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# Raindrop Momentum Triggers Growth of Leaf-Associated Populations of *Pseudomonas syringae* on Field-Grown Snap Bean Plants

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**Observational and microclimate modification experiments were conducted under field conditions to determine the role of the physical environment in effecting large increases in phyllosphere population sizes of *Pseudomonas syringae* pv. syringae, the causal agent of bacterial brown spot disease of snap bean (*Phaseolus vulgaris* L.). Comparisons of daily changes in population sizes of *P. syringae* on three plantings of snap bean cultivar Cascade and one of cultivar Eagle with weather conditions indicated a strong association of rainfalls with periods of 1 to 3 days in duration during which increases in bacterial population sizes were greater than 10-fold and up to 1,000-fold. The effects of rain on populations of *P. syringae* were explored further by modifying the microclimate of bean plants in the field with polyethylene shelters to shield plants from rain and fine-mesh inert screens to modify the momentum of raindrops. After each of three separate intense rains, the greater-than-10-fold increases in population sizes of *P. syringae* observed on plants exposed to the rains did not occur on plants in the shelters or under the screens. The screens decreased the velocity and, thus, the momentum of raindrops but not the volume or quality of rainwater that fell on plants under the screens. Thus, the absence of increases in population sizes of *P. syringae* on plants under the screens suggests that raindrop momentum plays a role in the growth-triggering effect of intense rains on populations of *P. syringae* on bean plants under field conditions.**

Fluctuations in relative abundance are a common attribute of populations in nature, including bacteria that inhabit the phyllosphere (i.e., leaves) (6, 10, 12, 33). To begin to identify and understand the factors that bring about or contribute to changes in phyllosphere bacterial population sizes, we have focused our studies on the bacterium *Pseudomonas syringae. P. syringae* may be found as an epiphyte and pathogen on leaves and other aerial parts of numerous plant species (15). As an epiphyte, *P. syringae* grows on leaves without causing disease as do the numerous other saprophytic bacteria that colonize the phyllosphere. On leaves of snap bean (*Phaseolus vulgaris* L.) plants, the pathogenic interaction leads to the formation of bacterial brown spot lesions. *P. syringae* may also cause frost injury as a result of its ability to nucleate supercooled water to form ice (2, 23–25). The probability of occurrence of bacterial brown spot disease (on snap bean plants) and frost injury (on frost-sensitive plants in general) has been shown to be related quantitatively to population sizes of *P. syringae* (15, 22, 23, 29). Hence, it is important to understand the factors that affect the dynamics of *P. syringae* populations under field conditions.

In previous studies, we examined the magnitudes of changes in means and variances of *P. syringae* population sizes on two time frames, i.e., during a few 26-h periods with bihourly samplings (14) and over the approximately 50-day life span of a bean crop with daily samplings (12). We found that most day-to-day changes in population sizes of *P. syringae* on snap bean leaflets were relatively small (i.e., fivefold or less). However, on a few occasions, substantial increases in numbers were observed. The net increases of 10- to 1,000-fold that occurred within 24 h or that accrued over 3 to 5 consecutive days were attributed to exponential growth of *P. syringae* (12, 14, 16). Hence, exponential growth of *P. syringae* on bean leaflets occurred sporadically. The leaf habitats colonized by *P. syringae* and other phyllosphere bacteria are characterized by a continuously fluctuating physical environment. Superimposed on the diurnal changes in temperature, relative humidity, radiation, wind speed, and other physical parameters are the cyclical wetting and drying of leaves due to dew and the periodic occurrence of rain. The objective of this study was to examine the role of rain in effecting the observed large increases in phyllosphere population sizes of *P. syringae.*

## MATERIALS AND METHODS

**Plot design and sampling procedure for determination of seasonal patterns in bacterial population sizes.** Experimental plots of snap bean (*Phaseolus vulgaris* L.) cultivars Cascade (Sunseeds Genetics, Inc., Hollister, Calif.) and Eagle (Asgrow Seed Co., Kalamazoo, Mich.) were established at the University of Wisconsin Experiment Station, Arlington, Wis. Cultivar Cascade is less susceptible to bacterial brown spot disease than cultivar Eagle (30). There were three plantings of cultivar Cascade (23 May, 6 June, and 2 July), each 45.7 by 45 m. Each large area was divided into a six-block by five-block (6 × 5) array (i.e., 30 plots), with each plot consisting of 12 rows spaced 76 cm apart and 7.62 m in length. Six plots per planting were selected at random for monitoring daily changes in bacterial population sizes. The additional plots were available for the microclimate modification experiments (see below). Cultivar Eagle was planted to an area 45.7 by 35.8 m on 6 June. The area was divided into 24 plots in a 6 × 4 array, with each plot being of a size similar to that described for cultivar Cascade. Six plots were selected at random for daily sampling of leaflets.

To obtain seasonal profiles in bacterial population sizes for each of the plantings, 15 leaflets of similar size were taken at random from each of the six plots per sampling time. Daily sampling was conducted between 0800 and 0830 from plant emergence to pod harvest. All leaflets from all six plots per planting were assayed with a tube ice nucleation test (11, 13) to rapidly assess relative population sizes of *P. syringae*. A subset of the samples was processed by dilution plating to obtain more precise measurements of bacterial population sizes. This subset consisted of leaflets collected from the 6 June plantings of cultivars

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Cascade and Eagle. Of the six plots for each of the cultivars, three were selected at random and 10 of the 15 leaflets from each of the selected plots and for each sampling time were processed by dilution plating.

**Microclimate modification experiments.** The microclimate modification experiments consisted of three treatments. Plants were (i) exposed to rain, (ii) shielded from rain with polyethylene shelters, or (iii) exposed to rain that passed through inert fiberglass screens before falling on the canopy. The shelters were designed and constructed by A. H. Alberga (1). Clear polyethylene film (0.1 mm thick, PS 17-69; Poly-Tech, Minneapolis, Minn.) covered all sides and the top of a wooden frame 1.8 m long and 0.75 m wide. The top of the shelter functioned as a lid which could be opened or closed as necessary. The side of the shelter to which the top was hinged was 0.7 m, while the opposite side was 0.6 m. Hence, when closed, the lid of the shelter sloped slightly to prevent accumulation of rain. Each shelter covered a 1.8-m segment of a single row. The screens were constructed by securing inert fiberglass (12-mesh) window screens on a wooden frame (1.52 by 1.93 m). Additional support was provided by a strip of wood that ran lengthwise through the middle of the frame. The frame with screen was secured to wooden legs on all corners with removable screws and bolts. A series of holes was drilled through each leg to allow positioning of the screen 15 cm above the top of the bean canopy, regardless of plant height. Each screen covered 1.93 m of two adjacent rows of plants.

For a given experiment, 3 of the 24 plots which were not used for the seasonal profile experiments were selected at random from one of the plantings of cultivar Cascade. Four shelters and two screens covering a total of 7.2 m and 7.72 m of row, respectively, were deployed in each of the three plots. Within each plot, 2-m segments in each of four adjacent rows were delineated as controls. The plants in these segments were fully exposed to rain and served as the control for the shelter and screen treatments. The shelters and screens were deployed on the forecast of rain. The lids of the shelters were closed during the rain and kept open when it was not raining for the duration of the experiment. Ten leaflets were taken for each treatment from each of the three plots (i.e., $n = 30$ per treatment). Samplings were done before and for 2 to 3 days after a rain.

**Determination of bacterial population sizes.** For all experiments, leaf samples were placed in no. 5 kraft paper bags and transported in a cooler to the field laboratory for immediate processing (i.e., within 5 to 10 min after completion of sampling). At the laboratory, each leaflet was submerged in 9.0 ml of sterile potassium phosphate buffer (0.01 M, pH 7.0) in a 16-mm test tube. The samples for the microclimate modification experiments were stored at −20°C until they could be processed by dilution plating. Population sizes of *P. syringae* on bean leaves immersed in buffer were found to be stable for up to 3 years when stored at −20°C (12).

The leaflets collected for determination of seasonal profiles of bacterial population sizes were assayed with a tube ice nucleation test as described previously (11, 13). Briefly, the test temperatures were −2.0, −2.2, −2.5, and −2.7°C, each maintained with a separate refrigerated constant-temperature bath (Neslab Instruments, Inc., Portsmouth, N.H.). The leaflets, immersed in ice-nucleus-free buffer in test tubes, were first subjected to −2.0°C. After 30 min, the number of tubes in which the buffer had frozen was counted and the remaining tubes were transferred gently to the bath maintained at −2.2°C. The process was repeated for the remaining test temperatures. The subset of leaves selected for dilution plating was stored at −20°C until they could be processed.

Population sizes of *P. syringae* were determined by dilution plating of leaflet homogenates as described previously (12). Briefly, the frozen samples were thawed at room temperature. Each leaflet was homogenized for 10 s with a Polytron equipped with a model PTA 20 TS probe (Brinkmann Instruments, Westbury, N.Y.). Tenfold serial dilutions were plated onto King's medium B (KMB) (20) and a selective medium for *P. syringae* (SM) (26). All media were supplemented with cycloheximide (100 μg/ml) to inhibit fungal growth. Colonies of *P. syringae* on KMB and SM were counted after 3 to 4 days of incubation at ambient temperature. It is not difficult for an experienced person to distinguish *P. syringae* colonies from other fluorescent pseudomonads. Colonies of pink-pigmented facultative methylotrophs (PPFM; *Methylobacterium* spp.) and all other non-*P. syringae* bacteria were counted 7 days after plating.

Bacterial population sizes were $\log_{10}$ transformed prior to calculations of population statistics. Samples with no detectable bacteria were assigned a value corresponding to the limit of sensitivity of the plating assay (i.e., 2.279 log CFU per leaflet).

**Measurement of weather parameters.** Parameters of the physical environment were sensed and recorded automatically with CR-7 and CR-21X data loggers (Campbell Scientific, Logan, Utah). The time of event for accumulation of each millimeter of rainfall was sensed with a Sierra-Misco tipping-bucket rain gauge (Campbell Scientific) and used to determine the volume, duration, and intensity of each rain event. The following parameters were recorded each half hour: air and leaf temperatures were sensed with 75-μm Evanohm/constantan thermocouples, dry and wet bulb temperatures from which relative humidities were calculated were sensed with two types of psychrometers (a ventilated psychrometer [Delta T Devices, Burwell, Cambridge, England] and 25-μm unventilated thermocouple psychrometers [1]), leaf wetness was sensed with electric grids (1, 9), global and diffuse radiations were sensed with Li-Cor silicon pyranometers (Campbell Scientific), and wind speeds and wind direction were sensed with a MET-ONE cup anemometer and windvane, respectively (Campbell Scientific).

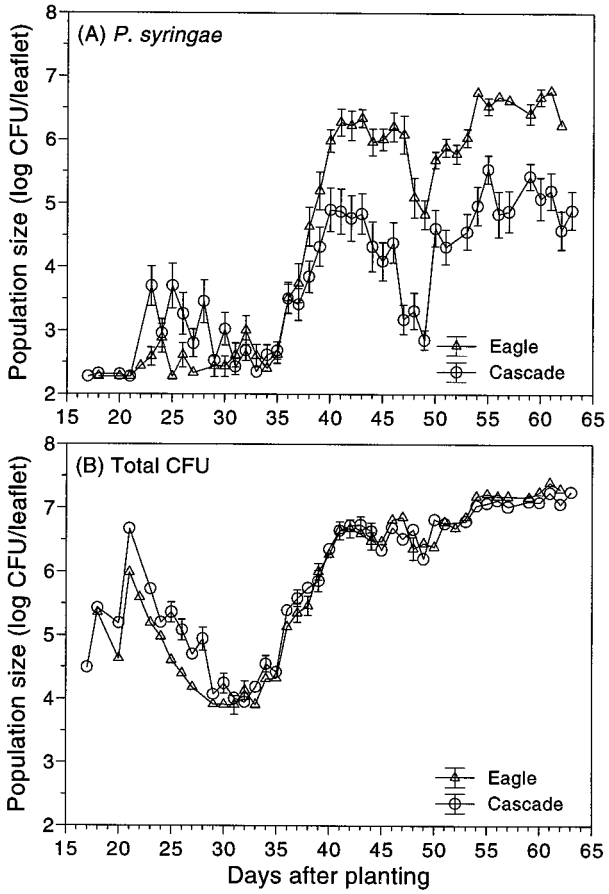


FIG. 1. Temporal changes in population sizes of *P. syringae* (A) and total culturable bacteria (B) associated with leaves of snap bean cultivars Eagle and Cascade. Bean seeds were planted on 6 June. For each sampling time, 30 leaflets were processed individually by dilution plating. Bacterial population sizes were $\log_{10}$ transformed prior to calculation of the means. Error bars represent the standard errors of the means.

## RESULTS

**Seasonal patterns in changes in bacterial population sizes.** Population sizes of *P. syringae* as estimated by dilution plating of leaflets of cultivars Cascade and Eagle as a function of days after planting (DAP; planting II, 6 June) are presented in Fig. 1A. In general, leaf-associated population sizes of *P. syringae* were larger from the brown-spot-susceptible cultivar Eagle than from the moderately resistant cultivar Cascade. There were three periods during which large increases in population sizes of *P. syringae* occurred on cultivar Cascade (i.e., at about 21, 35, and 49 DAP) (Fig. 1A) and two periods for cultivar Eagle. The absence of an increase for cultivar Eagle at about 21 DAP may be due to the observation that *P. syringae* population sizes were well below our limit of sensitivity on all leaves of cultivar Eagle before the rainfall. Thus, the magnitude of an increase, if it occurred, is uncertain. The increases that occurred between 35 and about 40 DAP were very large and resulted in changes in leaf-associated population sizes of *P. syringae* that were greater than 1,000-fold for cultivar Eagle and approximately 165-fold for cultivar Cascade. For both cultivars, the onset of the third period of increase at 49 DAP was preceded by several days in which population sizes of *P. syringae* declined. The duration of the period and magnitude of the decline appeared to be longer and larger, respectively, for

cultivar Cascade than for cultivar Eagle. Hence, periods of increases as well as decreases in population sizes of *P. syringae* appeared to be driven by the conditions that prevailed during the 6 June planting but were only quantitatively affected by cultivar. Although population sizes of *P. syringae* differed on the two cultivars, the population sizes of total bacteria culturable on KMB were similar (Fig. 1B). Hence, *P. syringae* represented a larger component of the bacterial communities associated with leaves of cultivar Eagle than of cultivar Cascade.

The rapidity with which large numbers of leaf samples can be assayed with the tube ice nucleation test relative to dilution plating provided a way to monitor population sizes of *P. syringae* across the three plantings of cultivar Cascade within a single growing season. *P. syringae* is the dominant ice-nucleation-active species on bean leaves, and we have found minimal, if any, interference from other $Ice^+$ species such as *Erwinia herbicola* (13). The relative population sizes of *P. syringae*, expressed as the cumulative percentage of leaflets frozen by −2.5°C, for all three plantings of cultivar Cascade are presented in Fig. 2. It is important to note that the overall profile in frequency with which leaflets froze by −2.5°C for samples taken from the second planting of cultivar Cascade (Fig. 2B) closely approximated the profile of actual population sizes of *P. syringae* determined by dilution plating (Fig. 1A). Hence, from this and previous findings (11, 13, 14), we are assured that the profiles for plantings I and III (Fig. 2A and C) are reliable estimates of relative population sizes of *P. syringae*. The periods during which large increases in the frequencies with which leaflets froze by −2.5°C coincided in calendar date across the three plantings, regardless of plant age (Fig. 2). The onsets of periods of increases occurred on approximately Julian days 177 (38 DAP for planting I and 20 DAP for planting II), 189 to 190 (50 to 51 DAP for planting I and 32 to 33 DAP for planting II), and 206 (49 DAP for planting II and 23 DAP for planting III). Thus, periods in which *P. syringae* population sizes increased occurred at the same time, regardless of plant age or stage of development.

In all cases, the periods of population size increases were associated with rain storms in which peak rainfall rates met or exceeded 1 mm/min (total rainfall per day is shown in Fig. 2; peak intensities for the rains which fell during the conduct of the microclimate modification experiments are shown in Table 1). The periods during which population sizes of *P. syringae* tended to decline were associated with several consecutive days without rain. At night, leaves were nearly always wet with dew during the periods of decline in population sizes.

Although the large increases in population sizes of *P. syringae* were associated with rainfalls, the converse was not found to hold true, i.e., not all rains led to increases in bacterial population sizes. There was no large increase in numbers of *P. syringae* on leaves from the first planting (Fig. 2A) in response to the 19- and 7-mm rains that fell on Julian days 161 and 162, respectively. Although a substantial amount of rain fell on these days, peak intensities remained below about 0.4 mm/min.

No obvious pattern in temperature, relative humidity, or leaf wetness duration that could explain the observed large increases in *P. syringae* population sizes was found. For example, there was no apparent coincidence between patterns in fluctuations in daily maximum and minimum temperatures (and hence in daily average temperatures) (Fig. 3A) and relative population sizes of *P. syringae* (Fig. 2). The average maximum daily temperature for those periods in which populations of *P. syringae* were increasing (i.e., Julian days 177 to 181, 190 to 196, 206 to 210, and 218 to 223) (Fig. 2) was roughly 27.3°C, as compared with 29.7°C for all other days from Julian days 170 to 230 (Fig. 3B). The average maximum temperatures for periods during which bacterial populations were increasing versus not increasing were significantly different ($P = 0.035$; $t$ test). It is important to note, however, the broad range of daily maximum temperatures that contributed to these averages (i.e., 17 to 35°C for periods when growth occurred and 20 to 37°C for all other Julian days). The average minimum temperatures were not different (i.e., 15.3 and 15.1°C for periods during which populations were increasing and not increasing, respectively).

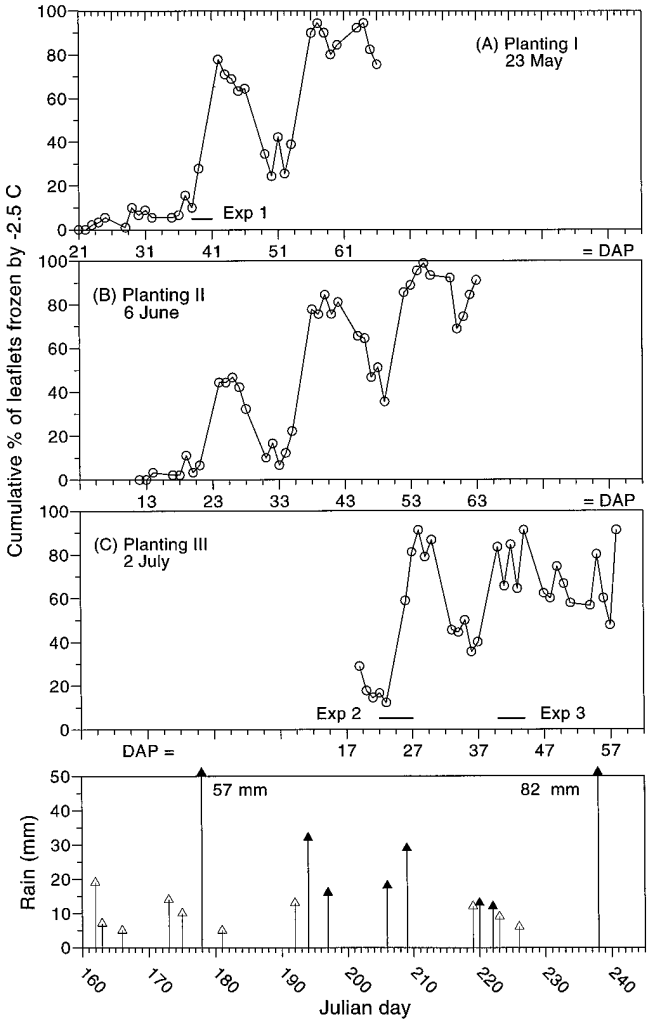


FIG. 2. Relative population sizes of *P. syringae* on each of three plantings of cultivar Cascade. Plantings were on 23 May (A), 6 June (B), and 2 July (C). For each sampling time and planting, 90 leaflets were assayed with a tube ice nucleation test. The data represent the cumulative percentage of leaflets that froze by −2.5°C (i.e., $[n_{-2.0} + n_{-2.2} + n_{-2.5}]/90 \times 100$, where $n$ is the number of leaflets that froze at the test temperature [°C] given as a subscript). The bottom panel indicates rainfalls of ≥5 mm total volume. To illustrate the amount of rain that fell during the interval from one leaf-sampling time to the next, rain amounts that fell between 0800 of one morning ($t_0$) to 0800 on the next ($t_1$) are plotted on the day on which the 24-h period ended (e.g., the amount of rain that fell between 0800 day 161 and 0800 day 162 is plotted on Julian day 162). Closed triangles represent rainfalls with peak intensities of ≥1 mm/min; open triangles represent rainfalls with peak intensities of ≤1 mm/min. The horizontal bars in panels A and C, labeled Exp 1, 2, and 3, indicate the plantings used and the times of conduct of the microclimate modification experiments. Julian day 160 = 9 June.

**Microclimate modification experiments.** Polyethylene shelters were used to shield plants from rains to demonstrate more definitively that rain triggers the onset of rapid growth of *P. syringae* on bean leaves. The screens were deployed to decrease

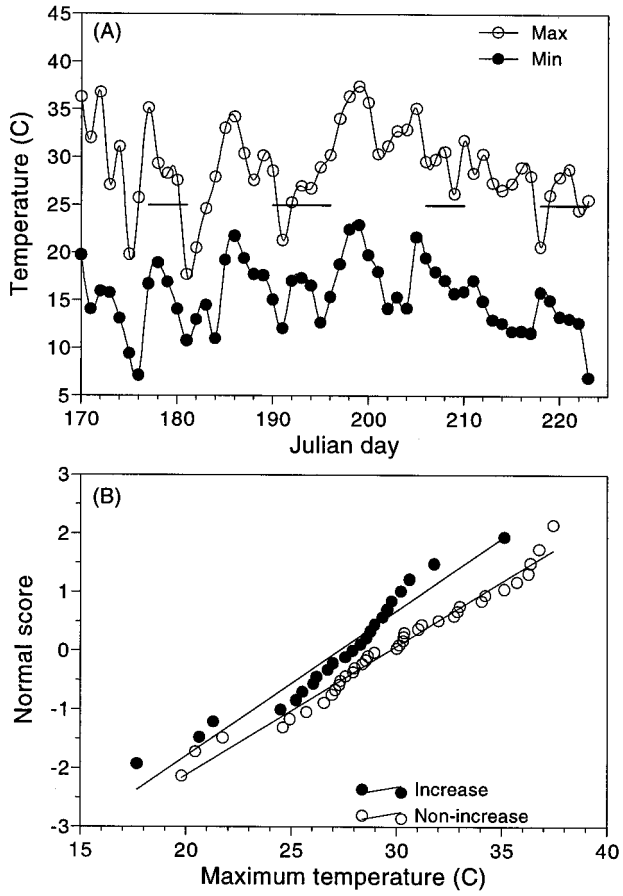


FIG. 3. (A) Fluctuations in daily maximum and minimum air temperatures. Temperatures were sensed with 75-μm Evanohm/constantan thermocouples and recorded every 30 min. The horizontal bars represent roughly those periods in which large increases in the frequency with which leaflets froze by −2.5°C were observed across the three plantings of cultivar Cascade shown in Fig. 2. (B) Frequency distributions of maximum temperatures for periods in which large increases in relative population sizes of *P. syringae* occurred (i.e., periods indicated by the horizontal bars in panel A) and for all other days (i.e., periods of nonincrease).

the velocity and, hence, the momentum or kinetic energy of raindrops falling onto the plants. The dates for the microclimate modification experiments relative to the seasonal patterns in *P. syringae* population sizes are indicated in Fig. 2. In experiment 1, a total of 57 mm of rain fell between 0155 and 0754 on Julian day 178 (27 June) (Table 1). Peak rainfall intensity exceeded 1 mm/min. Two days after the rainfall, population sizes of *P. syringae* had increased 78-fold on plants that were exposed to the rain (i.e., control) (Table 1 and Fig. 4). The changes in population sizes of *P. syringae* on leaflets sheltered from the rain and those under the screens were a 5.7-fold decrease and a 3-fold increase, respectively (Table 1 and Fig. 4). These changes were not significantly different from each other. However, they were significantly different from the change that was measured on plants that were rained on (Table 1).

Experiment 2 was confounded by two rainfalls. On the forecast of rain, the shelters and screens were deployed on Julian day 205 (24 July). On Julian day 206 (25 July), 18 mm fell between 0141 and 0833. Approximately 2 days later, an additional 29 mm fell between 2105 and 2345. Peak intensities exceeded 1 mm/min in both rain events. Population sizes of *P. syringae* increased 35-fold by 48 h after the first rain and nearly 70-fold by 96 h after the first rain, which corresponded to 32 h after the second rain. In contrast, no significant changes were measured on plants that were either sheltered from the rain or under the screens at all sampling times following both rains (Table 1 and Fig. 4).

To examine the possibility that the shelters and screens had an inhibitory effect on *P. syringae* populations, we conducted an experiment in which the shelters and screens were deployed after several rain events had occurred (Table 1, experiment 3). In one of the four rain events, peak intensities equaled 1 mm/min. The other three were only light rains. The positive and similar changes in bacterial population sizes on plants for all treatments indicated that the polyethylene shelters and screens did not have an inhibitory effect on *P. syringae* populations.

**The differential effect of rain on populations of *P. syringae* and PPFM.** In addition to enumerating *P. syringae* populations, we enumerated population sizes of PPFM for the leaf samples taken from the second microclimate modification experiment. The PPFM form very distinctive, small (ca. 1-mm) pink colonies on KMB that are easily distinguished from other bacteria. Population sizes of the PPFM were approximately 3.7 log CFU per leaflet on leaflets from the control, shelter, and screen treatments collected on Julian day 205, prior to the rainfalls that occurred on Julian days 206 and 208 (Fig. 5). On Julian day 210, population sizes of the PPFM had increased to 5.26, 5.87, and 6.27 log CFU per leaflet on leaflets from the control, screen, and shelter treatments, respectively (Fig. 5). In contrast to *P. syringae* populations, the largest increase in PPFM populations was measured on plants sheltered from the rains ($P = 0.073$ based on analysis of variance for three treatments with three replicates per treatment).

## DISCUSSION

The dynamics of *P. syringae* populations associated with bean leaves in the field are characterized by relatively small daily changes in population sizes punctuated by less-frequent large, rapid changes in numbers (12, 14) (Fig. 1 and 2). Both the kinetics and the magnitude of the large increases have led us to conclude that they are due to rapid growth of the bacterium (12, 14). Comparison of detailed weather records with daily changes in population sizes of *P. syringae* revealed that intense rainfall preceded the onset of most episodes of rapid bacterial growth.

Associations of large population sizes of *P. syringae* with rain and temperature have been reported for a number of plant species grown in different geographic areas (4, 5, 7, 8, 10, 21, 28, 32, 38). For example, large population sizes of *P. syringae* were found on leaves of stonefruit trees grown in Victoria, Australia, when rainfall was moderately high and temperatures ranged from 19 to 25°C (38), on buds and flowers of pome and stonefruit trees in the Pacific Northwest when rainfalls were frequent and temperatures were relatively cool (10), and on leaves of tomato plants grown in Georgia when moisture levels were high and temperatures were low (32). It has been generally assumed that the large population sizes of *P. syringae* associated with rains are due to the availability of moisture for bacterial growth. However, in our experiments, we observed periods when leaves were wet with dew for several consecutive nights, but no large increases in population sizes of *P. syringae* were measured in the absence of intense rains. Temperature did not appear to be a primary factor associated with the large increases in population sizes of *P. syringae.*

A. H. Alberga in our research group attempted to determine

TABLE 1. Effect of rain and microclimate modification on population sizes of *P. syringae* associated with snap bean leaflets

| Description of expts[a] | Sampling time (day, h relative to rain) | Change in population size [mean (SE)][b] | | |
|---|---|---|---|---|
| | | Control | Shelter | Screen |
| Expt 1 (planting I): shelters and screens deployed on JD 177; 57 mm of rain on JD 178 (peak intensity, 1.3 mm/min) | $t_0$ = JD 177, prior to rain | | | |
| | $t_{24}$ = JD 179, 24 h after rain | +1.11 (0.48) | −0.32 (0.26) | +0.46 (0.28) |
| | $t_{48}$ = JD 180, 48 h after rain | +1.85 (0.24) | −0.76** (0.31) | +0.50* (0.30) |
| Expt 2 (planting III): shelters and screens deployed on JD 205; 18 mm of rain on JD 206 (rain 1; peak intensity, 1.4 mm/min); 29 mm of rain on JD 208 (rain 2; peak intensity, 1.6 mm/min) | $t_0$ = JD 205, prior to rain 1 | | | |
| | $t_{24}$ = JD 207, 24 h after rain 1 | +0.88 (0.52) | −0.07 (0.17) | +0.25 (0.32) |
| | $t_{48}$ = JD 208, 48 h after rain 1 | +1.56 (0.42) | −0.01 (0.08) | +0.27 (0.52) |
| | $t_{72, 8}$ = JD 209, 72 and 8 h after rains 1 and 2, respectively | +0.90 (0.06) | −0.18* (0.13) | −0.28* (0.13) |
| | $t_{96, 32}$ = JD 210, 96 and 32 h after rains 1 and 2, respectively | +1.84 (0.33) | −0.27* (0.18) | −0.02* (0.42) |
| Expt 3 (planting III): JD 218, 10 mm of rain; JD 219, 12 mm of rain; JD 220, 1 mm of rain; JD 221, 6 mm of rain; JD 222, 15 mm of rain; shelters and screens deployed on JD 223 (after rains) | $t_0$ = JD 223 | | | |
| | $t_{24}$ = JD 224 | +0.38 (0.33) | +0.75 (0.17) | +0.58 (0.60) |
| | $t_{48}$ = JD 225 | +0.64 (0.37) | +0.92 (0.50) | +1.15 (0.50) |
| | $t_{72}$ = JD 226 | +0.22 (0.49) | +0.27 (0.04) | +0.50 (0.41) |
| | $t_{96}$ = JD 227 | +0.55 (0.81) | +0.17 (0.26) | +0.62 (0.33) |

[a] JD, Julian day.

[b] Change in population size is the difference in mean log CFU per leaflet at $t_n - t_0$. In all cases, the change in population size is relative to the population size at $t_0$. Population sizes [expressed as mean log CFU per leaflet (SE)] at $t_0$ for each of the three experiments are as follows: experiment 1, control, 2.94 (0.33); shelter, 3.38 (0.53); screen, 2.57 (0.29); experiment 2, control, 2.55 (0.14); shelter, 2.66 (0.22); screen, 2.81 (0.15); experiment 3, control, 3.94 (0.26); shelter, 3.10 (0.24); screen, 3.03 (0.12). ** and *, $P < 0.01$ and $P < 0.05$, respectively, for comparison of shelter or screen treatment with control (significance level protected $t$ test).

why *P. syringae* grew in response to rain but not when leaves were wet with dew (1). Polyethylene shelters equipped with heating tape were used to elevate nighttime temperatures when leaves were wet with dew. Leaves were kept wet by misting with water. Neither the duration of leaf wetness nor the temperature when leaves were wet was associated with the triggering phenomenon.

We applied 10 to 15 mm of water, enough to trigger growth of *P. syringae* if supplied by a thunderstorm, to bean plants in the field with garden sprinklers (18). *P. syringae* population sizes on treated and control leaves remained approximately equal. The sprinklers delivered moderate-size droplets at low velocity to the bean leaves. Thus, irrigation with these sprinklers differed from a thunderstorm in two important ways, namely, the chemistry of well water probably differed significantly from rainwater, and the kinetic energy or momentum of the water impacting on the bean leaves was much less from the sprinkler than it was from the thunderstorm. To separate these possibilities, we suspended fine-mesh inert screens above bean plants in the field to absorb most of the momentum of rain-

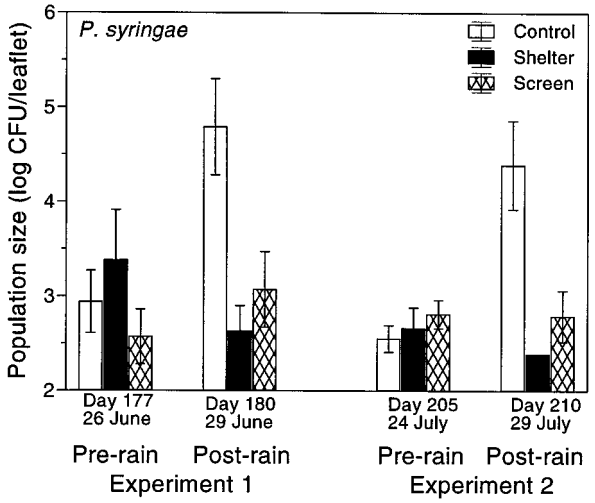


FIG. 4. Effects of rain and microclimate modification on population sizes of *P. syringae*. For each sampling time and treatment, 30 leaflets (i.e., 10 per replicate plot) were processed individually by dilution plating. The data are the means and standard errors of $\log_{10}$-transformed bacterial population sizes. Control plants were exposed to the rains, sheltered plants were covered with polyethylene shelters, and screened plants were covered with fine-mesh fiberglass window screens. Descriptions of the rain events, population sizes of *P. syringae* on additional sampling days, and changes in bacterial population sizes are summarized in Table 1.

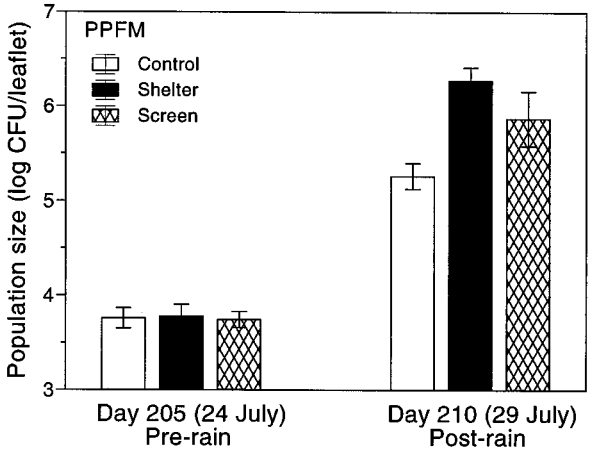


FIG. 5. Effects of rain and microclimate modification on population sizes of PPFM. For each sampling time and treatment in experiment 2, 30 leaflets (i.e., 10 per replicate plot) were processed individually by dilution plating. The dilution plates were scored for PPFM as well as *P. syringae*. The data are the means and standard errors of $\log_{10}$-transformed bacterial population sizes. Control plants were exposed to the rains, sheltered plants were covered with polyethylene shelters, and screened plants were covered with fine-mesh fiberglass window screens. A description of the rains that fell during experiment 2 is summarized in Table 1.

drops. Screens and mosquito gauze have been used by soil scientists to decrease the kinetic energy of falling raindrops in their studies on the effects of rainfall on soil erosion (19, 27). Population sizes of *P. syringae* increased on leaves exposed to the full momentum of the rain but not on leaves beneath the screens. Only the momentum and drop size distribution should be altered by the screens. The volume and quality of rainwater that fell on these plants were similar to that falling on plants fully exposed to rains. Thus, raindrop momentum is strongly implicated in triggering the onset of rapid growth of the bacterium on bean leaves.

Not all rains trigger increases in population sizes of *P. syringae* on bean plants in Wisconsin. Such a case occurred on Julian day 161, during the first planting of cultivar Cascade (Fig. 2). In this case, 21 mm of rain fell, but the intensity of the rain that fell remained below about 0.4 mm/min, insufficient to trigger growth of *P. syringae*. On Julian day 237, a rain with sufficient intensity fell on plants that carried *P. syringae* populations that were already at or near the carrying capacity for that cultivar, and a further increase was not detected by the ice nucleation assay. In addition, not all increases can be associated with an intense rain event. We have simultaneous measurements of population sizes of *P. syringae* and rainfall for six plantings of cultivar Cascade made over a 3-year period (12, 18). Pooled over all plantings, intense rains occurred within 48 h of the onset of 18 of 20 large increases in population sizes of *P. syringae.*

Under some conditions, growth of *P. syringae* on bean leaves occurs in the absence of intense rains. The application of high-momentum water drops is not necessary for growth of *P. syringae* on growth chamber or greenhouse-grown bean plants (18, 35–37). It is sufficient to maintain mist-inoculated growth-chamber-grown bean plants at moderate to high relative humidity for exponential growth of *P. syringae* to occur. When strains of *P. syringae* were sprayed onto primary leaves of newly emerged bean plants in an urban area in central California, they grew quite successfully (3, 35). However, in an area where snap beans are raised commercially in Wisconsin, large increases in sizes of natural populations of *P. syringae* associated with trifoliolate leaves of bean occur infrequently and almost always follow an intense rain event. Thus, although rain is not necessary for growth of the bacterium in association with bean leaves under all conditions, or even for every growth event in the field in Wisconsin, under conditions of commercial agriculture in the Midwest, the large bursts of growth following rain are probably very important to the interaction of *P. syringae* with bean plants in the field and are certainly important for the epidemiology of bacterial brown spot of bean plants.

What might intense rain actually be doing? What we know is that intense rain is needed to trigger growth of *P. syringae* in the field and that absorbing the momentum of intense rain on inert screens prevents triggering. Thus, the effect of rain is not due to factors that only require the presence of water, even relatively large quantities of water. In addition, the effect is on population sizes of *P. syringae* and not on total numbers of bacteria in the phyllosphere. Some explanations for the role of raindrop momentum in triggering rapid growth of *P. syringae* include effects on the plant, such as injury or water congestion. Increased quantities or qualities of nutrients may become available on leaves on which intense rain has fallen. If the normal lack of growth is due to some form of interaction with other microorganisms, washing of leaves by intense rain may remove sufficient bacteria to allow or promote growth of *P. syringae*. Intense rain may drive bacteria into leaves, through stomates or injuries. Thus, if internal growth is favored over external growth, this may be a factor. If this is the case, internal growth must occur frequently in the absence of disease. For example, population sizes of *P. syringae* on cultivar Cascade increased during two periods without reaching levels necessary for disease occurrence.

Some other mechanisms are not necessarily ruled out by our results but require additional explanation or conditions to remain possible. Among these are removal of some compound or compounds from the leaves (nutrients, antibiotics, or inhibitors produced by other microorganisms or the plant or regulatory compounds produced by *P. syringae* itself, the plant, or other microorganisms). Such compounds may be scrubbed from the leaves more efficiently by intense rain than by equal quantities of more gently applied water.

We cannot resist the opportunity to end with a speculative note. Since the discovery that strains of *P. syringae* are able to nucleate supercooled water to form ice, there have been ample discussions on the possible selective advantage(s) conferred upon ice-nucleation-active bacteria by the $Ice^+$ phenotype (17). The possibility that $Ice^+$ bacteria participate in some precipitation processes has been suggested (31, 34). If cells of $Ice^+$ *P. syringae* do participate in initiating precipitation processes in the troposphere, an outcome may be intense rains falling on leaf habitats. This, in turn, would perturb leaf habitats in such a way as to make them favorable for exponential growth of the species *P. syringae.*

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