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Detection of the Urban Release of a *Bacillus anthracis* Simulant by Air Sampling

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In 2005 and 2009, the Pentagon Force Protection Agency (PFPA) staged deliberate releases of a commercially available organic pesticide containing *Bacillus amyloliquefaciens* to evaluate PFPA's biothreat response protocols. In concert with, but independent of, these releases, the Department of Homeland Security sponsored experiments to evaluate the efficacy of commonly employed air and surface sampling techniques for detection of an aerosolized biological agent. High-volume air samplers were placed in the expected downwind plume, and samples were collected before, during, and after the releases. Environmental surface and personal air samples were collected in the vicinity of the high-volume air samplers hours after the plume had dispersed. The results indicate it is feasible to detect the release of a biological agent in an urban area both during and after the release of a biological agent using high-volume air and environmental sampling techniques.

A deliberate attack with an aerosolized biological agent is a low-probability, but high-consequence, event. Given the potential for significant morbidity, mortality, and socioeconomic disruption, the US government has implemented a number of programs to mitigate the bioterrorist threat. Two of the techniques commonly used in civilian (eg, BioWatch) and military (eg, Guardian) biodefense programs are environmental air monitoring and surface sampling. Both BioWatch and Guardian collect high-volume air samples, which are then analyzed for the presence of threat agents. Following detection of an aerosolized agent, surface samples may be collected for additional information. Although similar techniques are employed in other fields (such as air pollution monitoring) and modeling indicates they are effective, their application to a bioaerosol release is frequently criticized for a perceived lack of "operational data" supporting their use. However, "operational data" for a real or simulated terrorist attack on an urban area are difficult to obtain. The experimental evaluations of currently fielded systems are not publicly available for security reasons, and much of the relevant open literature is from studies that are outdated or were performed in nonurban settings.

In 2005 and 2009, the Pentagon Force Protection Agency (PFPA), in partnership with Arlington County, Virginia, staged deliberate releases of a commercially available organic pesticide containing *Bacillus amyloliquefaciens*.[1] These releases were designed to assess local biothreat response protocols by simulating a terrorist attack. In concert with the releases, separate studies, sponsored by the Department of Homeland Security, were conducted to determine whether air and surface sampling using common biodefense equipment and protocols could be used to detect an aerosolized release.

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Using an urban-aware transport and dispersion model to estimate the downwind plumes and deposition areas from the releases, air and surface samples were collected in and around the anticipated boundaries of the plumes. Results from these samples indicate it is feasible to detect the release of a dry powdered biological agent in an urban area both during and after the release using commonly employed environmental sampling techniques.

Methods

Simulant Release

Although some details of the PFPA release are closely held, it is publicly known that a commercially available pesticide containing *B. amyloliquefaciens* was released as a dry powder "by a blower system"[2] from the back of a truck driven along a road adjacent to the facility (Figures 1 and 2).[2,3] Multiple kilograms of pesticide were released,[2] but only a fraction of the pesticide material was viable *B. amyloliquefaciens*. Independent experiments were performed to characterize a sample of the material used in the testing, and it was determined that a small amount of viable *B. amyloliquefaciens,* consistent with what could reasonably be expected in an actual release, was released in each exercise.

Although most *Bacillus*-based pesticides contain *B. thuringiensis,* the material used by PFPA contained *B. amyloliquefaciens* as verified using genomic sequencing. *B. amyloliquefaciens* is a Group VI *Bacillus* species,[4] which, until recently, was classified as a subtype of *B. subtilis*, a common *B. anthracis* simulant.[5] *B. amyloliquefaciens* can therefore be viewed as a reasonable *B. anthracis* simulant. *B. amyloliquefaciens* is genetically distinct from and less ubiquitous than *B. thuringiensis* (another common *B. anthracis* simulant), making it easier to track a discrete release of *B. amyloliquefaciens* in environments where the background of other *Bacillus*-based pesticides may be high.

In 2005, the pesticide was dispersed as a "garden dust"–type formulation. Garden dust pesticides are intended to be

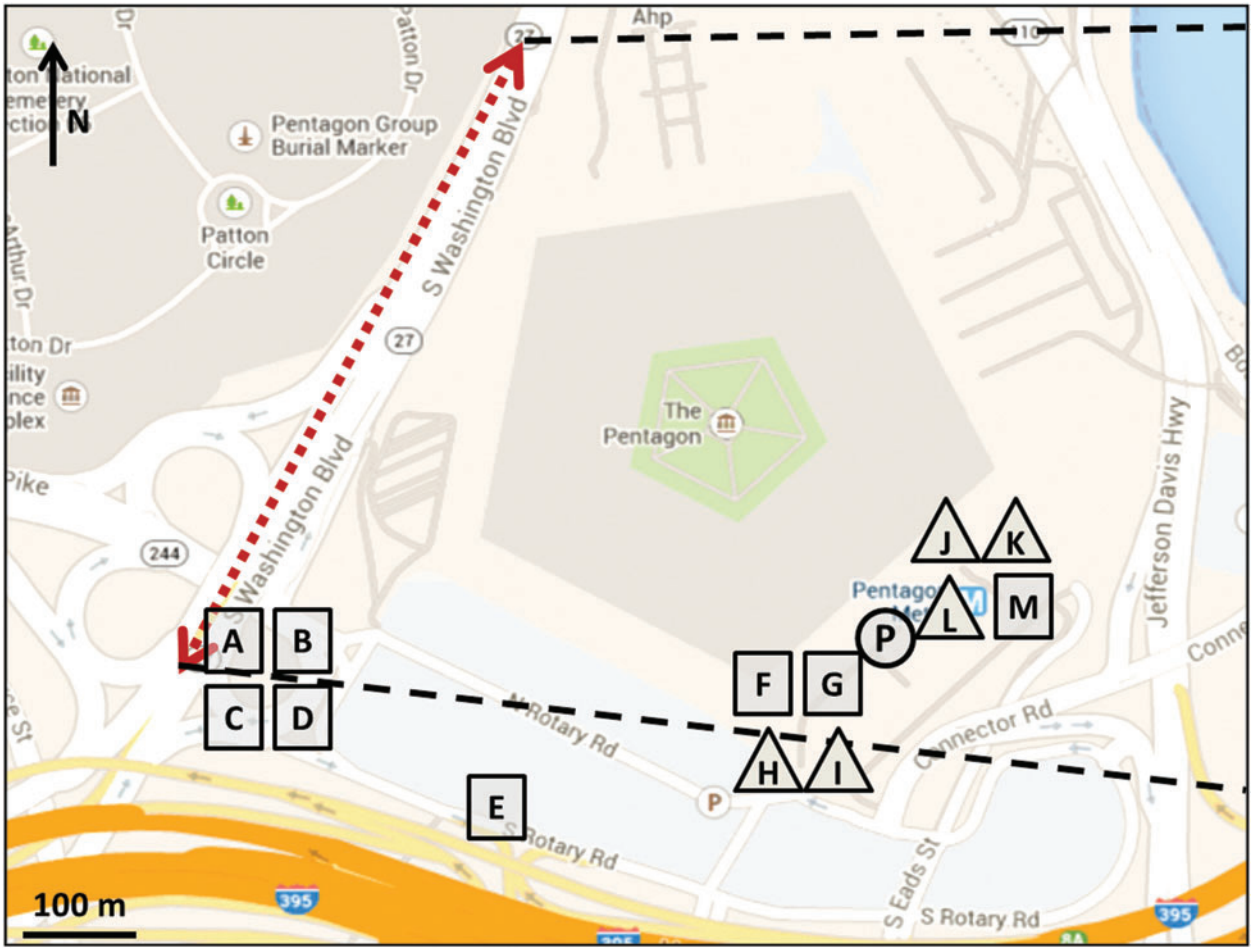

Figure 1. Predicted downwind dispersion area and sample collection locations for 2005 release. The approximate release location is shown by the red dotted line. The black dashed lines show the boundaries of the predicted downwind dispersion area. The PSU is shown as a circle. Outdoor environmental sample locations are shown as squares. Indoor (subway station) environmental sample locations are shown as triangles.

Figure 2. Predicted downwind dispersion areas and sample collection locations for 2009 release. The approximate release location is shown by the red dotted line. The black dashed lines show the boundaries of the predicted downwind dispersion area. The high-volume air sampler locations are shown as circles; environmental samples were collected in the immediate vicinity of the air samplers.

sprinkled onto foliage, and because of this, they are formulated as large particles of up to 150 microns in diameter.[6] Due to this large particle size, the transport and dispersion modeling results indicated that the 2005 release should have produced high levels of surface contamination in the immediate vicinity of the facility, with little material transported beyond the facility boundaries. In 2009, the material had a much finer particle size, with the majority of particles in the respirable range (0-10 microns). This release was expected to be more representative of a "typical" attack scenario, producing a plume that extended substantially downwind.

Assay Development

Lawrence Livermore and Los Alamos National Laboratories obtained a sample of the pesticide in advance of the releases. Its primary bacterial component was identified as *B. amyloliquefaciens* using genomic sequencing. Five real-time TaqMan® polymerase chain reaction (PCR) assays were then developed. These assays were validated against a panel of near neighbors (including *B. thuringiensis* var. kurstaki) and historical (background) high-volume air samples obtained from across the greater Washington, DC, area over a period of several years. No cross-reaction with near neighbors or reaction with background air samples was observed during validation.

Transport and Dispersion Modeling and Collector Placement

The releases were simulated using the Quick Urban and Industrial Complex (QUIC) transport and dispersion modeling system, a code that has been extensively evaluated

against urban tracer experiments[7-9] and is used by numerous universities and government agencies. QUIC rapidly computes 3-dimensional wind fields around buildings and calculates high-resolution gaseous and particulate plume dispersion and deposition around buildings using a building-aware random-walk transport and dispersion model.

In 2005, model results indicated little material was expected beyond the boundaries of the facility. Because of this, one collector was sited within the facility boundaries, inside the subway station, below ground level.

In 2009, historical (9-year averaged) wind data collected near the facility during the month of the release were obtained, and QUIC was used to estimate the plume's downwind extent. Neither the final amount of material to be released nor the expected conditions at the time of the release were known prior to the day of the release, so 3-g and 3,000-g plumes (arbitrarily chosen values) were generated using the historical data.

The 2009 plume modeling indicated the significantly smaller sized particles should be detectable further downwind than in 2005. Historical wind data additionally indicated that winds were likely to be from the south-southeast (between 180° and 225°) on the morning of the release. Because of these parameters, an approximately 45° arc was defined as the target region for sampling (Figure 2). One high-volume air sampler (a portable sampling unit, or PSU) was placed inside the subway station adjacent to the facility prior to the release. Three PSUs were placed outside the facility boundaries at 2.5, 3.2, and 3.5 km downwind (Figure 2). PSU #4, at 3.5 km downwind, was also located inside a subway station below ground level. Final locations were determined by the availability of power and access to the site and by local permitting requirements.

To better approximate the presumed amount released, 30-g plumes (Figures 3 and 4) were calculated postrelease using wind measurements from nearby stations for both the 2005 and 2009 release periods. A log-normal particle-size distribution with a mass median diameter of 177 microns and standard deviation of 2.5 was used in the simulation of the 2005 release.[6] A log-normal particle-size distribution

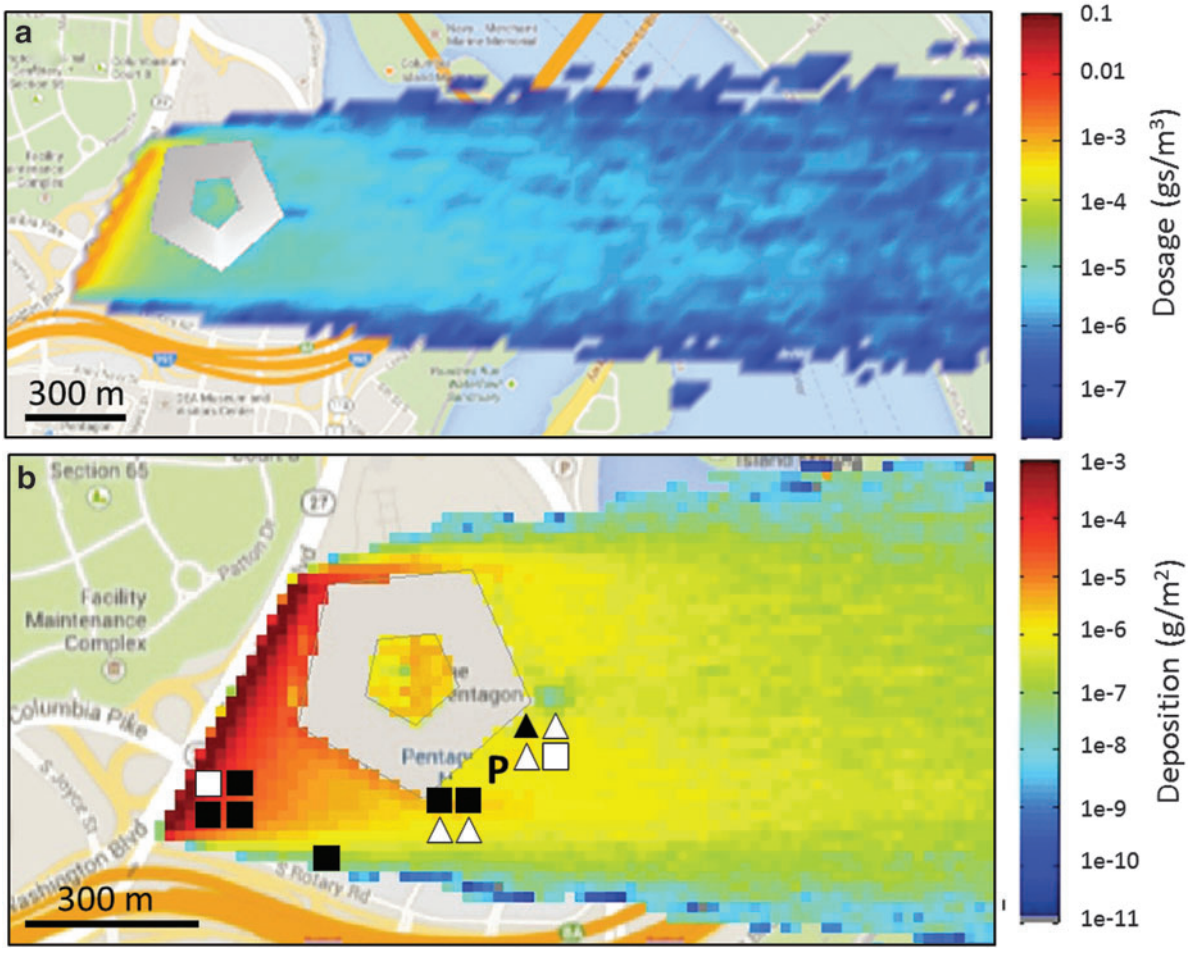

Figure 3. Calculated downwind dispersion (a) and deposition (b) footprints for the 2005 release. Sample locations are shown in (b). The PSU is shown as a P. Outdoor environmental sample locations are shown as squares. Indoor (subway station) environmental sample locations are shown as triangles. Positive environmental sample locations are solid black; negatives are white.

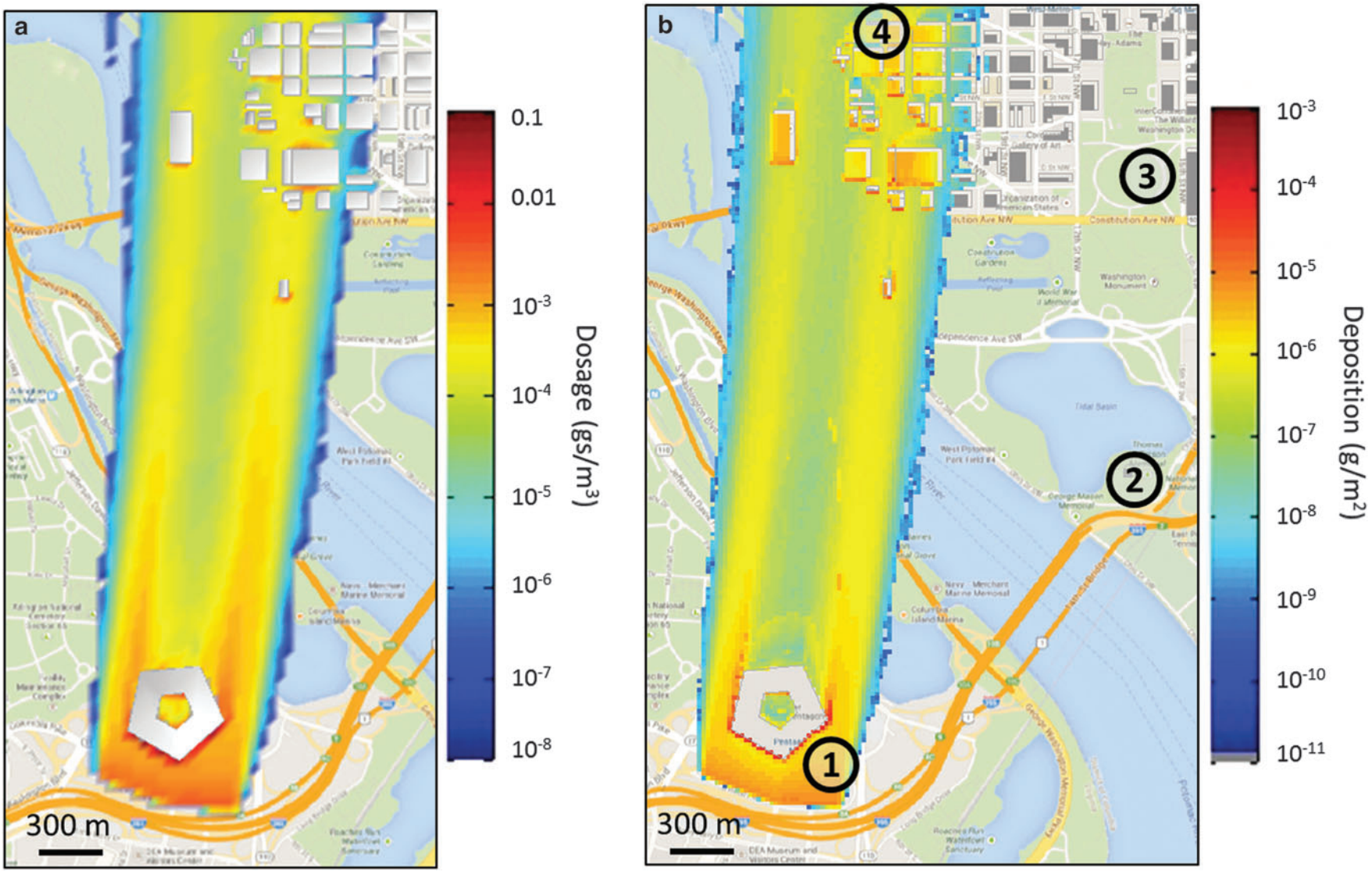

Figure 4. Calculated downwind dispersion (a) and deposition (b) footprints for the 2009 release. Sample locations are shown by site number in (b).

with a mass median diameter of 2.5 microns and standard deviation of 2.5 was used in the simulation of the 2009 release.

Air Sample Collection

High-volume air samples were collected using the Portable Sampling Unit-2 (PSU-2; Hi-Q Environmental Products) as described in Van Cuyk et al.[10] The PSU is equipped with a size-selective PM_{10} inlet, which excludes 50% or more of all particles greater than 10 microns in diameter, thereby selectively collecting particles in the respirable range. Each sampler was adjusted to a flow rate of 100 liters per minute at the start of the sampling period. PSU samples were collected using 47-mm Fluoropore membrane filters (Millipore) enclosed in sealed aluminum holders.

In 2005, background samples were collected 48 hours, 24 hours, and immediately before the release. Background air samples previously collected over a span of several years from other PSUs across greater Washington, DC (not discussed herein), were also analyzed during validation of the assay and were negative for *B. amyloliquefaciens*. A single sample was collected from the subway PSU 6 hours after the release.

In 2009, 1 set of 24-hour background air samples was collected 24 hours before the release. Samples were then collected at 12, 24, 48, and 72 hours postrelease, with the PSUs running continuously between samples.

In 2009, 4 personal air samples were collected to assess exposure to sampling personnel. At each sampling location, a preloaded mixed-cellulose ester (MCE) cassette (Zefon International) was attached via tygon tubing to a personal air sampling pump (SKC, Inc.) precalibrated to a flow rate of 3-4 liters per minute. The pumps were attached to sample collection personnel who wore them during their sampling activities (ie, these pumps moved around the sampling site with the sampling personnel). Following the completion of sampling activities, the used cassettes were capped.

Environmental Sample Collection

In 2005, swipe, soil, and water samples were collected 40 hours postrelease. A snowstorm occurred between the release and sample collection, and because of this, water samples were taken from standing water from the snowmelt. In 2009, postrelease swipes were collected 12 hours following the release.

Swipe samples were collected using procedures recommended by the Centers for Disease Control and Prevention (CDC),[11] with 1 exception: Sterile noncotton gauze wipes (3 in x 3 in) were moistened with 500 µL phosphate buffer solution (Fisher), rather than the 5 mL CDC recommends. A 1-m^2 area was wiped, making 2 passes of the entire sampling area using vertical then horizontal S-strokes. The swipe was placed in a sterile 50-mL vial that was capped and sealed with Parafilm.

Soil samples were collected with a sterile spatula, as described in Van Cuyk et al.[12] Between 30 and 50 mL of solid soil were collected from the top 5 cm and placed in a sterile 50-mL vial that was capped and sealed with Parafilm.

Water samples were collected via sterile pipette, as described in Van Cuyk et al;[12] 10 mL of standing water was collected and placed into a 50-mL vial that was capped and sealed with Parafilm.

Sample Handling and Transport

As described in Van Cuyk et al,[12] samples were collected by personnel wearing clean, disposable nitrile gloves and booties. Gloves were changed after collection of each sample. Sample data were recorded on log sheets and in an electronic database. All samples were placed in chain of custody bags after collection, and chain of custody procedures were followed. Prior to transport to the analytical laboratory, the chain of custody bags were wiped with a hospital-grade bleach wipe (Dispatch brand, Medline), allowed to air dry, and placed in a clean secondary container. Ten percent, or a minimum of 1 per day, of the total number of each sample type were collected as field blanks to verify that no cross-contamination occurred.

Sample Analysis

Upon receipt at the laboratory, all chain of custody bags were bleach-wiped and allowed to air dry. The bags were opened with a sterile scalpel, and personnel wearing clean nitrile gloves removed the primary sample containers, which were bleach-wiped and allowed to air dry.

Samples were extracted using a procedure similar to that described in EPA 2002.[13] Filters and swipes were placed into 30.0-mL screw cap conical tubes containing 20 mL PET buffer at 4°C (100 mM sodium phosphate with 10 mM EDTA and 0.01% Tween 20, Teknova) and vortexed for 20 minutes. The liquid was transferred to a 50-mL vial. The samples were washed again using 14 mL PET buffer, and the liquid from the second wash was combined with the liquid from the first. Samples were centrifuged for 30 minutes at 3,500 × *g* at 4°C. The supernatant was discarded and the pellet resuspended in 25 mL PET buffer by vortexing. The resuspended samples were centrifuged for 30 minutes at 3,500 × *g* at 4°C. 22 mL of supernatant was discarded, and the pellet was resuspended in the remaining 3 mL by vortexing. 0.5 mL suspension were transferred to a 2-mL bead beating tube containing 50 mg of ≤106-micron and 50 mg of 425-600 micron acid-washed glass beads (Sigma-Aldrich). Tubes were placed in a Mini-BeadBeater-96 (BioSpec Products, Inc), beaten at the highest setting for 5 minutes, then removed and cooled on ice for 2 minutes.

Tubes were centrifuged at 7,000 rpm for 4 minutes; then 250 µL supernatant was removed from each tube and extracted using a MultiScreen Vacuum Manifold and Millipore plates (EMD Millipore). Samples were loaded into a 0.22-µm 96-well filter plate. A vacuum of 17.5 mm Hg was applied until all wells in the plate were empty. The filtrate was collected into a MultiScreen PCR 96-Well filter plate. The

Table 1. 2005 Postrelease Sample Results by Sample Type and Location

Sample	*Type*	*Location Type*	*PCR Result (+ or −)*
P	PSU filter	subway	+
A	water	outdoor	−
B	water	outdoor	+
C	water	outdoor	+
D	water	outdoor	+
E	soil	outdoor	+
F	swipe	under bus shelter	+
G	swipe	under bus shelter	+
H	swipe	subway	−
I	swipe	subway	−
J	swipe	subway	+
K	swipe	subway	−
L	swipe	subway	−
M	swipe	outdoor	−

Note. The letter in the first column corresponds to the location in Figure 1. + indicates the sample produced a PCR positive; − indicates it did not amplify (PCR negative).

filtrate plate was placed on top of a 96-well filter-to-waste plate, and a vacuum of 17.5 mm Hg was applied until all wells in the plate were empty. 150 µL 1X TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0, Teknova) was added to each well, and a vacuum of 17.5 mm Hg was applied until all wells in the plate were empty; this step was repeated 3 additional times. The resulting samples, still in the Multi-Screen PCR 96-Well filter plate, were resuspended in 100 µL DNA- and DNase-free water and mixed using the "mix" feature on the multichannel pipette (Matrix Technologies Corp).

Erwinia herbicola DNA (American Type Culture Collection) was added to each extract as an internal PCR inhibition control. Samples that exhibited inhibition (defined as >3 cycle thresholds above the average value for the internal control) were diluted to 1:5 and 1:10 in DNA- and DNase-free water and reanalyzed. Samples were screened in duplicates against a panel of 5 *B. amyloliquefaciens* assays using an Applied Biosystems Prism® 7000 thermocycler (Life Technologies). Tests were considered positive according to standard thresholds employed by government testing programs and if both duplicates were positive for 4 or 5 (of 5 total) signatures.

In 2009, PCR-positive samples were cultured using a procedure similar to that in EPA 2002.[13] An aliquot of the original wash was heat-treated (80°C for 10 minutes) to kill vegetative cells. 100 µL of sample, or 3 serial dilutions (10^0 to 10^{-2}), were plated on tryptic soy agar medium (Becton Dickinson/Fisher Scientific) containing cycloheximide (50 mg/liter, Sigma-Aldrich). Plates were incubated at 36°C for 24 hours and then observed for colonies with *B. amyloliquefaciens* morphology. Colony identity was confirmed by PCR.

Results

All background PSU samples from the facility site and multiple samples from other PSUs across the region were negative for *B. amyloliquefaciens* prior to both the 2005 and 2009 releases.

In 2005, the sample collected 6 hours postrelease from the PSU in the adjacent subway station was positive for *B. amyloliquefaciens*. Seven of 13 environmental samples collected 40 hours postrelease also tested positive (Table 1).

In 2009, samples collected at 12 hours postrelease from the 2 PSUs closest to the release were PCR-positive for *B. amyloliquefaciens.* Within 24 hours, samples taken from all 4 PSUs were positive (Table 2).

Table 2. 2009 PSU Results

			Results (PCR, Culture)				
Location	*Type*	*Distance from Release*	*Background*	*12 hrs*	*24 hrs*	*48 hrs*	*72 hrs*
1	Subway	0 km	−	+,+	+,+	+,+	−
2	Outdoor	2.45 km	−	+, NG	+, NG	+,+	−
3	Outdoor	3.15 km	−	−	+,+	−	+, NG
4	Subway	3.45 km	−	−	+,+	−	−

Note. The number in the first column corresponds to the locations shown in Figure 2. + indicates the sample produced a PCR positive; − indicates it did not amplify. If the sample amplified, it was subsequently cultured. A second + indicates the culture was positive; NG indicates no growth of *B. amyloliquefaciens*–type colonies was observed.

A total of 36 surface swipe samples were collected 12 hours after the 2009 release: 6 at site 1, 10 at site 2, 15 at site 3, and 5 at site 4 (see Figure 2 for locations). Three of these swipe samples were PCR-positive. Of these, 2 were from site 3, which was outdoors, and 1 was from site 4, inside the subway. Cultures indicated about 10^2 colony-forming units per swipe. Four personal air samples were also collected. One personal air sample, from site 2, was PCR-positive, but no culture growth was observed.

All field blanks were negative for *B. amyloliquefaciens* by PCR. No-template controls were run on each PCR plate, and all were negative.

Discussion

An aerosolized biological agent release in an urban area has the potential to produce high morbidity, mortality, and social disruption. It is therefore incumbent on the US government to develop mitigating tactics, techniques, and procedures to protect the public. Environmental monitoring programs have been deployed in many locations by both the civilian and military communities to reduce morbidity and mortality by expediting the decision to distribute medical countermeasures. Typically, these programs involve continuous high-volume air sampling, with samples transported to a laboratory for analysis and, following a positive result, using surface sampling to obtain additional samples for culture or delineate the boundaries of the contaminated region.

Although environmental sampling methods are well established and accepted in other fields, their application for the detection of a biological release has come under scrutiny,[14-17] including in a recent multipart series in the *Los Angeles Times,* which alleged that "field tests and computer modeling … suggest it would have difficulty detecting [a release]. In an attack by terrorists or a rogue state, disease organisms could well be widely dispersed, at concentrations too low to trigger [the system] but high enough to infect thousands of people.… Even in a massive release, air currents would scatter the germs in unpredictable ways."[18]

One of the key arguments voiced by critics of environmental detection is the lack of publicly available "operational data." The PFPA releases provided an opportunity to obtain such data against a scenario similar to one often used in biothreat exercises: a line source release of a dry powder in an urban area. The material released was a reasonable surrogate for actual weapons based on the particle sizes used with the 2005 release representing a crude preparation and the 2009 material, a more refined one. The use of air and surface sampling was designed to replicate operational environmental sampling programs, such as BioWatch.

The data from the 2005 larger particle release demonstrated that a high-volume air sampler was able to detect the agent in the vicinity of the release (the high-volume air sampler, or PSU, is shown as a "P" in Figure 3). This technique was further validated in 2009 with multiple samplers, at distances up to 3.5 km downwind, detecting less than 50 g of released simulant.

The high-volume air sampler (PSU) in the subway station adjacent to the facility produced positive samples in both 2005 and 2009 and demonstrated that an above-ground release can be detected below the surface. This is not surprising given the proximity of this collector to the release location and known instances of bioagent transport into buildings.[19] However, the transport of a biological agent into the subway system has serious implications, including possible exposure of subway patrons who were not in the vicinity of the original release, and the need to decontaminate this critical urban infrastructure as part of restoration efforts.[20] The 2009 positive sample at PSU collector #4, inside a subway station 3.5 km downwind of the release, provides additional evidence of subway infiltration. However, due to the limited number of PSU collectors in this experiment, it is not evident whether the agent infiltrated into this particular subway station above ground or was transported through the subway tunnels.

The modeled 2005 and 2009 plume and deposition boundaries (generated using meteorological measurements taken in the vicinity of and at the target times of the releases) are shown in Figures 3 (2005) and 4 (2009). Although the 2005 model results are not in complete agreement with the experimental results, the model does estimate heavy deposition (Figure 3b), which corresponded with a large number of positive surface swipe, soil, and water samples collected postrelease (7 of 13 environmental samples, or 54%). Likewise, the 2009 model estimates a more substantial aerosol plume (Figure 4a) and less deposition (Figure 4b) because of the smaller particle size, and a correspondingly smaller number of surface positives were observed (3 of 36, or 8%). Some disagreement between the model and the surface sampling results is expected given the turbulent nature of the local winds in urban areas and the low recovery efficiencies expected from surface environmental samples (such as swipes),[21-23] particularly when the actual location of the target analyte (in this case, deposited *B. amyloliquefaciens*) is unknown.

PSU air samples collected in 2009 were positive up to 72 hours postrelease, the last aerosol samples collected. Air samples from all 4 PSUs were positive 1 or more times. Although reaerosolization is expected following a biological release,[24] the duration of the observed reaerosolization is notable since it is plausible that reaerosolization continued more than 3 days postrelease. The pattern of positives generated by reaerosolization is also interestingly erratic: For example, air samples from collector #3 were positive at 24 and 72 hours postrelease, but not at 48 hours. Reaerosolization may also explain the positive samples observed at collector #2, located outside the initial modeled plume. These results raise an intriguing question: How would continued, and possibly intermittent, positive air samples, at multiple sites, be interpreted in a real event? Bioaerosol

releases are often considered to be discrete events: A plume is released, moves through an area, and is dispersed. In this context, seemingly erratic positives on multiple days might be interpreted as evidence of "reload," or subsequent attacks, rather than reaerosolization. Reaerosolization of bioagents in urban areas is poorly understood. The 2009 data indicate that it is worthwhile to consider whether reaerosolization and "reload" could be distinguished and what policies should be developed in cases where they cannot.

In the 2009 release, *B. amyloliquefaciens* was successfully cultured from 6 of 9 PCR-positive PSU samples, including samples collected 48 hours postrelease. *B. amyloliquefaciens* is a spore-former and should be environmentally persistent. These data suggest that viability was maintained despite the relatively harsh aerosol sampling conditions. Following an event, obtaining viable sample will be important to understand specific characteristics of the agent released, such as antibiotic susceptibility, and for forensic and attribution purposes. One of the concerns that has been voiced regarding air sampling for bioagents is the perceived loss of viable agent and the information it could provide.[25] While this is likely true for less hardy agents, these data suggest that air sampling can produce viable spores for culture and analysis. Many plans to respond to an aerosolized biological attack include collecting environmental surface samples to obtain viable sample. However, this may not be necessary following a *B. anthracis* detection from an air sampler. This result deserves more study, because postevent sampling puts sampling personnel at risk and, as our data suggest, may not be as reliable as the primary aerosol sample.

Only 1 of 4 (25%) personal air samples collected in 2009 was PCR-positive postrelease. Some positives should be expected given the results of previous personnel exposure studies conducted by Van Cuyk et al[10] and Byers et al.[26] The number of nondetects is interesting, though, since all 4 samples were collected in the vicinity of positive PSU samples. The effectiveness of any air sampler is dependent on the amount of sample that can be collected in a given period of time—that is, the integrated exposure. Low-volume personal air samplers are inherently less sensitive than high-volume samplers when operated under similar conditions for a similar period of time, which likely explains the negative results. There has been debate about whether it would be more effective to use personal air samplers on first responders and law enforcement personnel as opposed to stationary high-volume samplers.[25] However, the performance of the personal air samplers in this study, particularly as compared to the PSUs, does not support this approach.

Conclusion

There has been skepticism surrounding the ability to detect an aerosolized bioagent released in an urban area, partly driven by the lack of operationally relevant experimental data in the open literature. Two field experiments conducted in concert with releases of *B. amyloliquefaciens,* designed to simulate real-world bioattack scenarios, demonstrate that high-volume air samples analyzed using commonly employed nucleic acid–based methods can detect a biological agent release over several kilometers downwind from the release. Environmental surface samples collected postrelease were also positive; however, their rate of detection was dependent on the size of the particles released, probably because of the direct relationship between size and amount of deposition. Viable *B. amyloliquefaciens* were cultured from several high-volume air samples. Sample collection equipment and procedures were deliberately designed to represent those used in civilian or military programs, and analytical techniques were directly adapted from US government protocols, so these results should give a reasonable representation of the way such systems would perform during a real bioterror event.

In the realm of bioterrorism, the key to a successful outcome is timely identification and mitigation. Traditional pathogen detection, whether by case recognition from an "astute clinician," biosurveillance using epidemiology and data analysis algorithms, or even point-of-care testing, all have a common starting point: an exposed patient who has become symptomatic. In the case of a large-scale attack, public health will be under extreme duress to muster resources and execute plans to mitigate impacts if response actions begin after the exposed have become symptomatic. Early detection through high-volume air sampling, followed by strategically collected surface samples, can play an essential part in the US government's biodefense programs by providing local public health agencies with additional time to mount a response before patients present to hospitals with symptomatic complaints and accompanying morbidity and mortality. Additional studies should be performed to test and understand the capabilities and limitations of environmental detection, as it is an important piece of our nation's ability to respond effectively to a biological attack.

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