



Decontamination of Vegetation to Inactivate Bacillus anthracis Spores

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Abstract

The Analysis for Coastal Operational Resiliency (AnCOR) program is an interagency collaboration between the U.S. Environmental Protection Agency (EPA), the Department of Homeland Security (DHS), and the United States Coast Guard (USCG). The overall purpose of this multiagency program is to develop and demonstrate capabilities and strategic guidelines to prepare the U.S. for a wide-area release of a biological agent, including mitigating effects on USCG facilities and assets. Following a wide-area release of *B. anthracis* spores, vegetation might become contaminated with the biological agent. Decontamination of vegetative materials such as trees, grass, and crops, especially on a large scale, will be a challenge, due to the organic nature of vegetative materials, and the potentially large and complicated surface areas of foliage. Other challenges include effectively decontaminating the plants without killing or damaging them.





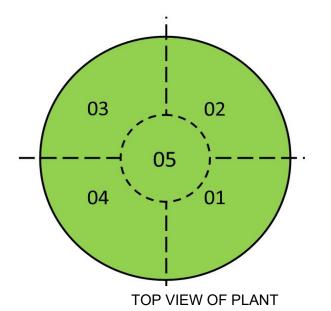




Small plant decontamination methods

- Bacillus atrophaeus as surrogate, 10⁷ colony forming units (CFU) inoculation per 3 plants
- 4 plants per test (1 used as negative control)
- 5 samples per plant, 3-hole punches per sample
- Positive control recoveries ~ 4 log CFU per sample
- Used 2-minute spray, 1 hr contact time, rinsed, sampled next day







Both electrostatic sprayers and back pack sprayers used in testing

Minimal decon conditions for highest efficacy achieved, for PAA and dichlor, by plant type

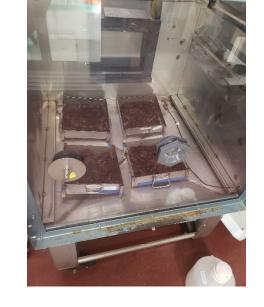
| Plant/bark | Plant Type | Decon solution | Amount sprayed for each plant (ml) | # Samples out of 15 that had at least one CFU |
|--------------------------|--------------|-------------------|------------------------------------|---|
| White Indian Hawthorn | Evergreen | PAA | 118 | 0 |
| Taber Indian Hawthorn | Evergreen | Dichlor | 130 | 2 |
| Blueberry shrub | Deciduous | PAA | 121 | 1 |
| Blueberry shrub | Deciduous | Dichlor | 120 | 8 |
| Japanese Spurge | Ground cover | PAA | 60 | 3 |
| Creeping Jenny | Ground cover | Dichlor | 130 | 0 |
| Pine bark nuggets | | PAA | 130 | 1 |
| Pine bark nuggets | | Dichlor | 1074 | 13 |

PAA = peracetic acid

Sod decon methods and results

- For each experiment, 5 sets of sod test "coupons" were used, 4 coupons per set (one of which was not inoculated); 20 sod coupons decontaminated in each experiment
- Each test coupon 12-inch X 12 inch, inoculated with 7 log CFU spores, settled overnight





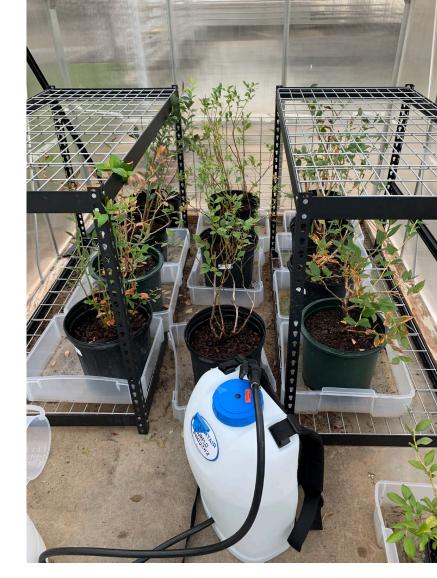
Number of sod coupons out of 15 that had CFU remaining after decon

| | Dichlor ~ 2% FAC | Jet Ag ~ 0.5% PAA |
|---------------|------------------|-------------------|
| Zenith Zoysia | 4 | 5 |
| Tall Fescue | 14 | 5 |
| Bermuda | 15 | 11 |

Phytotoxicity methods and results

- Four replicate plants sprayed with decontaminant or water, and monitored over 4 weeks
- Visual inspection of leaf color changes and loss of leaves
- Used Leaf Doctor app to quantify % diseased for sod
- No plants died
- Mixed results, no obvious trends
- No impacts to Indian Hawthorne plants
- More of an impact from dichlor on Creeping Jenny plants





Final thoughts and conclusions

- Good recovery of spores from vegetative material via standard liquid extraction
- Getting complete decon of plants/sod will be a challenge due to high surface area of foliage
- Further evaluation of electrostatic sprayers for applying decontaminants to vegetation may be warranted
- Cursory phytotoxicity tests showed amount of damage to plants from decontaminants varied by plant and decontaminant. Recommend rinsing ASAP after decon contact time if feasible.



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