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Population Sizes, Immigration, and Growth of Epiphytic Bacteria on Leaves of Different Ages and Positions of Field-Grown Endive (*Cichorium endivia* var. *latifolia*)

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Total, fluorescent, and pectolytic epiphytic bacterial population sizes were quantified on leaves of different age groups of broad-leaved endive during field cultivation from leaf emergence until harvest. Greater bacterial population densities (log_{10} CFU per square centimeter) were observed on outer leaves than on inner leaves of the plants throughout the growing season. These differences were statistically significant for total bacterial populations at all sampling times and were often significant for fluorescent and pectolytic bacterial populations. At harvest, a linear gradient of decreasing densities of epiphytic bacteria from outer (older) to inner (younger) leaves of the head was significant. Leaf age influenced the frequency distribution and variability of bacterial population sizes associated with leaves of broad-leaved endive. Total bacterial population sizes were greater at leaf emergence for leaves emerging during the second half of the cultivation period than for leaves emerging earlier. The size of fluorescent and pectolytic bacterial populations on newly emerged leaves increased throughout the season as plants aged. To assess the importance of plant age on bacterial immigration at leaf emergence, bacterial densities were quantified on leaves emerging simultaneously on plants of different ages. In two of the three experiments, greater bacterial population sizes were observed on leaves emerging on younger plants. This indicates that factors other than an increase in concentration of airborne bacteria can lead to increases in population sizes at leaf emergence as plants age in the field. Results of leaf pruning experiments suggested that adjacent leaves may act as a barrier for immigration of fluorescent bacteria on newly emerged leaves. Survival of an inoculated strain of *Pseudomonas fluorescens* on newly emerged leaves generally did not vary with the age of plants. However, these effects were not consistent among experiments, suggesting that interactions among micro- and macroenvironmental conditions, physiological condition of leaves, and accessibility of leaves to airborne bacteria are important in controlling epiphytic bacterial population sizes.

The aerial parts of plants are colonized by numerous microorganisms, including bacteria. Bacteria are generally the predominant initial colonists on newly expanded leaves, while yeasts and filamentous fungi dominate later in the growing season (6, 19). Dominant components of epiphytic bacterial populations and population dynamics vary with host genotype, age and position of leaves, and environmental conditions (1, 2, 9, 23, 28).

Leaf age and position have been shown to influence epiphytic population sizes on plants with a variety of morphologies (2, 17, 25, 29, 34). Nevertheless, leaf age is rarely considered in sampling procedures for determining microbial population sizes on vegetable crops. Factors accounting for positional variation in microbial population densities include accessibility of leaves to airborne microbes, microclimate, physiological condition of leaves, and the activity of vectors of foliar pathogens (2). In the case of broad-leaved endive (*Cichorium endivia* var. *latifolia*), the focus of the present study, we have previously shown that leaf physiology is one of the causes of variability of bacterial population size after harvest among leaf pieces of different ages in ready-to-use sachets (16). However, during field cultivation differences in accessibility of leaves to airborne bacteria could be of primary importance for population dynamics of epiphytic bacteria on leaves as a consequence of broad-leaved endive morphology: outer leaves are exposed to airborne bacteria, while inner leaves are covered by adjacent leaves and hence are more protected from this inoculum source.

The processes implicated in the dynamics of microbial populations on aerial plant parts are immigration, emigration, death, and growth of microorganisms. Immigration as a factor affecting microbial population sizes associated with leaves has rarely been studied experimentally (20, 21). It has been addressed in the test of the theory of island biogeography for phylloplane fungal communities on apple leaves (3, 4, 19). Inoculum sources of epiphytic bacteria include plant foliage, soil, and seed (11). The respective influences of airborne bacteria from distant sources and leaf-to-leaf bacterial movement as sources of immigrants for emerging leaves are not well known in general and, in particular, are poorly understood for epiphytic bacteria colonizing many vegetable crops. Growth of bacteria on aerial plant parts has been studied for introduced strains (15) or for naturally occurring components of bacterial populations (14). However, the relative importance of immigration versus growth in bacterial population dynamics has received little attention. The role of these processes in bacterial population dynamics is relevant to the development of novel strategies for controlling epiphytic bacteria.

We are examining the mechanisms implicated in colonization of broad-leaved endive during field cultivation by epi-

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phytic bacteria. Some of these epiphytic bacteria (*Pseudomonas fluorescens*) are implicated in postharvest decay of ready-to-use broad-leaved endive (16). Our present work focuses on (i) the effect of leaf position and age within a head of broad-leaved endive on the bacterial population dynamics associated with those leaves during field cultivation, (ii) the sources of bacterial immigrants for newly emerging leaves, and (iii) the influence of plant age on growth of bacteria associated with emerging leaves. To study these questions, two types of experiments were conducted. One type concerned description of the dynamics of bacterial populations associated with broad-leaved endive leaves of different ages in fields of plants of the same age. The other type of experiment allowed us to examine bacterial growth and immigration on newly emerged leaves of broad-leaved endive in fields of plants of different ages having different numbers of leaves under identical environmental conditions.

MATERIALS AND METHODS

Cultivation of plant material. All experiments were performed under traditional agricultural practices for broad-leaved endive in southeastern France. For description of population dynamics, broad-leaved endive (*C. endivia* var. *latifolia*) cv. Samy was cultivated in the autumn of 1991, 1992, and 1993 in experimental plots of the Interprofessional Technical Center for Fruits and Vegetables (CTIFL) at Bellegarde (department of Gard), France. After 4 weeks of greenhouse cultivation following sowing (19 August 1991, 12 August 1992, and 9 August 1993), plants were transplanted to the field (11 September 1991, 4 September 1992, and 25 September 1993). Field plots consisted of two to four beds (1.10 by 12 m each). Each bed consisted of three rows spaced 0.30 m apart. For about 2 weeks prior to harvest, the inner leaves of the head were covered with a plastic blanching cap as is standard practice for broad-leaved endive cultivation. Harvest occurred about 80 days after transplant (2 December 1991, 30 November 1992, and 22 November 1993). Soil fertilization and pesticide treatments were as described previously (16, 24). The plants received overhead irrigation as needed for about 2 weeks after transplant and received sufficient rain for the remainder of the growing season.

For assessment of bacterial immigration and growth rates on newly emerged leaves on plants of different ages, three additional experimental fields were set up. Seeds of *C. endivia* var. *latifolia* were sown at three dates for each of the three experiments, permitting simultaneous cultivation of plants of three different ages. For the first experiment, broad-leaved endive cv. Samoa was cultivated in the spring of 1993 in experimental plots of the National Institute of Agronomic Research (INRA) at Montfavet (department of Vaucluse), France. For experiments 2 and 3, *C. endivia* var. *latifolia* cv. Samy was grown in the autumn of 1993 in experimental field plots at INRA and at CTIFL, respectively. For plantings at INRA, the soil was fertilized with 180 kg of ammonium nitrate, 180 kg of phosphoric acid, and 180 kg of potassium oxide per ha prior to transplant. Other cultural methods were similar to those practiced at CTIFL. At INRA, plots consisted of 8-m-long rows spaced 0.3 m apart. At CTIFL, field plots consisted of nine beds (1.10 by 12 m each). Each bed consisted of three rows spaced 0.3 m apart. The spring planting at INRA was cultivated in a wire mesh (pore size, 0.8 mm) shelter. All plantings at INRA and CTIFL were cultivated in different experimental fields bordered by cypress hedges at the north and south edges.

Experimental design. For the description of bacterial population dynamics on leaves of different ages, leaves from individual plants were separated into different age groups (AGs) on the basis of their date of emergence in the field. Emergence date was determined by tagging three newly emerged leaves of individual marker plants at six dates over the growing season. Newly emerged leaves were defined as the three youngest leaves that were at least 5 cm long on each marked plant at the tagging date. These leaves were marked at 2- to 3-week intervals by depositing a few spots of acrylic paint on the midrib. Hence, the following leaf AGs were identified (followed by the number of weeks after sowing at which they emerged): AG 2 (6 weeks), AG 3 (8 weeks), AG 4 (10 weeks), AG 5 (13 weeks), and AG 6 (15.5 weeks). On average, these corresponded to the following positions on the plant (position 1 being the outermost and position 100 being the innermost leaves on the mature plant): leaves of AG 2 were at positions 1 to 5, leaves of AG 3 were at positions 6 to 16, leaves of AG 4 were at positions 20 to 30, leaves of AG 5 were at positions 40 to 60, and leaves of AG 6 were the innermost leaves on the plants. For all experiments, leaves were taken at each sampling date from plants that had not been used at previous sampling dates. Samples consisted of three leaves of each AG from each of 8 to 10 randomly selected plants at tagging dates in 1991 and 1992. In 1993, three leaves of AG 4, 5, and 6 were sampled at harvest only.

To determine the effect of leaf position on bacterial immigration and growth rates, three groups of newly emerged leaves were studied: those emerging at the beginning of field cultivation and corresponding to outermost leaves at harvest (AG 2 leaves), leaves forming the intermediate corona (AG 4 leaves), and innermost leaves emerging just before harvest (AG 6 leaves). Leaves of these three groups emerged simultaneously on different plants as plants were sown at three different dates. For the experiment conducted in the spring of 1993 at INRA, newly emerged leaves were sampled at 7, 12, and 16 weeks after planting for young, intermediate, and older plants, respectively. In the autumn of 1993, newly emerged leaves were sampled from the INRA field at 8, 10, and 14 weeks after planting and from the CTIFL field at 10, 12, and 14 weeks after planting for young, intermediate, and older plants, respectively. At each sampling time, three leaves were sampled from each of nine plants of each age.

To assess the influence of adjacent leaves on bacterial immigration at leaf emergence, two treatments were applied to plants from each of the three sowing dates. For treatment 1, inner leaves (about 6, 20, and 40 leaves on younger, intermediate, and older plants, respectively) were pruned from the center of broad-leaved endive heads. For treatment 2, leaves were pruned as for treatment 1 and a piece of polypropylene (40-μm thickness) was placed over the remaining older leaves to inhibit bacterial immigration from adjacent older leaves to emerging leaves. Treatment 3 was a control; plants were not pruned or dressed with plastic. Each treatment consisted of three adjacent plants replicated in two to three blocks per planting date. Three newly emerged leaves at the plant apex were sampled from each of the three adjacent plants in each block 7 days after leaf pruning.

To assess the effect of position of newly emerging leaves on bacterial growth, bacterial growth was quantified on leaves emerging on plants of different ages,

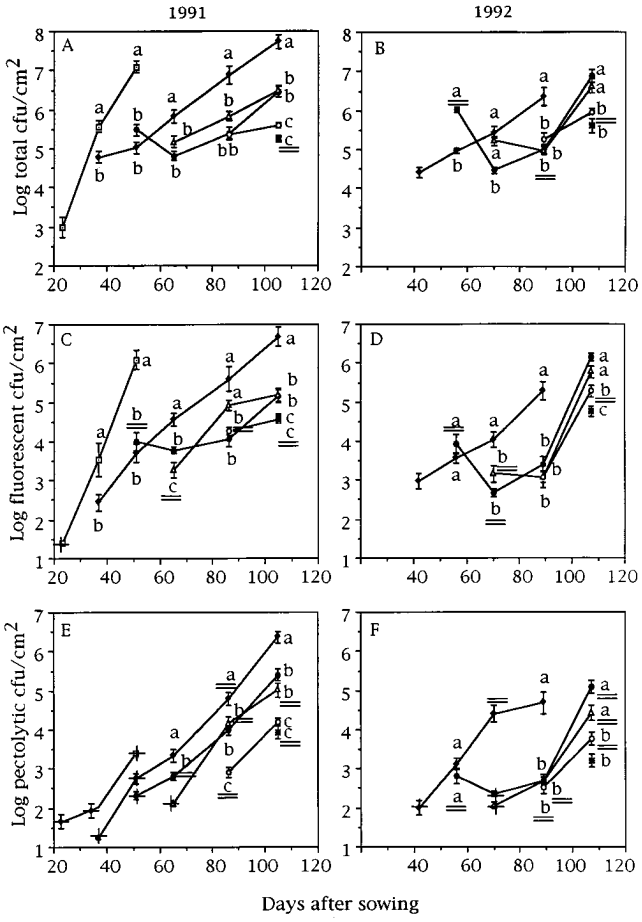

FIG. 1. Dynamics of mean densities of total (A and B), fluorescent (C and D), and pectolytic (E and F) bacteria on leaves of AG 1 (⊡), AG 2 (◆), AG 3 (●), AG 4 (△), AG 5 (○), and AG 6 (∗). Each point represents an average for about 30 and 24 individual leaves in 1991 and 1992, respectively. Points covered by a cross correspond to dates for which either no fluorescent or no pectolytic bacteria could be detected on at least 80% of the leaves sampled; hence, these points represent the mean level of sensitivity of the assay at that date. Error bars represent the standard error of the mean. For a given population at a given sampling date, mean densities followed by different letters are significantly ($P < 0.05$) different on the basis of Tukey's HSD test. Underlined letters indicate a significant effect of plant ($P < 0.05$) on bacterial densities for a given population and at a given sampling date.

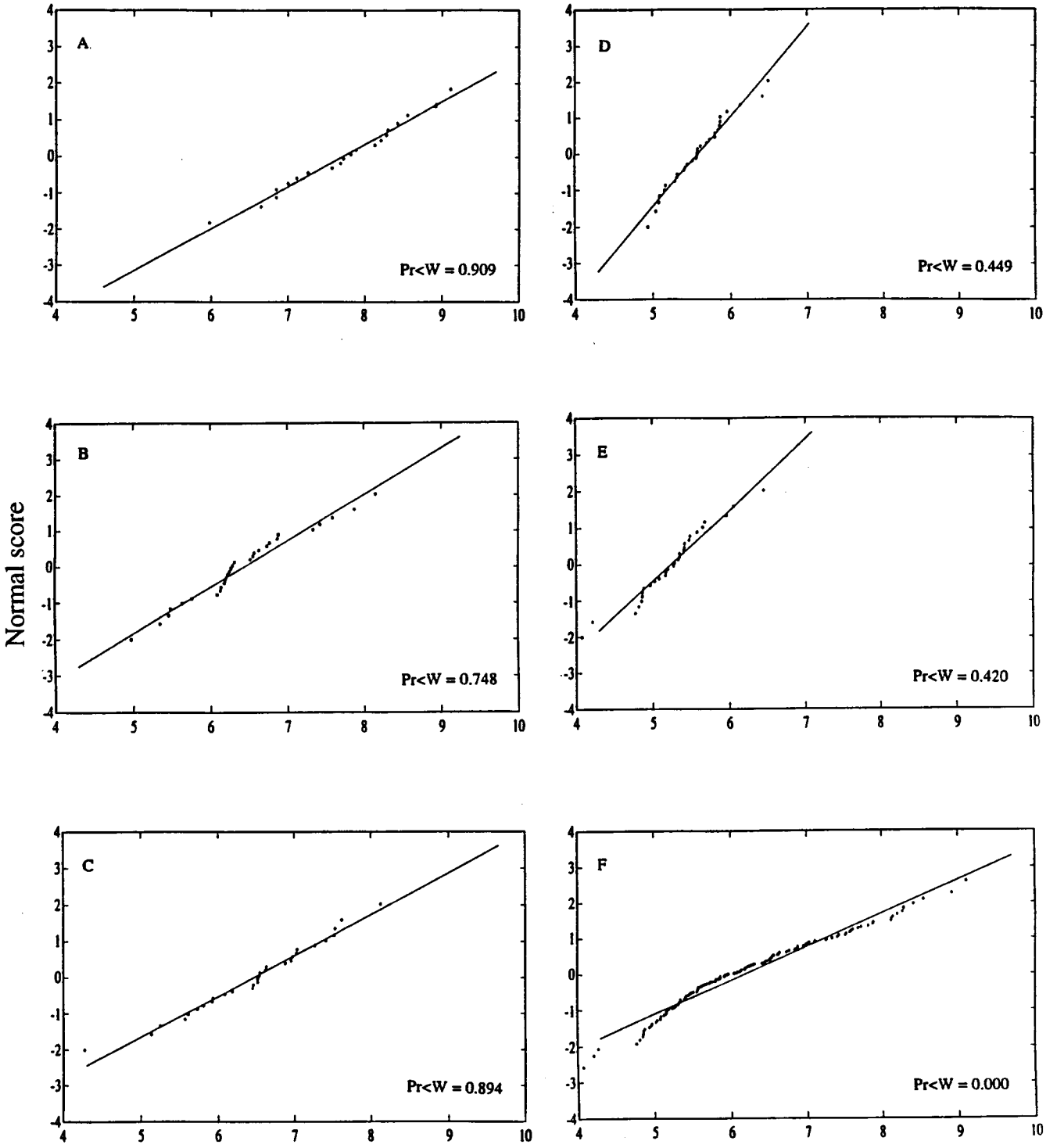

FIG. 2. Distributions of total bacterial population sizes at harvest 1991 on leaves of AG 2 (A), AG 3 (B), AG 4 (C), AG 5 (D), AG 6 (E), and all leaves pooled (F). The probability of observing a smaller W value (Pr<W) is shown on each graph. Values of Pr<W greater than 0.05 indicate a nonsignificant deviation from the normal distribution (31). Observations that follow a normal distribution approximate a straight line.

using the same field design as described above for the assessment of bacterial immigration. Plants were neither pruned nor dressed with plastic. Growth of a spontaneous rifampin-resistant mutant, PF130A, was assessed on three leaves from each of three adjacent plants immediately after inoculation and 24 h, 48 h, and 4 days after inoculation. To assess the influence of the pruning and plastic treatments on bacterial growth, growth of PF130A was determined on emerging leaves of 12-week-old plants from the spring planting at INRA.

Preparation of bacterial inoculum and inoculation of broad-leaved endive. *P. fluorescens* bv. 5 strain RTU 130 was isolated at harvest from broad-leaved endive grown at CTIFL in 1991. Strains of *P. fluorescens* bv. 5 were the predominant component of the fluorescent bacterial population isolated from broad-leaved endive at harvest in 1991 and 1992. A spontaneous rifampin-resistant mutant (PF130A) of RTU 130 was obtained by spread plating a suspension of the wild strain on diluted tryptic soy agar (TSA) (tryptone, 1.7 g; Bacto Soytone, 0.30 g; glucose, 0.25 g; NaCl, 0.50 g; K_2HPO_4, 0.25 g; agar, 15 g per liter) supplemented with 100 mg of rifampin per liter (TSA-rif). The mutation rate for this strain was about 5.8×10^{-7}. Individual mutant colonies were purified by several dilution platings onto TSA-rif. Physiological, biochemical, and biological properties of the mutant strains were analyzed and determined to be nondistinguishable from those of the wild strain. The in vitro growth rates at 4 and 25°C and the persistence of PF130A on broad-leaved endive leaves under field conditions were similar to those of the wild type.

In all experiments to determine growth of PF130A, the inoculum was produced by washing plates of a culture grown for 3 days at 25°C on TSA-rif with 0.05 M phosphate buffer (pH 7). The concentration of the inoculum was adjusted with a spectrophotometer to approximately 10^8 CFU/ml. A hand-held sprayer was used to apply inoculum to plants until runoff. Field inoculations were made at 0800 (autumn) and at 1900 (spring) on dew-covered plants on overcast days.

Quantification of bacterial populations. Collection of leaf samples, determination of their surface, and grinding of the individual leaves were as described previously (16). Dilutions of the leaf macerate suspension were spread plated on TSA to determine the size of the total bacterial population, on King's medium B (18) to quantify fluorescent pseudomonad populations, on Paton's polypectate double-layer medium (27) to quantify pectolytic bacteria, and on TSA-rif to track populations of PF130A. All media were amended with 50 mg of cycloheximide (Sigma) per liter. Colonies were counted after 3, 5, and 7 days of incubation at room temperature (ca. 25°C). Grindings and platings of leaf samples were completed within 24 or 48 h of sampling. Samples were processed in a random order for the description of bacterial population dynamics and per block for assessments of bacterial immigration and growth rates.

Data analysis. Regression analyses, determination of homogeneity of variances, determination of frequency distribution of bacterial populations, analyses of variance (ANOVA), and comparison of treatment means were conducted with SAS (SAS Institute Inc., Cary, N.C.), SYSTAT (Systat Inc., Evantson, Ill.), and STATISTICA (StatSoft Inc., Tulsa, Okla.) software. In order to address assumptions of ANOVA, the frequency distribution of bacterial population sizes on individual leaves was examined. The χ^2 test of goodness of fit (32) and the Shapiro-Wilk statistic (W) (31) were applied to population sizes expressed as $\log_{10}$ CFU per square centimeter. Skewness, kurtosis, and normal plots were also examined. Bartlett's test was used to detect nonhomogeneity of variances, and nonparametric ANOVA was used in cases of heteroscedasticity (32).

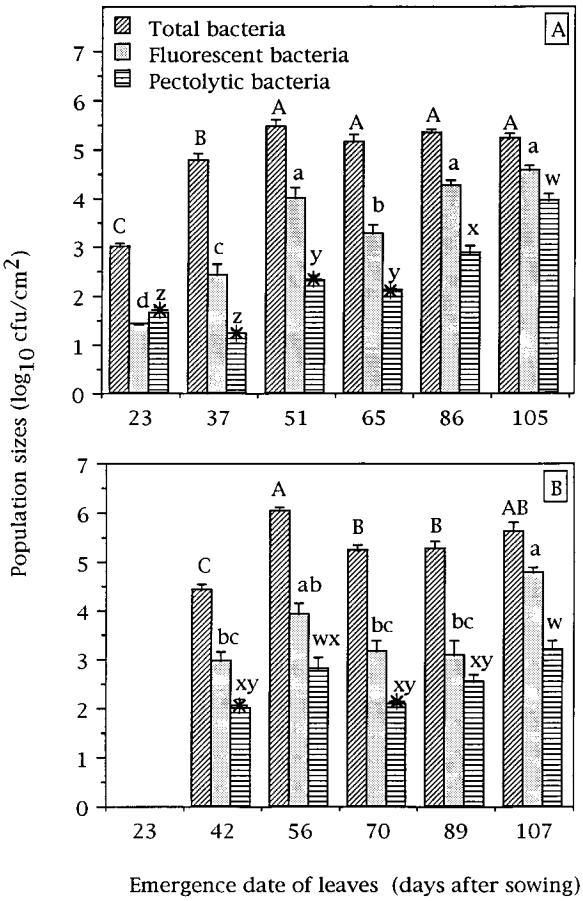

FIG. 3. Mean population sizes of total, fluorescent, and pectolytic bacteria on newly emerged leaves in 1991 (A) and 1992 (B). Newly emerged leaves were the youngest leaves on a plant that were completely unfurled and at least 5 cm long at the time of sampling. Each point represents an average for 27 leaves. Means associated with a star correspond to dates for which either no fluorescent or no pectolytic bacteria could be detected on at least 90% of the leaves sampled; hence, these points represent the mean level of sensitivity of the assay at that date. Error bars represent the standard error of the mean. For a given bacterial population per year, mean densities associated with different letters are significantly ($P < 0.05$) different on the basis of Tukey's HSD test.

RESULTS

Dynamics and sizes of bacterial populations on leaves of different AGs. Leaf AG had a significant effect ($P < 0.05$) on total bacterial population sizes at all sampling dates in 1991 and 1992 (Fig. 1). The effect of leaf AG on bacterial population sizes was significant for fluorescent and pectolytic bacteria in 1991 and 1992 for the majority of the sampling times (Fig. 1). At harvest in 1993, total, fluorescent, and pectolytic bacterial population sizes were significantly different on leaves of the three AGs present (AG 4, AG 5, and AG 6).

At each sampling time, the distribution of bacterial population sizes among leaves could generally be fitted by the normal distribution for population sizes expressed as $\log_{10}$ CFU per square centimeter of a single leaf AG (Fig. 2). The variances among the different age classes as indicated by the slopes of the lines in Fig. 2 are different. The log normal distribution provided a better fit for population sizes on leaves within individuals AGs than for the entire collection of leaves taken at a sampling date.

During the growing season, bacterial population sizes generally increased steadily over time, reaching densities of at least 2×10^4 total CFU/cm^2 at harvest (Fig. 1). Dynamics of a specific bacterial subpopulation differed among leaf AGs within each experiment. Dynamics of total, fluorescent, and pectolytic bacterial populations were generally not consistent among experiments for a given leaf AG. A gradient of decreasing bacterial densities from older leaves to younger leaves was statistically significant at harvest in all experiments ($P < 0.05$). At harvest in 1991, leaves could be separated into three groups that differed significantly in bacterial population sizes. These groups corresponded to three distinct parts of the head: the outer leaves (AG 2), the intermediate corona (AGs 3 and 4), and the inner leaves (AGs 5 and 6) (Fig. 1). Total, fluorescent, and pectolytic mean population densities were generally positively correlated. These correlations were rarely significant ($P < 0.05$) between fluorescent and pectolytic populations.

Bacterial population densities varied on newly emerged leaves throughout all growing seasons (Fig. 3). Bacterial population sizes on new leaves were the greatest at harvest (105 days after sowing in 1991; 107 days after sowing in 1992). Before the middle of the growing seasons, the lowest bacterial densities were quantified on leaves emerging on plants having less than 20 leaves. In 1991, total bacterial population sizes on new leaves stabilized after 50 days of cultivation, but fluores-

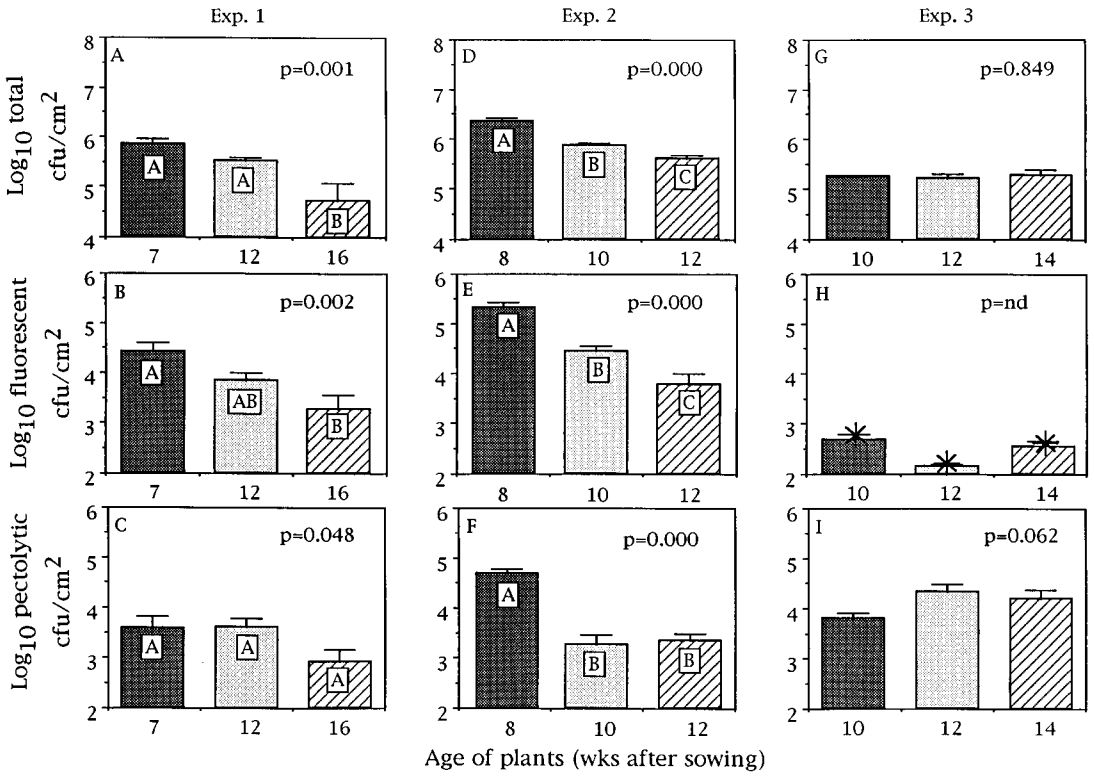

FIG. 4. Mean densities of total (A, D, and G), fluorescent (B, E, and H), and pectolytic (C, F, and I) bacteria on leaves emerging on plants of different ages of broad-leaved endive interplanted in the same field. Newly emerged leaves were the youngest leaves on a plant that were completely unfurled and at least 5 cm long at the time of sampling. All newly emerged leaves were sampled at the same time within an experiment. Plants were grown in experimental field plots at INRA in spring 1993 (experiment 1) and autumn 1993 (experiment 2) and in field plots at CTIFL in autumn 1993 (experiment 3). Each point represents an average for 18, 27, and 27 individual leaves for experiments 1, 2, and 3, respectively. Points covered by a star correspond to means for which no fluorescent bacteria could be detected on at least 90% of the leaves sampled; hence, these points represent the mean level of sensitivity of the assay at that date. Error bars represent the standard error of the mean. Mean densities associated with different letters are significantly ($P < 0.05$) different on the basis of Tukey's HSD test.

cent and pectolytic bacterial population sizes continued to increase until harvest. Pectolytic bacteria were not detected on newly emerged leaves until 3 weeks before harvest in 1991. Fluorescent and pectolytic bacterial densities were consistently greatest for leaves emerging late in the season just before harvest.

Bacterial immigration and growth rate at leaf emergence. The age of plants had a significant effect ($P < 0.01$) on total, fluorescent, and pectolytic bacterial population sizes on newly emerged leaves at INRA in the fall of 1993 (experiment 2) and on total and fluorescent bacterial population sizes at INRA in the spring of 1993 (experiment 1) (Fig. 4). Population sizes on leaves emerging on the youngest plants were significantly greater than population sizes on leaves emerging on the oldest plants. At CTIFL in the fall of 1993 (experiment 3), plant age did not have a significant effect on bacterial population size on newly emerged leaves (Fig. 4).

Removal of adjacent leaves resulted in significant ($P < 0.05$) increases in fluorescent bacterial population sizes on emerging leaves in the fall of 1993 at CTIFL (Fig. 5). On average, the older the plants, the greater the increase in fluorescent bacterial population sizes on those emerging leaves exposed to air contamination. Covering the remaining older leaves with a piece of polypropylene did not result in a constant modification of population sizes on newly emerging leaves. For other bacterial populations and experiments, differences among bacterial population sizes associated with newly emerged leaves from plants that received the three different treatments were generally small. No treatment had a significant effect in all experiments on a given type of leaf or component of the bacterial population.

PF130A population sizes generally did not increase after inoculation (Tables 1 and 2). PF130A population sizes on leaves sampled 7 days after inoculation were not affected by treatments performed on 12-week-old plants (Table 2). The age of plants influenced population sizes of PF130A associated with newly emerged leaves for two of the three experiments (Table 1). Dynamics of PF130A population sizes on leaves of plants of different ages was not consistent among the three experiments. For the experiment in the autumn of 1993 at INRA, the effect of plant age was significant ($P < 0.05$), with the highest populations occurring 4 days after inoculation on leaves emerging on the youngest plants. The mean population size of PF130A after inoculation was about 3×10^5 CFU/cm^2, similar to the size of the naturally occurring populations of total bacteria on newly emerged leaves of young and intermediate plants (Fig. 5).

DISCUSSION

This study presents evidence that leaf age affects densities of epiphytic bacterial populations of field-grown, broad-leaved endive and that immigration of bacteria to leaves may contribute significantly, albeit sporadically, to the sizes of the fluorescent epiphytic population. During field cultivation of broad-leaved endive, older leaves support more bacteria than

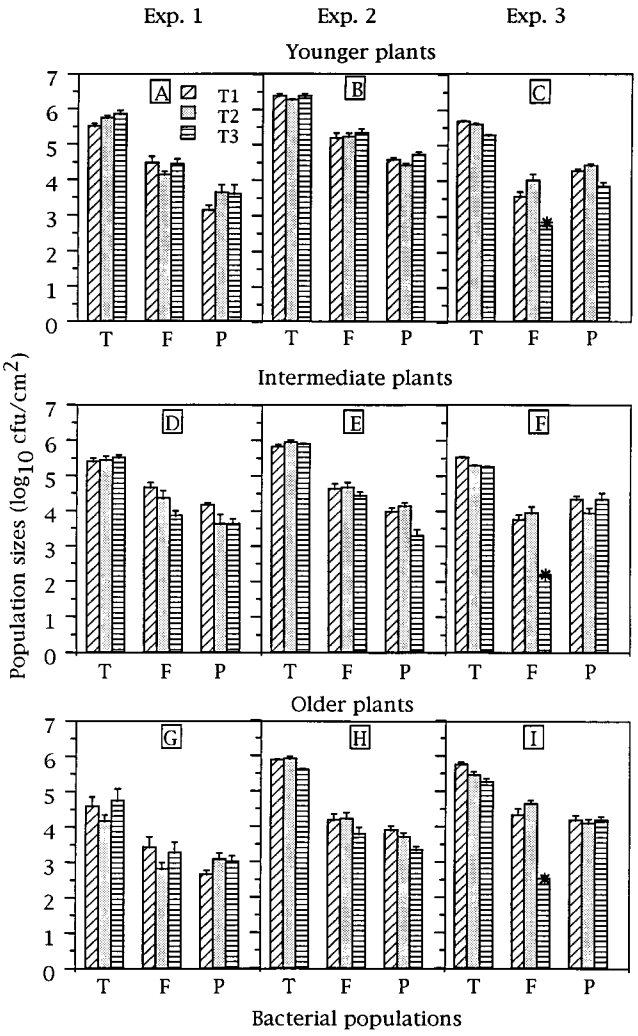

FIG. 5. Mean densities of total (T), fluorescent (F), and pectolytic (P) bacteria on newly emerged leaves on plants of different ages of broad-leaved endive interplanted in the same field. Newly emerged leaves were the youngest leaves on a plant that were completely unfurled and at least 5 cm long at the time of sampling. All newly emerged leaves were sampled at the same time within an experiment. The ages of plants at sampling times were 7, 12, and 16 weeks after sowing for experiment 1 (A, D, and G), 8, 10, and 12 weeks after sowing for experiment 2 (B, E, and H), and 10, 12, and 14 weeks after sowing for experiment 3 (C, F, and I). In treatment T1, inner leaves were excised 1 week before sampling to expose leaves that would emerge during the following week. For treatment T2, a piece of polypropylene was used to cover older leaves remaining after leaf excision. Treatment T3 plants were not groomed. Plants were grown in experimental field plots at INRA in spring 1993 (experiment 1) and autumn 1993 (experiment 2) and in field plots at CTIFL in autumn 1993 (experiment 3). Each point represents an average for 18, 27, and 27 individual leaves for experiments 1, 2, and 3, respectively. Points covered by a star correspond to means for which no fluorescent bacteria could be detected on at least 90% of the leaves sampled; hence, these points represent the mean level of sensitivity of the assay at that date. Error bars represent the standard error of the mean. The removal of adjacent leaves (T1 or T2 versus T3) had a significant ($P < 0.01$) effect on fluorescent bacterial population sizes in experiment 3 only (C, F, and I).

younger leaves. Our previous work has shown that these leaf age-associated differences at harvest affect the microbiological and market quality of stored ready-to-use broad-leaved endive (16). Here, we have also separated the effect of leaf age from that of plant age on bacterial population dynamics. We have shown that bacterial numbers increase as leaves age but that plant age also contributes significantly to bacterial population

TABLE 1. Mean population densities of *P. fluorescens* bv. 5 strain PF130A after inoculation on newly emerged leaves of broad-leaved endive on plants of different ages[a]

Expt[b]	Age of plants (wk after sowing)	Mean density (log_{10} CFU/cm^2) at given sampling time (SEM)[c]			
		0 h	24 h	48 h	4 days
1	7	5.67 (0.04)	5.65 (0.08)	5.42 (0.07)	5.29 (0.05)
	12	5.63 (0.10)	5.58 (0.11)	5.45 (0.08)	5.21 (0.09)
P[d]		0.713	0.604	0.795	0.447
2	8	5.77 (0.31)	5.72 (0.07)	5.80 (0.09)	6.17 (0.08)
	10	5.89 (0.03)	5.75 (0.05)	5.75 (0.07)	5.49 (0.08)
	12	5.66 (0.05)	5.81 (0.05)	5.66 (0.05)	5.71 (0.06)
P		0.000	0.540	0.444	0.000
3	10	5.68 (0.04)	5.65 (0.03)	5.70 (0.03)	5.53 (0.05)
	12	5.41 (0.04)	5.42 (0.04)	5.53 (0.04)	5.45 (0.06)
	14	5.51 (0.07)	5.57 (0.03)	5.82 (0.05)	5.65 (0.05)
P		0.002	0.000	0.000	0.030

[a] Newly emerged leaves were the youngest leaves on a plant that were completely unfurled and at least 5 cm long at the time of inoculation.
[b] Plants were grown in experimental field plots at INRA in spring 1993 (experiment 1) and autumn 1993 (experiment 2) and in field plots at CTIFL in autumn 1993 (experiment 3).
[c] Each mean represents an average for 18, 27, and 27 individual leaves for experiments 1, 2, and 3, respectively. Leaves were sampled immediately and 24 h, 48 h, and 4 days after inoculation.
[d] Probability of observing a greater *F* value from an ANOVA of the effect of plant age on bacterial population sizes for each sampling time of each experiment.

sizes. Leaf age affects the variance of epiphytic bacterial population sizes: those on younger leaves are less variable than those on older leaves.

Inner leaves of cabbage, lettuce, witloof chicory, and broad-leaved endive have been reported to carry smaller bacterial population densities than outer leaves at harvest (8, 12, 24, 33). Consistent with these findings, our work shows a gradient of decreasing contamination from outer (older) leaves to inner (younger) leaves of the head of broad-leaved endive. The regression describing this gradient has been shown to be signif-

TABLE 2. Mean population densities of *P. fluorescens* bv. 5 strain PF130A inoculated on emerging leaves of groomed broad-leaved endive plants

Treatment[a]	Mean density (log_{10} CFU/cm^2) at given sampling time (SEM)[b]			
	0 h	24 h	4 days	7 days
T1	5.46 (0.07)	5.37 (0.06)	4.67 (0.09)	4.28 (0.21)
T2	5.46 (0.07)	5.46 (0.09)	4.99 (0.07)	4.53 (0.13)
T3	5.61 (0.03)	5.45 (0.08)	5.37 (0.16)	4.52 (0.12)
P[c]	0.054	0.638	0.008	0.428

[a] In treatment T1, inner leaves were excised 1 week before sampling to expose leaves that would emerge during the following week. For treatment T2, a piece of polypropylene was used to cover older leaves remaining after leaf excision. Treatment T3 plants were not groomed.
[b] Each mean represents an average for 18 individual leaves emerged on 12-week-old plants grown in experimental field plots at INRA in spring 1993. Leaves were sampled immediately and 24 h, 4 days, and 7 days after inoculation.
[c] Probability of observing a greater *F* value from an ANOVA of the effect of plant age on bacterial population sizes for each sampling time of each experiment.

icant for total bacterial populations and fluorescent and pectolytic components at harvest (16).

We suggest that the effect of plant age observed in our experiment is due to the interaction of bacterial immigrants with plants at different morphological stages. In our experiments in fields of plants of the same age, there were greater bacterial population sizes on newly emerging leaves later than earlier in the growing season. This is consistent with the hypothesis that the density of airborne immigrants increases during the growing season as crops mature. Plant foliage is the principal source of airborne bacteria in agricultural areas as reported by Lindemann et al. (22), who measured greater bacterial densities above cropped fields than above bare soils.

Plant morphology also had a significant influence on phylloplane populations. In fields of plants of three different ages, bacterial population sizes on emerging leaves were smallest on the oldest plants. In broad-leaved endive heads, outer leaves are exposed to air whereas inner leaves are protected by adjacent leaves. In one of our experiments, fluorescent pseudomonad densities substantially increased on emerging leaves after adjacent leaves were cut off, indicating that the adjacent leaves could act as a barrier for contamination of emerging leaves. However, in two other experiments leaf pruning did not lead to an increase in bacterial densities on emerging leaves. It is possible that airborne inoculum concentrations were different among the three experiments and that this difference led to an inconsistency in the effect of leaf pruning on bacterial immigration. The hypothesis of accessibility to airborne spores was suggested by Andrews et al. (2) to explain the differences in microbial populations at the periphery of the canopy versus inside the canopy, but this hypothesis was not tested in their work. Our experiments provide further evidence that differences in bacterial population sizes on leaves of different positions could be attributed in part to variations in accessibility of leaves to airborne bacteria.

The importance of bacterial immigration for bacterial population dynamics on broad-leaved endive is further supported by the results of experiments on growth of a marked strain of *P. fluorescens* introduced on emerging leaves. Differences in growth or survival of the introduced bacterium PF130A on emerging leaves on plants of different ages were small compared with differences observed for naturally occurring populations on corresponding leaves. Furthermore, growth and survival of PF130A were not influenced by pruning of leaves in our studies of bacterial immigration. The population size of the introduced strain of *P. fluorescens* did not increase during the first week after inoculation, suggesting either that populations were not growing or that growth was balanced by losses due to emigration and death. Inoculation of PF130A was not followed in the first 24 h by a rapid decline of the population, as shown by Hirano and Upper (15) and Pruvost and Gardan (30). Though populations did not increase significantly over time, the level of persistence of PF130A was similar to that of an inoculated strain of *P. syringae* on beans (15). Perhaps emergent leaves are not the preferential site of colonization for strain PF130A. Differences in the ability of bacteria to colonize mature versus young leaves have been noted in other systems (26).

The differences we have observed in the variance of the bacterial population sizes among leaves of different ages and positions have important implications for sampling strategies and statistical analyses, experimental designs of tests for biological control of foliar diseases, and studies of the ecology of the interactions between host plants and bacteria. When we consider the morphology of broad-leaved endive plants, it is likely that there is substantial heterogeneity among leaves as habitats for bacteria. As we have illustrated here, differentiation of leaves by AG is one means to increase precision in determining population size and hence would increase the sensitivity of statistical tests aimed at comparing the effects of treatments on epiphytic bacteria. The precise determination of microbial population dynamics as influenced by host factors is required for successful prediction and enhancement of biological, chemical, and integrated control methods. As reported in our previous work, leaf age influences the likelihood that leaf tissue will decay in storage. Differences in this likelihood are partly a function of bacterial population size (16).

Overall, this work emphasizes the importance of leaf and plant age on bacterial population dynamics and how age may interact with bacterial immigration and growth. Physiological condition of leaves, micro- and macroenvironmental conditions, and accessibility of leaves to airborne bacteria are also likely to be important in the variations of total, fluorescent, and pectolytic bacteria among leaves of different ages and positions of broad-leaved endive. Leaf temperature and relative humidity (7), anatomic features (10), leachate composition and quantity (5), and wax composition and ultrastructure (13) are known to vary for leaves of different ages. We suggest that positional and leaf age variations influence the quality of habitats for bacteria within a plant canopy.

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