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Reaerosolization of *Bacillus* spp. in Outdoor Environments: A Review of the Experimental Literature

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Reaerosolization or resuspension—that is, the reintroduction of previously airborne particles into the atmosphere—is a complex phenomenon. Microbial reaerosolization is particularly poorly understood because few studies have been done in this area, and many of the studies that have been performed are not in the peer-reviewed literature. The reaerosolization of *Bacillus anthracis* in outdoor environments is of particular concern because of its stability and potential for use as a biological weapon. This review pulls together data from more than 30 publications, spanning field and laboratory experiments, to summarize the current state of our understanding of *Bacillus* spp. reaerosolization in outdoor environments.

In the literature about radionuclides and soil, the reintroduction of particles that have settled onto a surface back into the air is called *resuspension.* The biology literature terms it *reaerosolization,* but the fundamental phenomenon is the same. A reasonable body of data has been assembled on radionuclide and soil resuspension,[1,2] but the literature on microbial reaerosolization is sparse.

The reaerosolization of *Bacillus anthracis* is of particular concern because of its stability[3,4] and potential for use as a biological weapon. Numerous experiments, including indoor studies during the restoration of the Hart Senate Office Building,[5] have demonstrated that *Bacillus* spp. reaerosolize.[5-27] However, a quantitative understanding of the phenomena governing this process is lacking,[10,15,21,24] which makes assessment of the associated public health risks difficult.[15,28] The understanding of microbial reaerosolization in outdoor, urban areas is especially incomplete.

Much of the foundational information on the reaerosolization of *Bacillus* spp. is contained in older government documents that are difficult to obtain. Perhaps as a result, these documents are often cited in the peer-reviewed literature as references for conclusions that are not, in fact, consistent with their content or the conclusions of the authors. This review pulls together data from 31 publications to summarize the current state of our understanding of *Bacillus* spp. reaerosolization in outdoor environments so that future research can address the gaps that serve to confound our understanding of the potential public health risks.

Field Studies

Weather-Induced Reaerosolization

In the 1990s, a study conducted in Etosha National Park, Namibia, measured the aerosolization of *B. anthracis* spores from undisturbed wild animal carcasses.[25] Although this is technically initial, or primary, aerosolization (ie, aerosolization of spores that originated from the source), not

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re-aerosolization, the study is important in that it demonstrates the introduction of spores from a surface into the air under ambient conditions. Cyclone or gelatin air filter samples were collected at downwind distances of 6, 12, and 18 meters from the sites. Three of 43 total air samples collected were positive, with colony counts exceeding 100 colonies per plate. These positive samples were collected during the highest winds measured during the experiment (3.4 to 6.2 m/s^{-1}). Additional samples were collected during mechanical disturbances at the sites designed to imitate animal movement (ie, gently running a post along the top of soil); 9 samples were found to be positive during the disturbance, with an additional 5 positive post-disturbance.

The dissemination of *B. thuringiensis* for pest control has facilitated additional outdoor reaerosolization studies under ambient conditions. Wet slurries of *B. thuringiensis* var. *kurstaki* are commonly released to suppress populations of gypsy moth.[12] In a 2007 study, reaerosolized viable spores in the respirable range (<10 μm) were observed up to 2 weeks post-dissemination—the full duration of the study using a filter-based portable air sampling unit. Samples were collected daily and analyzed by real-time PCR and then by plating to assess viability. Thirteen days post-release, concentrations had decreased by 2 to 3 orders of magnitude, but air samplers still collected 10^4 DNA copies (measured by PCR) over 24 hours.[13] Additional studies by the same group using similar methodologies have found that viable reaerosolized spores can be collected up to 48 weeks post-release.[26]

Mechanically Induced Reaerosolization

Several military experiments have been conducted to characterize mechanically induced resuspension of *Bacillus* spp. In 1949, 2 grassy plots were loaded with either a dry powder or wet slurry of *B. atrophaeus* (formerly referred to as *B. globigii*) to a concentration of 10^8 spores/cm^{-2}, and reaerosolization by troop activity was measured using air samplers located at 3 feet above ground level.[23] More reaerosolization was observed from the dry powder plot than from the slurry plot; following the first troop passage, air samples averaged 10^5 spores for the powder and 10^4 spores for the slurry. After 75 days, the spores from the slurry had penetrated 4 cm into the soil, but the dry spores penetrated only 1 cm, and troop-induced reaerosolization was minimal for both preparations.[23]

Peck et al also found that reaerosolized spores deposited on the troops in a manner that could be explained by wind speed and direction, vegetation height, and marching order.[23] Similar conclusions were reached by Harper et al following studies of deposition onto and reaerosolization from a variety of fabrics worn by the Royal Navy; the reaerosolization of spores during the removal of contaminated clothing, collected using Aerojet General all-glass cyclones with analysis by culture, was also found to pose a significant aerosol hazard to personnel.[17] Harper et al also reported that *B. atrophaeus* was less likely to reaerosolize from hair than from clothing, probably as a result of the increased surface area and shielding effect of hair.[17]

In the 1980s, the Canadian military conducted field tests with *B. atrophaeus* to evaluate reaerosolization.[14] A wet slurry of 2×10^7 spores/m^{-2} was disseminated. Subsequent troop activity produced reaerosolization throughout the 9-day study. After 9 days, spore concentrations at a downwind distance of 46 m were reduced to 30% of the level measured 24 hours post-dissemination. As in the Harper study,[17] reaerosolization from contaminated clothing was found to present a non-negligible respiratory hazard.[14]

In 1970, the US military investigated troop- and vehicle-induced reaerosolization in the Utah desert using *B. atrophaeus.* Spores were released in a slurry-filled bomblet, as an aerial slurry, and as dry spores.[18] Troop- or truck-induced reaerosolization was observed for up to 7 days post-release and as far as 10 km downwind from release sites. All dispersal methods and temporal conditions studied produced reaerosolization, with dry spores producing the largest hazards. Nighttime dissemination was observed to spread secondary aerosols further from the source than dissemination during daytime, probably because of greater atmospheric stability.[18]

A 1998 Utah study also examined vehicle-induced reaerosolization. Asphalt and gravel roads were seeded with *B. atrophaeus*. Subsequent reaerosolization by a truck and trailer driving at 30 mph was found to be a function of deposition density and downwind distance. Viable reaerosolized spores were measured 600 m downwind after trucks drove through the deposited spores between 10 and 29 hours after deposition. The percentage of reaerosolized spores was comparable between the 2 road types.[10] Both the 1970 and 1998 Utah studies used the same instrumentation and detection methods: all-glass impingers and culture.

A second analysis of the 1998 study was performed by Jensen and Fagan.[21] For roadways seeded with 0.5 g/m^{-1} of *B. atrophaeus*, truck-induced reaerosolization produced an aerosol concentration of 600 spore minutes per liter. The hazard area formed a narrow plume (which is expected, since the seeded area was not large) that extended approximately 300 m downwind of the deposition area. Almost all of the reaerosolized particles collected more than 100 m downwind had diameters < 5 μm (ie, were in the respirable range).

Laboratory Studies

Laboratory studies using *B. anthracis* and simulants have corroborated the reaerosolization observed in the field experiments. A relatively recent study of potential post-release fixatives found that reaerosolization from *B. anthracis*–inoculated sand using a 3-second burst of air at 100 mL/sec^{-1} produced colony counts on agar plates that were too numerous to count. The amount of reaerosolization was

reduced by 33% following the application of water and by 89% following the application of a water-based polysaccharide fixative.[29]

Grasso et al used a model jet engine to investigate reaerosolization of *B. atrophaeus* spores from a metal surface by engine exhaust using 3 types of air samplers followed by culture.[16] The majority of spores were reaerosolized during the initial moments of operation; only a small percentage of the seeded spores (0% to 0.4%) were reaerosolized secondary to the initial engine start-up.[16]

Finally, Byers et al measured the reaerosolization produced by human activity using a dry-milled aerosol of *B. thuringiensis* var. *kurstaki* dispersed in an ambient breeze tunnel.[7] Air samplers captured reaerosolized spores that were liberated by personnel performing routine air monitoring activities. Personnel contamination and resulting transfer of spores to clean areas were also observed.[7] Similar personnel contamination and fomite transport were observed by Van Cuyk et al in a field study following the outdoor dissemination of *B. thuringiensis* var. *kurstaki*.[30] Byers et al also noted that reaerosolization increased with decreasing humidity, although this effect diminished for humidity greater than 40%.[7] The latter observation is not surprising, as it holds true for resuspension of other particulate matter.

Use of Simulants in Reaerosolization Experiments

Almost all of the studies of *Bacillus* spp. reaerosolization were performed using near neighbors, even though they were clearly intended to evaluate the effects of *B. anthracis.* Many of the authors acknowledge that there may be differences in simulant and agent behavior, but they also generally note that simulants, even nonmicrobial simulants such as silica or glass, have been demonstrated to behave comparably.[15,21]

In a side-by-side comparison using compressed air to liberate *B. atrophaeus* spores and fluorescent particles from fabric swatches, Byers et al found no significant difference in the concentrations of reaerosolized spores and fluorescent particles.[8] The affinity of spores for soil and other debris[31] may further validate the use of simulants in these experiments. A review of particulate reaerosolization by Sehmel concluded that resuspended contaminants are typically attached to soil particles.[2] If this is true for *B. anthracis*, it is reasonable to approximate all *Bacillus* spp. reaerosolization as the resuspension of much larger soil particles. Garland et al note that many of the physical processes governing resuspension are thought to be similar for particles in general,[15] and in a review on airborne bacteria, Burrows et al note that meteorological influences on bacterial spores are similar to those for other particles,[28] reinforcing the plausibility of applying simulant data to *B. anthracis* spores.

Conclusions

The literature unambiguously indicates that *Bacillus* spp. are reaerosolized by wind under ambient conditions, by pedestrian or vehicle traffic, and by other types of mechanical action. Since it is not feasible to conduct field tests using *B. anthracis*, the majority of tests have been conducted using simulants, though the literature generally validates the use of simulants for these studies. While the historical studies summarized above are interesting, it is difficult to draw quantitative conclusions from them because of the use of diverse simulants, multiple preparation and dissemination techniques, disparate collection and detection methods, and incomplete information on experimental conditions.

Gaps in the biological reaerosolization literature exist in a number of areas. While the broader transport and dispersion literature clearly identifies the importance of particle size in reaerosolization, very few of these studies involved any particle sizing. Those that did found that the reaerosolized particles were larger than those originally dispersed, probably as a result of spore-to-spore or soil-to-spore aggregation.[16] This phenomenon is commonly observed throughout the broader reaerosolization literature; as micron-sized particles become larger, they are more easily aerosolized, until they reach 100 microns or greater, at which point aerosolization becomes more difficult.[1] It is probable that similar conclusions would have been reached in the other studies if size-fractionated particles had been collected.

William C. Patrick is widely cited for postulating that a crude (ie, viscous, hydrophobic, nonuniform size distribution) *B. anthracis* slurry would produce little reaerosolization because of large initial particle sizes, but a weapons-grade powder (ie, milled to be free of charge, free-flowing, and hydrophobic) would continuously produce secondary aerosols due to small particle size.[32] The broader reaerosolization literature implies that this is incorrect,[1] but to our knowledge, Patrick's hypothesis has not been extensively investigated.

The broader transport and dispersion research also typically characterizes particles over a large range of sizes (<1 to 1000 microns). This is partly because, within the 1-100 micron range, larger particles are more easily aerosolized, but it is also because larger particles induce the aerosolization of other particles when they resettle on the ground.[1] None of the studies included above characterized particles outside the respirable range, probably because of a common belief in the biothreat community that holds that particles outside the respirable range are of little interest. Although this is true in terms of the public health impact, it does not provide a complete understanding of reaerosolization. There has not been enough investigation of larger spore-containing particles and their environmental fate to rule out their potential to cause infection, especially given the evidence that larger particles facilitate smaller particle resuspension[2,33] and may even be broken up into smaller particles during reaerosolization-facilitated collisions.

The influences of the dissemination method on particle size distribution and subsequent environmental fate are also largely unexplored. Environmental conditions (eg, humidity, humic-rich soil, etc) may significantly influence the size of a spore-particle aggregate and thereby affect its potential for subsequent reaerosolization.

On the microscale level, there is a limited understanding of the effects of electrochemical or physical forces (eg, van der Waals, electrostatic, capillary, condensation reactions) on spore-particle aggregation or spore-particle attachment to various surfaces. There is also a limited understanding of how other governing factors of reaerosolization, such as meteorology and soil/surface conditions, might affect microscale aggregation. More fundamentally, throughout the broader reaerosolization literature, there is no clear understanding of how microscale properties directly influence field experiment observations, probably because of the complexity of microscale interactions present in an outdoor environment.

A reasonable and cost-effective approach to furthering our understanding of biological reaerosolization would be to mine the broader reaerosolization literature and design targeted experiments to determine whether spores reaerosolize similarly to other, better characterized particles such as radionuclides. This would allow an assessment of the plausibility of using information and models derived from other particulates to understand *Bacillus* spp. reaerosolization. This type of information could be combined with information on dose-response to more accurately determine whether microbial reaerosolization will present a public health hazard following an outdoor release and, if so, of what magnitude, under what conditions, and for how long.

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