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**Special Feature: Remediation**

# Transport of *Bacillus Thuringiensis* var. Kurstaki Via Fomites

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The intentional and controlled release of an aerosolized bacterium provides an opportunity to investigate the implications of a biological attack. Since 2006, Los Alamos National Laboratory has worked with several urban areas, including Fairfax County, VA, to design experiments to evaluate biodefense concepts of operations using routine spraying of *Bacillus thuringiensis* var. kurstaki (*Btk*). *Btk* is dispersed in large quantities as a slurry to control the gypsy moth, *Lymantria dispar*. Understanding whether personnel and equipment pick up residual contamination during sampling activities and transport it to other areas is critical for the formulation of appropriate response and recovery plans. While there is a growing body of literature surrounding the transmission of viral diseases via fomites, there is limited information on the transport of *Bacillus* species via this route. In 2008, LANL investigated whether field sampling activities conducted near sprayed areas, post-spray, resulted in measurable cross-contamination of sampling personnel, equipment, vehicles, and hotel rooms. Viable *Btk* was detected in all sample types, indicating transport of the agent occurred via fomites.

The gypsy moth, *Lymantria dispar*, is a major forest pest along the eastern seaboard and in the midwestern United States. Over the past 20 years, many thousands of acres of land have been sprayed with pesticides to suppress or eradicate gypsy moth populations. The most common pesticide used is a water-based slurry containing *Bacillus thuringiensis* var. kurstaki (*Btk*). This bacterium produces a toxin that is lethal to gypsy moth caterpillars.

The intentional and controlled release of an aerosolized bacterium provides an opportunity to investigate the implications of a biological attack. While the application of *Btk* does not resemble a typical state-sponsored release scenario involving a dry, weaponized powder, analysts have noted it is a "technically feasible" and effective (albeit simplistic) means of producing inhalational exposures.[1] Since 2006, Los Alamos National Laboratory (LANL) has worked with several urban areas, including Fairfax County, VA, to design experiments to evaluate biodefense concepts of operations using normal *Btk* spray activities.

In formulating appropriate response and recovery plans, it is critical to understand whether personnel and equipment pick up residual contamination during sampling activities and transfer it to other areas. There is a growing body of literature surrounding the transmission of viral diseases

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via fomites. Boone and Gerba have reported "proven" or "accepted" transmission of respiratory syncytial virus, rhinovirus, influenza virus, rotavirus, hepatitis A virus, and adenovirus via fomites.[2] Lederman and colleagues reported the suspected transmission of vaccinia virus via fomites.[3] However, there is limited information in the peer-reviewed literature on the transport of *Bacillus* species via this route. In a 2008 review of the persistence of Category A select agents, Sinclair and colleagues noted, "Knowledge of the survival of *B. anthracis* on fomites is fairly limited,"[4(p558)] citing only 2 papers in the peer-reviewed literature.[4]

To address this gap, in conjunction with *Btk* spraying in Fairfax County, VA, in 2008, LANL collected samples from the backpacks, vehicles, and hotel rooms of personnel who performed aerosol sampling near a spray area after spraying had occurred, as well as dry gauze that had been attached to their persons, to determine whether viable *Btk* contaminates personnel, accessories, and secondary spaces at detectable levels.

## Experimental Conditions

In 2008, Fairfax County sprayed 46 blocks with *Btk*, covering 3,500 total acres (Figure 1). Aerial spraying began on May 2 and continued through May 10. LANL conducted an experiment in Mason District Park, immediately adjacent to Spray Block 20 (Figure 2), between April 30 and May 8 to determine whether field sampling activities conducted in close proximity to sprayed areas resulted in measurable cross-contamination of sampling personnel, backpacks, vehicles, and hotel rooms. In coordination with Fairfax County, Spray Block 20 was the first block sprayed on May 2, 2008.

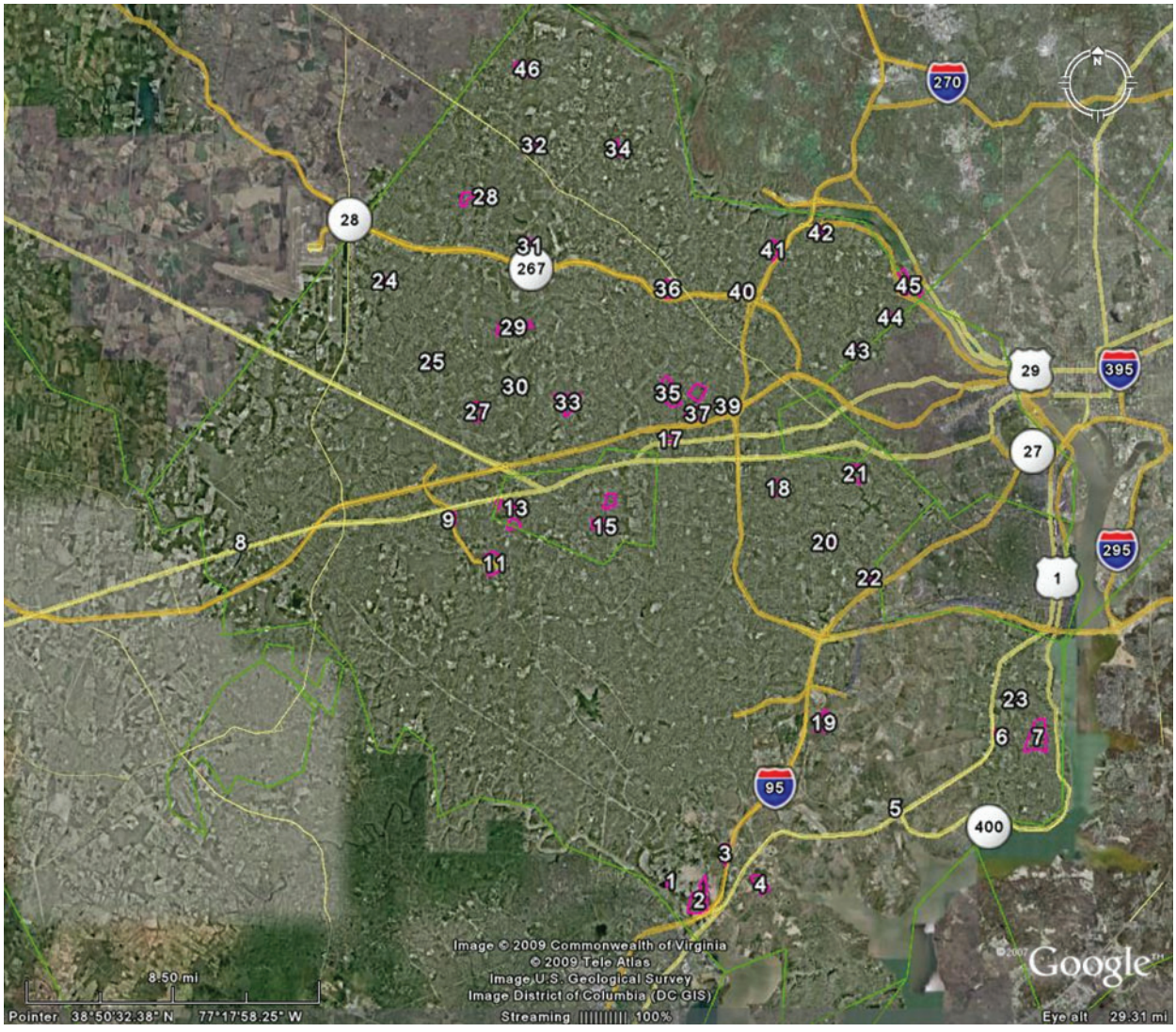


Figure 1. Map of Fairfax County 2008 *Btk* Aerial Spray Blocks. Counties are outlined in green; Fairfax County spray blocks are outlined in pink.

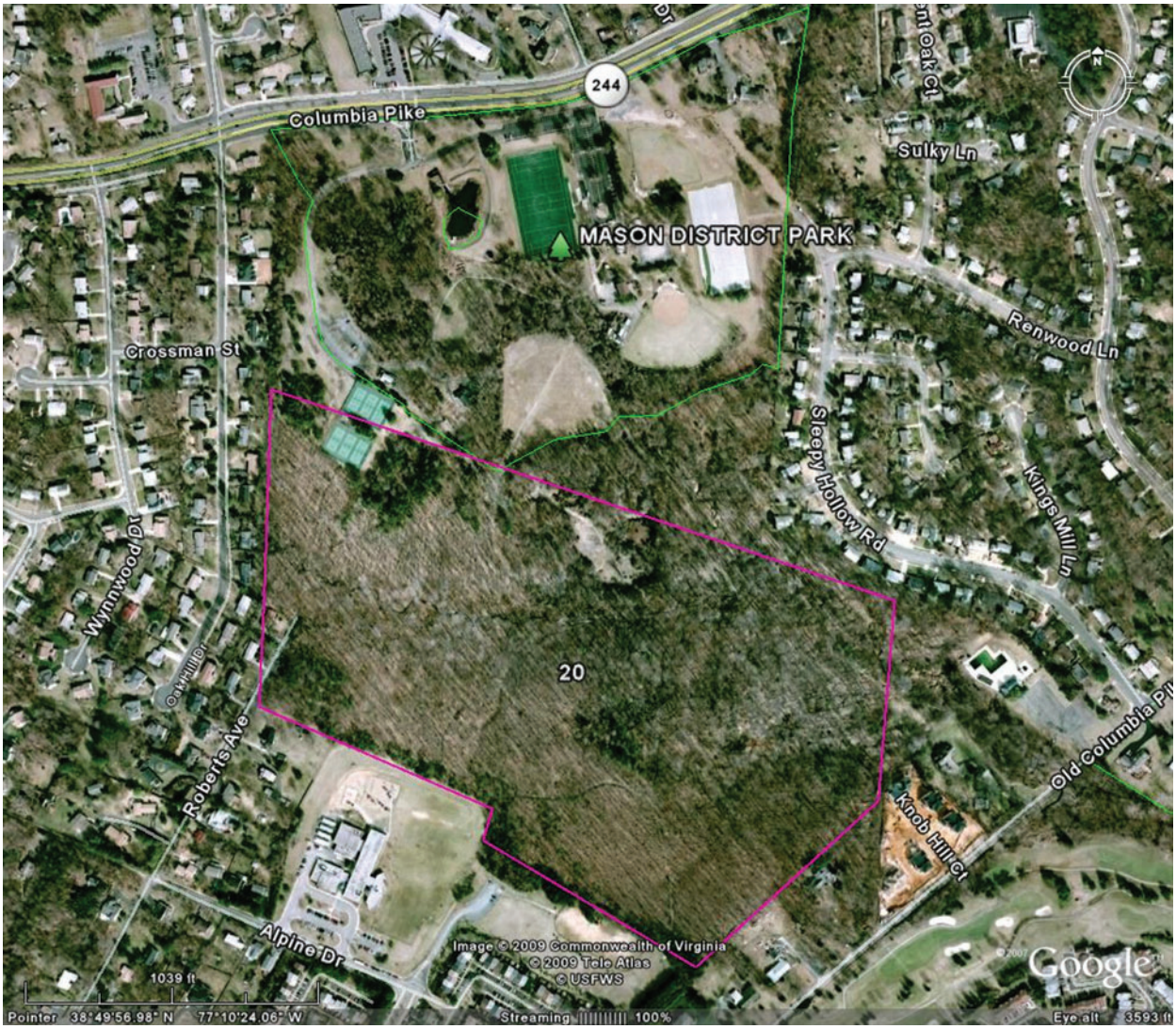


Figure 2. Mason District Park (outlined in green) and Spray Block 20 (outlined in pink).

A sample of the spray suspension (Foray 76B, Valent Biosciences) was obtained from the Fairfax County Gypsy Moth Suppression Program authorities for analysis using culture methods. (The manufacturer optimizes the suspension for toxin activity and does not perform direct measurements of *B. thuringiensis* concentration.) An aliquot of the sample was diluted 1,000-fold in phosphate buffer solution (PBS, Fisher Scientific) and heat-treated at 80°C for 10 mins. Serial dilutions of this preparation (100 µL aliquots) in PBS were plated onto trypticase soy agar (Becton Dickinson and Co., Fisher Scientific) with cycloheximide (50 mg/L, Sigma-Aldrich), and colony counts were obtained after overnight incubation at 37°C. Triplicate measurements gave an average viable *Btk* density of $1.3 \times 10^9$ colony-forming units (CFU) per ml suspension with a standard deviation of $5 \times 10^8$. Additional studies with Foray 76B demonstrated no significant difference in viable *Btk* density with or without heat treatment, indicating the presence of vegetative (ie, nonsporulated) *Btk* in the spray suspension is negligible.

Fairfax County applies the Foray 76B via spraying from a helicopter at a rate of 0.5 gallons per acre. Based on the measured concentration of viable agent, this equates to $2.5 \times 10^{12}$ CFU applied per acre. Spray Block 20 is approximately 63 acres; an estimated $1.6 \times 10^{14}$ total CFU were therefore applied to Block 20. Using a value of $3.3 \times 10^{-11}$ grams/wet spore,[5] an estimated 5.1 kg viable *Btk* were applied in Block 20. The material is distributed in large droplets using a rotary atomizer and is intended to stick to the foliage; measured at the canopy height, the droplets have a median volume diameter of 125 µm and median number diameter of 80 µm. The distribution of the droplet sizes, however, can be reasonably approximated as log-normal, which means at least a portion of droplets produced are in the respirable (1-10 µm) range.

Figure 3 shows the 24-hour precipitation levels measured at Ronald Reagan National Airport, located approximately 7 miles east of Block 20, and Dulles International Airport, located approximately 16 miles northwest of Block 20,

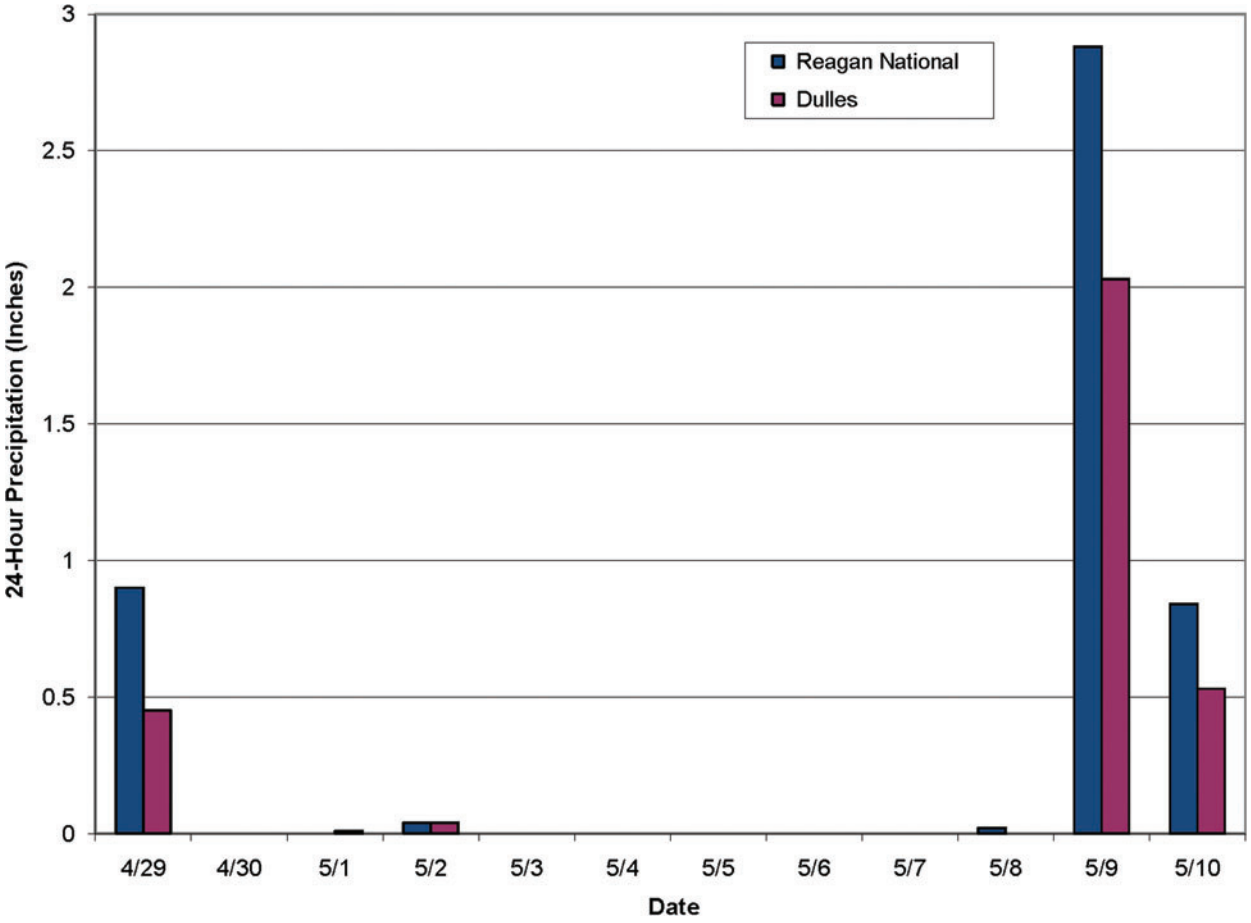


Figure 3. 24-hour integrated precipitation (measured at 7:52 AM each day) over the experimental period. Color images available online at www.liebertonline.com/bsp

during the study period. Precipitation for each 24-hour period was measured at 7:52 AM local time, and the value shown (in inches) is for accumulation over the previous 24 hours. The experimental period was generally dry, but there was heavy rain on the last day of the study.

Figure 4 shows the wind rose measured at Ronald Reagan National Airport over the period May 2-9, 2008. Winds were typically from the southwest during the experimental period, indicating a high probability of airborne transport of *Btk* from the spray zone to Mason District Park, where sampling activities were conducted. However, a significant occurrence of winds from the northwest is also observed. This pattern of winds is typical for this region and time period, as seen in Figure 5, the 9-year average wind rose.

## Methods

### *Aerosol Sample Collection*

Five aerosol samplers were placed in Mason District Park, just north of the spray block, to determine whether *Btk* was transported to the sample collection area. Four of these were sited around the amphitheater, and the fifth unit was sited near the restroom (Figure 6). The number and configuration of the collectors were constrained by both the need to minimize the experiment's impact on routine use of the park as well as availability of electricity.

Aerosol samples were collected using the Portable Sampling Unit-2 (PSU-2; Hi-Q Environmental Products). The PSU-2 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. Aerosol samples were collected in 24-hour increments onto a 47-mm Fluoropore membrane filter (Millipore). The filters were enclosed in sealed aluminum holders. The holders were placed in a chain of custody bag. Prior to transport to the analytical laboratory, the chain of custody bag was wiped with a hospital-grade bleach wipe (Dispatch brand, Medline), allowed to air-dry, and placed in a clean secondary container.

One set of samples was collected before spraying began as background. Post-spray, samples were collected each day

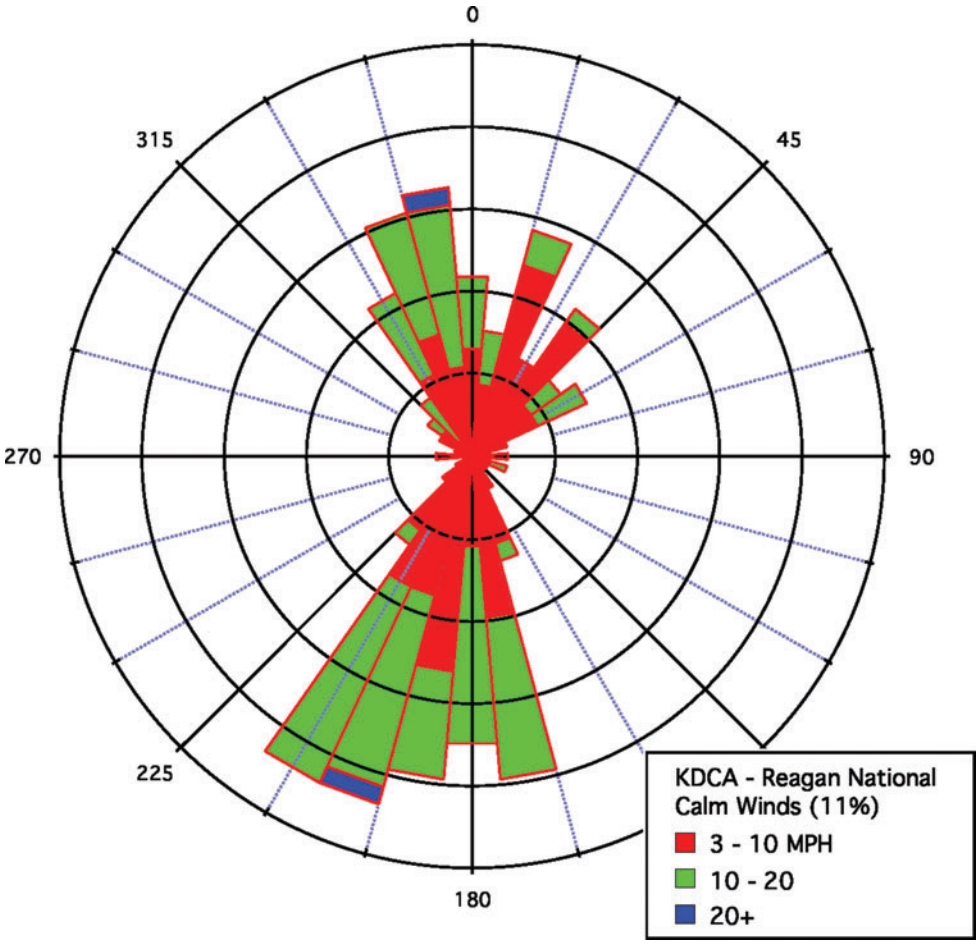


Figure 4. Wind rose measured at Ronald Reagan National Airport, May 2-9, 2008. Color images available online at www.liebertonline.com/bsp

for 1 week, May 3-9, 2008. All 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.

Each PSU-2 was covered with a large plastic bag during *Btk* spraying to minimize deposition on the unit. The bags were removed on May 2 after aerial spraying was completed. The size-selective $PM_{10}$ inlets were replaced at the start of each sampling cycle to reduce the likelihood of sample contamination from prior deposition.

## *Vacuum Sample Collection*

Vacuum samples were collected from the vehicle and from hotel rooms using the 3M Trace Evidence Collection Filter (3M) inserted into a HEPA Dirt Devil Classic handheld vacuum cleaner. Team members collected 1-2 tablespoons of vacuumed debris over a 1-$m^2$ surface, making 2 passes of the entire sampling area at a slow rate (12 in per 5 sec) using vertical then horizontal S-strokes. The 3M Trace Evidence Collection Filters were recapped, taped shut with tamper-indicating tape, and placed in a chain of custody bag. Prior to transport to the analytical laboratory, the chain of custody bag was wiped with a hospital-grade bleach wipe (Dispatch brand, Medline), allowed to air-dry, and placed in a clean secondary container. One set of samples was collected before spraying began as background. Post-spray, samples were collected each day for 1 week, May 3-9, 2008. All 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.

## *Swipe Sample Collection*

Swipe samples were collected using sterile noncotton gauze wipes (3 in × 3 in) moistened with 500 µL phosphate buffer solution (Fisher). 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 conical vial, which was capped and sealed with Parafilm. The sealed vial was placed in a chain of custody bag. Prior to transport to the analytical laboratory, the chain of custody bag was wiped with

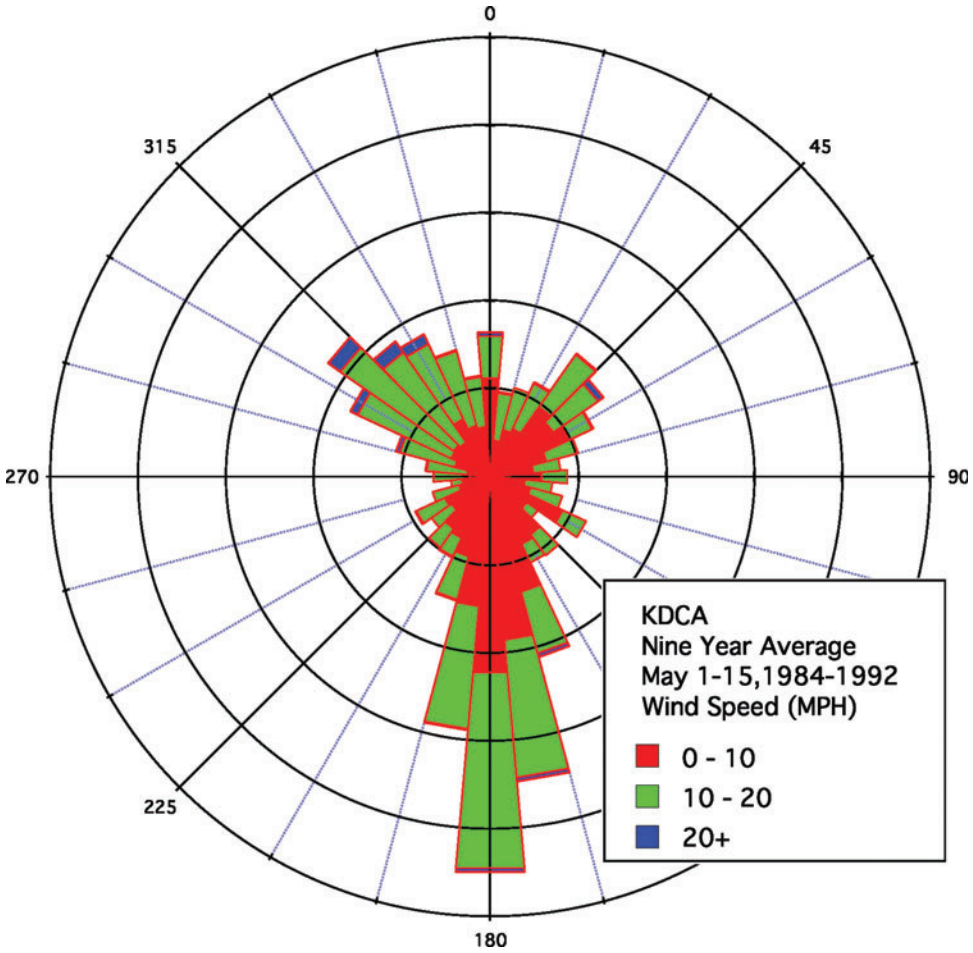


Figure 5. Wind rose measured at Ronald Reagan National Airport, 9-year average (1984-1992), May 1-15, 2008. Color images available online at www.liebertonline.com/bsp

a hospital-grade bleach wipe (Dispatch brand, Medline), allowed to air-dry, and placed in a clean secondary container.

One set of samples was collected before spraying began as background. Post-spray, samples were collected each day for 1 week, May 3-9, 2008. All 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.

## *Personnel Sample Collection*

Three dry gauze wipes (3 in×3 in) were secured to sampling personnel (1 on the arm, 1 on the knee, and 1 on the back between the shoulder blades; Figure 7) using tape prior to aerosol sample collection activities in Block 20. At the end of sampling activities, each wipe was placed in a sterile 50-mL conical vial, which was capped and sealed with Parafilm. The sealed vial was placed in a chain of custody bag. Prior to transport to the analytical laboratory, the chain of custody bag was wiped with a hospital-grade bleach wipe (Dispatch brand, Medline), allowed to air-dry, and placed in a clean secondary container.

One set of samples was collected before spraying began as background. Post-spray, samples were collected each day for 1 week, May 3-9, 2008. All 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.

## *Additional Sampling Precautions*

The hotel sampled was located in Merrifield, VA. The distance from the hotel to the nearest spray block (block 37 in Figure 1, located northwest of the hotel) was 1.3 miles. The distance from the hotel to the next nearest spray block (block 39 in Figure 1, located north of the hotel) was 1.4 miles. No spray blocks were located south or southwest of the hotel. As indicated in Figure 4, the winds during the experiment were predominantly from the south and southwest, with non-negligible contributions from the north-northwest.

LANL negotiated with their lodging provider to sample a "new" (ie, previously unused by sampling personnel) hotel room each day to minimize the possibility that material collected was deposited by the previous day's activities.


Figure 6. Location of aerosol samplers in Mason District Park (red icons). Spray Block 20 is outlined in pink.

As noted above, sample collection personnel wore clean gloves and nitrile booties while collecting all samples. No other personal protective equipment was worn, and no special precautions were taken when entering or leaving the sampling area (Mason District Park).

Of the total number of samples, 5% were taken as field blanks. At least 1 field blank for each medium was delivered to the analytical lab each day to ensure no cross-contamination of samples occurred.

## *Sample Analysis*

Upon receipt at the analytical laboratory, all sample bags were bleach-wiped and allowed to air-dry. The bags were cut open with a sterile scalpel, and the primary sample containers were removed by personnel wearing clean nitrile gloves. The containers were then bleach-wiped and allowed to air-dry.

All sample media were extracted using PET buffer (100 mM sodium phosphate, 10 mM EDTA, 0.01% v/v Tween-20, pH 7.4). A 100-µL aliquot of the extract was plated onto trypticase soy agar (Beckton Dickinson and Co., Fisher Scientific) with cycloheximide (50 µg/L, Sigma-Aldrich), and colony counts were obtained after overnight incubation at 37°C. Colonies exhibiting *B. thuringiensis* morphology were analyzed using polymerase chain reaction (PCR) to confirm their identity as *Btk.*

Five percent of the total number of samples, and at least 1 of each type of medium per day, were analyzed as field blanks. All field blanks were negative for viable *Btk.*

## Results

Table 1 presents aerosol sample results. One of 5 background aerosol samples (collected on May 1, 2008, prior to

Figure 7. Photograph of a gauze wipe attached to a sampler's arm. Color images available online at www.liebertonline.com/bsp

spraying) tested positive by culture. Four of 5 background aerosol samples were negative by culture. While it is not possible to unambiguously determine the source of the material that resulted in 1 background aerosol positive, it is not likely due to cross-contamination as all blanks tested were negative. Fairfax County has had an active gypsy moth suppression program since 1982, and *Btk* has been extensively disseminated in the county, including areas near Mason District Park. Recent studies indicate *Btk* can persist for 4 years in urban environments,[6] so it would not be unexpected if background *Btk* was detected from previous sprays. Post-spray, between 4 and 5 (of 5 total) samples each day tested positive by culture, with viability in CFUs typically 1 to 2 orders of magnitude higher than the concentration of the positive background sample.

Table 1. Aerosol Sample Results

| *Collection Date* | *Number Aerosol Samples Positive (N = 5)* | *% Aerosol Samples Positive* | *CFU Range* |
|---|---|---|---|
| 5/1/2008 (background) | 1 | 20 | $4.0\times10^2$ |
| 5/3/2008 | 5 | 100 | $1.3\times10^3$–$9.2\times10^3$ |
| 5/4/2008 | 5 | 100 | $9.8\times10^3$–$5.7\times10^4$ |
| 5/5/2008 | 5 | 100 | $1.8\times10^3$–$2.9\times10^4$ |
| 5/6/2008 | 5 | 100 | $1.5\times10^3$–$4.1\times10^4$ |
| 5/7/2008 | 4 | 80 | $2.0\times10^2$–$2.4\times10^3$ |
| 5/8/2008 | 5 | 100 | $8.0\times10^2$–$5.8\times10^3$ |

Table 2 presents the results from the dry gauze wipes attached to sampling personnel. Four of 31 background samples (collected on April 30, 2008, prior to spraying) tested positive by culture (13%); 27 of 31 background samples were negative by culture. While it is not possible to unambiguously determine the source of the material that resulted in 4 background gauze positives, it is not likely due to cross-contamination as all blanks tested were negative.

Post-spray, between 17% and 78% of each day's samples were positive by culture, with viability in CFUs typically 1 to 2 orders of magnitude higher than the concentration of the positive background samples.

Table 3 presents the results from wet swipes obtained from samplers' backpacks. Both of the 2 background samples (collected on April 30, 2008, prior to spraying) tested negative by culture. Post-spray, the majority of samples were positive by culture, with viability results ranging from $2\times10^2$ to $4.4\times10^3$ CFUs.

Table 4 presents the results from wet swipes and vacuum samples obtained from the vehicle used as primary transport for the sampling team and their supplies. One of 6 background samples (collected on April 30, 2008, prior to spraying) tested positive by culture; it was a vacuum sample collected from the rear compartment of the sport utility vehicle. While it is not possible to unambiguously determine the source of the material that resulted in 4 background gauze positives, it is not likely due to cross-contamination as all blanks tested were negative. Post-spray, the majority of samples were positive by culture, with viability results ranging from $1\times10^2$ to $4.5\times10^4$ CFUs (due to a processing error, no samples collected on May 4, 2008, were tested for viability). The final sample, a swipe of the vehicle roof collected on May 8, 2008, was positive despite being collected in the rain.

Table 5 presents the results from vacuum samples obtained from sample collectors' hotel rooms. A "new" (ie, previously unused by LANL staff) hotel room was sampled each day to minimize the possibility that material collected was deposited by the previous day's operations. Hotel vacuum samples were collected from the carpet, starting from the entrance to the room to a sofa that was located in the approximate middle of the room. One (of 1) background sample (collected on April 30, 2008, prior to spraying) was negative by culture. Post-spray, all samples but 2 were positive by culture, with viability results ranging from $4.0\times10^2$ to $6.2\times10^3$ CFUs.

## Discussion

Mason District Park, where the experiment was conducted, is located just north of Fairfax County's 2008 spray block number 20 (Figure 2). The expected and observed wind conditions (Figures 4 and 5) indicate a high probability of transport of *Btk* from the spray block to the park. The aerosol sample results are consistent with transport of *Btk* to

Table 2. Personnel (gauze) Sample Results

| *Collection Date* | *Total Number Samples* | *Number Samples Positive* | *% Samples Positive* | *CFU Range* |
|---|---|---|---|---|
| 4/30/2008 (background) | 31 | 4 | 13 | $1.0\times10^2$–$2.0\times10^2$ |
| 5/2/2008 | 8 | 4 | 50 | $1.0\times10^2$–$1.0\times10^3$ |
| 5/3/2008 | 12 | 2 | 17 | $1.2\times10^3$–$6.5\times10^3$ |
| 5/4/2008 | 9 | 5 | 55 | $4.9\times10^3$–$4.1\times10^4$ |
| 5/5/2008 | 9 | 7 | 78 | $2.8\times10^2$–$1.7\times10^4$ |
| 5/6/2008 | 8 | 3 | 38 | $1.0\times10^2$–$5.0\times10^3$ |
| 5/7/2008 | 9 | 3 | 33 | $3.0\times10^2$–$7.9\times10^3$ |
| 5/8/2008 | 6 | 4 | 67 | $4.0\times10^2$–$4.4\times10^3$ |

the park; while 1 aerosol sample was positive prior to spraying, the incidence of positive samples increases post-spray, as does the concentration of CFUs per sample.

Dry gauze samples were obtained from the aerosol sample collection personnel. It is important to note that the sample collection personnel were not in the area during spraying; all activities took place after the application of *Btk* was complete. The dry gauze sample results indicate *Btk* accumulated on sample personnel while they were performing normal aerosol sample collection activities. While several gauze samples were positive prior to spraying, the incidence of positive samples increases post-spray, as does the concentration (CFUs per sample).

Wet swipes were obtained from a backpack used by sample collection personnel. The backpack was not in the area during spraying but was introduced after the application of *Btk* was complete. The swipe results indicate *Btk* accumulated on the backpack during the course of normal aerosol sample collection activities (Table 9).

Wet swipes and vacuum samples were obtained from the primary transport vehicle used by sample collection personnel. The vehicle was not in the area during spraying but was introduced after the application of *Btk* was complete. The swipe and vacuum results indicate *Btk* accumulated on and in the vehicle during the course of normal aerosol sample collection activities. While 1 vacuum sample (the vacuum of the rear compartment of the sports utility vehicle) was positive prior to spraying, the incidence of positive samples increases post-spray, as does the number of CFUs per sample. The final sample, a swipe of the vehicle roof collected on May 8, 2008, was collected during a rainstorm, as noted by the sample collection personnel, yet is still positive by culture.

Table 5 presents the results from vacuum samples obtained from sample collectors' hotel rooms. A hotel room previously unused by LANL staff was sampled each day to minimize the possibility that material collected was

Table 3. Backpack (wet swipe) Sample Results

| *Collection Date* | *Total Number Samples* | *Number Samples Positive* | *CFU Range* |
|---|---|---|---|
| 4/30/2008 (background) | 2 | 0 | No growth |
| 5/2/2008 | 1 | 1 | $4.3\times10^3$ |
| 5/3/2008 | 1 | 0 | No growth |
| 5/4/2008 | 1 | 1 | $2.5\times10^3$ |
| 5/5/2008 | 1 | 1 | $2.0\times10^2$ |
| 5/6/2008 | 1 | 1 | $9.0\times10^2$ |
| 5/7/2008 | 1 | 0 | No growth |
| 5/8/2008 | 1 | 1 | $4.4\times10^3$ |

Table 4. Vehicle Sample Results

| *Collection Date* | *Sample Type* | *Sample Location* | *CFU* |
|---|---|---|---|
| 4/30/2008 (background) | Vacuum | Inside rear compartment | $1.0\times10^3$ |
| | Swipe | Steering wheel | No growth |
| | Swipe | Steering wheel | No growth |
| | Swipe | Exterior back | No growth |
| | Swipe | Exterior roof | No growth |
| | Swipe | Exterior hood | No growth |
| 5/2/2008 | Vacuum | Inside rear compartment | No growth |
| | Swipe | Steering wheel | $1.0\times10^2$ |
| | Swipe | Exterior roof | $5.0\times10^2$ |
| 5/3/2008 | Vacuum | Inside rear compartment | $4.0\times10^4$ |
| | Swipe | Steering wheel | No growth |
| 5/5/2008 | Vacuum | Inside rear compartment | $4.0\times10^3$ |
| | Swipe | Steering wheel | $3.0\times10^2$ |
| | Swipe | Exterior roof | $6.7\times10^3$ |
| 5/6/2008 | Vacuum | Inside rear compartment | $4.5\times10^4$ |
| | Swipe | Steering wheel | $1.0\times10^2$ |
| 5/7/2008 | Vacuum | Inside rear compartment | $3.1\times10^4$ |
| | Swipe | Steering wheel | $2.0\times10^2$ |
| | Swipe | Exterior roof | $1.0\times10^2$ |
| 5/8/2008 | Swipe | Steering wheel | No growth |
| | Swipe | Exterior roof | $1.1\times10^2$ |

Table 5. Hotel Room (vacuum) Sample Results

| *Collection Date* | *CFU* |
|---|---|
| 4/30/2008 (background) | No growth |
| 5/2/2008 | No growth |
| 5/3/2008 | $5.0\times10^2$ |
| 5/4/2008 | $6.2\times10^3$ |
| 5/5/2008 | $5.0\times10^2$ |
| 5/6/2008 | $4.0\times10^2$ |
| 5/7/2008 | No growth |
| 5/8/2008 | $4.0\times10^2$ |

deposited by the previous day's activities. The vacuum sample results indicate *Btk* was transported to the hotel rooms in measurable amounts as a result of normal aerosol sample collection activities.

There are a few notes to highlight related to the results presented here. First, there are a number of positive background samples. While the source of these positives cannot be unambiguously determined, there is a statistically significant increase in the incidence of positive samples post-spray, as well as the relative concentration (CFU/sample), indicating substantial transport of *Btk* via fomites during the 1-week experiment. Figure 8 summarizes a statistical analysis of the results for aerosol, vehicle, and personnel sampling prior to spraying (background) and after. The analysis indicates significantly higher concentrations of *Btk* in samples collected post-spray.

Second, the sample collection methods used are extremely inefficient. Several studies have found the recovery efficiency for wet swipes to be low.[7,8] The dry gauze used to collect samples from sampling personnel is not a well-characterized method, but its efficiency is likely lower than wet gauze, as it relies solely on the adhesive forces of the gauze and the aerosolized agent. The sample types were chosen because the analysis laboratory had protocols to process them. However, the low expected efficiencies indicate a high likelihood that more agent was transported than was detected.

Third, none of the sample collection personnel, their vehicles, or accessories were in a spray block during *Btk* application. All activities (except the collection of background samples) occurred post-spray, indicating that it is likely that secondary aerosolization, or reaerosolization, and subsequent transport of the *Btk* occurred post-application. The most convincing evidence of reaerosolization is the positive aerosol samples obtained up to 5 days post-spray. Following *Btk* dissemination, the majority of the particles (which have large median diameters) are expected to settle quickly onto foliage and other surfaces. The smaller diameter particles, which do not settle quickly, will be transported outside the spray area rapidly by even light winds.[9] It is therefore extremely unlikely that the material collected by aerosol filters near the spray area 5 days post-spray was due to the initially aerosolized material. The detection of viable *Btk* on gauze wipes collected from

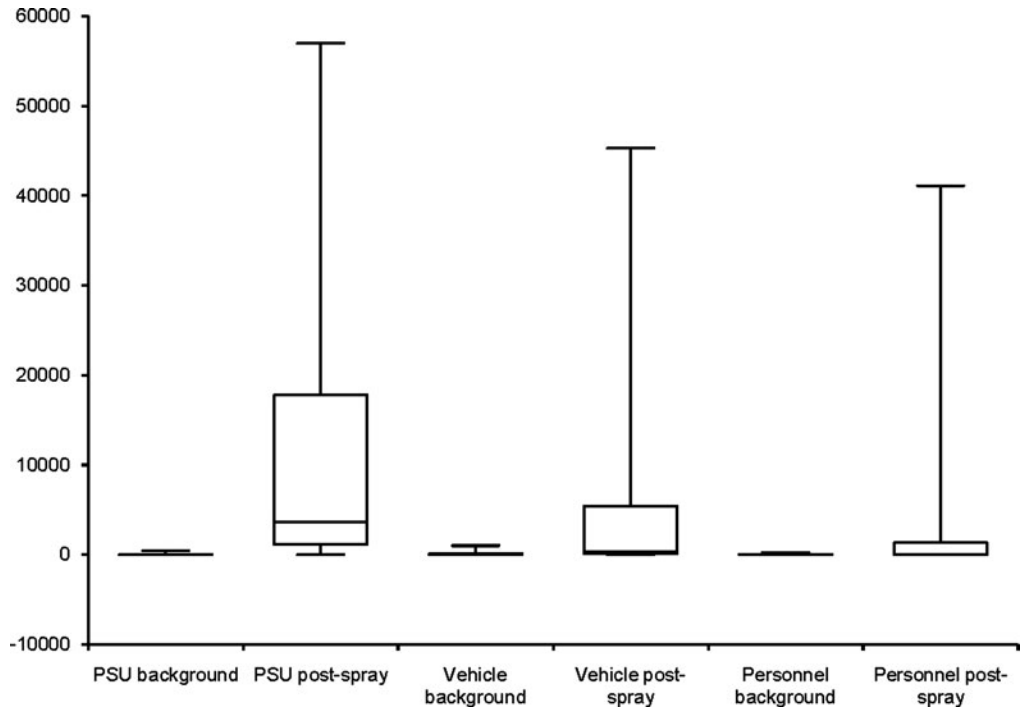


Figure 8. Results for aerosol, vehicle, and personnel sampling (CFU per sample) prior to spraying (background) and after spraying. The outer edge of box shows from the first to the third quartile, and the median values is marked in the center. Whiskers extend to the maximum and minimum observed value, showing the tails of the distribution.

sampling personnel's backs (between the shoulder blades) provides an additional indication that reaerosolization occurred. As no samples were collected immediately adjacent to preexisting structures or vegetation, it is unlikely that material was deposited due to direct contact with a contaminated surface.

Finally, it is useful to note that while sampling team members did not wear full personal protective equipment, they did use standard sampling techniques including changing gloves after the collection of each sample and wearing booties over their footwear. The *Btk* that was transported to the vehicles and hotel rooms was therefore likely transported by clothing and accessories.

These results have significant implications for response and restoration following a biological attack. Personnel carrying out routine sampling activities post-spray accumulated detectable contamination. This contamination was tracked outside the initially contaminated zone to their vehicles and hotel rooms, which were in an area that had not been sprayed. Following a biological attack, first responders or sample collection personnel working in a contaminated or "hot zone" would likely experience the same phenomena: without a rigorous regimen of personal protective equipment and decontamination, they would pick up contamination from routine activities and transport it out of the "hot zone" into areas previously deemed "clean." While extensive personal protective equipment was not used, and personnel decontamination was not performed in this experiment, the data obtained indicate it will be critical to prevent personnel operating in a contaminated zone from inadvertently transporting it outside the "hot zone" and, perhaps most disturbing, taking the agent home with them.

Transport of the agent outside the initially contaminated zone has additional implications for response and restoration. With current technologies, detection of a biological attack is likely to occur hours to days following the initial release. By the time it has been determined an attack occurred, people and vehicles that were in the release zone may have transported contamination significant distances away from the original release. Secondary exposures may have occurred, and individuals who were not in the area of the original release may become infected, complicating epidemiologic investigations and distribution of prophylactic treatment. In addition, unless restrictions on movement into and out of the "hot zone" are implemented, dynamic transport of the agent may continue to occur after the detection of the attack, changing the boundaries of the areas that need to be decontaminated in unpredictable ways. The data also indicate evacuation of the "hot zone" may be a counterproductive strategy, as transporting residents and peripherals out of this area may only serve to spread contamination further.

Finally, secondary aerosolization, or reaerosolization, of the agent was detected throughout the experiment using aerosol samplers. Much like fomite transport, reaerosolization post-attack may alter the boundaries of the areas that need to be decontaminated in unpredictable ways. In addition, while this experiment does not provide direct insight into the impacts of reaerosolization on public health, the fact that reaerosolization was observed indicates that longer-term, low-level aerosols may be generated following a biological attack and should be considered in planning response and restoration activities. Individuals who were not exposed to the initial release (eg, responders or contractors brought in from other areas) could be exposed due to reaerosolization and become ill, depending on the accumulated dose and their immune status. Most studies of dose-response for biothreat agents have focused on acute, high-level exposures. The effects of chronic, low-level exposures have been the subject of limited studies—for example, in wool mills.[10,11] More investigation of their impacts will be required to determine the health significance of reaerosolization.

## Conclusion

An understanding of the potential transport of *Bacillus* species via fomites is critical for the formulation of appropriate bioterrorism response and recovery plans. This experiment was not able to study all possible mechanisms of fomite transport; however, it did study aspects of the problem as it relates to field sampling personnel and equipment. The results indicate that routine sample collection activities can result in significant transport of *Bacillus* species via fomites.

While there is little information in the peer-reviewed literature on the transport of *Bacillus* species via fomites, the results of this study are not inconsistent with studies by other investigators in which transport of *Bacillus* species via fomites has been observed. Davids and Lejeune detected viable *B. subtilis* on clothing following both an initial release and subsequent activities in the contaminated area.[12] Bales and colleagues reported that a vacuum sample from a mill worker's home tested positive for *B. anthracis* during the investigation of a mill-associated anthrax outbreak, "suggesting that workers carried spores on their clothes from the mills to their homes."[13(p1169)] Krauter and colleagues have noted detectable contamination on personnel following chamber experiments with *B. atrophaeus*.[14] And Byers and colleagues have observed contamination of clothing following sampling activities in a *Btk*-contaminated breeze tunnel, with subsequent transport of *Btk* via personnel clothing to a nearby building.[15]

There is also little information in the peer-reviewed literature on secondary aerosolization, or reaerosolization, of *Bacillus* species following an initial dissemination. However, the results of this study are not

inconsistent with studies from other investigators in which reaerosolization of biological particles has been observed. Miguel and colleagues reported the reaerosolization of pollen, pollen fragments, animal dander, and mold from paved surfaces.[16] Turnbull and colleagues reported the detection of *B. anthracis* in a small number of samples surrounding carcass sites in the Etosha National Park.[17] And Inglesby and colleagues referenced a study on reaerosolization that "showed that in areas of ground contaminated with 20 million *Bacillus subtilis* spores per square meter, a soldier exercising actively for a 3-hour period would inhale between 1000 and 15000 spores."[18(p2249)]

These results have implications for the formulation of response and recovery strategies following a biological attack, as they demonstrate it is possible for routine activities in a contaminated area to result in transport of the biological agent to other (potentially "clean") locations. The public health implications of these results should be considered, with a focus on containing the initial contamination and tracing its possible spread to the greatest extent possible.

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