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Plants as Sources of Airborne Bacteria, Including Ice Nucleation-Active Bacteria

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Vertical wind shear and concentration gradients of viable, airborne bacteria were used to calculate the upward flux of viable cells above bare soil and canopies of several crops. Concentrations at soil or canopy height varied from 46 colony-forming units per m^3 over young corn and wet soil to 663 colony-forming units per m^3 over dry soil and 6,500 colony-forming units per m^3 over a closed wheat canopy. In simultaneous samples, concentrations of viable bacteria in the air 10 m inside an alfalfa field were fourfold higher than those over a field with dry, bare soil immediately upwind. The upward flux of viable bacteria over alfalfa was three- to fourfold greater than over dry soil. Concentrations of ice nucleation-active bacteria were higher over plants than over soil. Thus, plant canopies may constitute a major source of bacteria, including ice nucleation-active bacteria, in the air.

Airborne bacteria over land are considered to originate from soil on the basis of three lines of evidence. (i) Relative to terrestrial sources, oceans are weak sources of airborne bacteria (5, 9, 12, 18, 22, 28). (ii) Large increases in the concentration of airborne bacteria are associated with dust storms (3, 20) and with human activities, such as harvesting, which disturb soil (2). (iii) The genera of bacteria found in upper air aerosols are frequently also found in soil (11, 12, 20). However, plant surfaces also harbor most of the bacterial genera recovered from both soil (25) and air samples. If soil is the major source of airborne bacteria, bacterial aerosol concentrations should be very high in the spring planting season, when vast acreages are disturbed by tillage. However, concentrations of airborne bacteria over land are consistently least during winter and spring, whereas they are greatest during summer and autumn, when most agricultural soil is covered by plant canopies (2, 11, 17). Soil is virtually always covered by plant materials in most natural temperate habitats.

Bacterial plant pathogens have been found in aerosols near infected plants exposed to simulated raindrops in the laboratory (7, 8; J. R. Venette and B. W. Kennedy, Proc. Am. Phytopathol. Soc. 3, 1976), during rain and overhead irrigation in the field (21, 27), and during harvesting (19). However, harvests occur only one or a few times on each field throughout the growing season and tend to occur too late to explain the high concentrations in summer. Rain tends to decrease the bacterial aerosol concentration rather than increase it (2, 17). Thus, the majority of bacteria found in upper air aerosols are probably liberated from the earth's surface during dry weather, but it has not yet been determined whether most of these airborne bacteria specifically originate from the soil or from plant surfaces.

Although the total significance of natural bacterial aerosols is unknown, the recent discovery that certain bacteria are active as ice nuclei suggests that airborne bacteria could have a role in precipitation formation (26). The most active, abundant, naturally occurring ice nuclei known, other than ice itself, are those associated with intact cells of the bacterial species *Pseudomonas syringae* van Hall (16), *Pseudomonas fluorescens* biotype G Migula (L. R. Maki and D. M. Garvey, Trans. Am. Geophys. Union 56:994, 1975), and *Erwinia herbicola* (Löhnis) Dye (15). Ice nucleation-active (INA) bacteria are normal residents of living leaves of many plant species (14; unpublished data). Ice nuclei of biological origin enter the atmosphere from plant debris (23, 24, 26). Whether these airborne ice nuclei are or are not derived from living INA bacteria remains to be determined.

The objectives of this study were to assess the quantitative importance of living plant surfaces

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as sources of natural bacterial aerosols and to determine whether INA bacteria are present in those aerosols.

MATERIALS AND METHODS

The concentration of viable bacteria in the air was measured with, initially one and later two, Andersen six-stage viable microbial impaction samplers. When two samplers were used, they were separated either vertically by 2 m, with the lowest positioned at or slightly above the top of the vegetative canopy for the purpose of flux calculations, or horizontally for determination of local sources of airborne bacteria. Stage six was omitted from the samplers. Air samples were drawn at 28.3 liters/min for 10 to 30 min, depending on the experiment. Particles impacted onto a nutrient glycerol agar medium supplemented with 200 μg of cycloheximide per ml (NGA + C). Colonies were counted after 3 days of incubation at ca. 21°C, and the counts were corrected for coincidence by the method of Andersen (1). Colonies containing ice nuclei were located by a plate replica freezing technique (14). Corrected counts on each stage were summed to determine total concentrations of airborne bacteria per sample.

INA bacteria were isolated, and the ice nucleation activity of axenic cultures was verified. Fluorescent INA bacteria were identified on the basis of bacteriological characteristics (4), but nonfluorescent INA bacteria were not identified.

Horizontal wind speeds were measured with two totalizing four-cup anemometers which were vertically separated by 3 m, with the lowest anemometer positioned less than 1 m above the vegetative canopy. Wind speeds were averaged over the sampling period at each position.

The upward flux of viable bacteria, F_B, was estimated by a modification of the procedure of Gillette et al. (6):

$$F_B = -0.16\,\rho \left[\frac{\Delta z_u \Delta u}{\left(\ln \frac{z_2}{z_1}\right)_u^2}\right]\left[\frac{\Delta c}{\Delta z_c}\right]$$

where Δu (meters per second) represents the difference between wind speeds at two anemometers at heights of $z_{1,u}$ and $z_{2,u}$ and separated vertically by Δz_u meters, Δc represents the differences between bacterial concentrations (colony-forming units per cubic meter) at two samplers separated vertically by Δz_c meters, and ρ represents the density of air.

This assumes that bacterial cells have a small terminal velocity and that the wind speed followed a logarithmic vertical profile. We assumed ρ to be unity, which probably results in a slight underestimate of total bacterial flux but will not affect estimates of relative source strengths determined simultaneously. Small differences between heights at which wind speeds and bacterial concentrations were measured were assumed to have a negligible effect on flux estimates. When vertical gradients of wind speed and bacterial concentration were measured, the wind 4 m above the canopy exceeded 3 m/s, indicating that there was good mixing in the boundary layer of air. The lapse of temperature was probably close to adiabatic near the surface on these days, making the assumption of a logarithmic wind speed profile reasonable.

Leaf surface populations of total and INA bacteria were determined according to the methods of Lindow et al. (14) by using NGA + C and King's medium B (13). Total and INA bacterial populations in the soil were determined in the same way by using replicates of ca. 20 g of soil suspended in 0.1 M phosphate buffer (pH 7.0).

Three experiments centered around a 24-Ha field of heading winter wheat (*Triticum aestivum* L.) on which INA bacteria were a large fraction of the epiphytic bacterial flora. All land up to 3 km west and southwest (upwind on 1 and 9 June 1978) of the wheat had only very young corn (*Zea mays* L.), grassy weeds, or bare ground. Samplers were situated so that no farm buildings or groups of farm animals were in the path of the air flow toward our air samplers. On 1 June, one measurement was made in the upwind corn field and one on the far downwind side of the wheat field. On 9 June, three upwind and four downwind samples were taken in alternating fashion. On 19 June, land upwind (south) of the wheat contained young corn, peas, quackgrass (*Agropyron repens* L.), and foxtails (*Setaria* spp.), and the land downwind (north) contained peas (*Pisum sativum* L.). Two upwind samples, two samples each at 0.6 and 2.6 m above canopy height in the wheat field, and two samples at the downwind side of the pea field were taken in random sequence. On 7 August, we sampled air at two sites in a 64-Ha snapbean (*Phaseolus vulgaris* L.) field which supported a substantial population of epiphytic INA bacteria, *P. syringae* in particular. The concentration gradient of airborne bacteria used in flux calculation was deduced from a series of sequential measurements, five at canopy height and five at 2 m above the canopy.

On 6 September, we sampled at a chisel-plowed field with a dry surface and at the adjacent, downwind field of alfalfa (*Medicago sativa* L.). Two Andersen samplers were operated simultaneously at two heights to obtain the vertical concentration gradients over soil and alfalfa.

RESULTS AND DISCUSSION

In two experiments, the concentration of airborne bacteria was higher on the downwind side of a field of heading winter wheat than in a field of young corn, upwind from the wheat (Table 1, experiments of 1 and 9 June 1978). Thus, some local source of airborne bacteria was stronger in the wheat field than in the corn field. If soil were the source of the airborne bacteria, then bacterial concentrations should have been higher over the corn field, where the widely spaced rows of 0.4-m-high corn plants allowed much greater exposure of the soil to wind than the dense, 0.9-m-high wheat canopy. Since wind speeds decrease rapidly with depth in a dense plant canopy, very little wind energy, if any, would have been available to generate aerosols at soil level under the wheat. Furthermore, on both 1 and 9 June, the soil surface was wet. Gillette et al. (6)

TABLE 1. Populations of leaf and soil surface bacteria, concentrations of airborne bacteria, and vertical flux of bacteria over plant canopies and soil[a]

Date (1978)	Source	Surface bacteria			Airborne bacteria[c]			Mean canopy-level wind speed (m/s)	Vertical bacterial flux (CFU/m² per s)
		No. of samples	log (CFU/g)		No. of samples	CFU/m³			
			Total	INA[b]		Total ± SE	INA		
1 June	Corn				1	46	0		
	Winter wheat	9	6.04	4.97	1	2,408	56.5	4.5	
9 June	Corn	5	6.12	3.79	3	141 ± 85	1.2		
	Winter wheat	5	6.01	4.88	4	6,500 ± 1437	178.0	1.7	
19 June	Upwind composite[d]	9	4.86	4.30	2	51 ± 5.3	0		
	Winter wheat	4	5.77	5.22	2	506 ± 243	19.9	2.8	57
	Downwind pea	3	5.16	4.46	2	92 ± 32	1.7		
7 August	Bean	4	7.72	6.55	5	2,240 ± 725	7.5	1.3	499
6 September	Bare soil	5	7.75	5.34	2	663 ± 123[e]	0.9		124
	Alfalfa	8	7.04	4.84	5	2,690 ± 167[f]	1.1	3.5	543

[a] CFU, Colony-forming units.
[b] Bacteria that produce colonies active as ice nuclei at −10°C.
[c] Air samples taken at canopy height or at soil level.
[d] Upwind composite contained young corn, peas, quackgrass, and foxtails.
[e] Values represent the mean of samples taken 10 and 20 m upwind of the bare soil-alfalfa interface.
[f] Values represent the mean of samples taken 10, 20, 110, and 120 m downwind of the interface.

have reported that vertical fluxes of aerosols over wet soils are negligible. Thus, the most likely explanation of the differences in airborne concentrations is that epiphytic bacteria present on the wheat were a stronger source of airborne bacteria than those on the corn.

The experiment on 6 September was designed to contrast a plant canopy and a dry soil surface as sources of airborne bacteria. Canopy-level bacterial concentration and upward bacterial flux were four times greater over alfalfa than over bare ground, even though bacterial populations in the near-surface soil were approximately five times greater than those on the alfalfa plants (Table 1). Therefore, although it is clear from the literature that large numbers of bacteria enter the atmosphere from soil during severe but relatively infrequent conditions such as dust storms, under more common daytime weather conditions, plants are quantitatively more important than soil as sources of airborne bacteria (Fig. 1).

On 19 June, concentrations of bacteria over a pea field were lower than those over the wheat field just upwind (Table 1). The large differences between concentrations of airborne bacteria at three sampling sites separated by only 200 to 300 m illustrates the importance of recognizing differences between types of plant canopies as aerosol sources. On 6 September, the concentrations of airborne bacteria only 10 m inside the alfalfa field were indistinguishable from those 100 m into the field (Fig. 1). Thus, the strong local influence of the alfalfa canopy took effect in less than 10 m. Aerosol samples taken at sites "representative" of rural areas (2) may represent very local sources.

Although wheat was a stronger aerosol source than young corn plants on three consecutive occasions, the bacterial aerosol concentration over winter wheat varied more than 10-fold in samples taken 10 days apart. Thus, relative source strengths of different plant species and soils should be deduced only from simultaneous or nearly simultaneous samples, and not from measurements made on different days.

On 7 August, since INA bacteria were detected at 2 m above a bean canopy, we were able to estimate vertical fluxes of INA bacteria that were 0.4 and 1.2 bacteria per m² per s active at −5 and −10°C, respectively. The ratio of airborne INA bacteria to total bacteria at canopy level was approximately equal to the ratio of the INA bacterial flux to the total bacterial flux. Thus, even when the concentration of INA bacteria above a plant canopy was below the level of detection of our sampling method, an upward flux of INA bacteria can be inferred from the total bacterial flux and the fraction of INA bacteria in the aerosol at canopy height.

Fluorescent bacteria, identified as *P. syringae*, accounted for 75% of the 28 INA bacteria isolated from air samples. These were all active at −5°C. The remaining INA bacteria from aerosols resembled *E. herbicola* on the basis of yellow pigmentation and colony morphology.

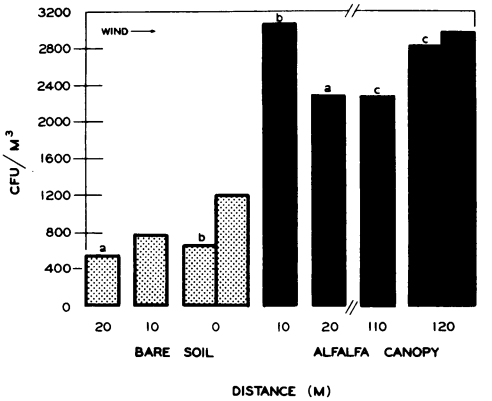

FIG. 1. Concentrations of airborne bacteria at soil (stippled) and alfalfa canopy (solid black) levels at several distances from the interface between the two fields on 6 September 1978. Bars with the same letter indicate measurements taken simultaneously. Concentrations over soil and alfalfa are significantly different by analysis of variance ($F = 32.9$; $P < 0.01$). CFU, Colony-forming units.

These isolates were not characterized further. The INA bacteria isolated from plant surfaces were predominantly *P. syringae*, whereas the INA bacteria from bare soil and air samples above soil (6 September) resembled *E. herbicola*.

Bacteria impacted on petri plates in the Andersen sampler are not detected until they have formed colonies. Thus, airborne bacteria capable of producing INA colonies were detected, but it was not possible to determine how many of the cells entering the sampler were actually active ice nuclei, since the ratio of ice nuclei to cells is usually $\ll 1$ in cultures of these bacteria (10). In addition, cells of INA bacteria may remain active after the cells are no longer viable. Thus, our measurements represent the numbers of viable bacteria in the air capable of producing ice nuclei when grown in culture, not the numbers of bacterial ice nuclei in the air.

Our findings indicate that plants constitute a major source of airborne bacteria, including those that are INA. Even a dry, bare soil, which characteristically releases a greater number of particles than a wet soil, yielded a bacterial flux much smaller than that of an adjacent field of alfalfa. Rain was not necessary for the generation of bacterial aerosols from plants, since all of our measurements were made when the leaves were dry and there was no rain in the vicinity. In addition, the flux calculations indicate that viable bacteria, including INA bacteria, were being transported well into the surface boundary layer and could be mixed throughout the depth of the troposphere. Viable INA bacteria in the troposphere are potentially in a suitable position to initiate precipitation processes, although their involvement in these processes remains to be demonstrated.

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