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Characterization of the Environmental Fate of *Bacillus thuringiensis* var. *kurstaki* (*Btk*) After Pest Eradication Efforts in Seattle, WA and Fairfax County, VA

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Abstract

Understanding the fate of biological agents in the environment will be critical to recovery and restoration efforts after a biological attack. Los Alamos National Laboratory (LANL) is conducting experiments in the Seattle, WA and Fairfax County, VA areas to study agent fate in urban environments. As part of their gypsy moth suppression efforts, Washington State and Fairfax County have sprayed *Bacillus thuringiensis* var. *kurstaki (Btk)*, a common organic pesticide for decades. Many of the spray zones have been in or near urban areas. LANL has collected surface and bulk samples from historical Seattle spray zones to characterize how long *Btk* persists at detectable levels in the environment, and how long it remains viable in different environmental matrices. This work will attempt to address three questions. First, how long does the agent remain viable at detectable levels? Second, what is the approximate magnitude and duration of resuspension? And third, does the agent transport into buildings? Data designed to address the first question will be presented. Preliminary results indicate *Btk* remains viable in the environment for at least two years.

Background

This effort characterizes the extent and duration of *Btk* contamination. The Gypsy Moth spraying programs offer unique opportunities to acquire information regarding the persistence of a biological agent with properties similar to *Bacillus anthracis* in a wide area environment. Much work has been accomplished over recent years to understand the initial exposure and response phases of a biological release event in the areas of detection, characterization and coordination. However, little has been explored concerning the wide area recovery and restoration of Department of Defense installations and affected adjacent civilian areas.

Surface, bulk, and air samples are collected and analyzed with culture and DNA-based analysis methods. Urban transport and dispersion modeling is being added to the laboratory analysis for characterization of detectable *Btk*. This characterization looks at persistence, dispersal and resuspension of *Btk*. Persistence depends on degradation due to environmental decay and dilution due to resuspension. Dispersal includes transport into and deposition on buildings and approximate extent of contamination.

Methods

Spray areas sampled in the Seattle metropolitan area were sprayed with *Btk* in 2007, 2006 (two spray areas), 2005 and 2004. These areas range from 5.5 acres to 60 acres and were sprayed using truck-mounted hydraulic sprayers or aerial, helicopter-mounted sprayers. In addition a 25 acre control area (one that has never been sprayed by WA Department of Agriculture) near downtown Seattle was sampled.

Sampling occurred in May 2008 in Fairfax County, VA immediately following aerial spraying of a 182 acre, largely residential area. In addition, this spray area was sampled at 6 and 12 weeks following spraying. Week 24 and 48 samples will be collected in future months. Just prior to spraying, background samples were taken in the spray block to understand the natural presence of *Btk* in this area.

Sampling plans were designed for each spray area to allow a statistical statement to be made about the persistence of *Btk*. At all locations, samples were collected using three sampling schemes: probabilistic, secondary samples and targeted or discretionary samples. The probabilistic scheme assumes uniform distribution of *Btk* and employs a grid technique.

Sample sizes were selected so as to have high confidence (99% bound) that at least 95% of the area was without contamination using a hypergeometric model. This high confidence bound allows for combining multiple years of data. An additional ten percent of the probabilistic samples will be taken as "close" or secondary samples. These samples are taken within a foot of the probabilistic samples and are designed to understand if the assumption of uniform distribution of *Btk* is correct. An additional ten percent were taken as targeted or discretionary samples. These samples were taken from areas where *Btk* spores are expected to persist as suggested by a review of current literature.

Soil and surface swipe samples were collected from spray areas located in Seattle, while in Fairfax County, leaves and grass samples were collected in addition to soil and surface swipes samples. These samples are analyzed using DNA-based analysis (polymerase chain reaction) to screen for the presence of *Btk* DNA and culture-based techniques to look for viable spores.

Preliminary Results and Future Work

Samples taken from Seattle 2007 and 2006 spray sites indicate residual viable *Btk* exists, but suggest a decrease in viability over time (Figure 1). Results to date suggest that *Btk* does persist, under common urban conditions, for years. A higher percentage of soil samples were positive for viable *Btk* than surface swipes. Samples from areas in Seattle that were sprayed with *Btk* in 2005 and 2004 have been collected and are currently being analyzed. Results from samples collected from areas in Seattle show all field blanks to be negative for viable *Btk*. Background samples taken from the Seattle control area showed no natural presence of viable *Btk*.

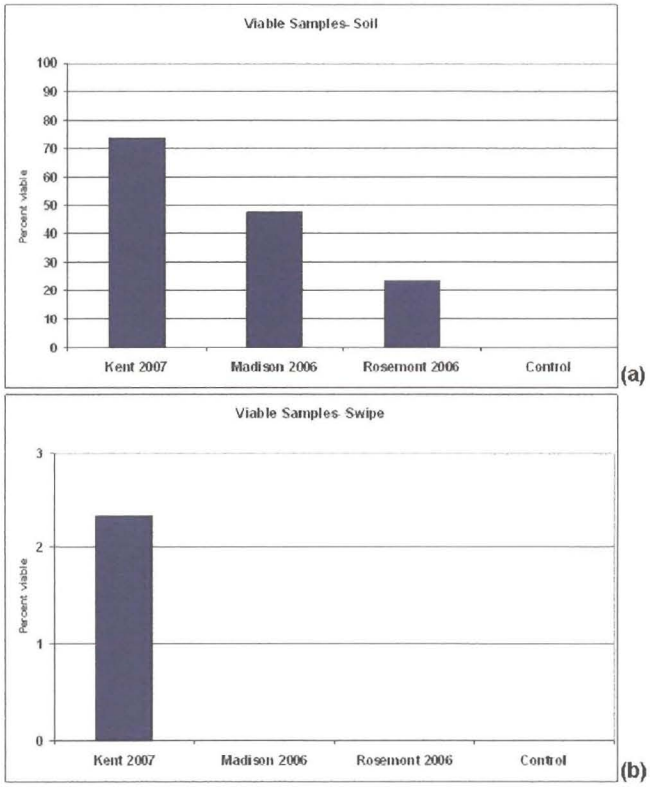

Figure 1. Viability results for samples collected in Seattle (2007, 2006 and control). (a) Percentage of viable positive soil samples for Kent 2007, Madison 2006, Rosemont 2006 and control area. (b)

Percentage of viable positive surface swipe samples for Kent 2007, Madison 2006, Rosemont 2006 and control area.

Ongoing sampling in Fairfax County, VA is designed to address the question of the magnitude and duration of resuspension. Background (pre-spray) samples collected from soil, surface swipes, grass and leaves in Fairfax suggest that no viable *Btk* was present prior to the 2008 spraying. Samples collected post-spraying are currently being analyzed. Samples for 24 weeks and 48 weeks post-spraying will be collected and analyzed in future months.

A building infiltration study is being conducted to characterize the transport of contamination from an outdoor release into nearby buildings. This study involves collecting air samples, surface swipes and vacuum samples from fresh air intake HVAC filters prior to, immediately following and for ten weeks after spraying. Two high-rise office buildings, a split-level residential home and a US Postal Service distribution center located in Fairfax County, VA were all sampled. Analysis of 2008 data from these four Fairfax buildings, in addition to results from experiments to be conducted in 2009, will help better understand the indoor transport of *Btk* following its release into the environment.