Estimation of and Temporal Changes in Means and Variances of Populations of *Pseudomonas syringae* on Snap Bean Leaflets

Susan S. Hirano, Murray K. Clayton, and Christen D. Upper

First author: Department of Plant Pathology, University of Wisconsin, Madison 53706; second author: Departments of Plant Pathology and Statistics, University of Wisconsin, Madison; and third author: Department of Plant Pathology and USDA ARS Plant Disease Resistance Research Unit, University of Wisconsin, Madison.

Cooperative research of the College of Agriculture and Life Sciences, University of Wisconsin, Madison, and the Agricultural Research Service, U.S. Department of Agriculture.

Supported in part by USDA Competitive Grant 84-CRCR-1-1422.

We thank P. Fischer, N. Grieshop, M. Holt, B. Hudelson, W. Kittel, T. Klusmeyer, S. Lietzke, M. Ruppel, and E. Scherb for valuable technical assistance and S. Vicen for preparation of the figures. We also thank Sunseeds Genetics, Inc., Hollister, CA, for the bean seeds used in this research.

Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Accepted for publication 25 May 1994.

ABSTRACT

Hirano, S. S., Clayton, M. K., and Upper, C. D. 1994. Estimation of and temporal changes in means and variances of populations of *Pseudomonas syringae* on snap bean leaflets. Phytopathology 84:934-940.

Temporal changes in means and variances of populations of *Pseudomonas syringae* were examined in experimental field plots (30 m × 30 m) of snap bean cultivar Cascade. For each of two plantings, 30 individual leaflets were collected 5 days/wk from plant emergence to pod harvest and processed by dilution plating to estimate population sizes of *P. syringae*. Because population sizes of *P. syringae* were below the limit of detection for our method on some of the leaflets from several of the sampling times, the effect of censoring on data analysis was examined. A maximum likelihood (ML) method was compared to assigning censored values the limit of sensitivity (LS). If fewer than 10% of the values were censored, there was little difference between the mean or the variance estimated by either method. However, differences between the two methods increased as the amount of censoring increased, and at about 80% censoring, use of the LS method resulted in an estimated mean about 100 times larger, and an estimated variance about four to eight times smaller than the ML method. Temporal changes in population sizes of *P. syringae* differed substantially between the two plantings established about 1 mo apart when time was expressed as days after planting but not when time was expressed as calendar date. This suggests that the weather or other physical factors may have a relatively greater influence on the population size of this bacterium than the growth stage of the bean plants on which they reside. During periods when population sizes of *P. syringae* were relatively large, the bacterium was the dominant component of bacterial communities on bean leaflets. Changes in population size of *P. syringae* were relatively small (less than fivefold) in most 24-h periods but were very large (as great as 2,000-fold) on relatively fewer occasions. The largest change within 24 h corresponded to a doubling time of 2.1 h, which is of similar order of magnitude to the optimal doubling time for this bacterium in culture.

Pseudomonas syringae is a common inhabitant of leaves of a diversity of plant species, where it may be present in epiphytic and/or pathogenic association(s) with its habitat (3). On leaves of snap bean plants (*Phaseolus vulgaris* L.), epiphytic populations of *P. syringae* pv. *syringae* serve as an immediate source of inoculum for bacterial brown spot disease (12). It has been demonstrated that the establishment of relatively large epiphytic population sizes of *P. s. syringae* is necessary for the likelihood of occurrence of bacterial brown spot (2,8,12). Lindemann et al (8) reported that the frequency with which population sizes of the pathogen exceeded 10^4 colony forming units (cfu) per symptomless bean leaflet was predictive of brown spot disease incidence. From infectivity titration experiments conducted under field conditions, Rouse et al (12) estimated an ED_{50} value of approximately 3×10^5 cfu per leaflet for bacterial brown spot.

To determine the likely rates at which populations of *P. syringae* may increase to a level that places the crop at hazard to disease, population sizes of *P. syringae* on leaflets from field-grown bean plants were estimated every 2 h during each of three 26-h periods (4). The overall diel changes in *P. syringae* population sizes differed during each of the 26-h periods. In one 26-h period, there was a continuous increase in *P. syringae* population size that led to a net 28-fold increase in bacterial density. During the other two 26-h periods, changes in population sizes of *P. syringae* were a fivefold increase and no detectable change. The 26-h periods sampled represent only 3 days in the approximately 50-day life span of a bean crop from plant emergence to pod harvest. Thus, the primary objective of this study was to determine the magnitudes of temporal changes in means and variances of *P. syringae* populations over the approximately 50-day life span of a bean crop as a step toward identifying those factors that regulate the dynamics of *P. syringae* population sizes. In the process of calculating population means and variances, we were faced with the issue of how to handle censored observations (i.e., leaflets with numbers of *P. syringae* below our limit of sensitivity of approximately 190 cfu per leaflet [2.279 log cfu per leaflet]). Thus, a second objective was to assess the effect of such censored observations on estimations of population means and variances.

MATERIALS AND METHODS

Plot design and sampling procedure. Experimental plots of snap bean cultivar Cascade (Sunseeds Genetics, Inc., Hollister, CA) were established on the University of Wisconsin Experiment Station, Arlington. Seeds were planted in a 30 × 30 m block on each of two planting dates: 23 May and 19 June 1984. Rows were spaced 76 cm apart and contained approximately 10 plants

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 1994.

per meter. The seeds had had prior commercial treatment with captan. At the time of planting, the seeds were treated additionally with Agrox D-L Plus (Wilbur-Ellis, Fresno, CA).

One hundred individual leaflets of approximately equivalent size were collected from the top of the plant canopy on each of 5 days/wk (i.e., Monday to Friday) from plant emergence to pod harvest. Leaflets were collected between 0730 and 0830 on each sampling day. An attempt was made to select leaflets randomly from the top of the canopy although no formal randomization procedure was used. The samples were placed in No. 5 Kraft paper bags and transported in a cooler to the field laboratory for immediate processing (i.e., within 5–10 min after completion of sampling).

Each leaflet was submerged in 9.0 ml of sterile, ice nucleus-free potassium phosphate buffer (0.01 M, pH 7.0) in a 16-mm test tube. All leaflets were assayed with a tube ice-nucleation test to rapidly assess the frequency with which bean leaflets harbored relatively large population sizes of *P. syringae* (2). The results of the tube ice nucleation tests were obtained for other related experiments and will not be reported in this manuscript. Thirty of the 100 tubes were randomly marked before the leaflets were placed in them. The leaflets in the marked tubes were stored frozen at −20 C until they could be processed by dilution plating for quantitation of population sizes of *P. syringae* and total culturable bacteria.

To determine the effect of storage at −20 C on bacterial population sizes, approximately 500 leaflets were collected at a single time at the end of the growing season. Each leaflet was placed in a sterile test tube with buffer and stored at −20 C as described above. Sets of 20 leaflets were thawed and dilution plated at approximately 3- to 4-wk intervals during the time required to complete processing of all of the leaflets collected daily during the 1984 growing season.

Determination of bacterial population sizes on individual bean leaflets. For processing by dilution plating, each leaflet was thawed at ambient temperature (about 22–24 C). The leaflet and 9 ml of buffer in each tube were transferred to a sterile 50-ml beaker. The test tube was rinsed with 10 ml of sterile potassium phosphate buffer (0.1 M, pH 7.0) supplemented with Bacto-Peptone (0.1% w/v). The rinse buffer was added to the beaker for a total volume of 19 ml. The leaflet was minced with sterile scissors then homogenized for 10 s with a Polytron equipped with a model PTA 20 TS probe (Brinkmann Instruments, Westbury, NY) set at speed 5. The Polytron was cleansed and sterilized between samples by running the probe in two changes each of hot water and 95% ethanol. Tenfold serial dilutions were prepared in sterile phosphate buffer (0.01 M, pH 7.0), and portions from the original homogenate and serial dilutions were plated onto King's Medium B (KMB) (7) supplemented with cycloheximide (100 μg/ml) to inhibit the growth of fungi. Fluorescent colonies with the appropriate colony morphology for *P. syringae* were counted after 3–4 days of incubation at ambient temperature. Colonies of all other bacteria were counted after 7 days of incubation. Total bacteria refer to the sum of *P. syringae* and all other bacteria that grew on KMB.

For each set of 30 leaflets collected on a given day, bacterial population sizes were $\log_{10}$-transformed and expressed as log cfu per leaflet prior to calculations of population means and variances. Population statistics for sets of leaflets that included censored observations (i.e., bacterial population size less than 2.279 log cfu per leaflet) were estimated in two ways. In the first, censored observations were assigned the limit of sensitivity of 2.279 log cfu per leaflet, a statistical process known as imputation (10) (hereafter referred to as the LS procedure). In the second, population means and variances were estimated using the maximum likelihood procedure of Rouse et al (12) (hereafter referred to as the ML procedure).

RESULTS AND DISCUSSION

Effect of storage at −20 C on bacterial populations on snap bean leaflets. To obtain meaningful estimates of means and variances of bacterial population sizes, 30 leaflets were collected at each sampling time and processed individually by dilution plating. A total of 1,950 individual leaflets was processed for the two plantings. The leaflets were stored frozen to provide a way of handling the large number of samples in an efficient manner. Approximately 50% of the *P. syringae* on leaves survived the initial freezing event.

Population sizes of *P. syringae* on leaflets collected in 1984 were not adversely affected by prolonged storage at −20 C (Fig. 1). Total bacterial populations may have decreased slightly during storage. However, the mean total bacterial populations were not significantly different at 1 versus 298 days of storage at −20 C. We also examined the effect of storage at −20 C on bacterial population sizes for sets of leaflets collected during 1983 and 1986. Results from all three experiments (1983, 1984, and 1986) were similar. Indeed, the last time point examined for the leaflets collected in 1986 was 3 yr after leaf harvest. Population sizes of *P. syringae* and total bacteria on bean leaves immersed in buffer were stable for up to 3 yr at −20 C.

Estimation of bacterial population means and variances: Statistical considerations. On several occasions throughout the growing season, *P. syringae* was not detected on a proportion of the leaflets collected on a given day. *P. syringae* may have been present on many of these leaflets because our limit of detection was 190 viable bacterial cells per leaflet. Such points for which the actual numbers of *P. syringae* were below the limit of sensitivity and thus, not detected by our method were considered censored observations. A data set that illustrates a fairly high level of censoring is illustrated in Figure 2.

The issue is how to obtain meaningful estimates of means and variances of bacterial population sizes for data sets with censored observations. A common method is to assign the limit of sensitivity (or a proportion thereof) to the censored points (10). An alternative method that involves the use of a maximum likelihood algorithm was developed by Rouse et al (12). An assumption of the latter method is that the underlying frequency distribution of the observations approximates a lognormal (12). We previously reported that leaf-associated bacterial populations, including *P. syringae*, could be modeled with the lognormal frequency distribution (1). Ishimaru et al (6) reported that the Weibull distribution provided a better fit than the lognormal for populations of *Xanthomonas campestris* pv. *phaseoli* on bean leaflets. Since our original report (1), we have examined the frequency distribution

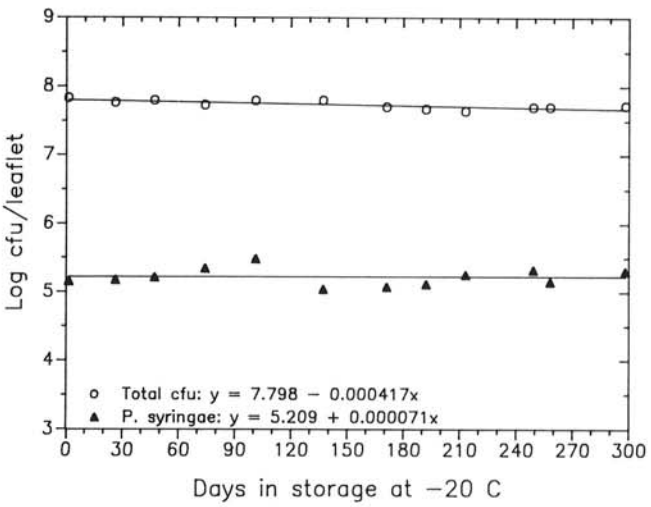

Fig. 1. Effect of storage at −20 C on bacterial populations on snap bean leaflets. At approximately monthly intervals, a subset of 20 frozen leaflets was selected at random from a set of approximately 500 leaflets collected at a single time at the end of the growing season and stored frozen at −20 C. Each leaflet was processed individually by dilution plating onto King's Medium B (KMB). The data are the mean log colony forming units (cfu) of *Pseudomonas syringae* and total bacteria culturable on KMB for each set of 20 leaflets.

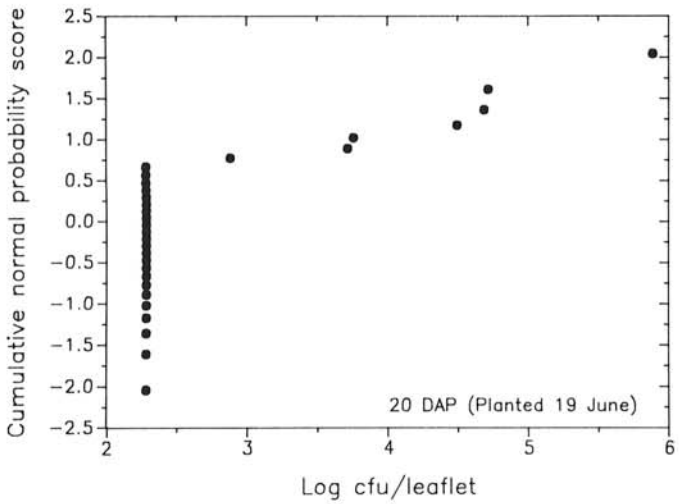

Fig. 2. Cumulative normal probability plot of population sizes of *Pseudomonas syringae* on individual bean leaflets collected 20 days after planting (DAP) for the 19 June planting. Each leaflet was processed individually by dilution plating of leaflet homogenates. Population sizes of *P. syringae* below our limit of sensitivity were assigned a value of 2.279 log colony forming units (cfu) per leaflet.

of an additional 100 data sets and found that for some sets the Weibull provided a better fit than the lognormal (S. S. Hirano and C. D. Upper, *unpublished data*). In more cases, however, the lognormal was superior.

For the data sets examined in this study, the lognormal distribution adequately described population sizes of *P. syringae* using the Wilk-Shapiro test for normality (13) for data sets with no censored observations. Because the Wilk-Shapiro test cannot be used on sets containing censored points, the appropriateness of modeling bacterial population sizes with the lognormal for such sets was evaluated graphically by the extent to which a plot of leaflets with detectable numbers of *P. syringae* approximated a straight line when population sizes were plotted against cumulative normal probability scores (Fig. 2). Even with the extreme censoring (75% of data points censored) found for the data set shown in Figure 2, the seven leaflets with detectable *P. syringae* population sizes appeared to fall on a straight line. Similar results were obtained for the other data sets that contained censored observations. Thus, we have assumed that the lognormal distribution is appropriate for these data sets as well.

Temporal changes in means and variances of populations of *P. syringae* are presented in Figures 3 and 4, respectively, for each of the two plantings. For the 23 May planting, there was a general increasing trend over time in means of *P. syringae* populations estimated with the LS and ML methods (Fig. 3A); for the 19 June planting, bacterial population means fluctuated over time, with little discernible overall trend (Fig. 3B). Means estimated by the ML method were always lower than those estimated by the LS method, although the discrepancy between

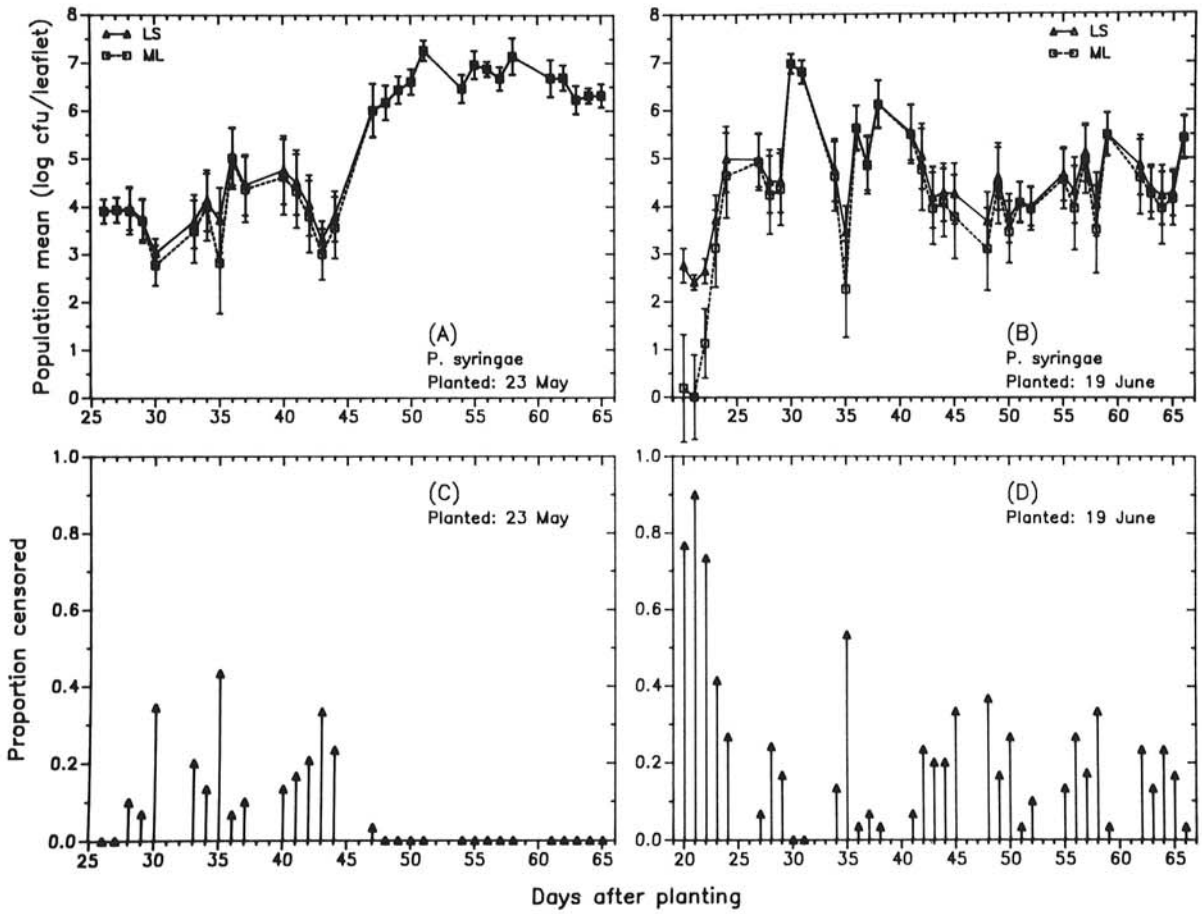

Fig. 3. Temporal changes in means of *Pseudomonas syringae* population sizes for two plantings of snap beans. Plantings were on **A,** 23 May and **B,** 19 June. Thirty leaflets were collected at each sampling time and processed individually by dilution plating. For each set of 30 samples, the proportion of leaflets with population sizes of *P. syringae* below our limit of sensitivity are shown in **C and D** for the first and second plantings, respectively. Means were calculated by 1) an imputation method (LS) wherein censored observations were assigned a limit of detection of 2.279 log colony forming units per leaflet and 2) the maximum likelihood procedure (ML) of Rouse et al (12). Error bars in **A and B** represent 95% confidence intervals.

the two methods was typically small (less than 10-fold) unless the extent of censoring was in excess of roughly 30% (Fig. 3C and D). When censoring was near 80%, the ML and LS methods differed by about 100-fold. The discrepancy between the two methods increased roughly linearly with the extent of censoring (Fig. 5). These discrepancies could potentially be diminished by assigning a value smaller than the limit of sensitivity to censored observations when employing the LS method. However, the actual effect of doing so is small. For example, if a value of one-half the limit of sensitivity is imputed, then the means estimated by the LS method for 20, 21, and 22 days after planting (DAP) for the 19 June planting become 2.46, 2.06, and 2.36 log cfu per leaflet, respectively, rather than 2.75, 2.40, and 2.63, respectively, when the limit of sensitivity is inputed.

The distinction between the LS and ML methods is even more striking in the resultant estimates of variance (Fig. 4A and B). Again the largest differences arose in situations in which censoring was largest, and again the discrepancy showed a roughly linear trend in the proportion censored (Fig. 5). The variances estimated by the ML method were four to eight times larger than those estimated by the LS method for five data sets with censoring greater than about 45–50%.

That the LS method should underestimate variance is not surprising. When the extent of censoring is large, a substantial fraction of the "data" consists of a single value that has been imputed numerous times. However, it is quite likely that at least some of the unobserved values for which these serve as surrogates lie further from the mean than the imputed values. Thus, we expect to see a reduction in the apparent variance in the modified data set. Modifying the imputed value has relatively little effect. For example, if a value of one-half the limit of sensitivity is imputed, then the variances estimated by the LS method for 20–22 DAP of the 19 June planting become 1.26, 0.29, and 0.73, respectively, as opposed to 0.95, 0.19, and 0.50, respectively, when the limit of sensitivity is imputed.

Because the LS method performs well when the extent of censoring is small, it may be preferred in such circumstances. For censoring above 10%, the ML method might provide more realistic estimates. It is important, however, to recognize that the ML method also has limitations, although they are less immediately obvious. First, as noted, the ML method as implemented here requires that the underlying (transformed) data be normally distributed (11). It is unclear how that method will perform when the data are not normal, and it is necessary, therefore, that the distribution of the data be examined before applying the method. It is possible to modify the ML method for other data distributions, but again this requires that the distribution be known.

Second, the ML method generally can produce biased estimates (11). This bias often arises in applications of maximum likelihood approaches. Nonetheless, methods based on maximum likelihood have considerable popularity in the statistical literature and its applications. The bias is not usually considered a serious fault of the method, in part because any potential bias diminishes to zero with increasing sample size (11).

Although the discrepancy between the ML and LS methods for estimating means is small except in extreme cases of censoring, this does not render the discrepancy in variance estimation less problematic. Recall that the estimation described above is on the log-transformed scale. In general, if μ and σ^2 represent the population mean and variance of data that are normally distributed on a log-transformed scale, then the mean and variance on the untransformed scale are given by $e^{\mu+\sigma^2/2}$ and $e^{2\mu+\sigma^2}(e^{\sigma^2}-1)$, respectively. Thus, in terms of describing the population in the original scale, an error in estimating the variance on the log scale can translate into a substantial error on the untransformed scale.

Changes in bacterial population means and variances: Biological considerations. The overall seasonal trends in population sizes of *P. syringae* differed significantly for the two plots of snap beans established 27 days apart during a single growing season (Fig. 3). On average, *P. syringae* was a dominant com-

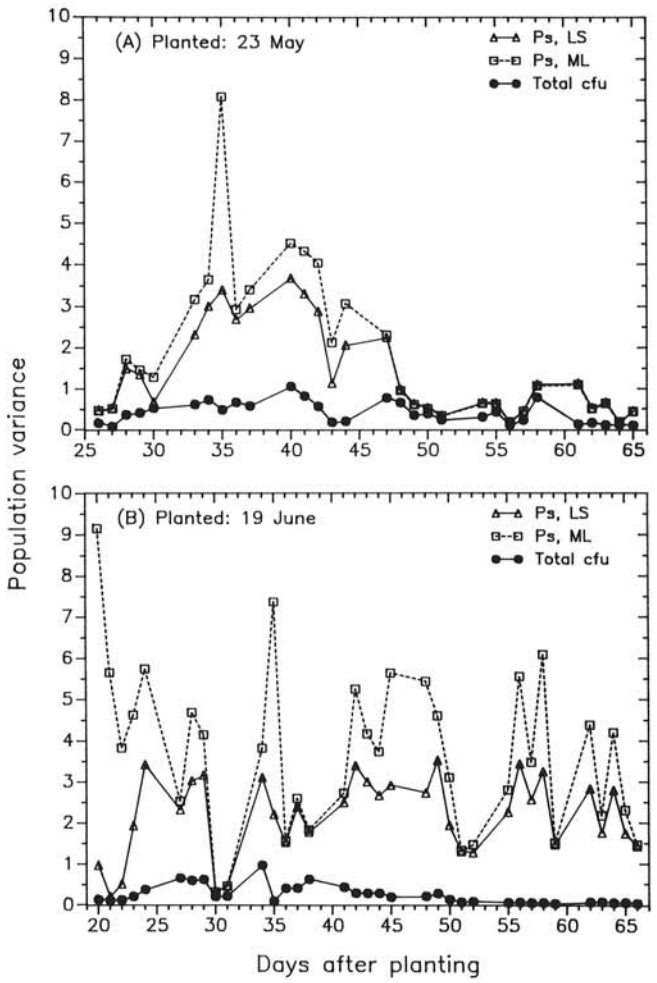

Fig. 4. Temporal changes in variances of populations of *Pseudomonas syringae* (Ps) and total bacteria culturable on King's Medium B (total colony forming units [cfu]) for two plantings of snap beans. Plantings were on **A,** 23 May and **B,** 19 June. Thirty leaflets were collected at each sampling time and processed individually by dilution plating. Variances were calculated by 1) an imputation method (LS) wherein censored observations were assigned a limit of detection of 2.279 log cfu per leaflet and 2) the maximum likelihood procedure (ML) of Rouse et al (12).

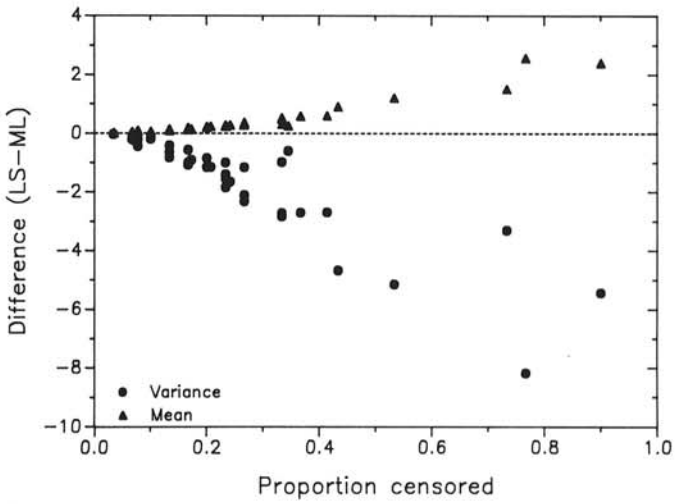

Fig. 5. Differences in estimates of means and variances of populations of *Pseudomonas syringae* using the maximum likelihood procedure (ML) of Rouse et al (12) and an imputation method (LS) wherein censored observations were assigned a limit of detection of 2.279 log colony forming units (cfu) per leaflet as a function of the proportion of leaflets with censored observations. The actual values for the means and variances are presented in Figures 3 and 4.

ponent of the bacterial community during the latter half of the growing season for beans planted on 23 May (Fig. 6A and C). However, on beans planted on 19 June, *P. syringae* was transiently dominant during the first half of the season (Fig. 6B and D). After approximately 42 DAP, it was a minor component of the bacterial communities present on bean leaflets. The period during which *P. syringae* was a substantial component of the bacterial community for the two plantings approximately coincided when time was expressed as calendar date, not DAP (i.e., 45–65 DAP for the first planting correspond to 18–38 DAP for the second planting). This suggests that, regardless of plant age, weather conditions were favorable for growth and maintenance of large population sizes of *P. syringae* during this period. The greater variability in mean population sizes on younger plants compared to older ones during this period further suggests that plant phenology may influence the dynamics of *P. syringae*. From this and other experiments, we have observed that large population sizes of *P. syringae* are frequently transient on bean plants that have not yet reached the pod-set stage of development.

Although *P. syringae* was a minor component during the latter half of the season for beans planted on 19 June, total bacterial populations were greater than 10^7 cfu per leaflet (Fig. 6B). Conditions were favorable for colonization of the leaflets by other species of bacteria. One of these components was *Methylobacterium organophilum*, a species of pink-pigmented facultative methylotrophs (PPFMs). Population sizes of the PPFMs comprised greater than approximately 50% of the total bacterial populations during the period when *P. syringae* was a minor component (i.e., 45–66 DAP for the second planting). The diverse species that comprise bacterial communities on bean leaflets have probably evolved different life strategies to be successful colonists of the phyllosphere.

The magnitudes of the changes in population sizes of *P. syringae* that occurred within 24- or 72-h (Friday to Monday) periods are presented in Figure 7. Most of the short-term changes were less than fivefold. The cumulative effect of relatively small increases accrued over several consecutive days may result in a significant net increase in bacterial population size. This may have been the case for the greater than 100-fold increase in *P. syringae* population size measured from 44 to 47 DAP (Friday to Monday) in the 23 May planting (Figs. 3A and 7A). There were several occasions on which *P. syringae* increased over 10-fold within 24 h. There were decreases of equivalent magnitude that occurred within the same time frame. Although our methods did not allow us to differentiate growth, death, immigration, and emigration processes, it is likely that the large net positive changes were due to rapid multiplication of the bacterium. Evidence supporting this assumption stems from an experiment in which population sizes of *P. syringae* were quantitated every 2 h during a 24-h period when the net increase in population size was 28-fold (4). A nearly continuously exponential increase in population sizes of *P. syringae* occurred during this period. Additionally, rates of immigration measured by Lindemann and Upper (9) are orders of magnitude too low to account for these very large increases in population sizes of *P. syringae* within 24 h (compare 5). If we assume that growth was the dominant process and that continuous exponential growth occurred during the 24 h period when population sizes of *P. syringae* increased 2,306-fold (Fig. 7B), then the estimated doubling time of approximately 2.1 h would be of the same order of magnitude as the doubling time of *P.*

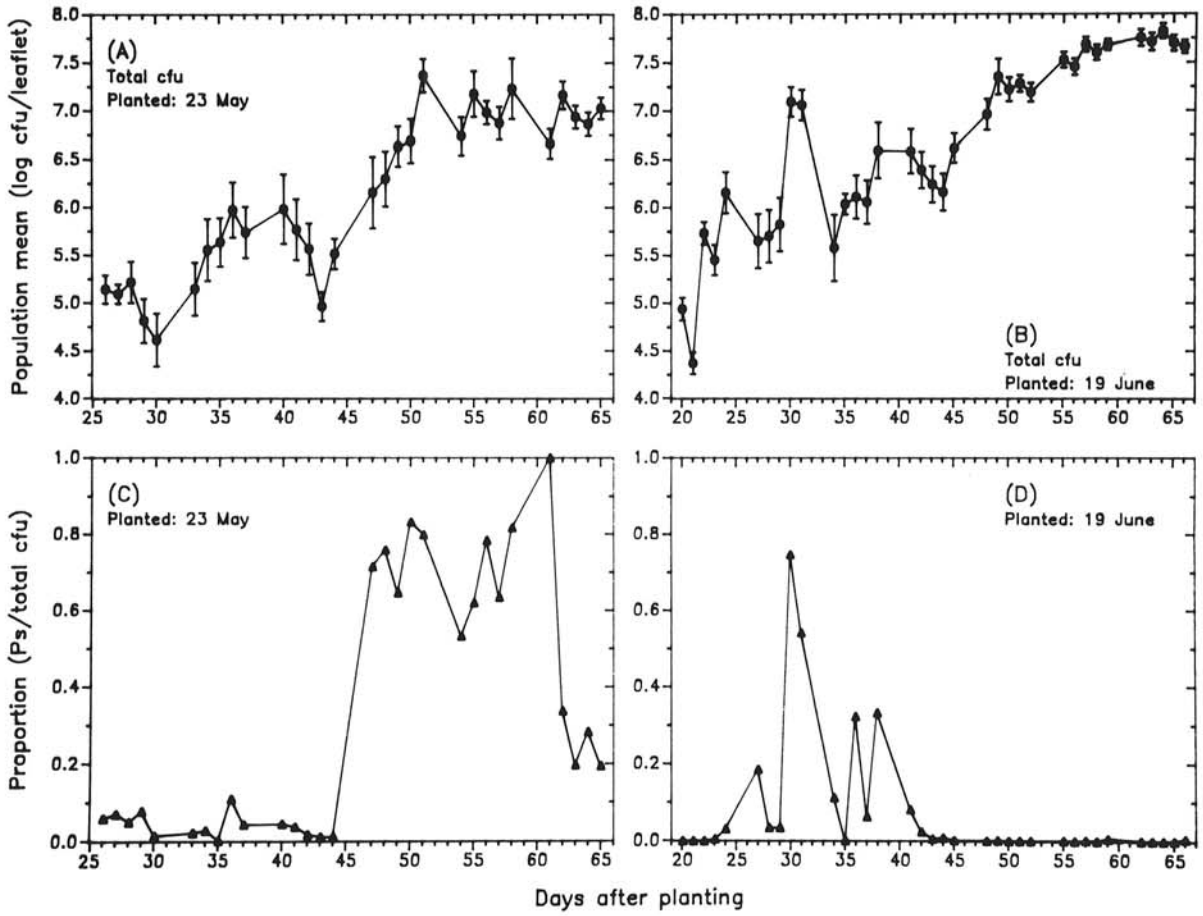

Fig. 6. Temporal changes in means of populations of total bacteria culturable on King's Medium B (total colony forming units [cfu]) for two plantings of snap beans. Plantings were on **A,** 23 May and **B,** 19 June. The proportion of the total bacterial population that was *Pseudomonas syringae* (Ps) for each sampling time is shown in **C and D** for the first and second plantings, respectively. Proportions were calculated by first untransforming the means of log-transformed bacterial population measurements. The means used for *P. syringae* were estimated with the maximum likelihood procedure of Rouse et al (12). The error bars in **A and B** represent 95% confidence intervals.

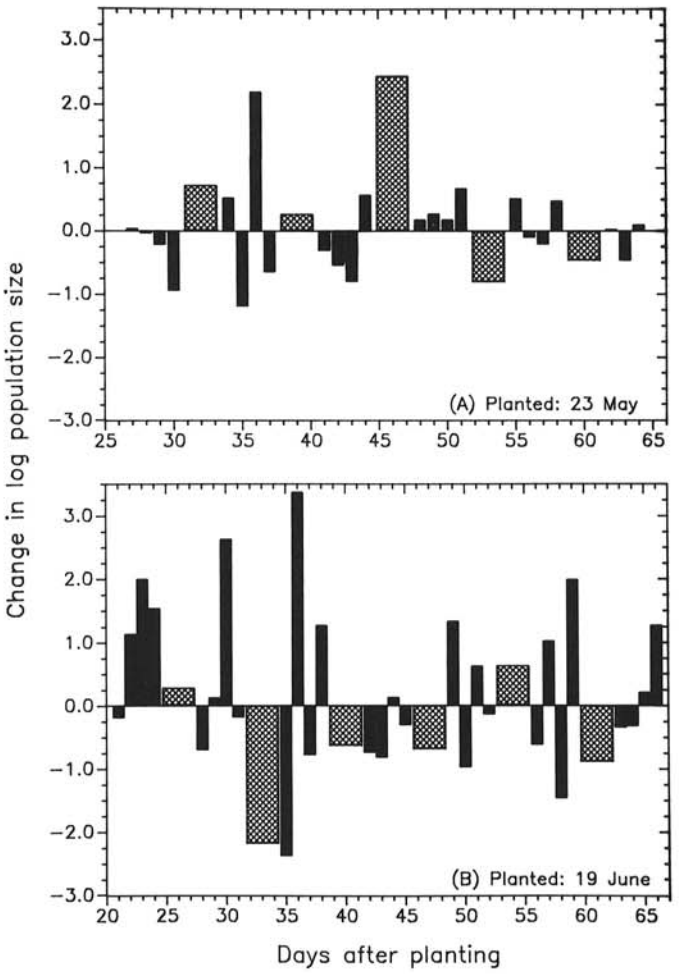

Fig. 7. Changes in mean population sizes of *Pseudomonas syringae* over periods of 24 (solid bars) and 72 h (hatched bars). Means for *P. syringae* populations were estimated with the maximum likelihood procedure and are shown in Figure 3A and B.

syringae measured in broth cultures under optimal and constant environmental conditions (i.e, 1.8 h; [15]).

Sundin et al (14) conducted daily samplings to monitor population sizes of marked strains of *P. s. syringae* and *P. s. morsprunorum* applied to sweet and sour cherry trees. Most daily changes in population sizes of the applied bacterial strains were small. However, a few large increases (i.e., greater than 10-fold) in population sizes of the bacteria occurred. Hence, at least for the two systems (snap beans and cherry trees), in which sampling frequencies were adequate to detect rapid changes, the dynamic nature of *P. syringae* populations on leaf habitats has been clearly demonstrated.

The magnitudes and changes in the variances of *P. syringae* populations further demonstrate the dynamic and inherently variable nature of leaf habitats (Fig. 4). Seasonal trends for the two plantings differed not only in the means but also in the variances of population sizes of *P. syringae*. It is worth noting that large differences between population sizes of *P. syringae* are quite common even when the variance may be considered relatively small. For example, from Figure 4A, the population variance of 0.45 at 65 DAP was small relative to that of 3.29 at 41 DAP. However, population sizes of *P. syringae* differed almost 1,000-fold on the leaflets collected at 65 DAP (variance = 0.45) (Fig. 8). For the samples collected at 41 DAP (variance = 3.29), population sizes of *P. syringae* ranged from nondetectable to 10^8 cfu per leaflet. Clearly, an understanding of the factors that regulate the development and dynamics of populations of *P. syringae* and microbes, in general, should include consideration not only of relative abundances (i.e., means) but also the variability (i.e., variances) in relative abundances.

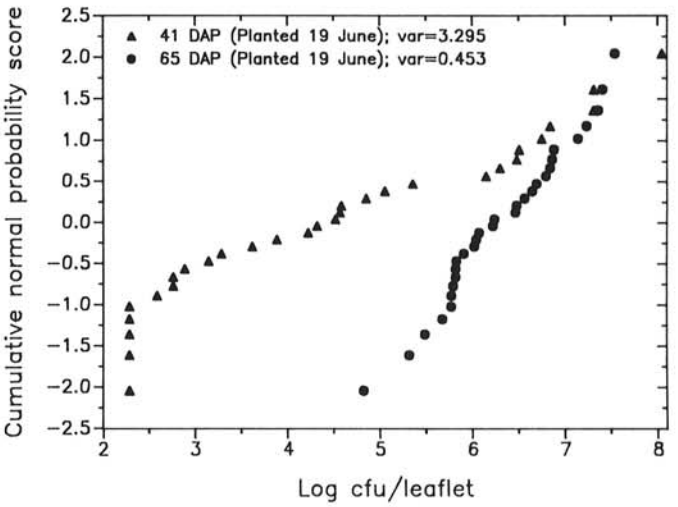

Fig. 8. Cumulative normal probability plot of population sizes of *Pseudomonas syringae* on individual bean leaflets collected at 41 and 65 days after planting (DAP) for the 19 June planting. Each leaflet was processed individually by dilution plating of leaflet homogenates. Population sizes of *P. syringae* that were below our limit of sensitivity were assigned a value of 2.279 log colony forming units (cfu) per leaflet.

Although the variances for populations of *P. syringae* were large, variances for total bacteria culturable on KMB were much smaller (Fig. 4A and B). This suggests that at any given time, bean leaflets harbored similar communities with regard to total numbers of individuals present but that the composition of these communities differed significantly among leaf habitats.

In summary, the way in which data were analyzed affected the actual estimates of means and variances of populations of *P. syringae*. However, population parameters estimated from both the ML and LS methods illustrated the inherent leaf-to-leaf variability and day-to-day dynamics in population sizes of *P. syringae* on snap bean leaflets. Population sizes of the bacterium tended to change little from day to day most of the time and to change very rapidly on less frequent occasions, presumably in response to changes in the physical environment. In a subsequent manuscript, we will describe experiments designed to determine how the physical environment affects the population dynamics of *P. syringae* on snap bean leaflets.

LITERATURE CITED

1. Hirano, S. S., Nordheim, E. V., Arny, D. C., and Upper, C. D. 1982. Lognormal distribution of epiphytic bacterial populations on leaf surfaces. Appl. Environ. Microbiol. 44:695-700.
2. Hirano, S. S., Rouse, D. I., and Upper, C. D. 1987. Bacterial ice nucleation as a predictor of bacterial brown spot disease on snap beans. Phytopathology 77:1078-1084.
3. Hirano, S. S., and Upper, C. D. 1983. Ecology and epidemiology of foliar bacterial plant pathogens. Annu. Rev. Phytopathol. 21:243-269.
4. Hirano, S. S., and Upper, C. D. 1989. Diel variation in population size and ice nucleation activity of *Pseudomonas syringae* on snap bean leaflets. Appl. Environ. Microbiol. 55:623-630.
5. Hirano, S. S., and Upper, C. D. 1991. Bacterial community dynamics. Pages 271-294 in: Microbial Ecology of Leaves. J. H. Andrews and S. S. Hirano, eds. Springer-Verlag, New York.
6. Ishimaru, C., Eskridge, K. M., and Vidaver, A. K. 1991. Distribution analyses of naturally occurring epiphytic populations of *Xanthomonas campestris* pv. *phaseoli* on dry beans. Phytopathology 81:262-268.
7. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
8. Lindemann, J., Arny, D. C., and Upper, C. D. 1984. Use of an apparent infection threshold population of *Pseudomonas syringae* to predict incidence and severity of brown spot of bean. Phytopathology 74:1334-1339.
9. Lindemann, J., and Upper, C. D. 1985. Aerial dispersal of epiphytic bacteria over bean plants. Appl. Environ. Microbiol. 50:1229-1232.
10. Little, R. J. A., and Rubin, D. B. 1987. Statistical Analysis with

Missing Data. John Wiley & Sons, New York.
11. Mood, A. M., Graybill, F. A., and Boes, D. C. 1974. Introduction to the Theory of Statistics. McGraw-Hill, New York.
12. Rouse, D. I., Nordheim, E. V., Hirano, S. S., and Upper, C. D. 1985. A model relating the probability of foliar disease incidence to the population frequencies of bacterial plant pathogens. Phytopathology 75:505-509.
13. Shapiro, S. S., and Wilk, M. B. 1965. An analysis of variance test for normality (complete samples). Biometrika 52:591-611.
14. Sundin, G. W., Jones, A. L., and Olson, B. D. 1988. Overwintering and population dynamics of *Pseudomonas syringae* pv. *syringae* and *P. s.* pv. *morsprunorum* on sweet and sour cherry trees. Can. J. Plant Pathol. 10:281-288.
15. Young, J. M., Luketina, R. C., and Marshall, A. M. 1977. The effects of temperature on growth in vitro of *Pseudomonas syringae* and *Xanthomonas pruni*. J. Appl. Bacteriol. 42:345-354.