Distribution Analyses of Naturally Occurring Epiphytic Populations of *Xanthomonas campestris* pv. *phaseoli* on Dry Beans

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ABSTRACT

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The distribution of naturally occurring epiphytic populations of *Xanthomonas campestris* pv. *phaseoli* on individual leaves of dry beans was assessed by various graphical and statistical techniques. Population sizes of *X. c. phaseoli* were estimated from the number of characteristic colonies formed on MXP medium. Data sets that contained 20 or 100 leaves and supported detectable numbers of *X. c. phaseoli* departed significantly from normality. Logarithmic transformation failed to result in normal distribution of data. The flexible Weibull distribution was selected as a possible alternative to normal and lognormal distributions for fitting censored and uncensored data sets. Kolmogorov-Smirnov goodness-of-fit tests indicated that a Weibull distribution fit in all but five of 23 data sets. Bacterial counts of *X. c. phaseoli* were plotted as straight lines in Weibull but not in normal or lognormal cumulative probability plots. The ability of Weibull and lognormal distributions to fit data sets was compared by the ratio of the maximized likelihood test statistic ($RML^{1/n}$). In the majority of data sets that contained several (>50) data points and low levels of censoring, a lognormal distribution was rejected and a Weibull distribution was accepted regardless of the cultivar's susceptibility to common blight. When data sets contained few uncensored data points, the distinction between Weibull and lognormal distributions for describing data was unclear. Explanations other than the general nature of the Weibull distribution may account for the superiority of Weibull for fitting data sets. The Weibull density function was derived from a model based on the assumption that bacterial numbers are related to the length of time bacteria have been on a leaf. However, further investigations are needed to elucidate the biological interpretation of Weibull parameters as they pertain to epiphytic population dynamics of *X. c. phaseoli*.

Epiphytic plant pathogenic bacteria have been examined for their impact on plant disease in several host-pathogen systems (3,7–9,16,21,28). Population size has been positively correlated with subsequent disease severity or incidence on several host plants (15,24,27). A general stochastic model relates epiphytic bacterial populations to disease incidence (24) and is based on the assumptions that population sizes are lognormally distributed and that disease incidence is dose-dependent. The conclusion that epiphytic populations can be described by a lognormal distribution was supported by previous measurements of total and fluorescent pseudomonad epiphytic population sizes isolated from a variety of plant species (14). By making a simple logarithmic transformation of bacterial counts obtained from single leaf samples,

individual data points approximated a normal distribution by both graphical and statistical analyses (14). However, a biological rationale for using the lognormal distribution has not been formally developed beyond a hypothesis that bacterial counts are the result of unidentified multiplicative variables. Departures from lognormality have also been observed (14,24).

Epiphytic populations of *X. c. phaseoli* are an important phase in the epidemiology of common blight on dry beans (*Phaseolus vulgaris* L.) and are greatly affected by host cultivar (3,31). However, evidence that correlated population size with disease rating was obtained by bulk leaf sampling methods and by the establishment of epiphytic populations by inoculation of leaves with antibiotic-resistant mutants of *X. c. phaseoli* (3,30,31). Since these earlier reports, a semiselective medium for isolation of *X. c. phaseoli* was developed that enabled the detection of small numbers of naturally occurring populations of *X. c. phaseoli* on host tissues (5). The apparent importance of single leaf observations and the availability of MXP prompted our examination of epiphytic population sizes of *X. c. phaseoli* on dry beans.

The objectives of this study were to assess the normality of log-transformed data sets and to compare the Weibull distribution to the lognormal distribution for fitting data sets.

MATERIALS AND METHODS

Bacterial cultures. Reference culture *X. c. phaseoli* LB2 was isolated from Charlevoix Dark Red Kidney beans grown in Lincoln, NE. Laboratory stock cultures were stored at −20 C in sterile 40% (v/v) glycerol buffered with 25 mM potassium phosphate buffer, pH 7.1, and were lyophilized.

Purification and characterization of epiphytes. Several colonies with morphologies characteristic of *X. c. phaseoli* were purified at each collection date. Subcultures were streaked on MXP medium for a maximum of two times to obtain single colonies, and then were streaked on a solid yeast extract-dextrose-calcium carbonate medium (YDC) (29). Restreaking of strains after purification was kept to a maximum of three transfers to reduce the chance of phenotypic changes due to subculturing.

Pathogenicity of strains was determined by both a detached (2) and an intact leaf bioassay. Inoculum for bioassays was taken from a small streak of each strain that had grown for 48 hr at 26 C on YDC. For intact leaf bioassays, Charlevoix Dark Red Kidney bean plants were grown in a greenhouse and inoculated when the first trifoliate leaves were one-half expanded. Leaflets were inoculated by dipping a pipet tip (200 µl) into a patch of the test strain and then touching the surface of a leaflet at five sites along one side of the midrib on the adaxial surface. Disease symptoms were recorded after 14 days.

In 1985, a randomized complete block design that contained six blocks and five cultivars of dry beans of different degrees of common blight susceptibility was established at North Platte, NE, and designated NPI-85. Each plot consisted of five 4.5-m rows planted 0.6 m apart, Olathe, a pinto bean, and four Great Northern types, Tara (tolerant), 1140 (susceptible), 59 (susceptible), and Harris (susceptible) (6) were included. Additionally, a 10-row plot of Pinto 114 was established at Lincoln, NE, with the same row and spacing dimensions as those in NPI-85.

In 1986, field experiments were planted at two locations in North Platte (NP1-86 and NP2-86) and one in Lincoln (UNL-86). The field design consisted of two large plots (eight rows, 9 m in length) of one each of cultivars Olathe and Tara separated from each other by 3 m.

To increase the probability that disease would occur, spreader rows of infested pinto bean seeds were sown around each experiment planted at NP2-86. Similarly, border rows of pinto beans at UNL-86 were water-soaked with a spray suspension of *X. c. phaseoli* LB2 (10^7 cfu/ml) in phosphate buffer 3 wk after planting. Care was taken to confine the inoculum to the border rows and, as a result, *X. c. phaseoli* was not detected in samples of leaflets collected from experimental plots immediately after inoculation of border rows (C. Ishimaru, *unpublished results*).

Sampling procedure. Symptomless leaflets of a similar size were selected at random from the plant canopy, because preliminary studies indicated no differences attributable to position. Leaflets were excised with sterile scissors and dropped into self-sealing freezer bags held below the cut leaflet. Thus, cross-contamination caused by handling was reduced. Leaflets were stored on ice immediately after collection and until they were processed.

Sampling began 3 wk after planting. At this time, bulk leaf samples of 10 leaflets were collected at weekly intervals and analyzed for *X. c. phaseoli*. Bulk sampling continued until populations of *X. c. phaseoli* were detected or disease was observed on susceptible cultivars. Then, both bulk and single leaf samples were collected. In 1986, 100 single leaf samples were collected from the large unreplicated plots of Olathe and Tara. This large-scale sampling of individual leaflets was repeated at weekly intervals and yielded a total of eight data sets for each cultivar, two from NP2-86 and three each from NPl-86 and UNL-86. Ten single leaf samples were collected from two of the replicates of the randomized complete block field design of 1985. In addition, two data sets were obtained from UNL-85 by processing 50 single leaflets at each of two sampling dates.

Isolation of epiphytic bacteria. Leaves contained in the freezer bags were shaken for 2 hr in 10 ml of phosphate buffer (12 mM potassium phosphate buffer, pH 7.1) per leaf, supplemented with 10 mM $MgSO_4$. Serial 10-fold dilutions of the wash fluids in phosphate buffer were plated onto MXP medium in duplicate. After incubation at 26 C for 5 days, the number of starch-hydrolyzing yellow colonies were recorded.

Estimation of model parameters and goodness-of-fit testing. Maximized likelihood functions and maximum likelihood estimates (MLE) of distribution parameters were computed by CENSOR, a computer program developed for analysis of machine life-testing and reliability experiments (Meeker and Duke, 1979, Iowa State University, Ames). Maximum likelihood estimates were those parameters that gave the largest probability of observing the actual data. MLEs calculated by CENSOR accounted for left-censored data, which were present in data sets in which bacterial counts were below the detection limit (i.e., 150 colony-forming units per leaf). For ease of computation, CENSOR computed MLEs on log-transformed values (18,22).

For complete data sets with fewer than 50 observations, normality was tested by the Shapiro-Wilk test statistic W (25) as computed by the Statistical Analysis System (SAS) User's Guide (1982 Ed., SAS Institute, Raleigh, NC). Goodness-of-fit for normal, lognormal, and Weibull distribution models was determined by the Kolmogorov-Smirnov test statistic D (19) on data sets with 50 or more observations. For censored data sets, corrected values of D (1) were obtained and then compared to tabular values (1) for assessing the goodness of fit to a Weibull distribution.

Visual inspection of cumulative probability plots for each data set was a subjective criterion for determining goodness of fit (22). Probability plots were obtained by plotting data points against their cumulative frequency on normal and on Weibull probability graph papers (Technical and Engineering Aids for Management, Tamsworth, NH). In these analyses, a distribution was considered an appropriate model if the data points approximated a straight line in a cumulative probability plot.

To compare Weibull and lognormal models for fitting data sets, transformed ratios of the maximized likelihood functions (11) were calculated. The transformed ratio $RML^{1/n}$ was calculated by substituting the maximum likelihood parameter estimates into each distribution's likelihood function and computing a ratio of these two values. If the value of the ratio raised to the $1/n$th power (n = the number of observations) was larger than a tabular value (10), then the null hypothesis (H_0: lognormal) was rejected in favor of the alternative hypothesis (H_1: Weibull).

RESULTS AND DISCUSSION

Pathogenicity of isolates. Of the 1,016 colonies presumptive of *X. c. phaseoli* that were purified and tested for pathogenicity, 78% were pathogenic on leaves of Charlevoix Dark Red Kidney

TABLE 1. Normality tests for untransformed and log-transformed values of epiphytic populations of *Xanthomonas campestris* pv. *phaseoli* on pinto bean cv. Olathe from complete data sets

Year	Location[a]	Days after planting	Total no. of leaves sampled	No. of leaves with >150 bacteria[b]	Statistic	Colony-forming units per leaf		ln (colony-forming units) per leaf	
						Value	Significance[c]	Value	Significance
85	NP	78	20	20	W[d]	0.65	<0.01	0.90	0.05
86	UNL	52	100	100	D[e]	0.260	<0.01	0.313	<0.01
86	UNL	59	100	100	D	0.284	<0.01	0.087	<0.05
86	NP1	55	100	100	D	0.288	<0.01	0.090	<0.05

[a] NP = North Platte, NE; UNL = University of Nebraska, Lincoln.
[b] Uncensored data points.
[c] Significance indicates probability of rejecting null hypothesis (normal distribution). Small significance indicates rejection of null hypothesis.
[d] Shapiro-Wilk test for less than 50 observations.
[e] Kolmogorov-Smirnov test for more than 50 observations.

bean. The percentage of pathogenic strains varied with location and sample date. These data support the usefulness of MXP medium in epidemiological studies of common blight of beans.

Distribution of epiphytic *X. c. phaseoli*. The finding that epiphytic pseudomonads and total bacterial population sizes are distributed lognormally (14) has impacted significantly on epidemiological studies of epiphytic plant pathogenic bacteria. Thus, quantification of bacterial population size was possible because logarithmic transformation of the data resulted in normally distributed data and satisfied one of the basic requirements for doing an analysis of variance. Although single leaf samples, instead of bulk leaf samples, were needed because of the inherent skewness of lognormally distributed data, these sampling modifications ensured an increase in the reliability of subsequent statistical analyses.

We were interested in quantifying population sizes of *X. c. phaseoli* on various dry bean cultivars, and therefore addressed the normality of the data sets first. By the simplest criterion, data sets that contained counts of *X. c. phaseoli* were not fitted to a normal nor, somewhat surprisingly, to a lognormal distribution, because they did not form straight lines in cumulative probability plots. Goodness-of-fit tests also rejected the normal and lognormal distributions for fitting complete data sets (Table 1). These results suggested that knowledge of population distributions of plant pathogenic bacteria on leaves could not be extended to *X. c. phaseoli*. Logarithmic transformation of the data did result in a straightening of lines in cumulative probability plots (Fig. 1; C. Ishimaru, *unpublished*), which suggests that the lognormal was an improvement over the normal distribution and that a logarithmic transformation might be sufficient, depending on the experiment. For example, when relating disease incidence to population sizes of *Pseudomonas syringae* on snap bean, logarithmic transformation of data was sufficient to predict disease incidence, even though data were not always distributed lognormally (24). However, the assumption of a distribution model is basic to subsequent population parameter estimates, and, theoretically, the power of the model that relates disease incidence to population size depends on the assumptions made about the population's distribution. If this model or a similar one were expanded to include *X. c. phaseoli*, an appropriate distribution for describing the pathogen's population would be required. Furthermore, most of the common statistical analyses for determining effects of cultivars on pathogen population size require normality of the data. Transformations other than the logarithm of the data might result in a normal distribution, but the distribution underlying populations of *X. c. phaseoli* could still be unknown. Therefore, our studies focused on selecting an alternative to the lognormal distribution for describing epiphytic populations of *X. c. phaseoli*.

Selecting a distribution that fits epiphytic bacterial populations is straightforward if data sets are complete (contain only values above the detection limit). There are several goodness-of-fit tests for selecting an appropriate distribution for complete data sets. The Shapiro-Wilk test (25) is routinely used for testing normality on small complete data sets, and the Kolmogorov-Smirnov goodness-of-fit test (19) is used for testing a variety of distributions on large complete data sets.

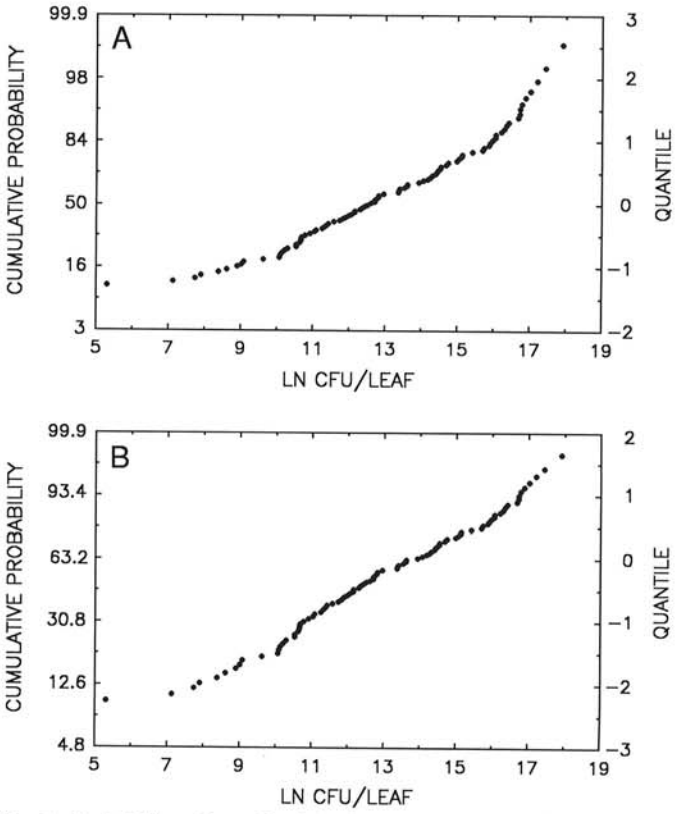

Fig. 1. Probability plots of epiphytic populations of *Xanthomonas campestris* pv. *phaseoli* isolated from leaves of Olathe grown at NP1-86 and collected 48 days after planting. **A,** Lognormal distribution assumed. **B,** Weibull distribution assumed. All bacterial counts are expressed as natural logarithmic transformed values (ln [cfu]).

Testing for goodness-of-fit becomes considerably more complicated when data sets are censored. There are two main reasons for this. First, parameter estimates for the assumed distribution must include the censored data points. Assigning a value, (e.g., 150 cfu) to the censored data points has been shown to affect parameter estimates of lognormally distributed data (24). Computer programs are available for calculating parameter estimates when censoring is present, and some, such as CENSOR, take into account the unique case of left-censored data, in which the censoring is below (left of) a detection limit. The second reason for complication of goodness-of-fit testing in the presence of censoring is that the power of a goodness-of-fit test is often affected by the level and type of censoring in data. Effects of censoring on the Shapiro-Wilk test are unknown and thus limit the application of this test to complete data sets. Effects of censoring on the Kolmogorov-Smirnov goodness-of-fit are known and tables

of adjusted D values are available for specific distributions and levels of censoring (1). One possible limitation in the use of these tables is that the type of censoring considered is type II and the type encountered in bacterial epiphyte studies is type I; values of censored bacterial counts are within a known range (zero to the detection limit). Tabular values based on type II censoring are, however, the best available approximations for determining goodness of fit with type I censored data.

The Weibull distribution was selected as an alternative to the lognormal distribution because the general nature of the Weibull allows it to fit distributions with a variety of shapes. The Weibull distribution has had applications in plant pathology (4,12,23,26), but was developed and is used mainly for machine life-testing and reliability (18). The Weibull distribution is based on a power function, and if defined in terms of epiphytic bacterial growth or spread, the Weibull parameters may provide insights into the population's dynamics (see Appendix). However, use of the Weibull was justified only if the Weibull model fit better than a lognormal one. Significance values for the Kolmogorov-Smirnov goodness-of-fit test indicated that the Weibull model was rejected in only five of the 23 data sets (Table 2), which suggests that the Weibull may be an appropriate model for describing epiphytic populations of *X. c. phaseoli*.

Data were analyzed by a ratio of the maximized likelihood test to discriminate between the two competing models (i.e., Weibull and lognormal distributions) for fitting data sets of *X. c. phaseoli*. Table 2 lists the $RML^{1/n}$ values and their associated significance values for testing the null hypothesis that the log-transformed values follow a normal distribution, or equivalently, that the untransformed data follow a lognormal distribution. The alternative hypothesis was that the data follow a Weibull distribution. When data sets contained 50 or 100 leaves collected from common blight susceptible cultivars, the lognormal distribution was rejected and the Weibull distribution accepted in eight of 10 cases. Data sets of 100 leaves collected from the tolerant cultivar, GN Tara, were heavily censored in four of the seven cases, but when censoring was low the lognormal was rejected in favor of the Weibull. Significance values failed to reject a lognormal distribution in most of the small data sets of 20 leaves (10 from each replication) from various dry bean hosts. In most cases in which the $RML^{1/n}$ test failed to reject the null hypothesis of a lognormal distribution, it failed to reject the null hypothesis of a Weibull distribution (data not shown). Thus, the power of the $RML^{1/n}$ test appeared to be affected by sample size (10) and censoring. In general, regardless of the host's susceptibility or tolerance to common blight, results indicated that the Weibull distribution was favored over the lognormal in most cases in which data sets contained several uncensored data points collected from a single, unreplicated plot. The distinction between a lognormal and a Weibull distribution was unclear when data sets contained few uncensored observations.

Rejection of the lognormal distribution and acceptance of the Weibull was also supported by simple graphical analyses (Fig. 1). It was noted that log-transformed counts of *X. c. phaseoli* curved upward in normal cumulative probability plots, whereas these same data sets were relatively straight in Weibull cumulative probability plots.

Several Weibull probability plots are presented (Figs. 2 and 3) to illustrate differences between populations of *X. c. phaseoli* on a tolerant versus a susceptible cultivar. The number of *X. c. phaseoli* that corresponded to 63% on cumulative probability plots, which is an estimate of the Weibull scale parameter b, was always greater on the susceptible cultivar than on the tolerant one (Figs. 2 and 3, Table 2). Other experimental designs and further analyses are required to test for significant differences in population sizes of *X. c. phaseoli* attributable to cultivar (see Lawless [18] for methods of testing equality of Weibull parameters with different treatments). However, visual inspection of cumulative probability plots of these data sets suggested that populations of *X. c. phaseoli* were affected by cultivar. It has been reported that cultivar affects populations of *X. c. phaseoli* (3,30). Our results were consistent with those reports. In addition, our results showed that the number of *X. c. phaseoli* on individual leaves ranged from undetectable to 6×10^7 colony-forming units per

TABLE 2. Maximum likelihood estimates of Weibull distribution parameters, Kolmogorov-Smirnov goodness-of-fit test, and transformed ratio of maximum likelihood ($RML^{1/n}$) for data sets of epiphytic populations of *Xanthomonas campestris* pv. *phaseoli*

Year	Location[a]	Cultivar	Days after planting	Total no. of leaves sampled	No. of leaves with >150 bacteria[b]	Weibull parameters[c]		D[d]	Significance[e]	$RML^{1/n}$	Significance[f]
						scale(b)	shape(c)				
1985	NP	Olathe	70	20	18	9.7×10^4	0.28	0.163	>0.10	0.989	>0.10
1985	NP	Olathe	78	20	20	6.5×10^5	0.42	0.115	>0.10	1.077	<0.10
1985	NP	GN Tara	70	20	5	1.8×10^1	0.15	0.077	>0.10	1.005	>0.10
1985	NP	GN Tara	78	20	14	2.0×10^3	0.12	0.377	<0.01	0.921	>0.10
1985	NP	GN 1140	70	20	15	8.9×10^4	0.25	0.099	>0.10	1.111	<0.05
1985	NP	GN 1140	78	20	18	1.6×10^5	0.34	0.095	>0.10	1.068	<0.10
1985	UNL	Pinto 114	49	50	40	1.3×10^5	0.24	0.087	>0.10	1.031	<0.05
1985	UNL	Pinto 114	55	50	34	1.0×10^5	0.19	0.125	<0.05	1.047	<0.05
1986	UNL	Olathe	43	100	55	2.6×10^3	0.16	0.050	>0.10	0.997	0.10
1986	UNL	Olathe	52	100	100	6.4×10^6	0.42	0.094	<0.05	1.104	<0.01
1986	UNL	Olathe	59	100	100	1.1×10^7	0.63	0.091	<0.05	1.050	0.01
1986	NP1	Olathe	48	100	91	9.5×10^5	0.34	0.051	>0.10	1.117	<0.01
1986	NP1	Olathe	55	100	100	1.2×10^7	0.63	0.720	>0.10	1.050	0.01
1986	NP2	Olathe	61	100	28	2.3×10^1	0.13	0.320	>0.10	1.004	<0.10
1986	NP2	Olathe	68	100	38	3.0×10^2	0.13	0.050	>0.10	1.024	<0.05
1986	NP2	Olathe	74	100	33	1.0×10^2	0.12	0.037	>0.10	1.016	<0.05
1986	UNL	GN Tara	43	100	7	1.2×10^0	0.20	0.035	>0.10	1.002	<0.10
1986	UNL	GN Tara	52	100	87	4.5×10^4	0.33	0.064	>0.10	1.036	<0.05
1986	UNL	GN Tara	59	100	66	1.5×10^3	0.29	0.101	<0.01	0.946	>0.10
1986	NP1	GN Tara	48	100	78	1.2×10^5	0.25	0.052	>0.10	1.085	<0.01
1986	NP1	GN Tara	55	100	95	4.0×10^6	0.50	0.083	>0.05	1.263	<0.01
1986	NP2	GN Tara	61	100	10	4.0×10^{-2}	0.10	0.021	>0.10	1.000	<0.10
1986	NP2	GN Tara	68	100	21	1.4×10^0	0.09	0.018	>0.10	1.009	<0.10

[a] NP = North Platte, NE; UNL = University of Nebraska, Lincoln.
[b] Uncensored data points.
[c] Scale has units of colony-forming units per leaf; shape is unitless.
[d] Kolmogorov-Smirnov goodness-of-fit test.
[e] Significance indicates probability of rejecting null hypothesis (Weibull distribution). Small significance indicates rejection of Weibull distribution.
[f] Significance indicates probability of rejecting null hypothesis (lognormal distribution) in favor of the alternative hypothesis (Weibull) when the null hypothesis is true. Small significance indicates appropriate use of the Weibull distribution (10,11).

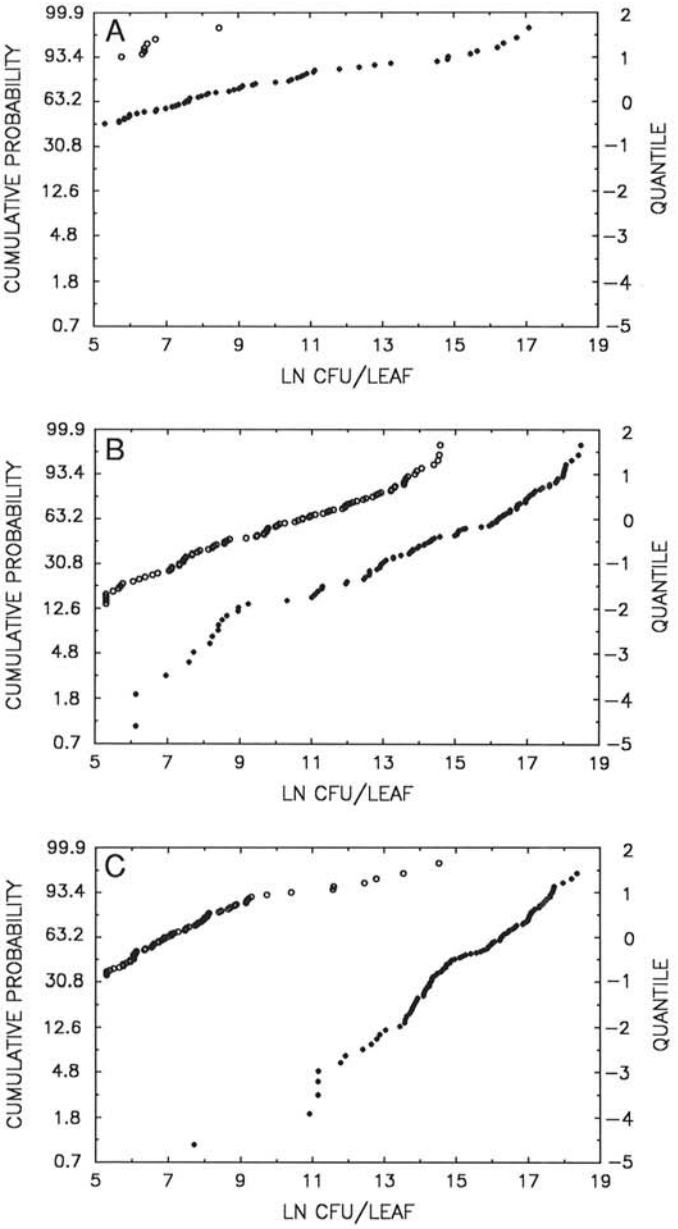

Fig. 2. Weibull probability plots of epiphytic populations of *Xanthomonas campestris* pv. *phaseoli* isolated from leaves of Olathe (●) and GN Tara (○) plants grown in 1986 at UNL. Leaves collected: **A,** 43 days after planting; **B,** 52 days after planting; and **C,** 59 days after planting.

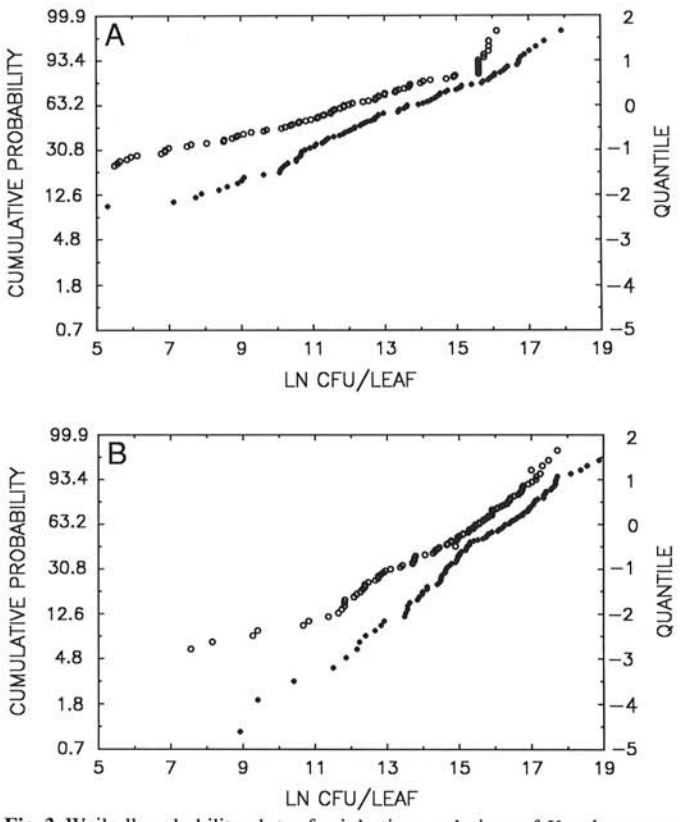

Fig. 3. Weibull probability plots of epiphytic populations of *Xanthomonas campestris* pv. *phaseoli* isolated from leaves of Olathe (●) and GN Tara (○) plants grown in NP1-86. Leaves collected: **A,** 48 days after planting; and **B,** 55 days after planting.

leaf. This kind of information was lost during the bulk leaf sampling process used in previous studies (3,30,31).

Our finding that data sets of epiphytic populations of *X. c. phaseoli* are fitted by a Weibull distribution presents interesting possibilities for future study and discussion. The Weibull distribution has found general application in machine life-testing and reliability, because the lengths of time before a machine fails are described by a Weibull distribution when the failure of a single component results in the failure of the machine (weakest link scenario) (18). In these cases, location and shape parameters of a Weibull distribution are valuable tools for predicting and assessing machine reliability. There has been interest in defining Weibull parameters as they pertain to plant diseases caused by fungi (4,23,26) and bacteria (12). Weibull parameters have not been defined in terms of the population dynamics of a bacterial plant pathogen. In the Appendix, we present one possible way of defining these parameters so that they are relevant to a discussion of population dynamics of *X. c. phaseoli.* In this model, the inverse of the Weibull shape parameter (c) is a relative growth rate (r) and the Weibull scale parameter (b) is the average population size of *X. c. phaseoli* at the time of sampling. The Weibull distribution can also be derived by using other assumptions and models. Validation of these assumptions is critical for future application of the Weibull distribution to elucidate population dynamics of *X. c. phaseoli.* The proposed model in the appendix assumes that the number of colonies of *X. c. phaseoli* on a leaf is dependent on the length of time bacteria have been on a leaf. Although this is a simplification of a very complicated and dynamic process, some trends in the data were revealed that support the use of this model. The scale (b) and shape (c) parameters increased over time at a given location. In other words, as the population size (b) increased, the relative growth rate ($r = 1/c$) decreased. This observation was consistent with the assumptions of the growth model used to derive the Weibull and may reflect a phenomenon common to many biological populations, i.e., as a population increases in size, its relative growth rate slows.

In conclusion, estimating population sizes of epiphytic plant pathogenic bacteria may not be as straightforward as proposed (15). At least in the case of *X. c. phaseoli*, data were inadequately described by a lognormal distribution. Although a high degree of censoring complicated distribution analyses and limited comparisons between competing distribution models, the Weibull distribution appeared to fit the data better than the lognormal. The flexibility of the Weibull may be the only reason for these results. However, after further examination, the parameters of the Weibull distribution may in the future be defined in terms meaningful to the population dynamics of an epiphytic plant pathogenic bacterium.

LITERATURE CITED

1. Aho, M., Bain, L. J., and Engelhardt, M. 1983. Goodness of fit tests for the Weibull distribution with unknown parameters and censored sampling. J. Stat. Comp. Simul. 18:59-69.
2. Arunakumari, A., and Vidaver, A. K. 1986. Identification of *Xantho-*

monas campestris pv. *phaseoli* mutants with altered pathogenicity using a detached bean leaflet bioassay. (Abstr.) Phytopathology 76:1136.
3. Cafati, C. R., and Saettler, A. W. 1980. Effect of host on multiplication and distribution of bean common blight bacteria. Phytopathology 70:675-679.
4. Campbell, C. L., Jacobi, W. R., Powell, N. T., and Main, C. E. 1984. Analysis of disease progression and the randomness of occurrence of infected plants during tobacco black shank epidemics. Phytopathology 74:230-235.
5. Claflin, L. E., Vidaver, A. K., and Sasser, M. 1987. MXP, a semiselective medium for *Xanthomonas campestris* pv. *phaseoli*. Phytopathology 77:730-734.
6. Coyne, D. P., and Schuster, M. L. 1974. 'Great Northern Valley' Dry Bean. HortScience 9:482.
7. Crosse, J. E. 1963. Bacterial canker of stone fruits. V. A comparison of leaf surface populations of *Pseudomonas syringae mors-prunorum* in autumn on two cherry varieties. Ann. Appl. Biol. 52:97-104.
8. Daniel, J.-F., and Baker, B. 1985. Epiphytic phase of *Xanthomonas campestris* pathovar *manihotis* on aerial parts of cassava. Agronomie 5:111-116.
9. Daub, M. E., and Hagedorn, D. J. 1981. Epiphytic populations of *Pseudomonas syringae* on susceptible and resistant bean lines. Phytopathology 71:547-550.
10. Dumonceaux, R., and Antle, C. E. 1973. Discrimination between the log-normal and the Weibull distributions. Technometrics 15:923-926.
11. Dumonceaux, R., Antle, C. E., and Haas, G. 1973. Likelihood ratio test for discrimination between two models with unknown location and scale parameters. Technometrics 15:19-27.
12. Ercolani, G. L. 1984. Infectivity titration with bacterial plant pathogens. Annu. Rev. Phytopathol. 22:35-52.
13. Gregory, F. G. 1928. Studies in the energy relations of plants, II. Ann. Bot. (London) 42:469-507.
14. 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.
15. Hirano, S. S., and Upper, C. D. 1983. Ecology and epidemiology of foliar bacterial plant pathogens. Annu. Rev. Phytopathol. 21:243-269.
16. Hirano, S. S., and Upper, C. D. 1985. Ecology and physiology of *Pseudomonas syringae*. Biotechnology 3:1073-1078.
17. Johnson, N. L., and Kotz, S. 1970. Distributions in Statistics Continuous Univariate Distributions I. Houghton Mifflin Co., Boston.
18. Lawless, J. F. 1982. Statistical Models and Methods for Lifetime Data. John Wiley & Sons, New York.
19. Lilliefors, H. W. 1969. On the Kolmogorov-Smirnov test for normality with mean and variance unknown. J. Am. Stat. Assoc. 62:399-402.
20. McKendrick, A. G., and Pai, M. K. 1910. The rate of multiplication of microorganisms: A mathematical study. Proc. R. Soc. Edinburgh 31:649.
21. Mew, T. W., and Kennedy, B. W. 1971. Growth of *Pseudomonas glycinea* on the surface of soybean leaves. Phytopathology 61:715-716.
22. Nelson, W. 1982. Applied Life Data Analysis. John Wiley & Sons, New York.
23. Pennypacker, S. P., Knoble, H. D., Antle, C. E., and Madden, L. V. 1980. A flexible model for studying plant disease progression. Phytopathology 70:232-235.
24. 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.
25. Shapiro, S. S., and Wilk, M. B. 1965. An analysis of variance test for normality (complete samples). Biometrika 52:591-611.
26. Thal, W. M., Campbell, C. L., and Madden, L. V. 1984. Sensitivity of Weibull parameter estimates to variation in simulated disease progression data. Phytopathology 74:1425-1430.
27. Thomson, S. V., Schroth, M. N., Moller, W. J., and Reil, W. O. 1975. Occurrence of fire blight of pears in relation to weather and epiphytic populations of *Erwinia amylovora*. Phytopathology 65:353-358.
28. Thomson, S. V., Schroth, M. N., Moller, W. J., and Reil, W. O. 1976. Efficacy of bactericides and saprophytic bacteria in reducing colonization and infection of pear flowers by *Erwinia amylovora*. Phytopathology 66:1457-1459.
29. Vidaver, A. K. 1967. Synthetic and complex media for the rapid detection of fluorescence of phytopathogenic pseudomonads: Effects of the carbon source. Appl. Microbiol. 15:1523-1524.
30. Weller, D. M., and Saettler, A. W. 1978. Rifampin-resistant *Xanthomonas phaseoli* var. *fuscans* and *Xanthomonas phaseoli*: Tools for field study of bean blight bacteria. Phytopathology 68:778-781.
31. Weller, D. M., and Saettler, A. W. 1980. Colonization and distribution of *Xanthomonas phaseoli* and *Xanthomonas phaseoli* var. *fuscans* in field-grown navy beans. Phytopathology 70:500-506.

APPENDIX

Growth model and distribution of the bacterial pathogen. The size of bacterial populations on bean leaves is undoubtedly affected by a multitude of conditions, e.g., humidity, temperature, canopy structure, growth stage of the plant, and stage of disease development. We assumed that the bacterial growth process is the same on all leaves of a particular cultivar and that the major source of variability of bacterial populations between plant leaves is attributable to the different lengths of time that bacteria have been on each leaf.

The exponential growth model has been used to describe growth in its initial stages, when growth is not limited by environment (13). This model assumes that the instantaneous growth rate at a particular time (dY/dt; Y = population size) is proportional to the size of the population; dY/dt increases at an increasing rate over time. This model may be adequate as long as environmental conditions allow for unconstrained growth. The later stages of growth, or when growth becomes increasingly limited by environment, have been modeled by a monomolecular growth function (20). This model assumes that dY/dt is proportional to the amount of growth yet to be achieved; dY/dt increases at a decreasing rate asymptotically approaching a growth maximum.

A problem with using one growth function for the first stage of development and another for the second stage is that a population's stage of development may not be known. A solution to this problem is to use a model with a simple integrated form that can approximate bacterial growth in both stages, provided that model parameters can depend on time. Such a model may be characterized by assuming the instantaneous growth rate is directly proportional to Y and inversely proportional to t:

$$dY/dt = r\ Y/t \tag{1}$$

or by integration,

$$Y = b\ t^r \tag{2}$$

in which t is the time bacteria have been on the leaf, b is the average density (size of bacteria on a leaf at $t = 1$), and r is the proportional growth parameter.

This model allows dY/dt to increase at an increasing rate ($r > 1$), or increase at a decreasing rate ($r < 1$), thus approximating either growth stage. Further, since some power function can usually be found to adequately fit either of these two curves, it appears that this model can approximate either exponential or monomolecular growth functions.

The model (eq. 2) could be used to predict the distribution of *X. c. phaseoli* if b, r, and t were known. Assuming bacterial counts were available for each of several time values, b and r could be estimated with regression if they could be assumed to be constant. However, in most field studies of bacterial epiphytes, as with this one, the length of time that bacteria have been on a particular leaf cannot be determined and it is therefore unknown.

One approach is to assume that the length of time (t) bacteria have been on a leaf has some known probability distribution, which would, in turn, establish the probability distribution of Y. If t has an exponential distribution with a mean parameter of one unit, then the amount of bacteria on the leaf has a Weibull distribution (17) with parameters b and $1/r$; $1/r$ = the shape parameter (c) of the two-parameter Weibull distribution and b = the Weibull scale parameter (b). The exponential distribution is commonly used to approximate the distribution of time until a critical event occurs, such as in engineering studies on machine life-testing and reliability (18). The exponential distribution is assumed to have a mean of one unit because we have no in-

formation on this parameter. The 'unit' can be thought of as the average amount of time the bacteria have been on the leaves until sampling.

Proof:

$$\text{From equation 2, it follows that } t = (Y/b)^{1/r} \tag{3}$$

$$\text{and } dt/dY = (1/r)\,(Y/b)^{(1/r)-1}\,(1/b). \tag{4}$$

If we assume t is exponentially distributed with a mean of one unit, then the density of t is

$$f_t(t) = e^{-t}, \text{ for } t > 0. \tag{5}$$

With a change of variable, the density of Y can be expressed as

$$f_y(Y) = f_t(t)dt/dY, \text{ for } Y > 0, \tag{6}$$

or by substitution as

$$f_y(Y) = (1/br)(Y/b)^{(1/r)-1}\exp(-(Y/b)^{1/r}), \text{ for } Y > 0 \tag{7}$$

which is the density function of the Weibull distribution (17), with shape parameter, $c = 1/r$, and scale parameter, b.