 36). Such inhibition of near-isogenic strains could not be explained by antibiosis; hence, it was concluded that preemptive exclusion of Ice^+ *P. syringae* by Ice^- *P. syringae* resulted from the prior utilization of limiting resources in the phyllosphere.

Ice^- *P. syringae* mutants were constructed, by means of deletions in the *inaZ* gene, in order to more rigorously test the hypothesis that optimal exclusion of Ice^+ *P. syringae* strains was related to genetic and hence ecological similarity (34). The ability of these Ice^- *P. syringae* strains to preemptively exclude parental and nonparental Ice^+ *P. syringae* strains was examined. While the Ice^- mutants were consistently effective in causing some level of preemptive exclusion of Ice^+ strains, the near-isogenic Ice^- *P. syringae* strains were not always superior to nonisogenic Ice^- *P. syringae* strains in the inhibition of a given Ice^+ *P. syringae* strain (24, 34, 35), suggesting that competitive interactions are strain specific. Furthermore, in tests of a near-isogenic Ice^- *P. syringae* strain against the parental Ice^+ *P. syringae* strain and against a mixture of indigenous Ice^+ *P. syringae* strains on plants in the field (39, 46), the population size of the near-isogenic Ice^+ *P. syringae* strain was reduced up to 10-fold more than the population size of the mixture of indigenous Ice^+ *P. syringae* strains at this field site (39). These results suggest that populations of Ice^+ *P. syringae* in the field are ecologically variable and differ in their degree of coexistence with a given Ice^- *P. syringae* strain. The efficacy of biological frost control with an Ice^- *P. syringae* strain could be compromised, therefore, by the coexistence of ecologically variable Ice^+ *P. syringae* populations.

The invasion and exclusion potential was determined for

* Corresponding author. Present address: Department of Plant Pathology, 209 Life Sciences Building, Auburn University, Auburn, AL 36849-5409. Phone: (205) 844-1956. Fax: (205) 844-1947. Electronic mail address: mwilson@ag.auburn.edu.

each strain in a group of *P. syringae* strains isolated from diverse hosts in several geographic areas (26). Some *P. syringae* strains were consistently less effective at excluding a challenge strain and more effective at invading an antagonist population. Among such strains, there was a significant positive correlation between the antagonist and challenge population sizes, suggesting that these strains were not competing for the same environmental resources. The observations indicated that the *P. syringae* strains in this group were ecologically variable and therefore exhibited different degrees of coexistence. Kinkel and Lindow (26) further suggested that the specificity in the interactions among individual strain pairs indicated that it is unlikely that any single *P. syringae* strain would be capable of excluding all possible challenge strains. If true, this would have important implications for biological frost control, and therefore recent investigations have concentrated on naturally occurring Ice$^-$ species, which may be capable of excluding ecologically variable Ice$^+$ *P. syringae* populations. *Pseudomonas fluorescens* A506 has been found to be effective as a biological frost control agent, capable of preemptively excluding mixtures of indigenous Ice$^+$ *P. syringae* strains under field conditions (33, 41, 58). *P. fluorescens* A506, the principal component of the biological frost control product Frostban B, has been registered by the U.S. Environmental Protection Agency for use on several crops.

Competitive interactions between plant species have been studied either by an additive design, in which the planting density of one species is held constant while that of the other is varied, or by a substitutive design, in which the two species are planted in mixtures of various proportions at a constant total density (14, 19, 52). The substitutive design, or replacement series, introduced by de Wit (11a), has the advantage that the effects of proportion and density are not confounded. The replacement series has been used extensively in plant ecology to study the nature of competitive interactions between plant species from natural and agricultural ecosystems (3, 10, 12, 20, 49). These studies have provided important insights into the relative competitive abilities of different species, niche differentiation, and differential resource utilization (14). There has been only one reported application of the replacement series in microbial ecology, in a study of the interaction between *Pyrenophora tritici-repentis* and *Septoria nodorum* (2).

The aims of this study were to demonstrate the utility of the replacement series method for the study of competitive interactions among populations of epiphytic microbes; to examine the nature of competitive interactions between Ice$^-$ *P. syringae* TLP2del1 and various nonisogenic Ice$^+$ *P. syringae* strains; to determine the extent of ecological niche differentiation between the Ice$^+$ *P. syringae* strains colonizing potato and the Ice$^-$ *P. syringae* strain; and to compare the intraspecific interactions between *P. syringae* strains with the interspecific interaction between *P. syringae* and the biological frost control agent *P. fluorescens* A506.

(A preliminary report of these findings has been published [57].)

MATERIALS AND METHODS

Bacterial strains. *P. syringae* TLP2 was isolated from symptomless potato leaves at the University of California Tulelake Field Station (Tulelake, Calif.). The construction of TLP2del1, the recombinant Ice$^-$ derivative of *P. syringae* TLP2, has been reported previously (34). In order to maximize the likelihood of selecting Ice$^+$ *P. syringae* strains exhibiting high levels of niche differentiation with respect to the Ice$^-$ *P. syringae* strain TLP2del1, Ice$^+$ *P. syringae* strains were isolated from potato plants which had been previously inoculated with strain TLP2del1 at the Tulelake Field Station. Ice$^+$ strains coexisting on leaves with strain TLP2del1 might be expected to show niche differentiation with respect to TLP2del1. *P. syringae* MS618, isolated from strawberry and provided by T. Suslow (DNA Plant Technologies, Oakland, Calif.), was used for comparison with the Ice$^+$ *P. syringae* strains isolated from potato. The source and characteristics of *P. fluorescens* A506 have been reported before (58). Two near-isogenic strain pairs were used in the validation of the replacement series method. A near-isogenic derivative of *P. syringae* Cit7, designated Cit7::*xylE*, was constructed by introducing the *xylE* gene, conferring the production of catechol-2,3-dioxygenase, into the chromosomally located *iceC* gene (6). *P. syringae* B728a was isolated from an asymptomatic bean leaf (47). A mutant of B728a, designated B728a::Tn*5*, which was not significantly reduced in epiphytic fitness (43), was selected.

Preparation of bacterial inocula. Bacterial strains were cultured on King's medium B (KB) for 18 h at 28°C. Bacterial cells were removed from the plate and suspended in phosphate buffer (0.01 M, pH 7.0). The cell suspensions were adjusted turbidimetrically to the appropriate concentration. Appropriate volumes of the cell suspensions were combined in 11 different proportions (strain A-strain B, 0:1 through 1:0), at a constant total concentration, for each strain pair.

Greenhouse experiments. Potato seed pieces (*Solanum tuberosum* cv. Russet Burbank) were surface sterilized in 0.5% formaldehyde for 5 min and then air dried in a fume hood. Seed pieces were sliced into pieces of about 4 cm in diameter and then planted in a peat-sand potting mix. Plants were maintained in the greenhouse under conditions that minimized the growth of epiphytic microorganisms. Four-week-old potato plants were spray inoculated with suspensions of the bacterial strain pairs in 11 different proportions, at a total concentration of 10^6 CFU/ml. Each inoculum mixture was replicated on five plants. The potato plants were covered with plastic bags to maintain a high relative humidity. The plants were randomized within the growth chamber and incubated for 72 h at 26°C. All replacement series experiments were repeated at least twice to ensure reproducibility.

Field experiments. Field experiments were performed at the University of California Tulelake Field Station. The experimental plot consisted of a randomized block design with two replications. Each replication consisted of about 10 plants. The experimental plot was surrounded by a buffer zone of approximately 10 m of fallow soil. Newly emerged potato plants were spray inoculated with suspensions of the bacterial strain pairs in 11 different proportions at a total concentration of 10^8 CFU/ml. Plants were sampled 5 days after inoculation.

Enumeration of bacterial populations. Twenty leaves were collected from plants treated with each inoculum mixture, and individual leaves were placed in 20 ml of sterile washing buffer (0.1 M potassium phosphate buffer, 0.1% Bacto-Peptone [pH 7.0]). The tubes were sonicated in an ultrasonic cleaning bath for 7 min (18), and serial dilutions of leaf washings were plated on selective medium. Ice$^-$ *P. syringae* TLP2del1 was enumerated on KB amended with 100 μg of cycloheximide per ml, 50 μg of benomyl (Benlate) per ml, and 100 μg of rifampin per ml (KBR). The Ice$^+$ *P. syringae* strains were enumerated on KB amended with 100 μg of cycloheximide per ml and 50 μg of benomyl (Benlate) per ml. The Ice$^+$ strains possessed distinctive colony morphologies which permitted then to be distinguished from TLP2del1, and contaminating pseudomonads present on the plant material were present at much lower population sizes than the inoculated organisms and hence were diluted out during the enumeration process. *P. syringae* Cit7

and Cit7::*xylE* were both enumerated on KBR; hence, to distinguish Cit7::*xylE*, the colonies were transferred onto filter paper and oversprayed with catechol (0.1 M). The production of a yellow chromophore distinguished colonies of Cit7::*xylE*. *P. syringae* B728a and B728a::Tn*5* were also enumerated on KBR and KBR amended with 25 μg of kanamycin per ml (KBRK). The count on KBRK (B728a::Tn*5*) was subtracted from the count on KBR (total) to determine the count of B728a.

The mean log-transformed population size of each strain pair was estimated from 20 individual leaves at each inoculum proportion. The arithmetic back-transformed mean $\log_{10}$ population size of each strain and the arithmetic back-transformed mean $\log_{10}$ total population size were plotted against the inoculum proportion.

Tests for in vitro antibiosis. Suspensions (~10^9 CFU/ml) of the five Ice$^+$ *P. syringae* strains (136, 485, 580, 881, 1063, and MS618) were spotted onto the center of one surface-dry plate of each of three different media (KB, nutrient broth-yeast extract agar, and potato dextrose agar). A suspension of *P. syringae* TLP2del1 (~10^9 CFU/ml) was spotted onto the center of six surface-dry plates of each of the three different media. The plates were incubated for 72 h at 28°C. Following incubation, the plates of the Ice$^+$ strains were oversprayed with a suspension (~10^8 CFU/ml) of *P. syringae* TLP2del1. Three plates, one of each medium, of *P. syringae* TLP2del1 were oversprayed with each Ice$^+$ *P. syringae* strain. The plates were dried for 5 min in a laminar flow unit and then incubated for 48 h at 28°C. Following incubation, the plates were examined for the presence of inhibition zones.

RESULTS

Validation of the replacement series method applied to interactions among epiphytic bacteria. In a replacement series between two strains which compete equally for all the same limiting resources, there is a linear relationship between population size and inoculum proportion (i.e., the expected population size can be predicted from the population size when inoculated alone and the inoculum proportion), and the total population size is the sum of the expected population sizes (19). This type of interaction, which represents a low level of coexistence (19), is indicated in the replacement series graphs by dashed lines (Fig. 1 to 6 and 8). Differential competition between two strains is indicated by (i) a significant positive deviation from linearity in the relationship between population size and inoculum proportion in one strain and (ii) a significant negative deviation from linearity in the relationship between population size and inoculum proportion in the other strain. An increased level of coexistence of one strain with respect to the other is indicated by (i) a significant positive deviation from linearity in the relationship between population size and inoculum proportion and (ii) a significantly higher total population size in the coinoculations than expected (based on the sum of the expected population sizes). To test the linearity of the relationship between population size and inoculum proportion, the following model was used: $\log_{10}(\text{population size}_{ij}) - \log_{10}(\text{inoculum proportion}_i) = \text{mean}_i + \text{normal error}_{ij}$, where i is the inoculum proportion and j is the leaf replicate. In this model, the population size is lognormally distributed, and the relationship is linear only if all the means are equal. With an analysis of variance (Proc GLM in SAS Rel. 6.01; SAS Institute, Cary, N.C.), equality of the means was determined by using an F test.

In order to establish the outcome of an interaction in which the strains were expected to exhibit a low level of coexistence (i.e., no niche differentiation) and to compete equally for limiting resources, the near-isogenic strain pair *P. syringae* Cit7 and Cit7::*xylE* was used. The population size of each strain in the equilibrium community was proportional to the proportion of the strain in the inoculum (i.e., one strain replaced the other as its proportion in the inoculum increased) (Fig. 1). The total population remained approximately constant and was independent of inoculum proportion (Fig. 1). All replications of the replacement series with this strain pair have shown similar interactions.

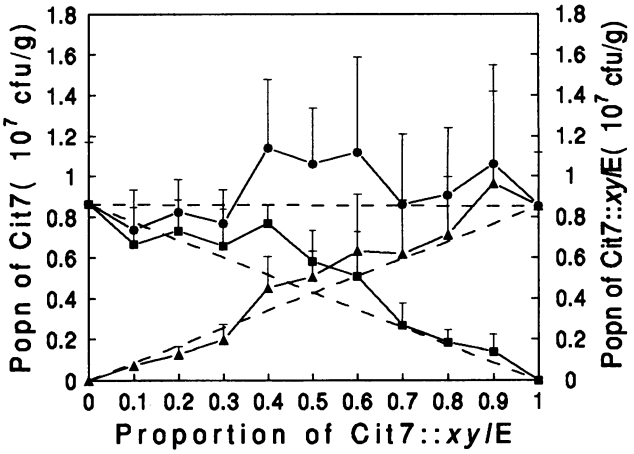

FIG. 1. Replacement series graph for near-isogenic strain pair *P. syringae* Cit7 and Cit7::*xylE* under constant environmental conditions. The population (popn) size of Cit7 (■) and Cit7::*xylE* (▲) and the total population size (●) are plotted against inoculum proportion. The dashed lines (shown in all of the replacement graphs) represent the expected populations based upon the population size when inoculated alone and the inoculum proportion. Bars represent 1 standard error of the mean (error bars are not included in subsequent graphs for clarity, but variances were similar in magnitude in all experiments). Neither population exhibited a highly significant deviation from linearity (Cit7: $F = 1.85$, $P = 0.062$; Cit7::*xylE*: $F = 2.10$, $P = 0.0311$).

To determine whether the interactions between the epiphytic bacteria had attained equilibrium by 72 h after inoculation, the incubation period chosen for the experiments, the replacement series with the nonisogenic strain pair *P. syringae* TLP2del1 and 1063 was sampled at 72 h and then again at 120 h (Fig. 2A and B, respectively). The nature of the interaction indicated by the replacement series was similar at 72 and 120 h. In both cases, the population size of *P. syringae* TLP2del1 was greater than expected for an equally competitive interaction, and the population size of *P. syringae* 1063 was less than expected for an equally competitive interaction. The population size of both strains at a given inoculum proportion was no higher after 120 h of incubation than after 72 h of incubation. All subsequent replacement series experiments were sampled at 72 h after inoculation.

In order to assess the effects of inoculum concentration on the assessment of competitive differences in a replacement series, the near-isogenic strain pair *P. syringae* B728a and B728a::Tn*5* was used. The replacement series was conducted at two different inoculum concentrations, 10^6 and 10^8 CFU/ml. The relative competitiveness of the strains indicated by the replacement series was similar at both inoculum concentrations (data not shown). At both concentrations, the population size of *P. syringae* B728a was similar to or greater than expected for an equally competitive interaction, and the population size of B728a::Tn*5* was less than expected for an

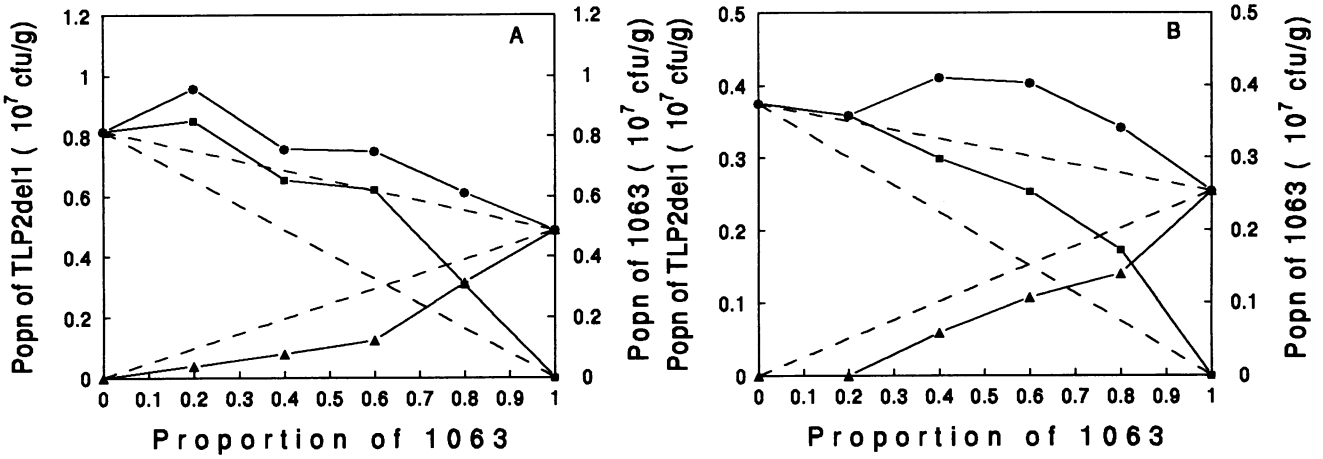

FIG. 2. Replacement series graph for nonisogenic strain pair Ice$^-$ *P. syringae* TLP2del1 and Ice$^+$ *P. syringae* 1063 sampled at (A) 72 h and (B) 120 h after inoculation. The population (popn) size of TLP2del1 (■) and 1063 (▲) and the total population size (●) are shown. At both sampling times, TLP2del1 exhibited a significant positive deviation from linearity: (A) $F = 5.19$, $P = 0.0008$; (B) $F = 6.62$, $P = 0.0001$. At 72 h, *P. syringae* 1063 exhibited a significant negative deviation from linearity ($F = 7.19$, $P = 0.0001$) but at 120 h, the deviation was not significant ($F = 1.51$, $P = 0.2184$).

equally competitive interaction, although the extent of the depression was greater at 10^6 CFU/ml than at 10^8 CFU/ml. The total inoculum concentration of 10^6 CFU/ml was therefore used in subsequent replacement series to maximize the effect of competitive differences between the strains.

Replacement series experiments between nonisogenic Ice$^+$ and Ice$^-$ *P. syringae* strains. Replacement series were performed between the Ice$^-$ *P. syringae* strain TLP2del1 and various Ice$^+$ *P. syringae* strains, including 1063 (Fig. 2A), 485 (Fig. 3A), 881 (Fig. 3B), 136 (Fig. 4A), and MS618 (Fig. 4B), under constant environmental conditions in a growth chamber in order to determine the level of coexistence of each Ice$^+$ strain with the Ice$^-$ strain and to assess competitive differences between the strains under these conditions. In most of these experiments, the nature of the interaction indicated by the replacement series was similar. The population size of *P. syringae* TLP2del1 when inoculated alone (Ice$^-$-Ice$^+$, 1:0) was in most cases less than the population size of the Ice$^+$ *P. syringae* strains when inoculated alone (Ice$^-$-Ice$^+$, 0:1). The population sizes of *P. syringae* TLP2del1 in the coinoculations were similar to or more than expected for an equally competitive interaction, while the population sizes of the various Ice$^+$ *P. syringae* strains were less than expected for an equally competitive interaction. In the case of strains 485 and 881 (Fig. 3A and B), the depression in population size in the coinoculations was greater than for strains 136 and MS618 (Fig. 4A and B). The total population size was less than expected from the population sizes when the strains were inoculated alone in all interactions except TLP2del1 and 1063 (Fig. 2).

Some replacement series were performed in the field to determine whether competitive interactions also occurred under these conditions (Fig. 5). The replacement series with *P.*

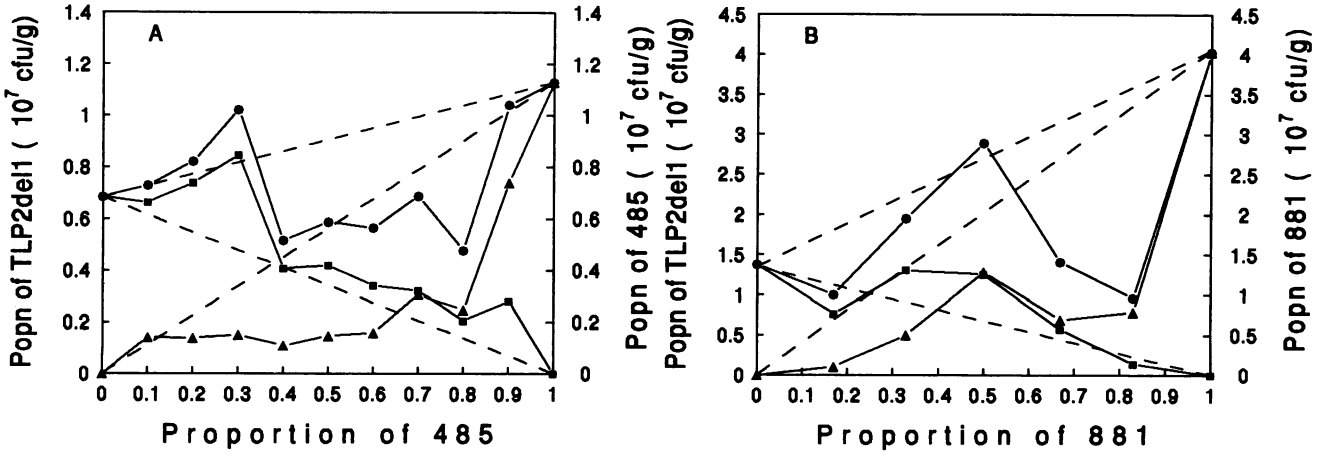

FIG. 3. Replacement series graph for nonisogenic strain pairs under constant environmental conditions. (A) Population size of Ice$^-$ *P. syringae* TLP2del1 (■) and Ice$^+$ *P. syringae* 485 (▲) and total population size (●) plotted against inoculum proportion. Both populations exhibited significant deviations from linearity (TLP2del1: $F = 5.37$, $P = 0.0001$; 485: $F = 3.20$, $P = 0.0022$). (B) Population size of Ice$^-$ *P. syringae* TLP2del1 (■) and Ice$^+$ *P. syringae* 881 (▲) and total population size (●) plotted against inoculum proportion. Both populations exhibited significant deviations from linearity (TLP2del1: $F = 6.22$, $P = 0.0001$; 881: $F = 3.78$, $P = 0.0028$).

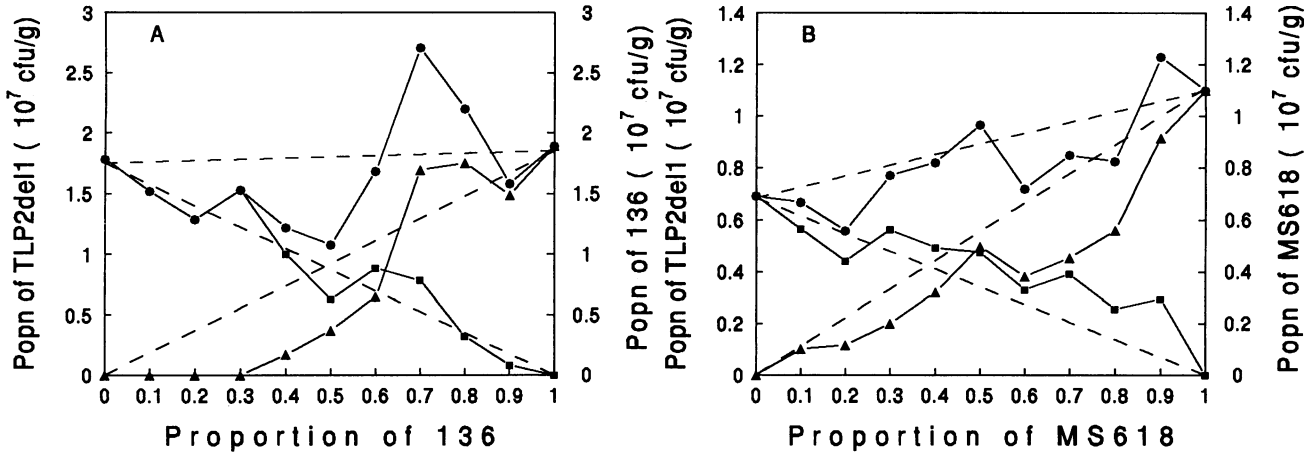

FIG. 4. Replacement series graph for nonisogenic strain pairs under constant environmental conditions. (A) Population size of Ice$^-$ *P. syringae* TLP2del1 (■) and Ice$^+$ *P. syringae* 136 (▲) and total population size (●) plotted against inoculum proportion. While the population of TLP2del1 did not deviate significantly from linearity ($F = 1.50$, $P = 0.154$), the population of 136 exhibited a significant deviation from linearity ($F = 5.96$, $P = 0.0001$). (B) Population size of Ice$^-$ *P. syringae* TLP2del1 (■) and Ice$^+$ *P. syringae* MS618 (▲) and total population size (●) plotted against inoculum proportion. Both populations exhibited significant deviations from linearity (TLP2del1: $F = 27.52$, $P = 0.0001$; MS618: $F = 7.63$, $P = 0.0001$).

syringae TLP2del1 and 580 (Fig. 5A) was repeated twice in the field with similar results, indicating that these interactions were reproducible under field conditions. The replacement series between *P. syringae* TLP2del1 and 580 (Fig. 5A) and between TLP2del1 and 881 (Fig. 5B) both indicated that competition for limiting resources also occurred under field conditions. The relative competitiveness of the strains, however, was not necessarily the same as in the growth chamber.

The tests for in vitro antibiosis indicated that *P. syringae* TLP2del1 exhibited antibiosis against two Ice$^+$ *P. syringae* strains, 580 and MS618, and one Ice$^+$ *P. syringae* strain, 1063, exhibited antibiosis against *P. syringae* TLP2del1.

Replacement series experiments between *P. syringae* TLP2del1 and *P. fluorescens* A506. In the replacement series between *P. syringae* TLP2del1 and *P. fluorescens* A506 (Fig. 6), the population sizes of TLP2del1 in the coinoculations were similar to or less than that expected for an equally competitive interaction (i.e., TLP2del1 was replaced by A506). The population sizes of A506 in the coinoculations, however, were greater than that expected for an equally competitive interaction, not significantly different from the population size when inoculated alone, and independent of inoculum proportion (i.e., A506 was not replaced by TLP2del1).

Resource limitation of phyllosphere populations. Various substrates providing carbon, nitrogen, or carbon and nitrogen were applied to the leaf surface at the time of inoculation with *P. syringae* TLP2del1 in order to determine the nature of resource limitation of the epiphytic populations. Nutrient amendments were performed both under controlled environmental conditions in the greenhouse and under fluctuating

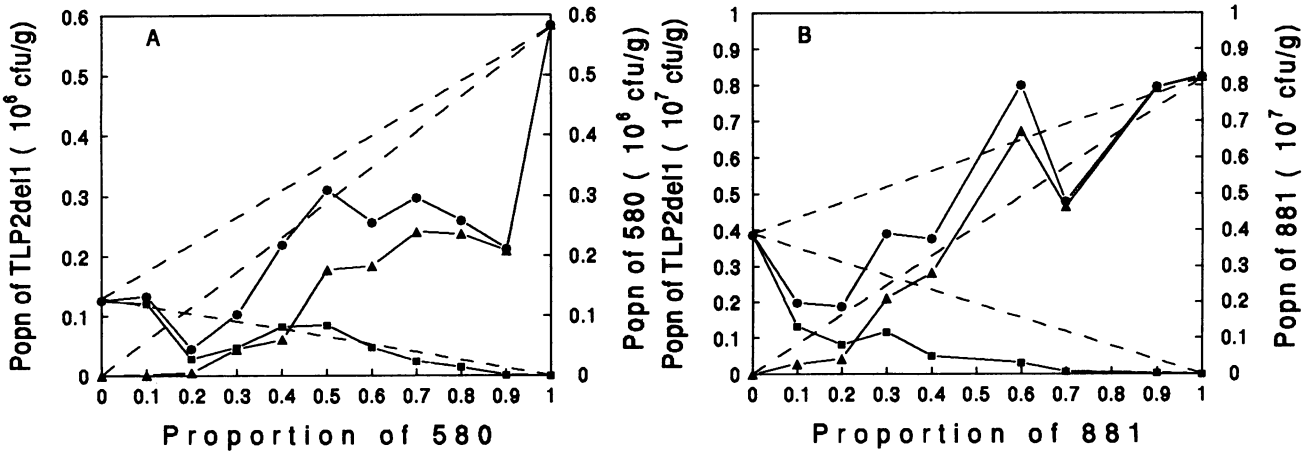

FIG. 5. Replacement series graph for nonisogenic strain pairs under field conditions. (A) Population size of Ice$^-$ *P. syringae* TLP2del1 (■) and Ice$^+$ *P. syringae* 580 (▲) and total population size (●) plotted against inoculum proportion. Both populations exhibited significant deviations from linearity (TLP2del1: $F = 4.02$, $P = 0.0001$; 580: $F = 4.19$, $P = 0.0001$). (B) Population size of Ice$^-$ *P. syringae* TLP2del1 (■) and Ice$^+$ *P. syringae* 881 (▲) and total population size (●) plotted against inoculum proportion. While the population of 881 did not deviate significantly from linearity ($F = 0.92$, $P = 0.503$), the population of TLP2del1 exhibited a significant deviation from linearity ($F = 11.88$, $P = 0.0001$).

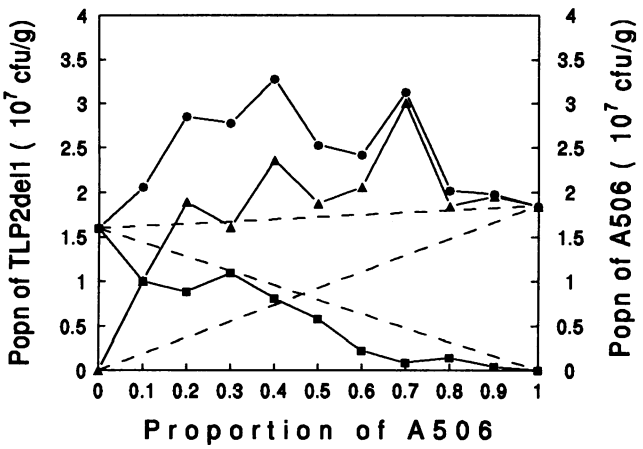

FIG. 6. Replacement series graph for the nonisogenic strain pair *P. fluorescens* A506 and *P. syringae* TLP2del1 under constant environmental conditions. The population size of *P. syringae* TLP2del1 (■) and *P. fluorescens* A506 (▲) and the total population size (●) are plotted against inoculum proportion. Both populations deviate significantly from linearity (TLP2del1: $F = 7.85$, $P = 0.0001$; A506: $F = 11.60$, $P = 0.0001$).

environmental conditions in the field. Addition of carbon or carbon plus nitrogen at the time of inoculation significantly increased the epiphytic population size under controlled environmental conditions ($P = 0.05$); however, addition of nitrogen alone had no significant effect ($P = 0.05$) (Fig. 7A). In the field, while additions of carbon alone increased the population size, this increase was not significant, and only additions of carbon plus nitrogen caused a significant increase in epiphytic population size ($P = 0.05$) (Fig. 7B).

In vitro carbon source utilization profiles and NOIs. BIOLOG GN 95 carbon source plates were used to generate carbon source utilization profiles for the Ice$^-$ *P. syringae* strain TLP2del1, for the Ice$^+$ *P. syringae* strains, and for *P. fluorescens* A506. Additionally, in vitro carbon source utilization profiles were generated by using carbon sources that have been reported in the literature to be found in the phyllosphere. Sixty-five different substrates were incorporated individually, at a concentration of 10 mM, into minimal A medium containing ammonium sulfate as a nitrogen source. The *P. syringae* strains and *P. fluorescens* A506 were tested for their ability to utilize each compound as a sole carbon source. In order to estimate the similarity of the niche of the Ice$^+$ strains to the niche of the Ice$^-$ strain in an ecologically significant resource dimension, the carbon source utilization profiles were compared. The niche overlap index (NOI) was defined as the number of carbon sources that both strains utilized as a proportion of the total number of carbon sources utilized by the Ice$^+$ strain (i.e., the proportion of carbon sources utilized by the Ice$^+$ strain that were also utilized by the Ice$^-$ strain). NOIs indicated a high degree of similarity between the Ice$^+$ strains and the Ice$^-$ strain even for Ice$^+$ *P. syringae* MS618, which was isolated from a different host (Table 1). The NOIs derived from the profile of 65 carbon sources were all 1, indicating that the Ice$^+$ strains were catabolically identical to the Ice$^-$ strain in this dimension of the ecological niche.

DISCUSSION

An understanding of competitive interactions among plant-associated bacteria is relevant to the study of the biological control of frost injury (29, 30, 35, 36, 39–42) as well as the biological control of bacterial plant diseases. While the replacement series has been used extensively in plant ecology (3, 10, 12, 20, 49) and on one occasion to study the competitive interactions among phytopathogenic fungi on wheat leaves (2), this is the first application of the method to study competitive interactions among plant-associated bacteria. For this reason, it was necessary to validate the replacement series for use with epiphytic bacteria. The near-isogenic strain pair *P. syringae* Cit7 and Cit7::*xylE* was used to establish the outcome of an

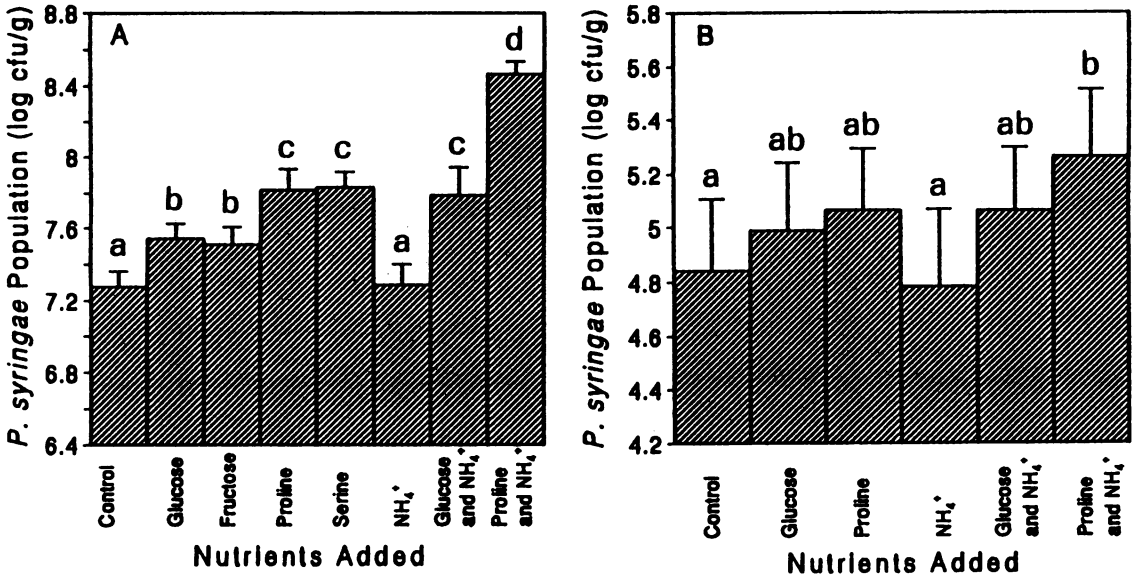

FIG. 7. *P. syringae* populations following addition of carbon and nitrogen sources, at 2.0 g/liter, at the time of inoculation and incubation under (A) growth chamber conditions and (B) field conditions. Values marked by the same letter were not significantly different ($P = 0.05$) when analyzed by analysis of variance and Duncan's multiple-range test.

TABLE 1. NOIs for target Ice$^+$ *P. syringae* strains with respect to the excluding Ice$^-$ strains (*P. syringae* TLP2del1 or *P. fluorescens* A506), derived from carbon source utilization data

Target strain[a]	NOI[b]	
	BIOLOG plate	Individual carbon sources
Ice$^+$ *P. syringae* 136	0.918	1.000
Ice$^+$ *P. syringae* 485	0.911	1.000
Ice$^+$ *P. syringae* 580	0.935	1.000
Ice$^+$ *P. syringae* 881	0.900	1.000
Ice$^+$ *P. syringae* 1063	0.905	1.000
Ice$^+$ *P. syringae* MS618	0.786	0.970
Ice$^-$ *P. syringae* TLP2	0.848	1.000

[a] Ice$^+$ *P. syringae* strains were paired with Ice$^-$ TLP2del1; Ice$^-$ *P. syringae* TLP2 was paired with *P. fluorescens* A506.

[b] NOIs were derived from carbon source utilization data generated by using BIOLOG 95 carbon source microwell plates or by the addition of 65 carbon sources individually to minimal A medium.

interaction between equally competitive strains. The *xylE* gene was inserted into the chromosomal *iceC* gene of *P. syringae* Cit7, a location which caused no loss of fitness; hence, Cit7::*xylE* and the parental strain Cit7 were expected to be equally competitive (6). The near-isogenic *P. syringae* strain pair behaved as expected for two strains competing equally for limiting resources in the phyllosphere (Fig. 8A). The population size of each strain in the equilibrium community could be predicted from its proportion in the inoculum and the population size it achieved when inoculated alone, and the total population size was constant and independent of inoculum proportion.

The substitutive or replacement series design has been criticized because of the density dependence of competitive interactions (7, 13, 23, 53). It has been suggested that the replacement series should be repeated at a range of initial densities (27). While the response-surface analysis employed by Law and Watkinson (27) may be more appropriate than one replacement series at a single planting density for the study of competing plant species, it does not appear to be necessary in the study of epiphytic bacterial populations. In this application, inoculum concentration is not analogous to planting density. An increase in inoculum concentration increases the number of cells deposited in each colonization site, reduces the number of generations needed to exhaust the resources of that site, and therefore reduces the period during which competition can occur. An increase in inoculum concentration would not be expected, therefore, to increase the intensity of competition, as would planting density, but would reduce the expression of competitive differences. The near-isogenic strain pair B728a and B728a::Tn*5* was used to test this. Strain B728a::Tn*5* contained a random chromosomal transposon insertion which reduced its epiphytic fitness. In the replacement series, the insertional mutant was less competitive than the parental strain at both inoculum concentrations, but at the high inoculum concentration (which did not permit as many generations of growth of the strain), the difference in competitive ability was apparently less than at the low inoculum concentration. Inoculum concentration did not therefore affect the qualitative interpretation of relative competitive differences between the epiphytic strains. Even with plants, there is little evidence that planting density affects the qualitative interpretation of relative competitive differences (8).

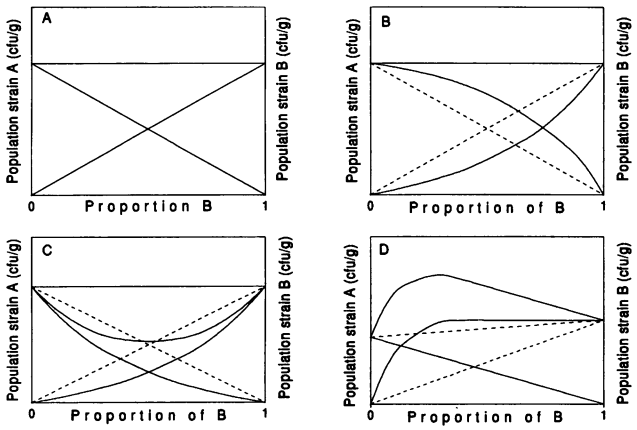

FIG. 8. Theoretical replacement series graphs indicating various types of interaction. (A) Strains of equal competitiveness which are limited by the same resource and exhibit a low level of coexistence. (B) Strains of unequal competitiveness which are limited by the same resource (strain A is more competitive than strain B) and exhibit a low level of coexistence. (C) Strains which are limited by the same resource, exhibit a low level of coexistence, and are subject to mutual antagonism. (D) Strains exhibiting niche differentiation. Strain B is not limited by all the same resources as strain A and exhibits a high level of coexistence with respect to strain A. (Panels A, B, and C are from Harper [19].)

Previous studies have suggested that competition, not antibiosis, plays the major role in mediating interactions between *P. syringae* strains in the phyllosphere (29, 30, 36, 37). In replacement series experiments, the Ice$^-$ *P. syringae* strain TLP2del1 and the various Ice$^+$ *P. syringae* strains replaced each other, indicating that these strains were indeed in competition for limiting environmental resources in the phyllosphere. The population sizes of *P. syringae* TLP2del1 in the equilibrium communities were generally similar to or greater than those expected for an equally competitive interaction, and the population sizes of the Ice$^+$ *P. syringae* strains were less than expected for an equally competitive interaction. These results indicate differences in the relative competitive ability of TLP2del1 with respect to the various Ice$^+$ strains (Fig. 8B). The total population sizes in the equilibrium communities were less than expected for several of the strain pairs, which is evidence that antagonism may have occurred in addition to the competition (Fig. 8C). The in planta antagonism exhibited by TLP2del1 was apparently lowest toward strains MS618 and 136 and greatest toward strains 485 and 881. The results suggest that both competition for limiting environmental resources and antagonism are involved in the interactions between the nonisogenic *P. syringae* strains. The importance of antagonism appears to be strain pair specific and cannot be quantified from these data. Even the precise nature of the antagonism remains unclear, as several possibilities exist: (i) that *P. syringae* TLP2del1 caused habitat modification which was detrimental to the Ice$^+$ *P. syringae* strains; (ii) that *P. syringae* TLP2del1 exhibited antibiosis toward the Ice$^+$ strains in planta; or (iii) that *P. syringae* TLP2del1 reduced the efficiency of resource use by the Ice$^+$ strains. *P. syringae* TLP2del1 did exhibit antibiosis against the Ice$^+$ *P. syringae* strains 580 and MS618 in vitro; however, antibiotic production in vitro is not proof of in planta antibiosis. In the context of these experiments, it seems most appropriate to consider antagonism to be just one of the attributes contributing to the competitive superiority of TLP2 del1 over the Ice$^+$ *P. syringae* strains, permitting TLP2del1 to acquire a greater proportion of the nutritional resources than would have occurred in a near-isogenic interaction.

The Ice^+ and Ice^- *P. syringae* strains replaced each other under field conditions in a manner similar to that observed under controlled environmental conditions in the growth chamber, indicating competition for limiting environmental resources. The relative competitiveness of the strains, however, was not the same under field conditions as was observed under controlled environmental conditions. This may be due to strain-dependent desiccation mortality in the field, environmental dependence of relative competitive ability, or different nutrient availability in the phyllosphere of field-grown plants. The replacement series conducted in the field did confirm, however, that even under the fluctuating environmental conditions of the field environment, the *P. syringae* strains competed for limiting resources.

P. syringae is genotypically and phenotypically variable (4, 11, 16, 17, 48), and recent experiments in the laboratory (25, 26) and the field (38–40) have suggested that *P. syringae* is also ecologically variable. Such ecological variability, or niche differentiation, among Ice^+ *P. syringae* populations on a particular host could lead to coexistence of Ice^+ populations with an Ice^- biological control agent and consequently lead to a reduction in the efficacy of biological frost control. Replacement series in which the population sizes of one or both strains are elevated above those expected for an equally competitive interaction and are independent of inoculum proportion would suggest niche differentiation (Fig. 8D). In none of the many replacement series conducted with *P. syringae* strain pairs during this study was there any indication of niche differentiation. These data suggest that the Ice^+ *P. syringae* strains on potato plants at the Tulelake Field Station were ecologically similar to the Ice^- *P. syringae* strain TLP2del1. The lack of niche differentiation between the *P. syringae* strains contrasts with the ecological differentiation observed between *P. syringae* TLP2del1 and *P. fluorescens* A506. Although TLP2del1 showed a low level of coexistence with A506 (i.e., TLP2del1 was replaced by A506), A506 showed a high level of coexistence with TLP2 del1 (i.e., A506 was not replaced by TLP2del1). This relationship between *P. syringae* TLP2del1 and *P. fluorescens* A506 has also been demonstrated on other host plants (unpublished data). In a greenhouse study, Lindemann and Suslow (30) similarly observed that while Ice^- *P. syringae* did not inhibit the population of *P. fluorescens* in strawberry blossoms, *P. fluorescens* did inhibit the population of *P. syringae* on those blossoms. The high level of coexistence of *P. fluorescens* A506 with respect to *P. syringae* TLP2del1 was probably achieved through partitioning of limiting environmental resources. Hence, although niche differentiation was not observed between the Ice^- and Ice^+ *P. syringae* strains in this study, niche differentiation can occur among epiphytic bacterial populations in the phyllosphere and can be demonstrated by the replacement series method.

Carbon and nitrogen sources added to the phyllosphere at the time of inoculation indicated that the epiphytic bacterial populations growing under controlled environmental conditions in the growth chamber were more limited by the availability of carbon than by the availability of nitrogen. This suggests that the C/N ratio in the bean phyllosphere under growth chamber conditions was lower than the optimum for the epiphytic growth of *P. syringae*. While other workers have reported that epiphytic population sizes can be manipulated by nutrient addition (15, 50), these reports have not indicated whether carbon or nitrogen was more limiting or demonstrated the significance of the C/N ratio of the phyllosphere. Under field conditions, the C/N ratio of the phyllosphere may have been closer to the optimum for growth of *P. syringae*, and hence additions of carbon had less effect and only the addition of carbon and nitrogen significantly increased the epiphytic population size. Presumably, the limiting environmental resource for which the *P. syringae* strains competed in the phyllosphere was carbon, suggesting that the epiphytic populations compete for carbon from a pool of substitutable sources derived from the leaf leachates (54–56).

While nutrient competition between plant-associated microorganisms has been reported previously (5, 9), the significance of ecological similarity or niche overlap in microbial competition has not been assessed. Despite several contradictory studies (22, 28, 51), it is generally agreed that niche overlap per se indicates nothing about the intensity of interspecific competition unless overlap is measured in a limiting-resource dimension (1). In the case of the growth chamber experiments, the limiting resource was carbon; hence, the NOIs were determined from carbon source utilization profiles. The NOIs derived from the in vitro carbon source data are probably more ecologically meaningful than those derived from BIOLOG plates, which contain several compounds not found in plants. The indices, however, were similar in both cases, and hence BIOLOG plates could be used for determinations of ecological similarity.

According to the NOIs, there was little ecological variation, or niche differentiation, between the Ice^+ *P. syringae* strains and the Ice^- *P. syringae* strain. Even *P. syringae* MS618, isolated from strawberry, exhibited a high degree of ecological similarity with TLP2del1 (NOI_{MS618} = 0.970). This lack of ecological variability would explain why the Ice^+ *P. syringae* strains all exhibited low levels of coexistence with the Ice^- strain. Hence, while the species *P. syringae* is known to be genotypically and phenotypically variable (4, 11, 16, 17, 48) and also ecologically variable (25, 26), all the Ice^+ *P. syringae* strains in this study (except for MS618) were derived from a single host at a single location and exhibited a high degree of ecological similarity. Although the genotypic and phenotypic variability observed for this species is not necessarily predictive of ecological variability, it is probable that in a sample of Ice^+ *P. syringae* strains from diverse hosts or locations, greater niche differentiation would be observed.

The NOI is significant to biological control achieved by preemptive exclusion because it indicates the proportion of carbon sources remaining for utilization by the Ice^+ strain after the biological control agent has colonized the leaf surface. While recent investigations have demonstrated variation in the ability of a given *P. syringae* strain to exclude other *P. syringae* strains (26), the ecological similarity of the Ice^+ strains and the Ice^- strain means that we would expect the recombinant biological frost control agent *P. syringae* TLP2 del1 to be equally effective in the preemptive exclusion of most Ice^+ *P. syringae* strains on potato at this location. This biological frost control agent might not be effective, however, on hosts other than potato, on which the Ice^+ *P. syringae* strains exhibit greater ecological differentiation with respect to TLP2del1. In this case, biological frost control might be achieved most effectively by using either a mixture of complementary Ice^- *P. syringae* strains (26) or a biological control agent which occupies a large nutritional niche capable of encompassing the ecological diversity of Ice^+ *P. syringae* strains. The biological frost control agent *P. fluorescens* A506 exhibited a more extensive nutritional niche than *P. syringae* TLP2del1. The ability of *P. fluorescens* A506 to replace *P. syringae* TLP2del1 is consistent with the high NOI ($NOI_{TLP2del1}$ = 1.00), which was equivalent to that observed for the nonisogenic *P. syringae* strain pairs. In field trials, A506 has consistently been at least as effective as *P. syringae* TLP2del1 in the prevention of frost injury on potato (43a) and

is also effective on other hosts (33, 41, 58). The efficacy of *P. fluorescens* A506 as a biological control agent for Ice$^+$ *P. syringae* strains probably relates to the nutritional versatility of the strain and the high probability that its nutritional niche would overlap to a high degree even with that of nutritionally variable Ice$^+$ *P. syringae* strains, as *P. fluorescens* A506 has not been demonstrated to exhibit in vitro antibiosis against Ice$^+$ *P. syringae* strains.

This study demonstrates the utility of certain macroecological concepts applied to the microbial ecology of epiphytic bacteria. The replacement series appears to be a useful technique for studying the nature of the interactions between epiphytic bacteria, providing information about the relative competitiveness of strains, in planta antagonism, and partitioning of resources. The replacement series can provide useful information about the level of coexistence due to niche differentiation between epiphytic species, which can be correlated with niche overlap in an ecologically significant resource dimension. The replacement series could be equally useful for studying the interactions between plant-associated bacterial strains in the rhizosphere or on fruit or seed surfaces. The replacement series method may be particularly appropriate for the study of the relative competitiveness of near-isogenic pairs of recombinant and parental bacterial strains in risk assessment studies with genetically engineered microorganisms.

ACKNOWLEDGMENTS

This work was supported by National Science Foundation grant FD91-06782.

We acknowledge S. R. Adee for suggestions regarding the application of the de Wit replacement series to epiphytic bacteria; B. Larget for development of the statistical method used in these studies; and L. L. Kinkel and G. A. Beattie for critical review of the manuscript.

REFERENCES

1. **Abrams, P. A.** 1980. Some comments on measuring niche overlap. Ecology **61**:44–49.
2. **Adee, S. R., W. F. Pfender, and D. C. Hartnett.** 1990. Competition between *Pyrenophora tritici-repentis* and *Septoria nodorum* in the wheat leaf as measured with de Wit replacement series. Phytopathology **80**:1177–1182.
3. **Akey, W. C., T. W. Jurik, and J. Dekker.** 1991. A replacement series evaluation of competition between velvet leaf (*Abutilon theophrasti*) and soybean (*Glycine max*). Weed Res. **31**:63–72.
4. **Baca, S., M. L. Canfield, and L. W. Moore.** 1987. Variability in ice nucleation strains of *Pseudomonas syringae* isolated from diseased woody plants in Pacific Northwest nurseries. Plant Dis. **71**:412–415.
5. **Blakeman, J. P., and I. D. S. Brodie.** 1977. Competition for nutrients between epiphytic microorganisms and germination of spores of plant pathogens on beetroot leaves. Physiol. Plant Pathol. **10**:29–42.
6. **Clark, E., S. E. Lindow, and M. Wilson.** 1992. Chromosomal insertions of *xylE* in *Pseudomonas syringae* as identifiable markers for ecological studies (abstract). Phytopathology **82**:1179.
7. **Connolly, J.** 1986. On difficulties with replacement series methodology in mixture experiments. J. Appl. Ecol. **23**:125–127.
8. **Cousens, R., and M. O'Neil.** 1993. Density dependence of replacement series experiments. Oikos **66**:347–352.
9. **Couteaudier, Y.** 1992. Competition for carbon in soil and rhizosphere, a mechanism involved in biological control of *Fusarium* wilts, p. 99–104. *In* E. C. Tjamos, G. C. Papavizas, and R. J. Cook (ed.), Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York.
10. **Cudney, D. W., L. S. Jordan, J. S. Holt, and J. S. Reints.** 1989. Competitive interactions of wheat (*Triticum aestivum*) and wild oats (*Avena fatua*) grown at different densities. Weed Sci. **37**:538–543.
11. **Denny, T. P.** 1988. Phenotypic diversity in *Pseudomonas syringae* pv. *tomato*. J. Gen. Microbiol. **134**:1939–1948.

11a. **de Wit, C. T.** 1960. On competition. Versl. Landbouw. Onderz. **66**:1–82.

12. **Duncan, C. M., and R. M. Zimdahl.** 1991. Competitive ability of wild oats (*Avena fatua*) and barley (*Hordeum vulgare*). Weed Sci. **39**:558–563.
13. **Firbank, L. G., and A. R. Watkinson.** 1985. On the analysis of competition within two species mixtures of plants. J. Appl. Ecol. **22**:503–517.
14. **Firbank, L. G., and A. R. Watkinson.** 1990. On the effects of competition: from monocultures to mixtures, p. 165–192. *In* J. B. Grace and D. Tilman (ed.), Perspectives on plant competition. Academic Press, San Diego, Calif.
15. **Fokkema, N. J., J. G. den Houter, Y. J. C. Koselman, and A. L. Nelis.** 1979. Manipulation of yeasts on field-grown wheat leaves and their effects on *Cochliobolus sativus* and *Septoria nodorum*. Trans. Br. Mycol. Soc. **72**:19–29.
16. **Gaignard, J. L., and J. Luisetti.** 1993. *Pseudomonas syringae*, an epiphytic ice nucleation active and phytopathogenic bacterium. Agronomie **13**:332–370.
17. **Gross, D. C., Y. S. Cody, E. L. Proebsting, G. K. Radamaker, and R. A. Spotts.** 1984. Ecotypes and pathogenicity of ice nucleation-active *Pseudomonas syringae* isolated from deciduous fruit tree orchards. Phytopathology **74**:241–248.
18. **Haefele, D., and R. Webb.** 1982. The use of ultrasound to facilitate the harvesting and quantification of epiphytic and phytopathogenic microorganisms (abstract). Phytopathology **72**:947.
19. **Harper, J. L.** 1977. The population biology of plants. Academic Press, London.
20. **Hill, M. J., and A. C. Gleeson.** 1991. Competition between Clare and Seaton Park, and Clare and Daliak subterranean clovers in replacement series mixtures in the field. Aust. J. Agric. Res. **42**:161–173.
21. **Hirano, S. S., and C. D. Upper.** 1990. Population biology and epidemiology of *Pseudomonas syringae*. Annu. Rev. Phytopathol. **28**:155–177.
22. **Hurlbert, S. H.** 1978. The measurement of niche overlap and some relatives. Ecology **59**:67–77.
23. **Jolliffe, P. A., A. N. Minjas, and V. C. Runeckles.** 1984. A reinterpretation of yield relationships in replacement series experiments. J. Appl. Ecol. **21**:227–243.
24. **Kinkel, L. L., and S. E. Lindow.** 1989. The role of competitive interactions in bacterial survival and establishment on the leaf surface, p. 634–638. *In* T. Hattori, Y. Ishida, Y. Maruyama, R. Y. Morika, and A. Uchida (ed.), Recent advances in microbial ecology. Japan Scientific Society Press, Tokyo.
25. **Kinkel, L. L., and S. E. Lindow.** 1989. Dynamics of *Pseudomonas syringae* strains coexisting on leaf surfaces (abstract). Phytopathology **79**:1162.
26. **Kinkel, L. L., and S. E. Lindow.** 1993. Invasion and exclusion among coexisting *Pseudomonas syringae* strains on leaves. Appl. Environ. Microbiol. **59**:3447–3454.
27. **Law, R., and A. R. Watkinson.** 1987. Response-surface analysis of two-species competition: an experiment on *Phleum arenarium* and *Vulpia fasciculata*. J. Ecol. **75**:871–886.
28. **Lawlor, L. P.** 1980. Overlap, similarity and competition coefficients. Ecology **61**:245–251.
29. **Lindemann, J., L. Joe, and A. Moayeri.** 1985. Reciprocal competition between INA$^+$ wild type and INA$^-$ deletion mutant strains of *Pseudomonas* on strawberry blossoms (abstract). Phytopathology **75**:1361.
30. **Lindemann, J., and T. V. Suslow.** 1987. Competition between ice nucleation-active wild type and ice nucleation-deficient deletion mutant strains of *Pseudomonas syringae* and *P. fluorescens* biovar I and biological control of frost injury on strawberry blossoms. Phytopathology **77**:882–886.
31. **Lindow, S. E.** 1983. The role of bacterial ice nucleation in frost injury to plants. Annu. Rev. Phytopathol. **21**:363–384.
32. **Lindow, S. E.** 1983. Methods of preventing frost injury caused by epiphytic ice-nucleation-active bacteria. Plant Dis. **67**:327–333.
33. **Lindow, S. E.** 1985. Integrated control and role of antibiosis in biological control of fireblight and frost injury, p. 83–115. *In* C. E. Windels and S. E. Lindow (ed.), Biological control on the phyllo-

plane. American Phytopathological Society, St. Paul, Minn.
34. **Lindow, S. E.** 1986. Construction of isogenic Ice$^-$ stains of *Pseudomonas syringae* for evaluation of specificity of competition on leaf surfaces, p. 509–515. *In* F. Megusar and M. Gantar (ed.), Microbial ecology. Slovene Society for Microbiology, Ljuvljana, Yugoslavia.
35. **Lindow, S. E.** 1986. Specificity of epiphytic interactions of *Pseudomonas syringae* strains on leaves (abstract). Phytopathology **76:** 1136.
36. **Lindow, S. E.** 1987. Competitive exclusion of epiphytic bacteria by Ice$^-$ *Pseudomonas syringae* mutants. Appl. Environ. Microbiol. **53:**2520–2527.
37. **Lindow, S. E.** 1988. Lack of correlation of in vitro antibiosis with antagonism of ice nucleation active bacteria on leaf surfaced by non-ice nucleation active bacteria. Phytopathology **78:**444–450.
38. **Lindow, S. E.** 1990. Use of genetically altered bacteria to achieve plant frost control, p. 85–110. *In* J. P. Nakas and C. Hagedorn (ed.), Biotechnology of plant-microbe interactions. McGraw-Hill Publishing Company, New York.
39. **Lindow, S. E.** 1991. Tests of specificity of competition among *Pseudomonas syringae* strains on plants using recombinant ice$^-$ strains and use of ice nucleation genes as probes of *in situ* transcriptional activity, p. 457–464. *In* H. Hennecke and D. P. S. Verma (ed.), Advances in molecular genetics of plant-microbe interactions, vol. 10. Kluwer Academic Publishers, Dordrecht, The Netherlands.
40. **Lindow, S. E.** 1992. Ice$^-$ strains of *Pseudomonas syringae* introduced to control ice nucleation active strains on potato, p. 169–174. *In* E. C. Tjamos, G. C. Papavizas, and R. J. Cook (ed.), Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York.
41. **Lindow, S. E.** 1992. Integrated control of frost injury, fire blight, and fruit russet of pear with a blossom application of an antagonistic bacterium. Phytopathology **82:**1129.
42. **Lindow, S. E.** 1993. Biological control of plant frost injury: the Ice$^-$ story, p. 113–128. *In* L. Kim (ed.) Advanced engineered pesticides. Marcel Dekker, Inc., New York.
43. **Lindow, S. E.** 1993. Novel method for identifying bacterial mutants with reduced epiphytic fitness. Appl. Environ. Microbiol. **59:**1586–1592.
43a. **Lindow, S. E.** Unpublished data.
44. **Lindow, S. E., D. C. Arny, and C. D. Upper.** 1983. Biological control of frost injury: an isolate of *Erwinia herbicola* antagonistic to ice nucleation active bacteria. Phytopathology **73:**1097–1102.
45. **Lindow, S. E., D. C. Arny, and C. D. Upper.** 1983. Biological control of frost injury: establishment and effects of an isolate of *Erwinia herbicola* antagonistic to ice nucleation active bacteria on corn in the field. Phytopathology **73:**1102–1106.
46. **Lindow, S. E., and N. J. Panopoulos.** 1988. Field tests of recombinant Ice$^-$ *Pseudomonas syringae* for biological frost control in potato, p. 121–138. *In* M. Sussman, C. H. Collins, F. A. Skinner, and D. E. Stewart-Tull (ed.), Release of genetically-engineered micro-organisms. Academic Press, London.
47. **Loper, J. E., and S. E. Lindow.** 1987. Lack of evidence for in situ fluorescent pigment production by *Pseudomonas syringae* pv. *syringae* on bean leaf surfaces. Phytopathology **77:**1449–1454.
48. **Malvick, D. K., and L. W. Moore.** 1988. Population dynamics and diversity of *Pseudomonas syringae* on maple and pear trees and associated grasses. Phytopathology **78:**1366–1370.
49. **Marshall, D. R., and S. K. Jain.** 1969. Interference in pure and mixed populations of *Avena fatua* and *Avena barbata*. J. Ecol. **57:**251–270.
50. **Morris, C. E., and D. I. Rouse.** 1985. Role of nutrients in regulating epiphytic bacterial populations, p. 63–82. *In* C. E. Windels and S. E. Lindow (ed.), Biological control on the phylloplane. American Phytopathological Society, St. Paul, Minn.
51. **Pielou, E. C.** 1972. Niche width and overlap: a method for measuring them. Ecology **53:**687–692.
52. **Snaydon, R. W.** 1991. Replacement or additive designs for competition studies? J. Appl. Ecol. **28:**930–946.
53. **Taylor, D. R., and L. W. Aarsen.** 1989. On the density dependence of replacement-series competition experiments. J. Ecol. **77:**975–988.
54. **Tukey, H. B.** 1966. The leaching of metabolites from above-ground plant parts and its implications. Bull. Torrey Bot. Club **93:**385–401.
55. **Tukey, H. B.** 1970. The leaching of substances from plants. Annu. Rev. Plant Physiol. **21:**305–324.
56. **Tukey, H. B.** 1971. Leaching of substances from plants, p. 67–80. *In* T. F. Preece and C. H. Dickinson (ed.), Ecology of leaf surface micro-organisms. Academic Press, London.
57. **Wilson, M., and S. E. Lindow.** 1991. Resource partitioning among bacterial epiphytes in the phyllosphere (abstract). Phytopathology **81:**1170–1171.
58. **Wilson, M., and S. E. Lindow.** 1993. Population dynamics of *P. fluorescens* A506 in pear flowers following inoculation in relation to strategies for biological control of fire blight and frost injury. Acta Hort. **338:**331–332.