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Mark P. Buttner
University of Nevada, Las Vegas, mark.buttner@unlv.edu

Linda Stetzenbach
University of Nevada, Las Vegas

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Evaluation of Four Aerobiological Sampling Methods for the Retrieval of Aerosolized Pseudomonas syringae

MARK P. BUTTNER AND LINDA D. STETZENBACH*

Environmental Research Center, University of Nevada, Las Vegas, Nevada 89154

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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-release 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 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 spreading 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 18°C (benches A to C, left to right from the spray source) °C at the initiation of spraying and 24°C (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 correlation 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 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 spreading, 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
TABLE 1. Quantitation ranges for four aerobiological sampling methods

<table>
<thead>
<tr>
<th>Sampler</th>
<th>Flow rate (liters/min)</th>
<th>Run time (min)</th>
<th>CFU/m³</th>
<th>LQLᵃ</th>
<th>UQLᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGI-30</td>
<td>12</td>
<td>5</td>
<td>16.7</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Andersen six stage</td>
<td>28</td>
<td>5</td>
<td>7.1</td>
<td>1.0 x 10⁶ᵇ</td>
<td></td>
</tr>
<tr>
<td>SAS</td>
<td>180</td>
<td>1</td>
<td>5.6</td>
<td>7.3 x 10⁶ᵇ</td>
<td></td>
</tr>
<tr>
<td>Gravity plates</td>
<td></td>
<td></td>
<td>65.0</td>
<td>1.3 x 10⁵</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ Assumes enumeration of a single colony. LQL, Lower quantitation limit.
b Assumes 100% "positive holes."

³ Data given in CFU per square meter.

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 10⁷-CFU/ml spray trials with the AGI-30 samplers.

![FIG. 1. Retrieval of B. subtilis (A) (10⁷ CFU/ml, spray concentration) and P. syringae (B) (10⁷ CFU/ml, spray concentration) by using gravity plates. Bars represent data (mean ± 1 standard error) at A (C); B (C); C (C); D (C); E (C); F (C); benches D to F) m.](http://aem.asm.org/)

TABLE 2. Sampler comparison for the retrieval of P. syringae and B. subtilis (10⁷ CFU/ml, spray concentration)

<table>
<thead>
<tr>
<th>Sampler</th>
<th>Run time (min)</th>
<th>4 m</th>
<th>8 m</th>
<th>4 m</th>
<th>8 m</th>
<th>Ratio, P. syringae/B. subtilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGI-30</td>
<td>5</td>
<td>3.40 ± 0.01ᵃ</td>
<td>2.74 ± 0.14</td>
<td>4.36 ± 0.04</td>
<td>4.26 ± 0.11</td>
<td>0.78 ± 0.05 (10⁷ CFU/ml)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3.02 ± 0.02ᵇ</td>
<td>2.32 ± 0.12</td>
<td>4.26 ± 0.04</td>
<td>4.18 ± 0.06</td>
<td>0.71 ± 0.05 (10⁷ CFU/ml)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.66 ± 0.06ᵇ</td>
<td>1.96 ± 0.11</td>
<td>4.12 ± 0.54</td>
<td>4.07 ± 0.02</td>
<td>0.64 ± 0.48 (10⁷ CFU/ml)</td>
</tr>
<tr>
<td>Andersen</td>
<td>5</td>
<td>3.90 ± 0.06ᵇ</td>
<td>3.52 ± 0.20</td>
<td>&gt;UQL</td>
<td>&gt;UQL</td>
<td>NDᵃ (10⁷ CFU/ml)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3.55 ± 0.06ᵇ</td>
<td>3.22 ± 0.18</td>
<td>&gt;UQL</td>
<td>&gt;UQL</td>
<td>NDᵃ (10⁷ CFU/ml)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.28 ± 0.08ᵇ</td>
<td>2.93 ± 0.14ᵇ</td>
<td>&gt;UQL</td>
<td>&gt;UQL</td>
<td>NDᵃ (10⁷ CFU/ml)</td>
</tr>
<tr>
<td>SAS</td>
<td>1</td>
<td>&gt;UQL</td>
<td>&gt;UQL</td>
<td>&gt;UQL</td>
<td>&gt;UQL</td>
<td>ND (10⁷ CFU/ml)</td>
</tr>
</tbody>
</table>

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.

We thank Stephen Hern of the Environmental Monitoring Systems Laboratory-Las Vegas, U.S. Environmental Protection Agency, for providing encouragement and technical advice throughout this project. We also thank James Meldrum and Michelle McIntyre for technical assistance and Shirley Burns for preparation of graphics.

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REFERENCES
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.”