Susan S. Hirano and Douglas I. Rouse
Department of Plant Pathology, University of Wisconsin, Madison

Murray K. Clayton
Department of Plant Pathology and Statistics, University of Wisconsin, Madison

Christen D. Upper
USDA Agricultural Research Service and Department of Plant Pathology, University of Wisconsin, Madison

Pseudomonas syringae pv. *syringae* and Bacterial Brown Spot of Snap Bean:

A Study of Epiphytic Phytopathogenic Bacteria and Associated Disease

In 1959, J. E. Crosse published the rather revolutionary finding that *Pseudomonas syringae* pv. *morsprunorum*, the causal agent of bacterial canker and leaf spot of stone-fruit trees, could be readily recovered in large numbers from the *surfaces* of *healthy* cherry leaves (7). Crosse suggested that populations of the pathogen on healthy cherry leaf surfaces, rather than those in leaf spot lesions, were the main source of inoculum for the infection of stems and branches in autumn that leads to canker development. This finding was revolutionary because phytopathogenic bacteria had never before been recognized as coexisting with healthy leaves. That leaf surfaces are colonized by a diversity of bacteria was known since the early 1900s (Fig. 1), but the role of phytopathogenic bacteria in these microbial communities had not been recognized previously. The classical view was that growth of pathogenic bacteria occurred in the intercellular spaces of leaves and invariably resulted in disease; in other words, growth was synonymous with lesion formation. Further, lesions were considered the likely source of inoculum for foliar bacterial diseases, as had been well demonstrated for many fungal diseases of plants. The terms "epiphytic" and "resident" phase have been used to refer to foliar phytopathogenic bacteria growing on the surface of healthy plants (7,32). The resident phase was later expanded to include growth of microbes on all parts of healthy plants (i.e., on surfaces, in internal parts, above and below ground) (33).

Dr. Hirano's address is: Department of Plant Pathology, 1630 Linden Dr., University of Wisconsin, Madison 53706. E-mail: SSH@PlantPath.wisc.edu

Numerous reports over the past three decades have supported the concept that many pathovars of phytopathogenic bacteria in the *P. syringae, Xanthomonas campestris,* and *Clavibacter michiganense* groups exist as epiphytes on susceptible host plants (cf. reviews in 15,23). Epiphytic phytopathogens have also been found on nonhost plants (e.g., weeds) and resistant cultivars, leading to the suggestion that these populations may serve as reservoirs of inoculum for susceptible host plants (cf. 23). Although epiphytic populations of phytopathogenic bacteria appear to be commonly and ubiquitously distributed, the *quantitative* importance of these populations in the epidemiology of the diseases they cause remains somewhat unresolved (17,25,27).

In the early 1980s, we too decided to examine the role of epiphytic pathogen populations in disease development. We chose to work with *Pseudomonas syringae* pv. *syringae* in relation to bacterial brown spot disease on snap bean plants (*Phaseolus vulgaris* L.) for a number of reasons. Wisconsin is the leading state in the United States in the production of snap beans for processing, and bacterial brown spot is a frequent problem for growers. Foliar symptoms of the disease are necrotic spots frequently surrounded by a narrow margin of chlorosis (Fig. 2A). The economically important phase of the disease is necrotic lesions on the pods, which lower the quality of the product (Fig. 2B). Entire fields may not be harvested if the frequency of pod infections exceeds the tolerances allowed by food processors. *P. s.* pv. *syringae* had been shown to be present as an epiphyte on its host as well as on nonhost plants (13,35). The ecology of *P. s.* pv. *syringae* was also of interest to us because *P. s.* pv. *syringae* is one of only a few bacterial species that is capable of ice nucleation at temperatures only slightly below 0°C (Fig. 2C) (2,40). In other words, the bacterium is able to catalyze crystallization of supercooled water to form ice. When this happens on frost sensitive plants, frost injury results (Fig. 2D) (38,39).

During the 15 years we have studied this system, our data have led us to a number of conclusions on the epidemiology of bacterial diseases that are contrary to the dogma that existed at the time we began. Given a strong tendency to be just as dogmatic about our new concepts as others have been about the older ones, we recently raised the question (to ourselves and our readers) as to whether the *P. s.* pv. *syringae*–snap bean–brown spot system is merely a case study or, indeed, a good

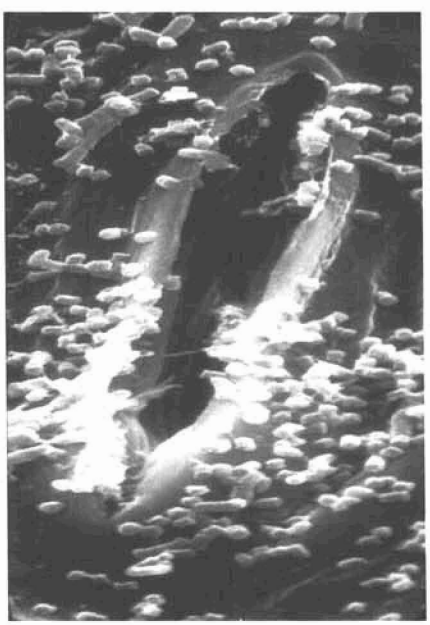


Fig. 1. Scanning electron micrograph of epiphytic bacteria on the surface of a corn leaf. The photograph was taken by J. Lindemann and appeared on the cover of the APS publication, *Biological Control on the Phylloplane* (44).

model system for general understanding of the ecology of foliar bacterial pathogens and the epidemiology of their associated diseases (27). With this question in mind, we present some of our findings and our views on the role of epiphytic populations of *P. s.* pv. *syringae* in the overall life strategy of the bacterium and in the epidemiology of bacterial brown spot. While the focus is on *P. s.* pv. *syringae* and disease, it is important to keep in mind that this bacterium is but one component of microbial communities associated with leaves (Fig. 3). A thorough understanding of the ecology of *P. s.* pv. *syringae* will require knowledge of the dynamics of phyllosphere bacterial communities in general.

Variability in Population Sizes of *P. syringae*: Boon or Bane for Research?

Each leaf or leaflet may be viewed as an individual habitat for bacterial colonization. The canopy of a crop in a large field is composed of millions of leaves or leaflets (Fig. 4). An issue that arose early in our research efforts was how to describe the numbers (i.e., population sizes) of bacteria that are present on populations of leaves. When we measured bacteria on several individual leaves from a number of different canopies, we found great leaf-to-leaf variability, as had Crosse (7). This variability is illustrated in Figure 5, where each petri dish represents the population size of *P. syringae* from an individual rye leaf. We wondered if there might be useful information in this variability.

It turned out that numbers of bacteria (expressed as CFU or colony-forming units per leaf) varied among leaves in a way that could be described by a particular type of asymmetrical frequency distribution—the lognormal (21). A useful outcome of knowing that bacterial populations are lognormally distributed is that we can take the logarithm of the original measurements (i.e., CFU per leaflet), and the log-transformed values (i.e., $\log_{10}$ CFU per leaflet) are seen to follow the well-known normal distribution. Ishimaru et al. (30) subsequently reported that the Weibull distribution provided a better fit than the lognormal to the distribution of epiphytic populations of *X. campestris* pv. *phaseoli* on bean leaflets. Since our original report (21), we have examined more than 100 data sets and found that for some sets, the Weibull provided a better fit than the lognormal. In more cases, however, the lognormal was superior. Most importantly, nearly all of the sets could be modeled adequately by one or the other of these closely related frequency distributions.

Why is it important to be able to describe the variability in bacterial popula-

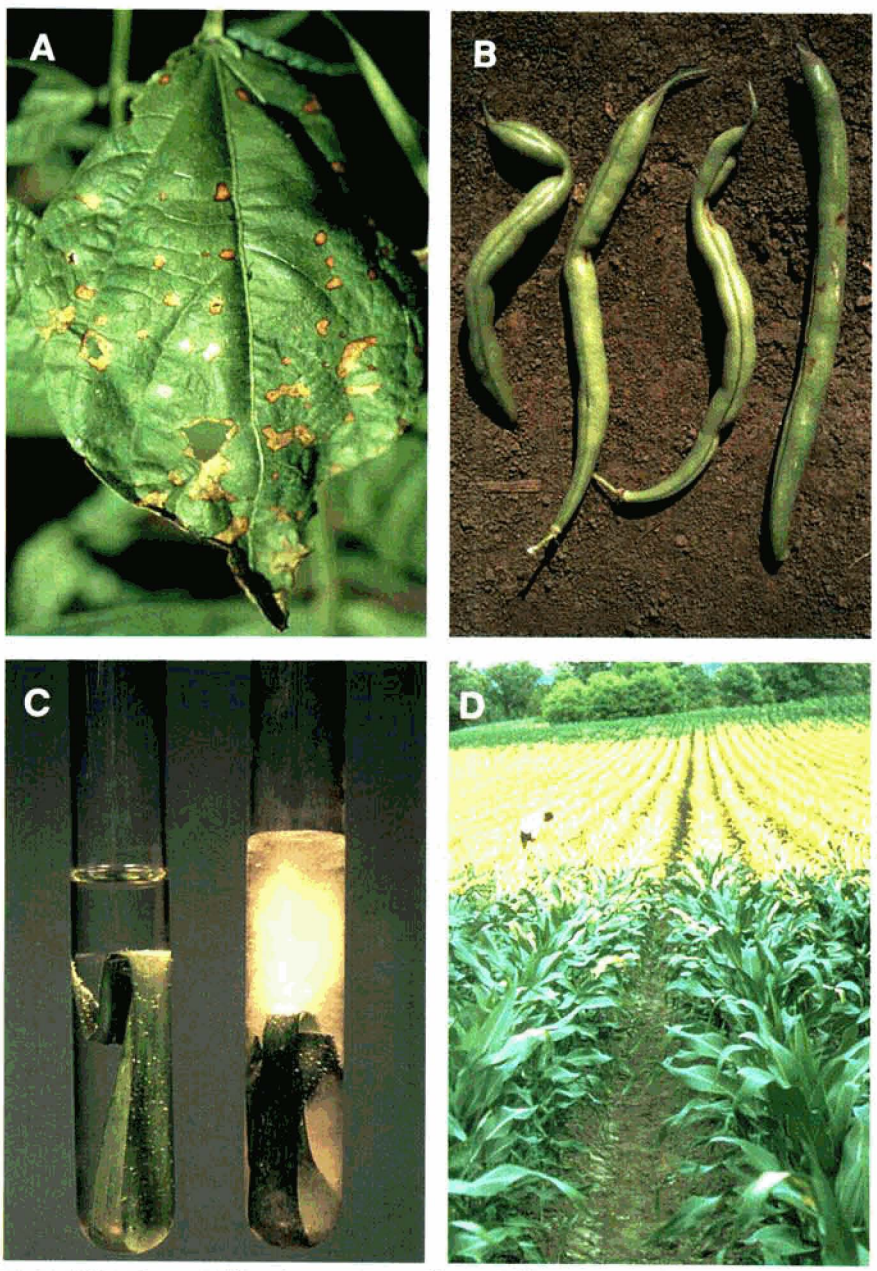


Fig. 2. (A) Foliar and (B) pod symptoms of bacterial brown spot disease on snap bean plants caused by *Pseudomonas syringae* pv. *syringae*. (C) An ice nucleation event occurred in the test tube on the right due to the larger numbers of ice nucleation active *P. syringae* present on the leaf. (D) Frost injury to corn due to a natural radiative frost. Photograph taken by D. C. Arny.

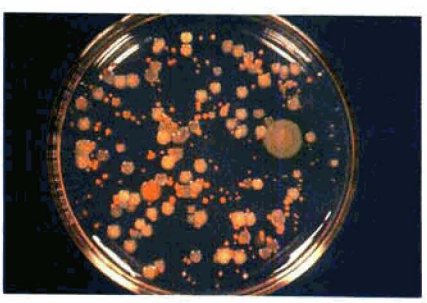


Fig. 3. Pigmented bacterial colonies on King's Medium B. The bacteria were removed from a bean leaflet by washing. The leaflet was taken from a bean plant grown in the field. *Pseudomonas syringae* is represented by the single large greenish colony. The very small pink colonies are pink-pigmented facultative methylotrophs (PPFM) of the genus *Methylobacterium*.

Fig. 4. Populations of leaf habitats in a snap bean canopy. How can we best describe numbers of epiphytic bacteria that are present on populations of leaves in a field?

tion sizes among populations of leaves? Why would any plant pathologist be interested in the arcane process of describing variability at all? The reason is simple, elegant, and of great practical importance. One important example of the utility of such information is based on the fact that both the lognormal and the Weibull are skewed distributions. Hence, when leaves are bulked to form a single sample (a common practice in the 1960s and 1970s), the estimated population parameters may be erroneous (21). Further, the variance will usually be underestimated from replicate bulked samples. Thus, statistical analysis of bacterial population sizes based on bulked samples from skewed distributions may lead to the conclusion that means are significantly different, regardless of whether or not they actually are different (31). And all because the experimental design ignored the appropriate model for variability among population sizes of bacteria on populations of leaves.

Another, perhaps more important, example comes from the epidemiology of foliar bacterial diseases. The lognormal and Weibull are closely related two-parameter probability distribution functions. Thus, the entire distribution of bacterial population sizes across a field can be summarized by only two parameters (for example, the mean and variance of the normally distributed log-transformed measurements). In the presence of great leaf-to-leaf variability in bacterial population sizes, we may miss important events on the vast majority of leaves if we think only in terms of the "average leaf." To illustrate this point, consider the distribution of population sizes of *P. syringae* on bean leaflets in two fields, as shown in Figure 6. The average of the $\log_{10}$-transformed pathogen population sizes is the same in fields A and B (about 5.0 expressed as log CFU per leaflet). However, the population variances are different (2.5 versus 0.23). Would the expectation be that similar amounts of brown spot disease are likely to develop in the two fields?

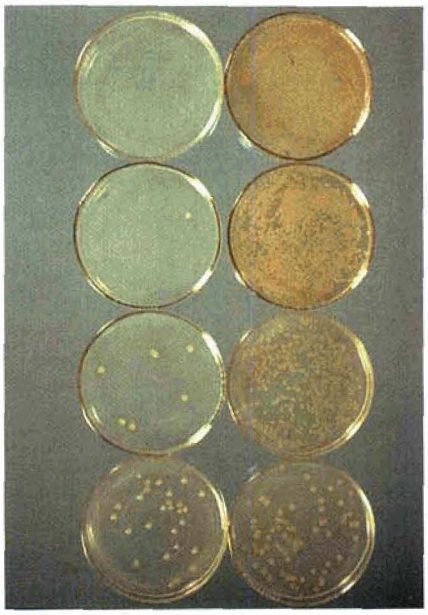


Fig. 5. Quantitative variability in population sizes of *Pseudomonas syringae* on individual rye leaves. Each plate represents an equivalent dilution from washings of different individual field-grown rye leaves.

Quantitative Relationship Between Population Sizes of *P. s.* pv. *syringae* and Brown Spot Disease Incidence

Given that *P. s.* pv. *syringae* grows in association with healthy leaves, is there a relationship between the number of pathogenic bacteria on a particular leaf and the probability that the leaf will become diseased? Can we relate numbers of epiphytic pathogenic bacteria on leaves to subsequent disease incidence or severity? Further, can we use the model describing the variability in population sizes as part of a more inclusive model that relates bacterial numbers to the amount of subsequent disease? As a starting point for this discussion, consider the hypothetical case in which disease is *not* a linear function of bacterial numbers. Suppose disease always occurs when at least a threshold number of bacteria, for example 10^4 CFU per leaflet, is present on a bean leaflet, but never when numbers of bacteria are below the threshold value. If the threshold model accurately predicts disease incidence and if we assume the threshold to be 10^4 CFU per leaflet, then the expected disease incidences for the hypothetical situations shown in Figure 6 would be roughly 67% for field A (i.e., pathogen populations are greater than the threshold on two-thirds of the leaflets) and 100% for field B (i.e., pathogen populations are greater than the threshold on all leaflets sampled). Note that disease incidence would differ in the two fields even though the mean pathogen population sizes are similar. This is because of the different variance or variability in pathogen populations on individual leaflets in the two fields. Indeed, in field experiments, Lindemann et al. (36) found that mean pathogen population sizes were not predictive of brown spot disease incidence. However, the frequencies with which epiphytic population sizes of *P. s.* pv. *syringae* were equal to or greater than a threshold of 10^4 on asymptomatic individual bean leaflets were predictive of disease (36).

According to the threshold model, the probability that a leaf will become diseased is either 0 (no disease) or 1 (disease present), depending on whether the number of bacteria on that leaf is below or above a given value, the threshold. Intuitively, it seems likely that this scenario is only a first approximation to reality. On the basis of infectivity titration studies done under controlled conditions, we know that an individual bacterial cell can cause visible disease (11,12). Although in many cases the probability of this occurring is quite small (e.g., 10^{-3} to 10^{-4}), it is not zero. Thus, in contrast with a threshold model, we might expect that in a large field at least a few leaves with one bacterial cell per leaf will become diseased. Likewise, we might expect that slightly more leaves with two bacterial cells per leaf will become diseased. In general, we might expect that, given any number of bacterial cells on a leaf, we could determine the probability of disease on that leaf. Indeed, the probit function does precisely this: given the number of bacterial cells, n, on a leaf, the probit function gives the probability of disease on that leaf (cf. 11,12). This probability is denoted $p(d \mid n)$. Note that for the threshold model, $p(d \mid n) = 0$ if n is below the threshold, while $p(d \mid n) = 1$ if n is above the threshold. For the probit model, $p(d \mid n)$ is a smoothly increasing function that goes from 0 to 1 as n increases.

Rouse et al. (41) combined the probit model with the lognormal model for bacterial numbers to give a more comprehensive model for the probability of disease in a given field. This model can be expressed in the form:

$$P(\text{disease}) = \int p(n)p(d \mid n)\, dn$$

The equation says that the probability of disease in a field P(disease) can be expressed in terms of the probability of a leaf having a particular number of bacteria $p(n)$ multiplied by the probability of disease occurring on that leaflet given that particular number of bacteria $p(d \mid n)$. The quantity P(disease) is given by the integral of this product. Again, the probability $p(n)$ is determined by the lognormal distribution, and the probability $p(d \mid n)$ is given by the probit model. This probit-lognormal model was tested with data from replicated field plots in which a range of *P. s.* pv. *syringae* population sizes had been achieved by various treatments (41). The model was used to estimate the dose of inoculum that corresponded to a brown spot disease incidence of 50% (i.e., ED_{50} value). Under field conditions, these values were approximately 3×10^5 CFU per leaflet (41).

Both of these examples, one as simple as understanding how to compare bacterial populations in different canopies (e.g., Fig. 6), the other as powerful as a clear quantitative relationship between bacterial numbers and subsequent disease (e.g., probit-lognormal model), require use of the information contained in the variability in bacterial population sizes among populations of leaves. Is variability a boon or a bane for research on this system? We do know that it is an intrinsic part of the system that needs to be considered—indeed, carefully examined in the process of studying the relationships between bacteria and plants.

Spatial Patterns in *P. s.* pv. *syringae* Populations and Bacterial Brown Spot Disease

The probit-lognormal model of Rouse et al. (41) provides an important link be-

tween *P. s.* pv. *syringae* population sizes and disease incidence. For example, with some level of confidence, it permits the forecasting of disease incidence in a region based on bacterial population numbers. Moreover, we thought that the model could be used to understand spatial patterns of disease. Specifically, it would seem that spatial patterns in disease could be predicted, via the probit-lognormal model, from spatial patterns in epiphytic pathogen population sizes. If this is not true, then it might be that other factors, for example aspects of the environment, may have an effect on the likelihood of disease given pathogen population sizes. In either case, there seemed to us to be clear motivation to study spatial patterns in this system, and the easiest place to start was with patterns of disease.

We started studying spatial patterns of brown spot disease by looking at disease incidence per plant in short (5 m) row segments (28) (Fig. 7). This represents a fairly detailed sampling scale, since it really amounts to trying to understand spatial patterns from plant to plant. Given our findings on the necessity of studying bacterial population variation on a leaf-to-leaf scale, this fine scale of spatial pattern study seemed appropriate. It required, however, the use of tools not commonly found in phytopathology. Specifically, we made much use of Auto-Regressive Moving Average (ARMA) models—a family of models frequently used in the study of time series data (3). An example of such a model is given by the formula:

$$Y_t = \phi Y_{t-1} + \varepsilon_t - \theta\varepsilon_{t-1} + \delta$$

Here, Y_t represents the (suitably transformed) disease incidence for a plant at position t in the row. (So, for example, Y_{42} represents the amount of disease on the 42nd plant in the row segment under study.) The term ε_t represents an "error" term, much like the error term in a regression model. The ARMA model above says that Y_t, the amount of disease on a given plant, can be related to Y_{t-1}, the amount of disease on its neighbor to the left, by a coefficient ϕ. Also involved in the relationship is ε_t, the error term for the tth plant, and the error term for the neighbor ε_{t-1}. The latter term is related to Y_t by a coefficient θ. The constant δ can be related to the mean amount of disease. Note that this model is not "mechanistic" in the sense that the disease level for a given plant Y_t probably depends on the level of disease for its neighbors at positions $t - 1$ and $t + 1$, and perhaps in adjacent rows. Nonetheless, the ARMA model might still be useful in terms of being "descriptive": it helps to summarize the pattern of disease values and also helps in the formulation of statistical tests of randomness.

Indeed, after studying several cultivars in several locations over many years, we found that the vast majority of spatial patterns of brown spot could be described in terms of ARMA models such as the one above (4,28,29). These models suggested at least two "scales" of spatial pattern within 5-m row segments, both of which are illustrated in Figure 7. The first is a general "undulating" pattern, which can be seen in the overall increases (e.g., around 1 and 4.75 m) and decreases in disease. The undulating pattern is suggestive of regionalized patches of disease (and modeled by the component ϕY_{t-1}). The second is a "jagged" pattern, suggesting that plants with high amounts of disease tend to have neighbors within the row that have relatively lower amounts of disease, and vice versa. This corresponds to the term $\theta\varepsilon_{t-1}$ in the model. The jagged pattern can be seen by comparing the disease incidence on adjacent plants in Figure 7.

To look at spatial patterns over larger scales and across rows, we have had to apply a variety of different sampling and analysis methods (4,5,29). The most elaborate of these is cyclic sampling: within a row segment of 1,550 plants (distance ranged from 65 to 147 m), we number the plants and divide them up into contiguous subsegments, each containing 31 plants. Then we sample the plants at positions 2, 5, 7, 11, 25, and 26 in each of these subsegments. This sampling plan permits us to learn about spatial patterns on a larger scale (up to 147 m) while conserving sampling effort and costs. Interestingly, the cyclic samplings revealed nonrandom spatial patterns in which brown spot disease was seen to occur at a regularity of every 400 to 600 plants (i.e., every 20 to 40 m) (4). Hence, we have found nonrandom patterns in disease that occur at several different scales within a given bean field.

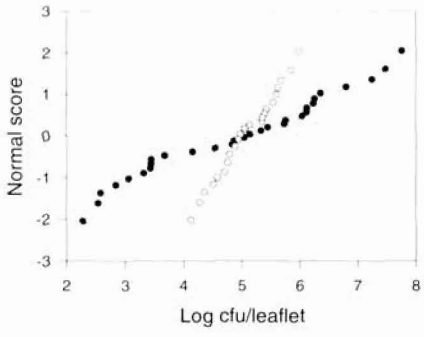


Fig. 6. Frequency distributions of population sizes of *Pseudomonas syringae* on individual leaflets from each of two hypothetical bean fields. The mean *P. s.* pv. *syringae* population size for both sets of leaflets is approximately 5.0 log CFU per leaflet. The variances are 2.5 for field A (closed circles) and 0.23 for field B (open circles).

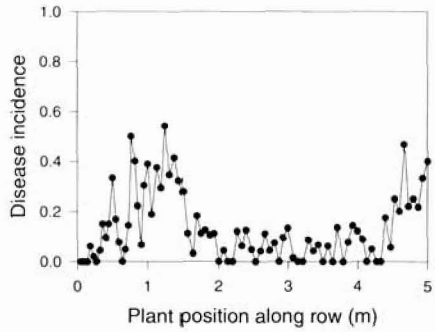


Fig. 7. Nonrandom spatial pattern in brown spot disease incidence. Each datum point represents the proportion of diseased leaflets per plant. The number of leaflets per plant ranged from 4 to 66 with a median of 23 leaflets.

The nonrandom spatial patterns in disease lead immediately to the question of the nature of the spatial patterns in epiphytic pathogen population sizes and whether the latter can be used to predict the former. Because determination of *P. s.* pv. *syringae* population sizes on every leaflet of every plant within even a single 5-m row segment by dilution plating is a daunting task (i.e., the number of samples may be as large as 2,000), we used an ice nucleation assay (22) to indirectly assess population sizes of *P. s.* pv. *syringae* on every leaflet of mature bean plants. The assay is based on the ability of *P. s.* pv. *syringae* to cause the formation of ice at temperatures just below 0°C, as previously noted (see Fig. 2C). The larger the population size of ice nucleating *P. s.* pv. *syringae* on a leaflet, the greater the likelihood that a nucleation event will occur at temperatures around –2 to –2.5°C. We have used the assay in previous experiments to successfully predict relative amounts of brown spot disease (22). Similar to disease, nonrandom spatial patterns in ice nucleation activity that could be described by ARMA models were detected within 5-m row segments of mature bean plants (10). We have also examined spatial patterns of *P. s.* pv. *syringae* populations during the early development of bean seedlings (10,18). Bean seeds were inoculated with a rifampicin-marked strain of *P. s.* pv. *syringae* at the time of planting. Immediately after planting and 1, 2, 4, and 9 days later, every seed within replicated 5-m row segments was carefully dug up, and population sizes of the marked strain were determined by dilution plating. The spatial patterns of *P. s.* pv. *syringae* were random on seeds sampled immediately after planting. Interestingly, the patterns were found to be nonrandom by the next day. The nonrandom spatial patterns, however, were ephemeral. By day 2 the patterns in *P. s.* pv. *syringae* population sizes were once again random. Our current thought is that soil moisture and possibly other environmental factors may be possible causes of the spatial patterns observed on germinating bean seeds.

Where does this leave us with our understanding of the relationship between epiphytic pathogen population sizes and disease? The similarity in spatial patterns

of disease and in *P. s.* pv. *syringae* population sizes is intriguing. Could it be that patterns in bacterial numbers are established very early in the growing season, and that these are manifest later in the season as patterns in disease? We think the process might be more complicated than that, but it nonetheless remains at least a curiosity that the patterns are so similar. Unfortunately, the destructive sampling necessary for determination of bacterial numbers precludes direct comparison of spatial patterns in *P. s.* pv. *syringae* populations and subsequent disease on the same leaves.

Knowing that disease occurs in a nonrandom fashion within a field has an impact on the effectiveness of various sampling procedures (4). For example, if a commercial processor is sampling a bean field to estimate disease levels to decide whether to bypass a field for harvest, then a precise estimate of disease incidence is critical for making that judgment. However, if the disease (such as brown spot) occurs in a spatially nonrandom fashion, typical sampling approaches might not be appropriate. In particular, a simple random sample can result in mean estimates of disease incidence that are more variable than estimates based on some other sampling plans. More specifically, when a disease exhibits the types of spatial patterns we have seen for brown spot, then a systematic sampling plan can be superior (4).

Dynamics of *P. s.* pv. *syringae* Populations

If the amount of disease in a canopy follows directly from the numbers of bacteria that are present on individual leaves, then the processes that influence bacterial numbers on leaves might be considered the driving forces in the epidemiology of the disease. Following this line of reasoning, we may ask: How rapidly can these large populations become established? What factors favor the buildup of the large pathogen population sizes that place the bean canopy at hazard to disease? Additionally, the occurrence of nonrandom spatial patterns of disease raises the related question of the nature of the spatial patterns in pathogen population sizes and how such patterns arise. These types of questions are encompassed within the broader framework of seeking an understanding of the extent to which the processes of growth, death, immigration, and emigration contribute to pathogen population sizes at any given time, and the physical and biotic factors that influence the rates with which these population processes occur. In our experimental approach to studying the dynamics of *P. s.* pv. *syringae* populations, we have placed great emphasis on the issues of time frame (i.e., time scaled to the relatively short generation times exhibited by bacteria) and of conducting experiments under field conditions in order to understand the dynamics of *P. s.* pv. *syringae* as it occurs naturally (cf. 19,23–27).

Temporal changes in *P. s.* pv. *syringae* population sizes. In our earlier experiments on the relationship between pathogen population sizes and disease, we sampled leaflets at 3- or 4-day intervals (22,41). While this sampling frequency was adequate to address the questions asked at the time, it became apparent that sampling on such a time frame was masking the inherent dynamics of *P. s.* pv. *syringae* populations. We assumed at the time, as others had before, that growth of *P. s.* pv. *syringae* on bean leaves in the field would more likely occur during the night than the day. At night, moisture would be present on leaves when dew formed, relative humidity would be high, solar radiation would be nil, and temperatures would not be excessively high. To confirm this assumption and with the loftier goal of obtaining estimates of *rates* of change in population sizes under field conditions, we decided to monitor population sizes of *P. s.* pv. *syringae* "around the clock" by sampling every 2 hours during each of three nonconsecutive days (24).

Population sizes of *P. s.* pv. *syringae* increased 5.8-fold during the first of these 24-hour periods. In the second (Fig. 8A), population size of *P. s.* pv. *syringae* decreased during the day and then increased during the night, more or less as we had expected. However, because the increase that occurred at night was of the same approximate size as the decrease that occurred during the day, no net increase occurred as would be necessary for development of the large population sizes that result in disease. In the third 24-hour period (Fig. 8B), we found what we were looking for but not what we expected. That is to say, we observed a large increase in population sizes of *P. s.* pv. *syringae,* but the increase continued throughout a day that was characterized by intense solar radiation, absence of free moisture on the leaf surfaces, and low relative humidity; conditions that were generally assumed to be unfavorable for growth of leaf-associated bacteria. The size of this increase (28-fold) in numbers of *P. s.* pv. *syringae* in 24 hours was orders of magnitude larger than the largest amount of bacterial immigration measured on a single day over a 3-year period (37). Hence, the increase was attributed to bacterial multiplication. We estimated an overall doubling time of approximately 4.9 hours from 0900 at the start of the experiment to 0900 the next day. During this period, it appeared that doubling times could have been as short as 3.3 and 1.9 hours from 1700 to 2300 and from 0100 to 0700, respectively (Fig. 8B). From these experiments we learned that (i) an appropriate sampling frequency will allow estimation of rates of change in bacterial population sizes under field conditions, (ii) growth rates of *P. s.* pv. *syringae* in association with bean leaflets in the field may on occasion be of magnitude similar to those measured in broth cultures in the laboratory (45), and (iii) the assumption that growth of *P. s.* pv. *syringae* in association with leaves would not occur during the hot, sunny, dry daytime was clearly incorrect.

Hourly changes in population sizes of *P. s.* pv. *syringae* sum to daily changes, which in turn result in seasonal trends. By sampling "around the clock," we saw examples of scenarios that might lead to no, small, or large net daily changes in *P. s.* pv. *syringae* population sizes. To examine how such daily changes may fit together to determine the seasonal dynamics of *P. s.* pv. *syringae*, we measured population sizes of *P. s.* pv. *syringae* on sets of individual bean leaflets which were collected either 5 or 7 days per week throughout the life span of the bean crop (19). Rates of change in *P. s.* pv. *syringae* population sizes can only be inferred from such a sampling frequency. Nonetheless, with simultaneous measurements of daily changes in *P. s.* pv. *syringae* population sizes, various parameters of the physical environment, host phenology, and brown spot disease incidence, we could begin to understand the extent to which pathogen population dynamics are affected by a number of abiotic and biotic factors. Our data include results from 11 plantings of beans representing two cultivars that differ in susceptibility to brown spot and span a

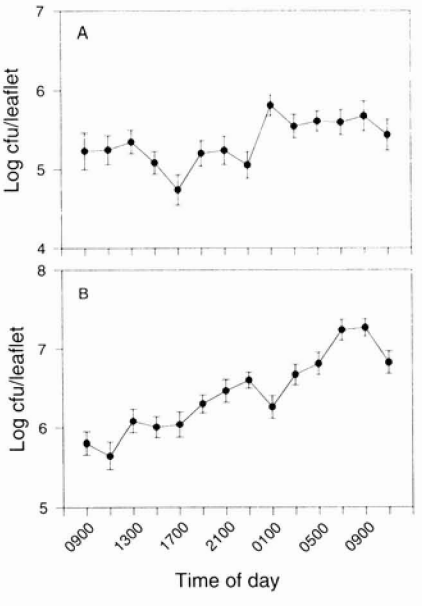


Fig. 8. Bi-hourly changes in population sizes of *Pseudomonas syringae* on snap bean leaflets. Bacterial population sizes were estimated for each of 30 individual bean leaflets per sampling time. The data are the means and standard errors for each set of 30 leaflets collected on (A) 58 to 59 days after planting and (B) 44 to 45 days after planting (24).

period of 6 years. In all cases, populations of *P. s.* pv. *syringae* were those that occurred naturally; i.e., none of the plantings were inoculated with the pathogen.

In two plantings of the moderately resistant cultivar Cascade, in 1987 and 1988, population sizes of *P. s.* pv. *syringae* remained near or below our limit of detection (Figure 9A illustrates the 1987 planting). Patterns of changes in *P. s.* pv. *syringae* population sizes characteristic of the remaining six plantings of this cultivar are presented in Figure 9B. Periods during which population sizes of *P. s.* pv. *syringae* increased or decreased significantly occurred during all six plantings. The magnitude of these increases ranged from greater than 10-fold to more than 1,000-fold within a 24-hour period. Note, for example, the nearly 100-fold increase that occurred from 35 to 36 days after planting (DAP) in Figure 9B. More frequently, the development of the large pathogen population sizes that place the crop at risk to disease was seen to accrue from smaller increases that occurred over several consecutive days.

Effect of the physical environment on changes in *P. s.* pv. *syringae* population sizes. A comparison of daily changes in *P. s.* pv. *syringae* population sizes and weather parameters across all plantings suggested a strong association between the occurrence of rain and the onset of periods of large increases in pathogen population sizes. For example, the nearly 100-fold increase that occurred from 35 to 36 DAP (Fig. 9B) followed a 26-mm rainfall on 34 DAP. The very large increase in *P. s.* pv. *syringae* population sizes that began about 44 DAP was associated with 18 mm of rain that fell on that day. The persistence of the large populations during the latter half of the growing season was associated with numerous rainfalls that totaled approximately 95 mm (Fig. 9B). Overall, 491 mm of rain fell between planting and harvest of the 1984 plot (Fig. 9B), but only 94 mm fell during the growth of the 1987 planting (Fig. 9A). Hence, the establishment of the large pathogen populations that lead to disease appears to depend on the frequency of rainfall events during a growing season and on their timing relative to plant development. When blooms in *P. s.* pv. *syringae* population sizes follow intense rains, it appears that rain triggers the onset of rapid multiplication of the bacterium on bean leaves. Indeed, the exponential increase in *P. s.* pv. *syringae* population sizes (Fig. 8B) that occurred during the 24-hour period in which samples were taken every 2 hours was preceded by a 22-mm rain event. Although blooms in *P. s.* pv. *syringae* population sizes were nearly always preceded by intense rains, not all rains resulted in large increases. For example, during the 1987 growing season (Fig. 9A), there was no measurable increase associated with the 23 mm of rain that fell 34 DAP. This may have been due to the near absence of *P. s.* pv. *syringae* on bean leaves, which in turn may have been related to the lack of rain during the 5 weeks from planting to the first intense rain on 34 DAP. Indeed, an increase may have occurred, but the final population may have remained below our level of detection.

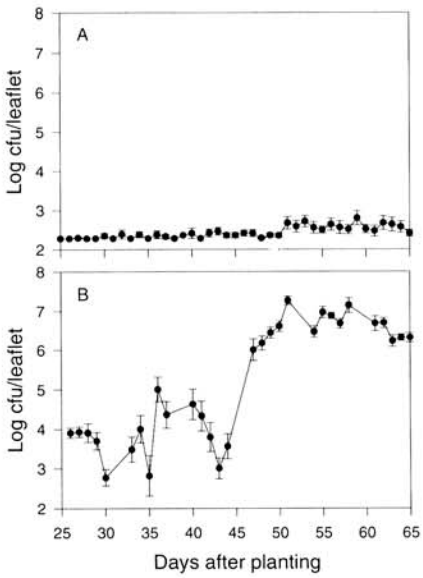


Fig. 9. Temporal changes in population sizes of *Pseudomonas syringae* on snap bean leaflets collected during the (A) 1987 and (B) 1984 growing seasons. Thirty leaflets were collected at 0800 at each sampling time. Each leaflet was processed individually by dilution plating of leaf homogenates. The data are the means and standard errors for each set of 30 leaflets.

Our attempts to address the mechanism by which rain triggers the onset of rapid growth of *P. s.* pv. *syringae* on bean leaves have been frustrated by the highly reproducible observation that when strains of *P. s.* pv. *syringae* are mist-inoculated onto growth chamber–grown bean plants, multiplication of the bacteria occurs. This is simply not the case for populations of *P. s.* pv. *syringae* on bean plants in the field. Hence, the approach taken was to attempt to modify the microclimate of bean plants under field conditions in various ways. Population sizes of *P. s.* pv. *syringae* were compared on plants that were naturally rained on versus those that were shielded from rain with polyethylene shelters as shown in Figure 10. In each of three experiments, population sizes of *P. s.* pv. *syringae* increased >100-fold on plants that were rained on. However, there was no increase on plants that were sheltered from the natural rains. The findings demonstrated more conclusively that rain does indeed trigger growth of *P. s.* pv. *syringae* on bean plants in the field.

The role of leaf wetness duration and volume, intensity, and acidity of rain was examined by Alberga (1) by applying simulated rain in various ways to bean plants. For example, the intensity of rain was examined by applying well water to bean plants using different types of spray nozzles attached to a tractor-mounted spray boom. Shelters (Fig. 10) equipped with heating tape were used to determine whether temperature may play a role, given that temperatures are generally higher when leaves are wet due to rain versus wetness due to dew. None of these factors was found to have a significant effect on population sizes of *P. s.* pv. *syringae*, at least in the way examined (1). In subsequent experiments, we found that the momentum of raindrops does appear to be important for initiating the onset of rapid growth of *P. s.* pv. *syringae*. Inert fiberglass screens were placed over rows of bean plants (Fig. 10) to modify the momentum of raindrops during natural rains (27). The idea here was that the velocity of the falling rain would be greatly reduced by the screens, resulting in drops with attenuated momentum or energy as they landed on the leaves. The volume and quality of water that fell on the leaves would be similar for plants under the screens and plants exposed to the rain. In each of three experiments centered around natural rainstorms, population sizes of *P. s.* pv. *syringae* increased as expected on plants that were exposed to the rains but not on plants under the screens. Hence, the momentum of raindrops is in some way associated with the rapid growth of *P. s.* pv. *syringae* following rains. Possible explanations for the phenomenon include the following: that intense rains may affect the plant in a way that makes nutrients more readily available, removes some sort of toxin or inhibitor, or even facilitates ingress of *P. s.* pv. *syringae* into the intercellular spaces of bean leaves where growth may occur. At this time, we continue to debate among ourselves as to what the mechanism might be and how to go about elucidating it. Whatever the mechanism might be, it is interesting to note that not all bacteria on bean leaves respond to rain as does *P. s.* pv. *syringae*. Bacteria referred to as pink-pigmented facultative methylotrophs (PPFMs) of the genus *Methylobacterium* (see Figure 3, small pink colonies) are consistently present in large numbers on bean leaves by pod harvest, regardless of the amount of rain (S. S. Hirano and C. D. Upper, *unpublished*). We have been intrigued by the seemingly very different life strategies that *P. s.* pv. *syringae* and the PPFMs have evolved to be successful inhabitants of the phyllosphere.

The role of rain in disease outbreaks. If the mechanism by which rains trigger growth of *P. s.* pv. *syringae* on bean leaves remains obscure, the role of rain in the epidemiology of bacterial brown spot disease is now quite clear. Rains have long been associated with outbreaks of foliar diseases caused by pathovars of *P. syrin-*

gae and *X. campestris* (8,13,14,43). The accepted explanation for the association of disease outbreaks with intense rains is that rain splash disseminates bacterial pathogens to susceptible tissue. After the pathogen has been dispersed to inoculum-deficient leaves or plants, disease follows. This classical explanation is inconsistent with our findings for the *P. s.* pv. *syringae*–brown spot system in two ways. First, detectable populations of *P. s.* pv. *syringae* may be found on nearly all of the leaves in a bean canopy in the absence of disease. (The model discussed above states this in a more formal way.) Hence, dispersal of *P. s.* pv. *syringae* (by rain splash or any other way) may merely move the pathogen to leaves on which it already resides. Second, although horizontal movement of leaf surface bacteria due to splash dispersal does occur during rain (13,37), this process is very inefficient. Large numbers of bacteria are washed off of leaf surfaces during rain, and only a few are redeposited on leaves. The result is a net *decrease* in population sizes of bacteria on leaves (6,37). A process that decreases pathogen population size should decrease, not increase, the hazard of disease. What intense rain does do is trigger the onset of rapid growth of *P. s.* pv. *syringae*. Although large numbers of bacteria are removed from leaves by rain, those that remain multiply to establish the population sizes that lead to disease. Thus, at least for bacterial brown spot of snap bean, disease follows rain not because of splash dispersal but because of pathogen multiplication. Whether the role of rain in outbreaks of other foliar bacterial diseases is due to its effect on the process of pathogen growth, not immigration (i.e., splash dispersal), remains to be determined.

Effect of biotic factors on *P. s.* pv. *syringae* population sizes. The biotic factors that we have found to have an effect on populations of *P. s.* pv. *syringae* are host related. Daub and Hagedorn (9) reported that breeding lines and cultivars of snap bean that differed in relative resistance to brown spot differed in the epiphytic populations of *P. s.* pv. *syringae* that they would support. In a comparison of the seasonal dynamics of *P. s.* pv. *syringae* population sizes on two cultivars that differed in susceptibility to brown spot, we found that the onset of periods of large increases following intense rains was similar for both (S. S. Hirano and C. D. Upper, *unpublished*). However, the magnitude of the increases was larger for the susceptible compared to the moderately resistant cultivar. Hence, host genotype has a significant effect on populations of *P. s.* pv. *syringae* on snap bean. Host phenology also appears to influence epiphytic populations of *P. s.* pv. *syringae*. During flowering, populations of *P. s.* pv. *syringae* tend to decline (Fig. 9B, ca. 40 to 45 DAP). Legard and Schwartz (34) also reported a similar finding. During this phase of plant development, population increases following rains are frequently transient in nature.

Dispersal and spread of *P. s.* pv. *syringae*. Thus far, we demonstrated that growth is the driving force in the development of the *P. s.* pv. *syringae* populations that lead to disease and that the onset of growth is triggered by intense rains. However, before growth can occur, immigrants of *P. s.* pv. *syringae* must arrive on leaf habitats; immigration is a required part of the process that leads to bacterial brown spot disease. The literature suggests many ways that bacteria can move between plants (cf. 42). In addition to splash dispersal by rain (or irrigation), movement has been shown to occur by aerosols generated during dry, sunny, windy weather, by human activities, and by aerosols dispersed into the troposphere followed by atmospheric scrubbing by rain (cf. 6,37,42). Dispersal between plant generations may be accomplished by bacteria surviving on seeds, on plant debris, or on other living plant material.

The literature is much less clear about which of these various routes make important quantitative contributions to successful spread of the bacteria. For the past few years, we have been working to determine the quantitative importance of immigration relative to growth in determining population size of *P. s.* pv. *syringae* on bean, and the relative quantitative importance of the various possible dispersal routes. These studies are as yet incomplete, and most of the conclusions that we have made are quite tentative. Our current view is that all of the possible routes of dispersal may be quantitatively important at one time or another. But the relative importance of the various dispersal routes probably depends very heavily on weather, stage of plant growth, and scale (in both time and space). For example, rain splash is probably unimportant at distances beyond one or a few meters but may be very important for delivering the initial inoculum to newly emerged leaves from older leaves on the same or adjacent plants. During extended periods of rainy weather, rain splash may be the major means of very local dispersal. On the other hand, when rain is infrequent, deposition from aerosols may effectively move bacteria very short distances. Washout of airborne bacteria onto crop canopies by rain is probably only a minor contributor in terms of numbers of bacteria delivered, but it has the potential to function at distances of hundreds or even thousands of kilometers. Thus, it may be important in the delivery of the first bacterium of a particular strain in a given field.

One of the points that has emerged from our research is the rapid infestation of very young bean plants with *P. s.* pv. *syringae* (18,20). Commercial bean seed is quite clean. In most cases, infestation rates are one seed in several hundred or lower. Yet, given wet conditions during germination and emergence, *P. s.* pv. *syringae* can be isolated from a relatively large proportion of first trifoliolate leaves in large plantings. Somehow the bacteria have spread from the few infested seeds (and other sources in the local environment) to all of the plants in an entire field quite rapidly (20). We know that *P. s.* pv. *syringae* grows very rapidly on germinating bean seeds in the field (18) and that primary leaves are heavily infested if seedborne bacteria are present. The surprising finding, however, has been that the bacteria may spread more than 6 m within the first 2 days after plant emergence (20). The most likely candidate for moving bacteria this distance soon after emergence is insects (16). Almost any insect that is active in the plant canopy when leaves are wet (frequently at or shortly after sunrise) may

Fig. 10. Modification of the environment with polyethylene shelters and inert fiberglass screens. The shelters were used to shield bean plants from rain; the screens were used to decrease the momentum of raindrops as they passed through the screen and dripped onto the plants.

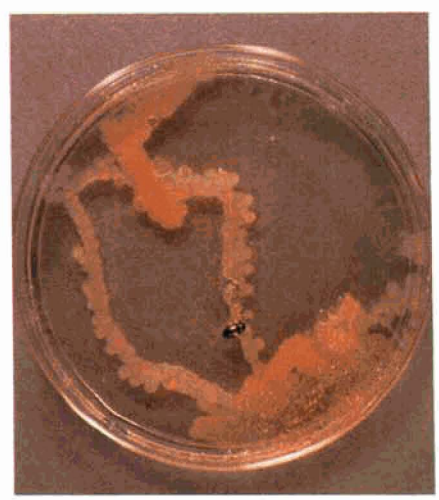
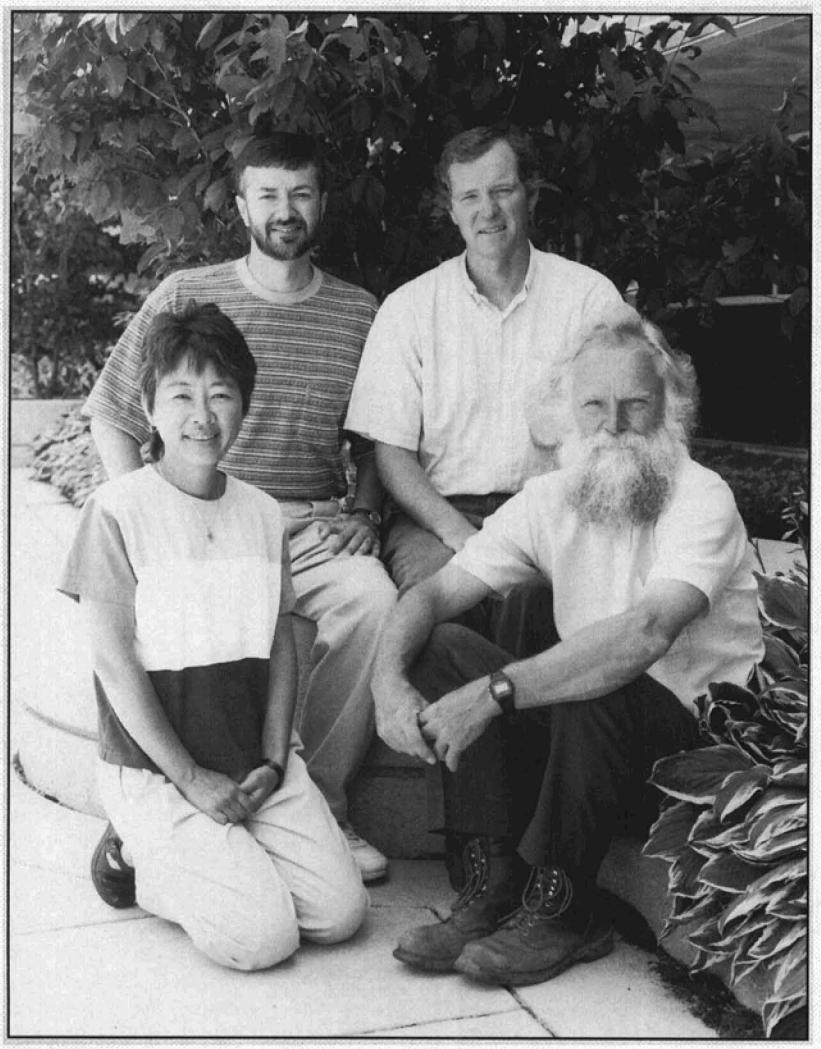


Fig. 11. An insect (*Glischrochilus quadrisignatus*) was trapped in a sterile empty petri dish exposed in a bean canopy in the early morning when leaves were wet with dew. The trapped insect was transferred to a petri dish containing an appropriate medium and allowed to walk over the surface of the medium. The white colonies are *Pseudomonas syringae*.



Murray K. Clayton Douglas I. Rouse
Susan S. Hirano Christen D. Upper

Dr. Hirano is a senior scientist in the Department of Plant Pathology, University of Wisconsin-Madison. She received her B.S. and Ph.D. in biology from the University of Oregon and UCLA, respectively. Her research interests center on the ecology of leaf-associated bacteria, in particular the population biology of *Pseudomonas syringae*.

Dr. Rouse is a professor in the Department of Plant Pathology, University of Wisconsin-Madison. He received his B.A. in mathematics from Ottawa University, his M.S. in botany and plant pathology from Colorado State University, and his Ph.D. in plant pathology from the Pennsylvania State University. As a quantitative epidemiologist, he maintains an interest in stochastic models. His current major research interest focuses on potato early dying, including specific projects ranging from modeling disease and yield loss to the application of epidemiological principles to practical disease control.

Dr. Clayton holds a joint appointment as a professor in the Department of Plant Pathology and in the Department of Statistics, University of Wisconsin-Madison. He received his B.Math. in statistics and pure mathematics from the University of Waterloo, Ontario, and his Ph.D. in statistics from the University of Minnesota. His research interests include the application of statistics to biology, in particular the study of spatial patterns of disease and plant disease epidemiology.

Dr. Upper is a research chemist in the United States Department of Agriculture, Agricultural Research Service Plant Disease Resistance Unit, and a professor in the Department of Plant Pathology at the University of Wisconsin-Madison. He received his B.S. in chemistry from Washington State University and his Ph.D. in biochemistry from the University of Illinois. His research interests are the ecology of foliar plant-associated bacteria and the epidemiologies of the diseases they cause.

move *P. s.* pv. *syringae*. Figure 11 shows an insect "caught in the act" of spreading *P. s.* pv. *syringae* to a petri plate. This insect landed on a petri plate in a bean field slightly after sunrise, while the leaves were still wet with dew. When leaves are dry during the day, insects appear not to be an effective mechanism for dispersal of *P. s.* pv. *syringae*. They may walk on petri plates but do not leave a trail of bacteria. We are currently investigating the relative quantitative contributions of all the various dispersal mechanisms discussed above relative to growth of *P. s.* pv. *syringae* in the dynamics of *P. s.* pv. *syringae* populations on bean plants.

Concluding Remarks

The *P. s.* pv. *syringae*–snap bean–brown spot system has been an exquisitely rich and amenable system for investigating the complexities and dynamics of epiphytic plant pathogens and the diseases they cause. While we have learned much, there remain several unanswered and intriguing questions. For example: What exactly are the causes of the nonrandom spatial patterns in disease and in pathogen population sizes? What is the mechanism by which raindrop momentum triggers the onset of rapid growth of *P. s.* pv. *syringae*? What dispersal mechanisms of *P. s.* pv. *syringae* are most important in subsequent disease development?

With respect to the broader issue of the role that epiphytic pathogen populations play in the epidemiology of their associated diseases and whether the *P. s.* pv. *syringae*–brown spot system may be viewed as a model system, our current perspective may be found in the following conjecture: The life strategies of foliar bacterial pathogens and the epidemiologies of the diseases they cause range along a continuum from those for which epiphytic populations are the primary source of inoculum to those for which lesions are the dominant inoculum source. The conjecture predicts that the relative importance of epiphytic growth and growth in lesions should differ among foliar bacterial pathogens and that these differences should be reflected in the epidemiologies of the diseases they cause. We speculate that the *P. s.* pv. *syringae*–brown spot system may fall on that part of the continuum for which epiphytic populations are the primary source of inoculum.

Perhaps, whether it is a case study or model system is not as important as recognizing that there is much more to the biology of bacteria such as *P. s.* pv. *syringae* than just disease causation. *P. s.* pv. *syringae* may grow for many tens or hundreds of generations in association with healthy plants without ever causing disease. Although much attention is currently focused on the mechanisms of pathogenesis by such bacteria, most of the remainder of their life strategies remains seriously

neglected. There is no question that pathogenesis is a requisite property of a bacterium that can cause economically important disease. However, such activities as dispersal, survival, and growth in locations other than lesions, while not directly involved in pathogenesis, may be equally important to development of economically important epidemics. As the majority of current strategies to control foliar bacterial diseases are designed to interfere with the activities of bacteria when they are not in lesions, we expect future control methods to be similarly based. Thus, we consider knowledge of the activities of pathogens when they are not in lesions essential for developing successful, environmentally benign strategies for the management of bacterial diseases.

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Literature Cited

1. Alberga, A. 1987. Microclimate influences on *Pseudomonas syringae* bacteria on snap beans. Ph.D. diss. University of Wisconsin, Madison.
2. Arny, D. C., Lindow, S. E., and Upper, C. D. 1976. Frost sensitivity of *Zea mays* increased by application of *Pseudomonas syringae*. Nature 262:282-284.
3. Box, G. E. P., Jenkins, G. M., and Reinsel, G. C. 1994. Time Series Analysis. Forecasting and Control. 3rd ed. Prentice Hall, Englewood Cliffs, NJ.
4. Clayton, M. K., and Hudelson, B. D. 1991. Analysis of spatial patterns in the phyllosphere. Pages 111-131 in: Microbial Ecology of Leaves. J. H. Andrews and S. S. Hirano, eds. Springer-Verlag, New York.
5. Clayton, M. K., and Hudelson, B. D. 1995. Confidence intervals for autocorrelations based on cyclic samples. J. Am. Stat. Assoc. In press.
6. Constantinidou, H. A., Hirano, S. S., Baker, L. S., and Upper, C. D. 1990. Atmospheric dispersal of ice nucleation-active bacteria: The role of rain. Phytopathology 80:934-937.
7. Crosse, J. E. 1959. Bacterial canker of stonefruits. IV. Investigation of a method for measuring the inoculum potential of cherry trees. Ann. Appl. Biol. 47:306-317.
8. Daft, G. C., and Leben, C. 1972. Bacterial blight of soybeans: Epidemiology of blight outbreaks. Phytopathology 62:57-62.
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. Dik, A. J., Clayton, M. K., Upper, C. D., and Hirano, S. S. 1993. Spatial patterns in populations of *Pseudomonas syringae* on snap beans. (Abstr.) Proc. Int. Congr. Plant Pathol., 6th.
11. Ercolani, G. L. 1973. Two hypotheses on the aetiology of response of plants to phytopathogenic bacteria. J. Gen. Microbiol. 75:83-95.
12. Ercolani, G. L. 1984. Infectivity titration with bacterial plant pathogens. Annu. Rev. Phytopathol. 22:35-52.
13. Ercolani, G. L., Hagedorn, D. J., Kelman, A., and Rand, R. E. 1974. Epiphytic survival of *Pseudomonas syringae* on hairy vetch in relation to epidemiology of bacterial brown spot of bean in Wisconsin. Phytopathology 64:1330-1339.
14. Faulwetter, R. C. 1917. Wind-blown rain, a factor in disease dissemination. J. Agric. Res. 10:639-648.
15. Gleason, M. L., Gitaitis, R. D., and Ricker, M. D. 1993. Recent progress in understanding and controlling bacterial canker of tomato in Eastern North America. Plant Dis. 77:1069-1076.
16. Groth, E. M., Hirano, S. S., Baker, L. S., and Upper, C. D. Insect-mediated dispersal of *Pseudomonas syringae* in a bean field. (Abstr.) Phytopathology. In press.
17. Henis, Y., and Bashan, Y. 1986. Epiphytic survival of bacterial leaf pathogens. Pages 252-268 in: Microbiology of the Phyllosphere. N. J. Fokkema and J. van den Heuvel, eds. Cambridge University, Cambridge.
18. Hirano, S. S., Clayton, M. K., Dik, A. J., and Upper, C. D. 1994. Spatial patterns in ice nucleation temperatures and population sizes of *Pseudomonas syringae* pv. *syringae* on germinating bean seeds. (Abstr.) Phytopathology 84:1135.
19. 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.
20. Hirano, S. S., Fourrier, K. D., Riely, B. K., Baker, L. S., and Upper, C. D. 1995. Spread of *Pseudomonas syringae* in the phyllosphere of snap bean seedlings. (Abstr.) Phytopathology. In press.
21. 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.
22. 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.
23. Hirano, S. S., and Upper, C. D. 1983. Ecology and epidemiology of foliar bacterial plant pathogens. Annu. Rev. Phytopathol. 21:243-269.
24. 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.
25. Hirano, S. S., and Upper, C. D. 1990. Population biology and epidemiology of *Pseudomonas syringae*. Annu. Rev. Phytopathol. 28:155-177.
26. 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.
27. Hirano, S. S., and Upper, C. D. 1994. Autecology of foliar Pseudomonads. Pages 227-243 in: Ecology of Plant Pathogens. J. P. Blakeman and B. Williamson, eds. CAB Int., U.K.
28. Hudelson, B. D., Clayton, M. K., Smith, K. P., Rouse, D. I., and Upper, C. D. 1989. Nonrandom patterns of bacterial brown spot in snap bean row segments. Phytopathology 79:674-681.
29. Hudelson, B. D., Clayton, M. K., Smith, K. P., and Upper, C. D. 1993. Modeling of superimposed spatial patterns of bacterial brown spot of snap bean. Phytopathology 83:430-438.
30. 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.
31. Kinkel, L. 1992. Statistical consequences of combining population samples. (Abstr.) Phytopathology 82:1168.
32. Leben, C. 1965. Epiphytic microorganisms in relation to plant disease. Annu. Rev. Phytopathol. 3:209-230.
33. Leben, C. 1974. Survival of plant pathogenic bacteria. Ohio Agric. Res. Devel. Ctr., Spec. Circ. 100.
34. Legard, D. E., and Schwartz, H. F. 1987. Sources and management of *Pseudomonas syringae* pv. *phaseolicola* and *Pseudomonas syringae* pv. *syringae* epiphytes on dry beans in Colorado. Phytopathology 77:1503-1509.
35. Lindemann, J., Arny, D. C., and Upper, C. D. 1984. Epiphytic populations of *Pseudomonas syringae* pv. *syringae* on snap bean and nonhost plants and the incidence of bacterial brown spot disease in relation to cropping patterns. Phytopathology 74:1329-1333.
36. 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.
37. Lindemann, J., and Upper, C. D. 1985. Aerial dispersal of epiphytic bacteria over bean plants. Appl. Environ. Microbiol. 50:1229-1232.
38. Lindow, S. E. 1983. The role of bacterial ice nucleation in frost injury to plants. Annu. Rev. Phytopathol. 21:363-384.
39. Lindow, S. E., Arny, D. C., and Upper, C. D. 1982. Bacterial ice nucleation: A factor in frost injury to plants. Plant Physiol. 70:1084-1089.
40. Maki, L. R., Galyan, E. L., Chang-Chien, M. M., and Caldwell, D. R. 1974. Ice nucleation induced by *Pseudomonas syringae*. Appl. Microbiol. 28:456-459.
41. 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.
42. Venette, J. R. 1982. How bacteria find their hosts. Pages 3-30 in: Phytopathogenic Prokaryotes, Vol. 2. M. S. Mount and G. H. Lacy, eds. Academic Press, New York.
43. Walker, J. C., and Patel, P. N. 1964. Splash dispersal and wind as factors in epidemiology of halo blight of bean. Phytopathology 54:140-141.
44. Windels, C. E., and Lindow, S. E., eds. 1985. Biological Control on the Phylloplane. American Phytopathological Society, St. Paul, MN.
45. 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.