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The Andersen six-stage impactor, the SAS (Surface Air System) impactor, the AGI-30 impinger, and gravity plates were evaluated for the retrieval of aerosol-released *Pseudomonas syringae*. The upper limits of the impactor samplers were exceeded at a spray concentration of 10^7 CFU/ml, indicating that these samplers are not appropriate for monitoring high airborne concentrations. Decreased cell concentrations were retrieved with increased sampling time for the Andersen and AGI samplers, indicating that a minimum sampling time is preferable for monitoring aerosolized vegetative cells.

The release of microorganisms onto an agricultural field to enhance productivity not only results in the inoculation of organisms to the target crop, but also produces airborne cells that may be transported to surrounding areas. Several samplers are commercially available (3) for monitoring the fate and transport of aerosol-released microorganisms; however, there is insufficient information on the relative proficiency of these samplers to design a comprehensive monitoring scheme. Four sampling methods used previously in field monitoring were compared in this study: the Andersen six-stage sampler (1, 6–8), the portable high-volume SAS (Surface Air System) impactor sampler (5), the AGI-30 all-glass impinger (6), and agar-filled 150-mm petri dishes serving as gravity plates (10). This study was designed to determine the relative retrieval capability of these methods for the retrieval of aerosol-released bacteria and to establish optimal run times for each sampler.

Release trials were conducted in a greenhouse (ca. 6 by 9 m) with aerosolized cells of rifampin-resistant *Pseudomonas syringae* Cit7, a nonrecombinant ice nucleation-active organism (9) (obtained from S. E. Lindow, University of California, Berkeley). *P. syringae* was cultured on King medium B (6), pH 7.0, supplemented with 100 μg each of rifampin and cycloheximide per ml and incubated at 28°C. *P. syringae* was prepared for aerosolization as described previously (2) and diluted to the desired concentration with sterile distilled water. *Bacillus subtilis* spores (obtained from A. J. Mohr, U.S. Army Dugway Proving Ground, Utah) were aerosolized as a physical tracer. Dry *B. subtilis* spores were suspended in 0.01 M phosphate buffer containing Tween 20 (1%, vol/vol; Sigma Chemical Co.), and the suspension was diluted with sterile distilled water to a final estimated concentration of 10^5 CFU/ml. The spores were cultured on nutrient agar (Difco Laboratories), pH 7.0, supplemented with 100 μg of cycloheximide per ml and incubated at 37°C. Actual determination of CFU per milliliter of spray suspensions was made by spread plating serial dilutions onto the appropriate agar.

A series of three Andersen six-stage (containing agar plates) and six AGI-30 samplers (containing 20 ml of phosphate buffer) were placed at both 4 and 8 m from the spray source and operated for 5, 10, or 20 min from initiation of spraying. Additional AGI-30 samplers operated for 2 min were included in some trials. One SAS sampler was placed at each distance and operated for a single run time of 1 min. A series of 24 gravity plates per trial were grouped at both the 4- and 8-m distances, with replicate plates exposed for 15, 30, 60, or 90 min.

Temperatures during the *P. syringae* release trials averaged 18m (benches A to C, left to right from the spray source) °C at the initiation of spraying and 24 (benches D to F) °C at the conclusion of the 90-min sampling period. Initial relative humidity measurements ranged from 42 to 48%, with an average relative humidity of 35% at the conclusion of the spray trials. Background concentrations of *Bacillus* spores and *P. syringae* in the greenhouse bay were determined prior to each spray release and were not statistically significant.

Spray equipment, described previously (10), was used for the aerosolization of microorganisms. *P. syringae* was sprayed at concentrations of 10^7 or 10^5 CFU/ml, and *B. subtilis* was sprayed at a concentration of 10^5 CFU/ml. A minimum of two spray trials were performed for each organism at each concentration.

At the conclusion of each spray trial, the necks of the AGI-30 samplers were washed with a known volume of phosphate buffer and the contents were either spread plated directly or concentrated by filtration with the filters placed onto agar plates and incubated with exposed gravity, SAS, and Andersen plates. Colony counts from the Andersen and SAS samplers were adjusted by using positive-hole correction charts (1, 11) which account for the probability of multiple "hits" through the sampling holes. Data were converted to CFU per volume of air for the forced-air flow samplers and to CFU per surface area for the gravity plate samples. Data were transformed to logarithms prior to statistical analysis.

Results in these greenhouse trials indicate that the quantitation ranges and length of operation of these samplers are important considerations for meaningful aerobiological monitoring. The quantitation ranges varied greatly between sampler types (Table 1). Because the AGI-30 collects particles in a liquid medium which can be diluted prior to spread plating, there is essentially no upper quantitation limit (UQL) for this sampler. The SAS and Andersen samplers collect particles through sieved openings and impel cells onto the agar surface, but as airborne concentrations increase, enumeration with these samplers becomes less accurate due to multiple impactions (1). In areas of high microbial concentration (e.g., close proximity to target

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TABLE 1. Quantitation ranges for four aerobiological sampling methods

Sampler	Flow rate (liters/min)	Run time (min)	CFU/m³	
			LQL[a]	UQL
AGI-30	12	5	16.7	None
Andersen six stage	28	5	7.1	1.0×10^{5}[b]
SAS	180	1	5.6	7.3×10^{3}[b]
Gravity plates[c]			65.0	1.3×10^{5}

[a] Assumes enumeration of a single colony. LQL, Lower quantitation limit.
[b] Assumes 100% "positive holes."
[c] Data given in CFU per square meter.

plants at the spray release site), the UQL of the Andersen and SAS would likely be exceeded, resulting in data above the quantitation limits as observed at both 4 and 8 m for the 10^7-CFU/ml *Pseudomonas* spray concentration (data not shown). The AGI-30 was the only sampler used which recovered *P. syringae* within quantitation limits at both distances in the 10^7-CFU/ml spray trials (data not shown). Mean recovery of *P. syringae* with gravity plates was 4.07 ± 0.09 CFU/m² ($\log_{10}$ ± standard error) at the 8-m benches, but concentrations exceeded the UQL at 4 m.

Results of the 10^5-CFU/ml spray trials are shown in Table 2, with gravity plate data shown separately (Fig. 1). Airborne concentrations of spores and vegetative cells were above the UQL of the SAS sampler for all trials. While the UQL of the Andersen sampler was exceeded in trials with *B. subtilis*, numbers of *P. syringae* cells were within quantitation limits (Table 2). The Andersen sampler retrieved higher numbers of *P. syringae* cells than the AGI-30 for all run times at both distances, although the difference was significant at only the 20-min run time.

Distance of samplers from the spray source had the effect of reducing the number of vegetative cells retrieved with all samplers. The difference was significant for gravity plate samples (Fig. 1) and for the AGI-30 (Table 2), but not for the Andersen sampler. Retrieval of spores, which are resistant to environmental stresses, was not affected by distance.

When expressed as CFU per square meter, gravity plate data were in the same order of magnitude as the data, in CFU per cubic meter, from the forced-air flow samplers (Fig. 1, Table 2). Because the relationship between the two units depends on particle size and physical factors affecting their settling velocities (3), comparison of the data is limited.

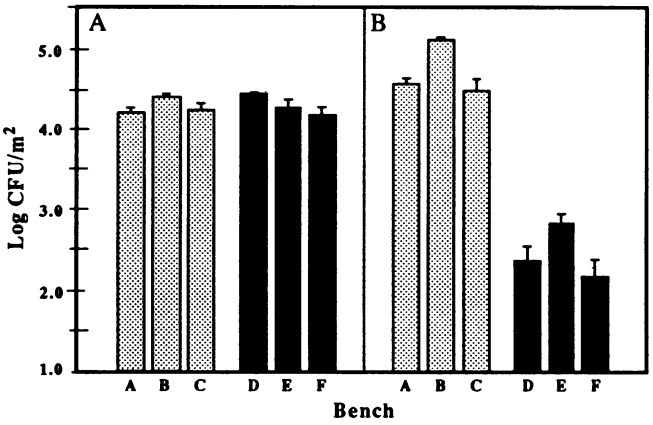

FIG. 1. Retrieval of *B. subtilis* (A) (10^5 CFU/ml, spray concentration) and *P. syringae* (B) (10^7 CFU/ml, spray concentration) by using gravity plates. Bars represent data (mean ± 1 standard error) at 4 (; benches A to C) and 8 (; benches D to F) m.

Nonetheless, gravity plates may serve as a low-cost indicator of the presence and approximate concentration of airborne cells and may be useful in providing additional information, such as distribution patterns, unattainable with the more costly forced-air flow samplers. This was demonstrated with the gravity plate data showing uneven distribution of *P. syringae* within the greenhouse bay as noted by the differences in recovery by sampling bench (Fig. 1).

Longer sampling time decreased the retrieval of both *B. subtilis* and *P. syringae*. Decreasing retrieval of spores with extended sampling time was expected due to dispersion and settling of the aerosol. In addition to physical factors, loss of vegetative cells may be attributed to environmental factors or sampling stress. The ratio of the retrieval of *P. syringae* to *B. subtilis* spores (Table 2) decreased with increasing sampler run time with the AGI-30 sampler at both distances, indicating that sampling stress was affecting the viability of the vegetative cells. Lower recovery of vegetative cells was also demonstrated with increasing run times with the Andersen samplers (Table 2). The corresponding spore data, however, were greater than the Andersen UQL, and ratio comparison was not possible. Decreased retrieval of vegetative cells with increasing sampling times was also observed in the 10^7-CFU/ml spray trials with the AGI-30 samplers

TABLE 2. Sampler comparison for the retrieval of *P. syringae* and *B. subtilis* (10^5 CFU/ml, spray concentration)

Sampler	Run time (min)	CFU/m³ (log ± SE)				Ratio, *P. syringae*/*B. subtilis*	
		P. syringae		*B. subtilis*			
		4 m	8 m	4 m	8 m	4 m	8 m
AGI-30	5	3.40 ± 0.01[a]	2.74 ± 0.14	4.36 ± 0.04	4.26 ± 0.11	0.78	0.64
	10	3.02 ± 0.02[a]	2.32 ± 0.12	4.26 ± 0.04	4.18 ± 0.06	0.71	0.55
	20	2.66 ± 0.06[a]	1.96 ± 0.11	4.12 ± 0.54	4.07 ± 0.02	0.64	0.48
Andersen	5	3.90 ± 0.06	3.52 ± 0.20	>UQL	>UQL	ND[b]	ND
	10	3.55 ± 0.06	3.22 ± 0.18	>UQL	>UQL	ND	ND
	20	3.28 ± 0.08[c]	2.93 ± 0.14[c]	>UQL	>UQL	ND	ND
SAS	1	>UQL	>UQL	>UQL	>UQL	ND	ND

[a] Significantly higher than corresponding AGI-30 data at 8 m.
[b] ND, Not determined.
[c] Significantly higher than corresponding AGI-30 20-min data.

(data not shown). In contrast to the forced-air flow samplers, significant decreases in retrieval were not observed with longer exposure times with gravity plates at 8 m (data not shown).

This evaluation of aerobiological sampling methods in a greenhouse provided a controlled setting for retrieval of airborne cells. The information on detection limits and sampling times obtained from these greenhouse trials may be used to assist in the design of field experiments to establish standardized monitoring methods for aerosol-released microorganisms. These data, however, should be verified under actual field conditions, and further studies should be conducted with additional samplers and other microorganisms targeted for field release to provide a comprehensive monitoring framework.

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ERRATUM

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Volume 57, no. 4, p. 1268, column 2, line 4: "4- and 8-m distances" should read "4-m (benches A to C, left to right from the spray source) and 8-m (benches D to F) distances."

Lines 7 and 8: "18m (benches A to C, left to right from the spray source) °C at the initiation of spraying and 24 (benches D to F) °C" should read "18°C at the initiation of spraying and 24°C."