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Aerial Dispersal of Epiphytic Bacteria over Bean Plants

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Plant canopies are strong sources of bacterial aerosols during sunny days when the leaves are dry. Bacterial concentration, upward flux, and deposition onto exposed petri plates were measured over snap beans during three growing seasons. A net upward flux of bacteria occurred only during the warm part of sunny days, not at night when leaves were wet with dew or when a thermal inversion was present. Aerosol source strength was positively correlated with wind speed. Upward fluxes were higher on days after rain than on days when the soil was dry. Other unidentified sources of variability in source strength probably exist. Canopy-level deposition, apparently due to intermediate-scale transport of bacteria in fairly concentrated clouds, can occur in the early evening.

Viable airborne bacteria in outdoor environments have been enumerated in several recent studies. Attempts to correlate the concentration of bacterial aerosols with prevailing environmental conditions or with the time of day have been hampered, either because data sets contain a mixture of measurements of aerosols of anthropogenic and nonanthropogenic origin, or because the source of the bacterial aerosols has not been determined. Bacterial concentrations measured in a suburban area between 0800 and 1700 h were not significantly correlated with either time of day or environmental conditions during or before sampling (11). High concentrations of airborne bacteria, detected during daylight hours and dry weather in both suburban and rural areas, tended to be associated with anthropogenic sources (3, 11, 14, 17), wind speeds of >8 m s^{-1} (3), or proximity to plant canopies (15). Bacterial aerosol concentrations measured during dry weather in an urban area were found to exhibit a diurnal periodicity with peak concentrations at 0800 and 2000 h (16). High concentrations of airborne plant-pathogenic bacteria were detected near plant canopies during rain and sprinkler irrigation (9, 18, 23). To our knowledge, no study relating bacterial aerosol concentrations to time of day and to environmental conditions during both day and night hours has been reported for an agricultural site.

In agricultural areas, away from anthropogenic sources of bacterial aerosols, plant-associated bacteria were the principal source of airborne bacteria (15). Crop-plant canopies were much stronger sources of airborne bacteria than was bare soil. The present study was undertaken to determine which of the physical environmental factors such as wind speed, time of day, air temperature, and leaf wetness affected the dispersal of epiphytic bacteria and to compare quantitatively dispersal during dry weather with dispersal during rainy weather at the same site.

MATERIALS AND METHODS

Measurements of airborne bacterial concentrations and estimates of upward flux of bacteria were performed as previously described (15), using six-stage viable samplers (Andersen Samplers Inc.). Aerosols were impacted upon plates of King B medium (12) supplemented with 100 mg of cycloheximide per liter. Concentrations were measured at canopy height and at ca. 1.5 m above the plant canopy. During rain, clean metal shields (33 by 57 cm) were suspended ca. 0.1 m above the Andersen samplers to prevent rain from entering the samplers. The deposition of airborne bacteria at canopy height was estimated by exposing open petri dishes filled to the top with King B medium supplemented with 100 mg of cycloheximide per liter. Although deposition of bacteria onto agar plates is probably not identical to deposition onto leaves, we felt that this method would give us a rough idea of how many bacteria from the air were being deposited onto leaves. Most aerosol and deposition sampling periods were of 15-min duration. Agar plates were incubated for 3 to 4 days at ambient laboratory temperatures (ca. 21°C) before the bacterial colonies were counted. Total bacterial concentrations were expressed as $\log_{10}$ CFU per cubic meter, and deposition and upward flux of total bacteria were expressed as $\log_{10}$ CFU per square meter per second.

In 1980, we took samples from the center of a plot (20 by 20 m) of snap beans. In 1981 and 1982, samples were taken at the downwind edge of small blocks of snap beans, measuring 7.6 by 6.1 m in 1981 and 4.6 by 4.6 m in 1982. Individual blocks were separated by 3-m-wide alleys. All plots were located on the Arlington experimental farm of the University of Wisconsin. The site is in an agricultural area several kilometers from the nearest population center.

Samples were taken during July, August, and September, beginning when the plants had at least one trifoliolate leaf and ending when the bean pods were large enough to harvest. In 1980 and 1981, sampling days were chosen based on the weather forecast. In 1982, samples were taken every Monday and Thursday to accumulate dispersal and deposition data that were not biased by preselection of a particular kind of weather. Data from samples taken when harvesting equipment was observed to be operating upwind were not included in these analyses.

Wind speeds were measured with totalizing four-cup anemometers. In 1980 and 1981, wind speeds were measured at ca. 1 and 4 m above the plant canopy. In 1982, wind speeds were measured at ca. 0.1 m above the canopy and at 4 and 8 m above the ground. Air temperatures were measured in 1981 and 1982 with thermocouples mounted near the bases of the anemometers. In 1982, an additional thermocouple was mounted 11 m above the ground. A data logger (CR5; Campbell Scientific) recorded data from thermocouples and

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anemometers every 5 min during the sampling periods and every 2 h when samples were not being taken.

In 1982, when samples were taken systematically twice per week regardless of the weather forecast on sampling days, wind speeds and temperatures at midday were not significantly different from those on days when no samples were taken (analysis of variance, F test). Thus, conditions on sampling days were fairly representative of conditions prevailing throughout the season.

RESULTS AND DISCUSSION

General characteristics. In 1982, canopy-level concentration, deposition, and estimated upward flux of total airborne bacteria appeared to follow a lognormal distribution as determined by the Shapiro-Wilk test for normality (19). Airborne concentrations of total bacteria measured in a suburban area were also lognormally distributed (11). Mean wind speeds at canopy height measured during the sampling times were normally distributed.

Diurnal variation of bacterial dispersal during dry weather. Concentrations of airborne bacteria measured during three growing seasons are shown in Fig. 1. The variability in concentrations from sample to sample was very large; however, concentrations of airborne bacteria exhibited a distinct diurnal pattern with peak concentrations during midday (Fig. 1 and 2). Deposition of total bacteria exhibited the same diurnal pattern. Concentration and deposition of airborne bacteria were highly correlated ($r = 0.92$; $P < 0.001$).

Although viable bacteria were present in the air at all times of day, significant upward fluxes of bacteria occurred only during the warmest part of sunny days. Upward flux was not detected until after the leaves dried in the morning, and it always declined by late afternoon (at ca. 1800 h). Upward fluxes of bacteria were consistently larger in 1980, when samples were taken over a continuous expanse of bean canopy, than they were in either 1981 or 1982, when beans were planted in small blocks separated by 3-m-wide alleys of bare soil. This is consistent with the earlier finding that plant canopies are a stronger source of airborne bacteria than is the soil (15). On the average, environmental conditions at the sampling times were not significantly different among the 3 years.

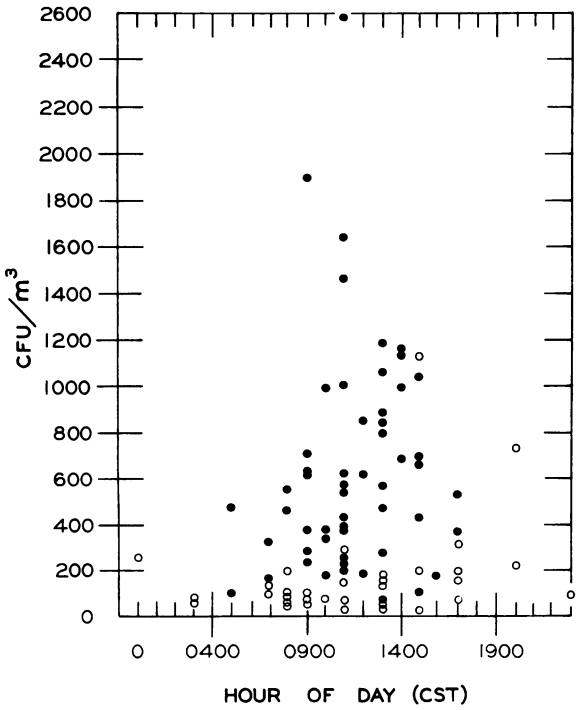

FIG. 1. Concentrations of airborne bacteria as a function of time of day. Concentrations were determined at canopy level during dry weather in 1980, 1981, and 1982. Symbols indicate the presence (●) or absence (○) of an upward flux at the time each sample was taken.

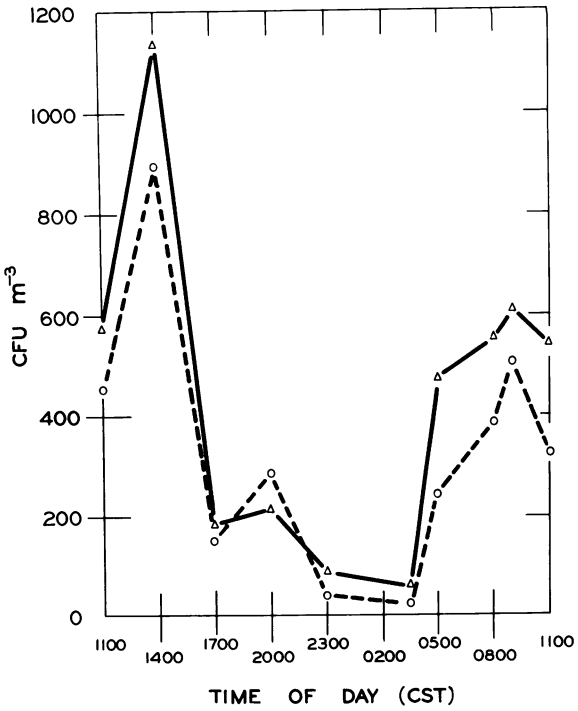

FIG. 2. Bacterial aerosol concentrations at bean canopy level (△) or above canopy level (○) during one 24-h period, 26 to 27 August 1982.

The midday mean log of the upward flux of total bacteria during 1980 was 2.29 CFU m^{-2} s^{-1}. If we make the simplifying assumption that the bacterial aerosol source is a monolayer of bean leaflets, each measuring 50 cm^2, and that the midday dispersal lasts about 6.66 h, then the mean daily upward flux occurring during a typical midday period is 4.36 (as log CFU $leaflet^{-1}$ day^{-1}). In contrast, although deposition occurs continuously during a 24-h period, the daily total deposition estimate is only 3.03 (as log CFU $leaflet^{-1}$ day^{-1}). Thus, by difference, we estimate a daily net loss of bacteria from the canopy to be 4.34 (as log CFU $leaflet^{-1}$), atmospheric conditions permitting. This corresponds to a daily net loss to the bean canopy of 10.65 (as log CFU ha^{-1}), or a net introduction to the atmosphere of $\sim 4.5 \times 10^{10}$ particles per ha per day bearing viable bacteria.

Atmospheric conditions appropriate for bacterial transport during dry weather. Upward fluxes of bacteria occurred only when neutral or unstable atmospheric conditions created shearing stresses in the surface boundary layer, as indicated by differences in wind speeds at different heights above the plant canopy. Such conditions commonly occurred during midday, but on only ca. 50% of sampling days. During midday periods of stable atmospheric conditions, when the air was stagnant or when a thermal inversion was present, concentration gradients were absent and no upward flux

TABLE 1. Correlation of bacterial aerosol measurements with wind speed at times of upward flux of bacteria from a bean canopy

Year	Deposition (log CFU m^{-2} s^{-1})		Upward flux (log CFU m^{-2} s^{-1})		Canopy-level concn (log CFU m^{-3})	
	r	*P*	*r*	*P*	*r*	*P*
1980	0.682	<0.01	0.798	<0.001	0.628	<0.05
1982	0.59	<0.01	0.63	<0.001	0.47	<0.05

occurred. No upward flux occurred when leaves were wet with dew, even during windy nights, or during conditions of light misty rain or fog.

On two occasions, high bacterial deposition and concentrations were detected at 2000 h, under conditions of atmospheric stability when there was no wind shear, when an inversion was present, and when the flux of bacteria was downward rather than upward (i.e., bacterial concentration increased as height above the canopy increased). In the experiment shown in Fig. 2, the typical upward flux and high concentration of airborne bacteria occurred during the sunny part of the day. However, at 2000 h, the concentration of airborne bacteria at the sampler above the canopy was higher than the concentration closer to canopy height, indicative of a net downward flux. Thus, the bean canopy at the sampling site was not the source of these bacteria. Such concentration peaks correspond closely to the evening peaks reported by Pady and Kramer (16). Aerosols detected under these conditions were qualitatively different from aerosols encountered when an upward flux from the bean canopy occurred, both in particle size distribution and in the types of bacteria present. These data may be evidence of intermediate-scale transport of viable bacteria. The peak of deposition occurred 6 to 9 h after the peak of upward flux for the day. Thus, with wind speeds of ~6 km h^{-1} in the lower atmosphere, we might expect this cloud of bacteria to have travelled 40 to 65 km from some source. The source of these concentrated aerosols may have been a number of intense point sources (e.g., harvesting operations) (14, 17) or a large area source (many square kilometers) where some plant canopy was serving as a source for a very strong upward flux of bacteria. Regardless of its nature, the source probably produced bacterial aerosols for several hours. Long-distance transport of bacteria has been reported (4).

Influence of wind. The evident diurnal periodicity in bacterial concentration can be partially explained by an association with net upward flux. Canopy-level bacterial concentrations were generally high (160 to 1,600 CFU m^{-3}) in the presence of upward fluxes and low (30 to 160 CFU m^{-3}) in their absence. Conditions conducive to upward flux occurred almost exclusively during midday. The large variability in concentration that occurred during midday needs further explanation. During periods when there was a measurable upward flux of airborne bacteria, we were confident that the canopy was a source of airborne bacteria (15). Thus, further analysis was performed on data gathered during these periods to attempt to understand better the factors that affect aerosol source strength from the bean canopy.

Bacterial concentration, deposition at canopy height, and upward flux were positively correlated with wind speed in 1980 and 1982 (Table 1) but not with canopy-level air temperatures or relative humidity. No upward flux occurred when mean canopy-level wind speeds were less than 1 m s^{-1}. Although we might suppose that entrainment of bacteria from leaves is an energy-requiring process, which is thus dependent on wind speed, there was a large amount of variation in both concentrations of airborne bacteria and estimated upward flux at any one wind speed, as indicated by the relatively small correlation coefficients. The reason for this variation is unknown. It may be related to some biological variables which have a large influence on the ability of wind to remove bacteria as it passes over the leaf surface (e.g., the strength of bacterial attachment to the leaf surface and the number of bacteria present). Biological variables are likely to be related to events before the sampling as well as to conditions during the sampling. For example, during 1982 upward fluxes were greater on days immediately after a rain (as indicated by soil surface moisture) than on days when the soil surface was dry (Table 2). Since wet soil is a poor aerosol source (8), these data may indicate that rain at some interval before the sampling time either causes the bacteria to be released more easily from leaves or promotes growth of the bacteria so that more are available for dispersal. The physiological state of a marine vibrio has been shown to affect the strength of its adherence to a surface (6).

Another possible explanation for the variability in the data is that dispersal is related to some physical parameter that we did not measure. Leaves acquire large positive electrostatic charges (13) under conditions identical to those correlated with significant upward fluxes of bacteria reported here (i.e., dry, sunny days). Thus, release of bacteria from leaf surfaces may be affected by electrostatic forces. Alternatively, bacterial dispersal may be related to fast gusts of wind (20, 2) rather than to mean wind speed.

Bacterial dispersal during rain. Bacterial aerosol concentrations increased dramatically during rain. In one 1981 experiment, canopy level concentrations increased ca. 25-fold, from 2.29 (as log CFU m^{-3}) at 1630 h, before the rain, to 3.68 (as log CFU m^{-3}) at 1730 h during the first 10 min of a rainstorm. Differences in bacterial concentrations at two sampling heights and the presence of a strong shearing stress indicated that there was an upward flux of bacteria from the canopy of 2.06 (as log CFU m^{-2} s^{-1}). At the same time, deposition of bacteria at canopy height was >2.85 (as log CFU m^{-2} s^{-1}). Thus, the net flux of bacteria during rain is strongly downward as observed by Constantinidou et al. (H. A. Constantinidou, S. S. Hirano, and C. D. Upper, personal communication). Similar results were obtained in other samples taken during rainstorms. In one experiment during 1982, rain was collected with funnel-topped test tubes. Rainwater 2 m above the canopy contained 0.30, rain at canopy height contained 2.89, and rain collected below the canopy contained >5.00 (as log CFU ml^{-1}). Thus, at canopy level, the net downward flux of bacteria is due to removal of

TABLE 2. Comparison of bacterial aerosols measured during rainless periods with aerosols measured on days after rain in 1982

Soil condition	Mean ± SE		
	Total bacterial upward flux (CFU m^{-2} s^{-1})[a]	Wind speed (ms^{-1})	Bacterial concn (CFU m^{-3})
Dry[b]	43 ± 9.2	3.5 ± 0.19	738 ± 198
Wet	154.5 ± 57	3.5 ± 0.12	1,545.5 ± 439

[a] Values of upward flux with wet versus dry soil were significantly different (F test; $P < 0.05$).

[b] Soil surface in alleys and between plants.

bacteria from leaves and deposition of them onto soil by rain. We estimate that a mean log of 5.00 CFU was lost per bean leaflet in 15 min of this moderate rainfall (0.4 cm h^{-1}). Others have also reported that large numbers of bacteria are washed off of leaves during rain (22, 10).

Substantial local lateral movement of bacteria also occurred during rain. In plots where a rifampin-resistant bacterium was present on leaves (but not detected in rain above the canopy), deposition of this bacterium at canopy height was measured to estimate lateral movement within the canopy during rain. Deposition increased ~5,400-fold during rain, from 0.9 during dry weather immediately before rain to 2.83 during rain (as log rifampin-resistant CFU per square meter per second). Lateral movement during rain is apparently important for the redistribution of bacteria from one plant to another (5, 7) or from leaf to leaf within the canopy. However, it is difficult to speculate on the number of new colonizers which could adhere to a leaf during a rainstorm because of the tendency for bacteria to be washed off of leaves during rain. We anticipate that a newly introduced bacterium might have a relatively higher probability of successfully colonizing a rain-cleansed leaf surface or a recently unfolded leaf, whereas bacteria deposited during dry weather onto heavily populated older leaves may have a smaller probability of becoming established because of competition from established resident bacteria.

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