United States Environmental Protection Agency

USGS/EPA Collaboration:
Bacillus anthracis in American Soils: From Sample Collection to Data Application

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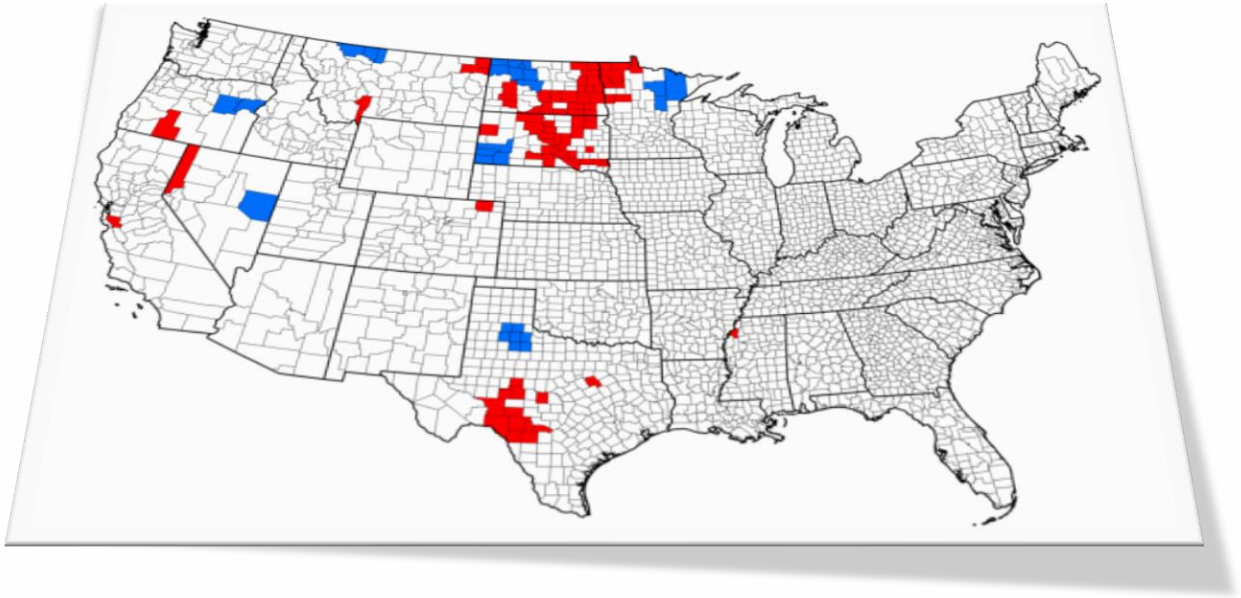
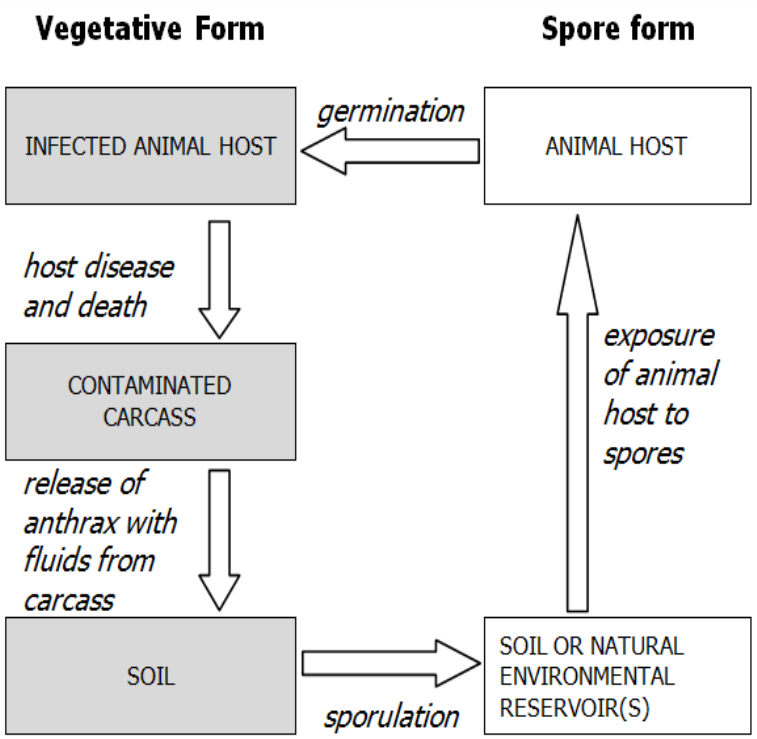


Outline

- Background
- Sample collection protocols
- Naturally occurring *Bacillus anthracis*
- Methods to recover and analyze *B. anthracis* from soils
- Spore germination
- Lessons learned and gaps

B. anthracis in Soil

- *B. anthracis* is the causative agent for anthrax.
- *B. anthracis* is naturally occurring in many soil environments and can persist in soil for many years.
- Outbreaks of anthrax in wildlife and livestock are often associated with old graves of anthrax stricken animals and suitable soil conditions.
- The presence of *B. anthracis* in the environment depends on many factors.



***B. anthracis* natural lifecycle; modified from Schuch and Fischetti (2009).**

B. anthracis and Incident Preparedness

- *B. anthracis* sent in the mail to intentionally inflict disease in 2001
  - Raised questions about persistence, recovery, and clean-up of *B. anthracis.*
- Knowledge of environmental background data for *B. anthracis* could help decision makers better prepare for an incident.
- Soils remains one of the most difficult materials to analyze for *B. anthracis.*

Potential for Collaboration

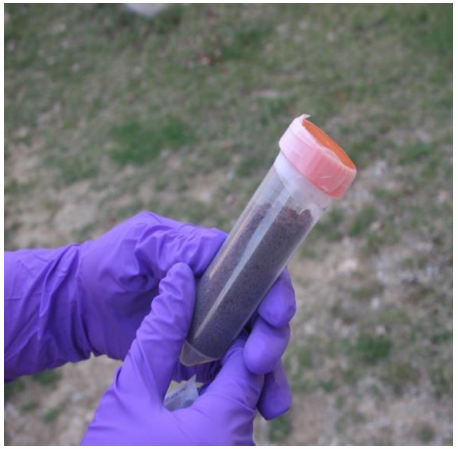
- USGS and EPA collaboration initiated in 2010.
  - Both agencies were interested in:
    - The natural background distribution of microbial pathogens.
    - Factors that influence disease prevalence.
    - Distinguishing natural outbreaks from those due to terrorism.
  - EPA also had interest in:
    - Improving sample collection and analysis of pathogens in soil.
  - USGS also had interest in:
    - Support for long term USGS personnel involvement.

Capabilities and Assets

- USGS:
  - Availability of USGS mineralogy and geochemistry data and soils samples.
  - Technician support for mapping various pathogen distributions and outbreaks.
  - Availability of a microbiology laboratory support facilities.
- EPA:
  - Available funding to support the research.
  - Availability of contract to support laboratory work at EPA facility.

Sample Collection Protocol for Bacterial Pathogens in Surface Soil

- USGS and EPA effort
- Protocol for collecting, handling, shipping of soil samples taken from the top 0-5 cm of soil (surface soil)
- Based on the procedures used by U.S. Geological Survey (USGS) during its North American Geochemical Landscapes Pilot Studies
- Guide for developing sampling plans and other site-specific documentation

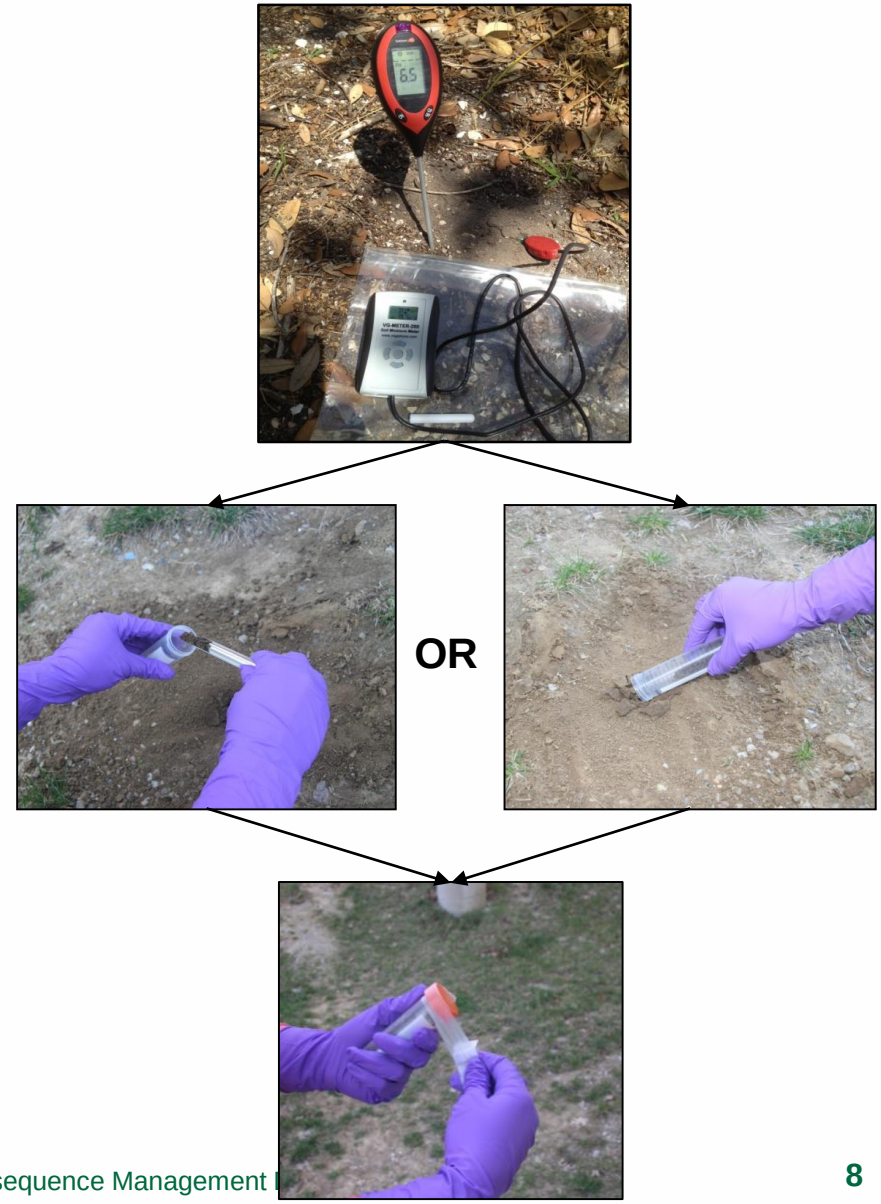


Use and Purpose

	Level 1 Protocol	Level 2 Protocol
Purpose	Background study or surveillance of surface soil	Suspected or known accidental contamination in surface soil
Sampling Team	1 person	2 people
Roles	All duties	Collector – collects samples
		Assistant – supplies and documentation
PPE	Gloves, dust mask, booties, safety glasses or goggles	Gloves, booties, full face respirator, and Tyvek suit

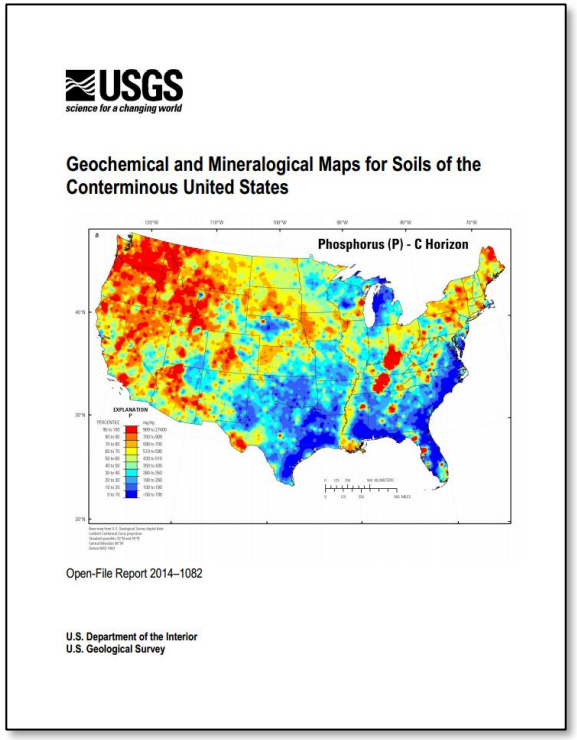
Protocol Summary

- 50 mL sterilized tubes
- Applicable for most types of soil
- Top 0-5 cm of soil
- Step-by-step instructions for Level 1 and Level 2 collection
- Field log and chain of custody forms provided
- Soil moisture, temperature, pH, and other landscape characteristics recorded



USGS North American Soil Geochemical Landscapes Project (NASGLP)

- Soil collected at a density of 1 site per 1600 km^2 to expand baseline geochemical and microbiology data for the U.S., Canada, and Mexico.
- Generalized random tessellation stratified design for sample site selection
- Pilot studies began in 2004 and sample collection ran from 2007-2010



http://pubs.usgs.gov/of/2014/1082/

USGS and EPA Sample Analysis

USGS and EPA collaborated on analysis of the NASGLP samples for presence of biological agents of interest in the 48 conterminous states

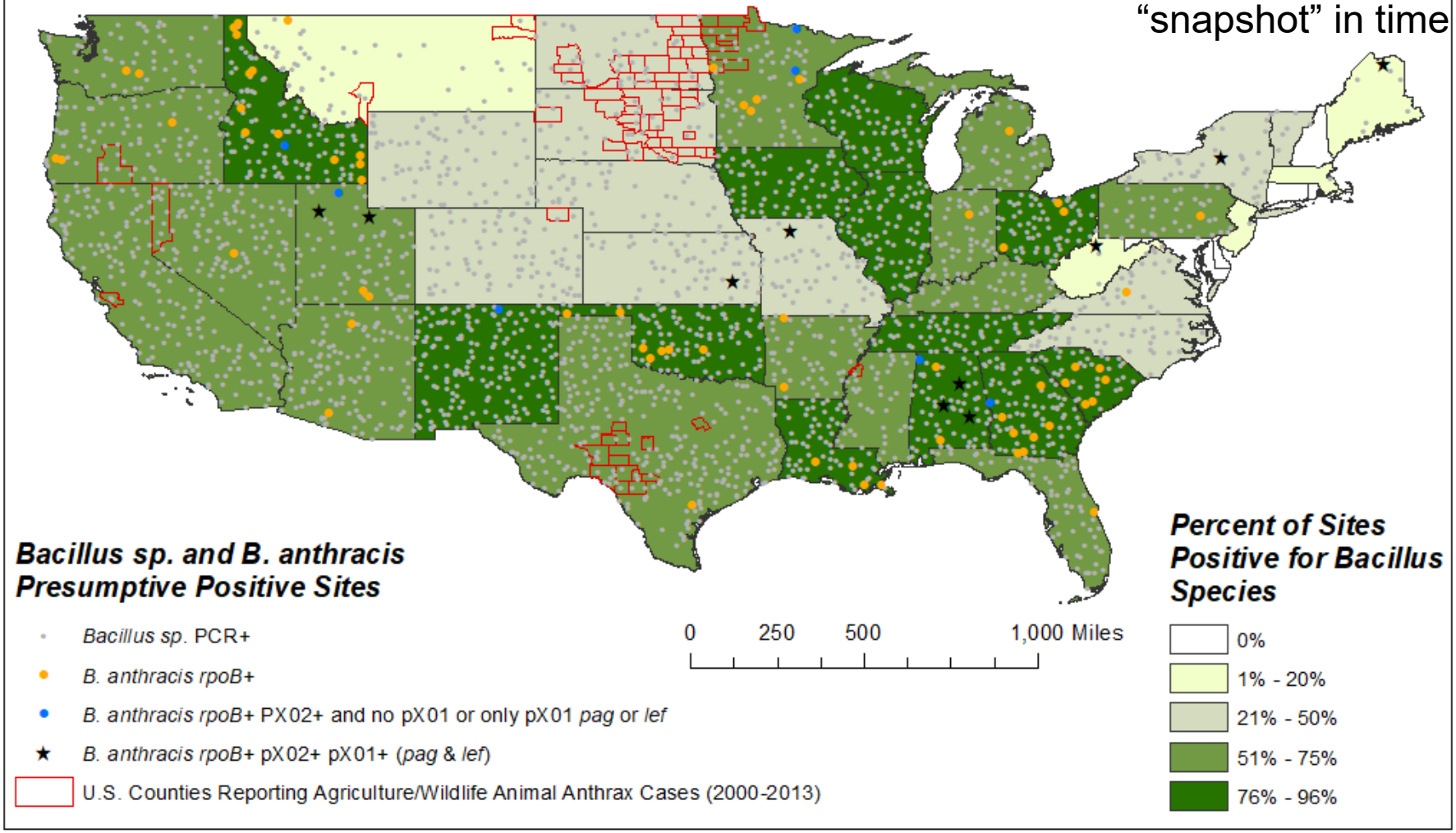
~ 4800 analyzed for *Bacillus* species and *B. anthracis*

Analyzed via MoBio Powersoil DNA Extraction Kit and standard multiplex PCR assay and gel electrophoresis

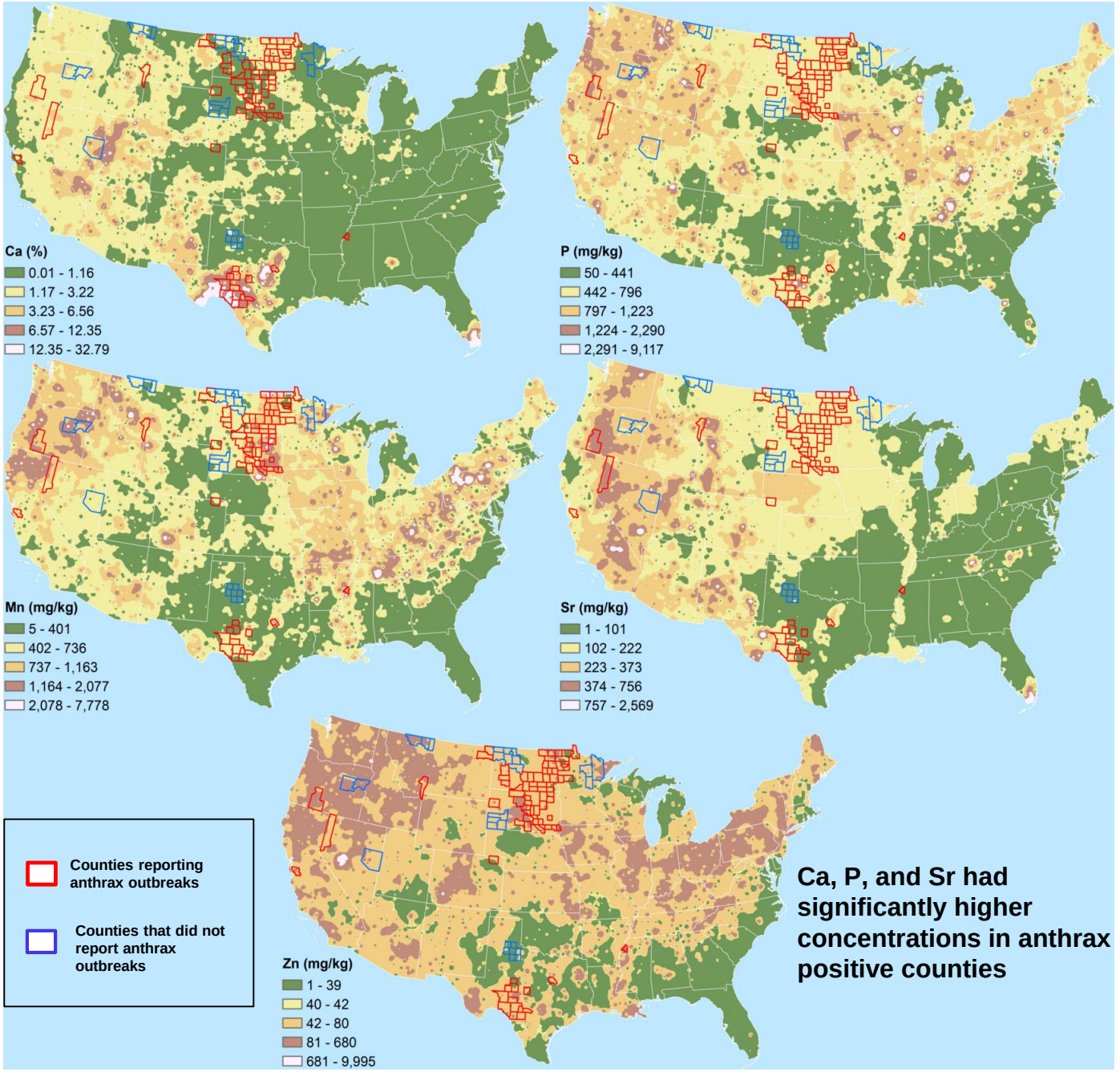
Detected Samples versus Outbreak Locations

Bacillus sp. were detected in 60.3% of the samples and 43 of 48 states

Represents a "snapshot" in time

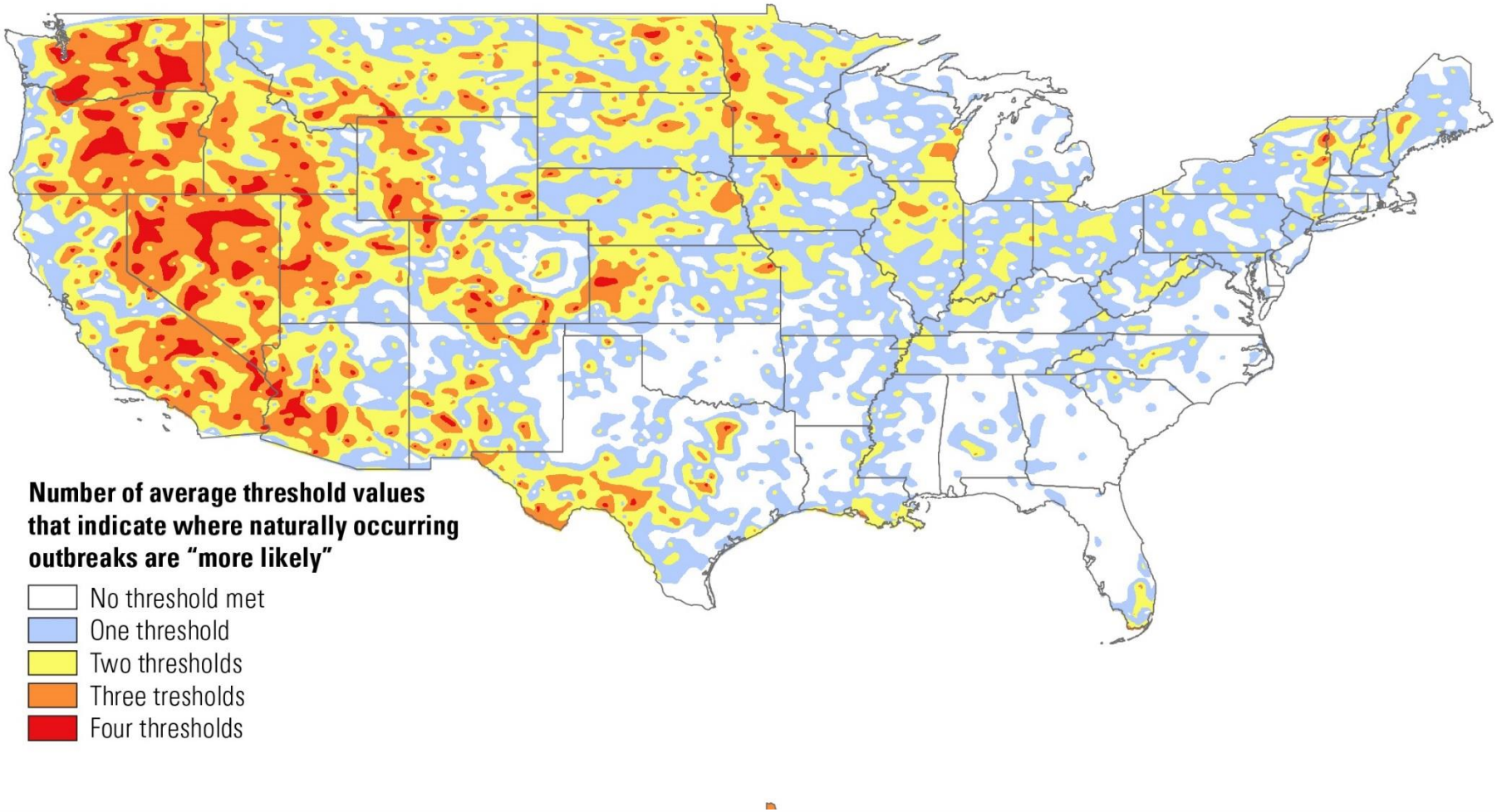


Elemental Data Compared to Counties Reporting/ Not Reporting Animal Anthrax Cases



Ca, P, and Sr had significantly higher concentrations in anthrax positive counties

Mapping Areas where Outbreaks are "More Likely" Based on Geochemical Thresholds



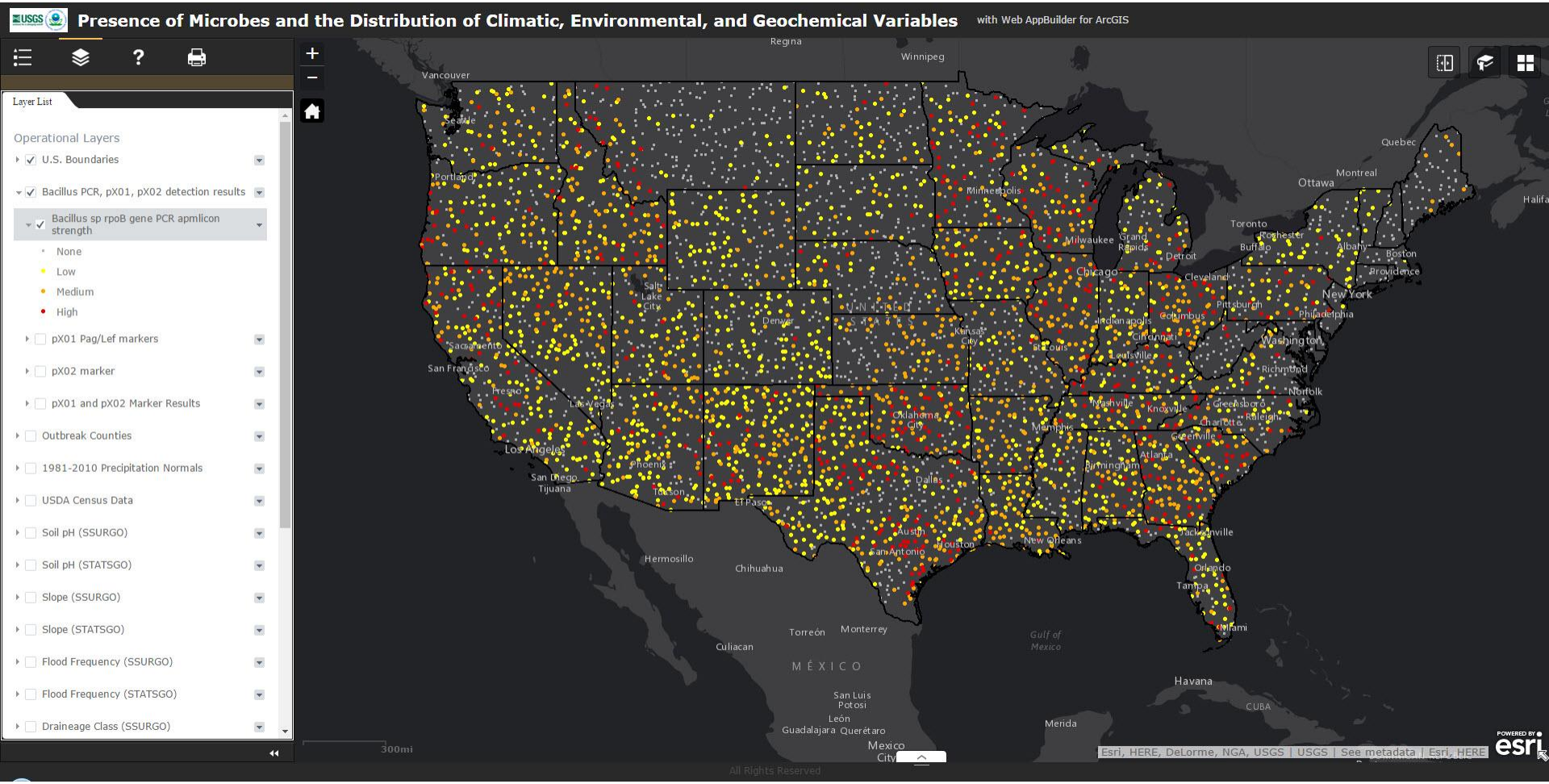
EPA United States Environmental Protection Agency

USGS science for a changing world

Joint EPA/USGS webpage to highlight collaborative projects

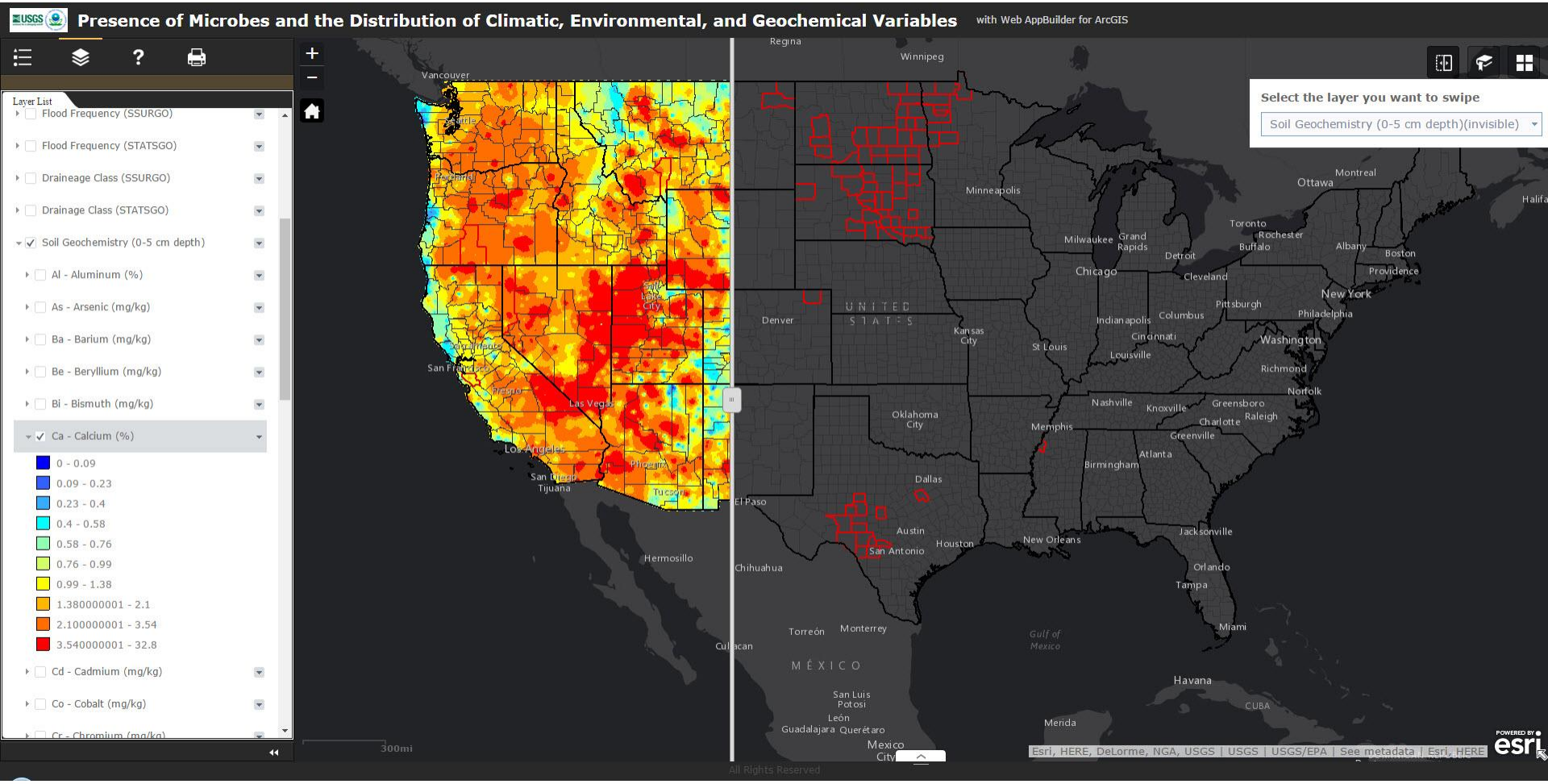
https://coastal.er.usgs.gov/development/gittens/soil-pathogens/

Online Interactive Maps Developed to Display Background Pathogen Data, Geochemical Data, and Climatic Data

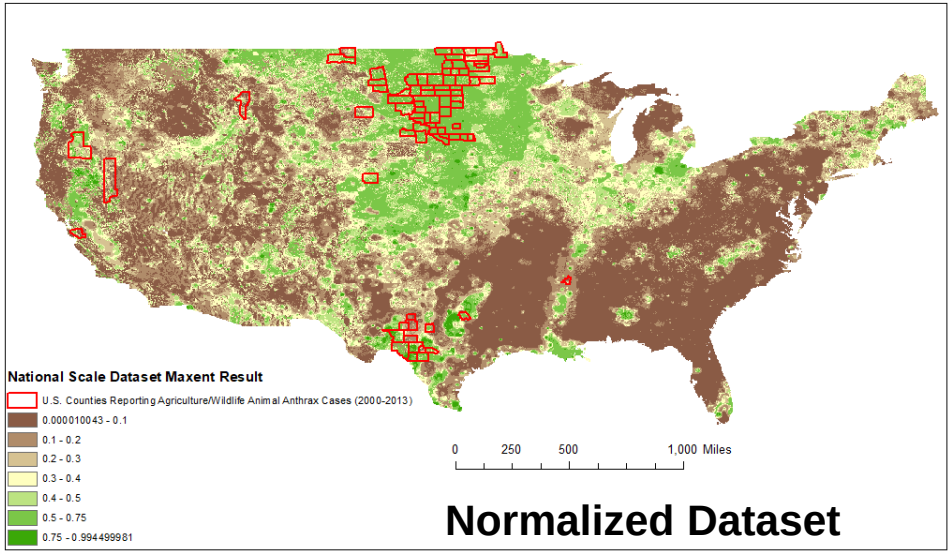


***Bacilllus* species - *rpo*B gene – PCR amplicon**

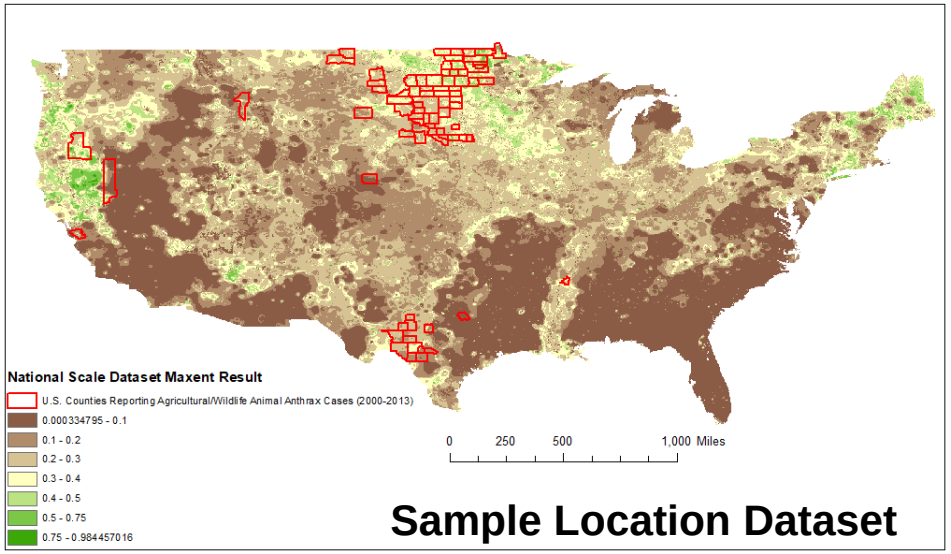
Web Maps Example Showing Slider Tool



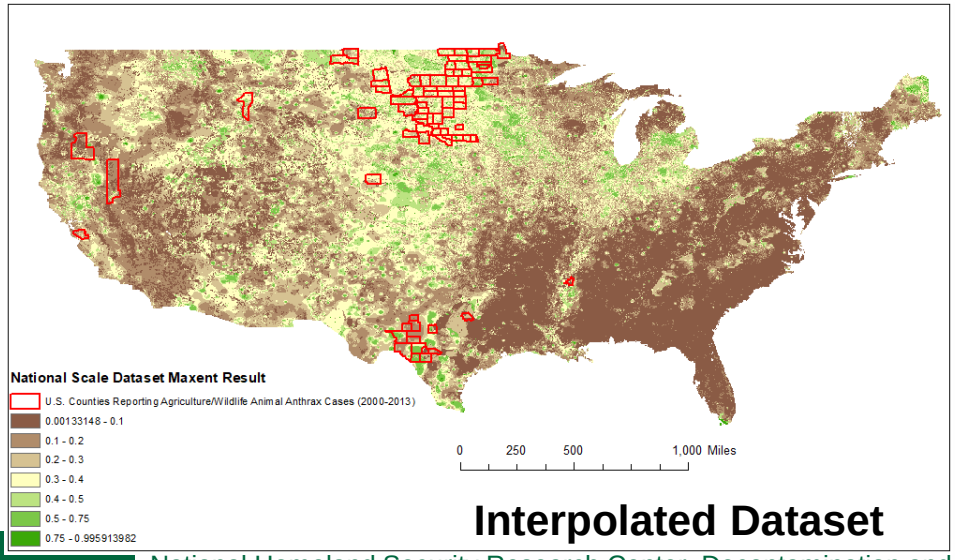
Maxent Modeling Using Anthrax Outbreaks as the Presence Variable



Normalized Dataset



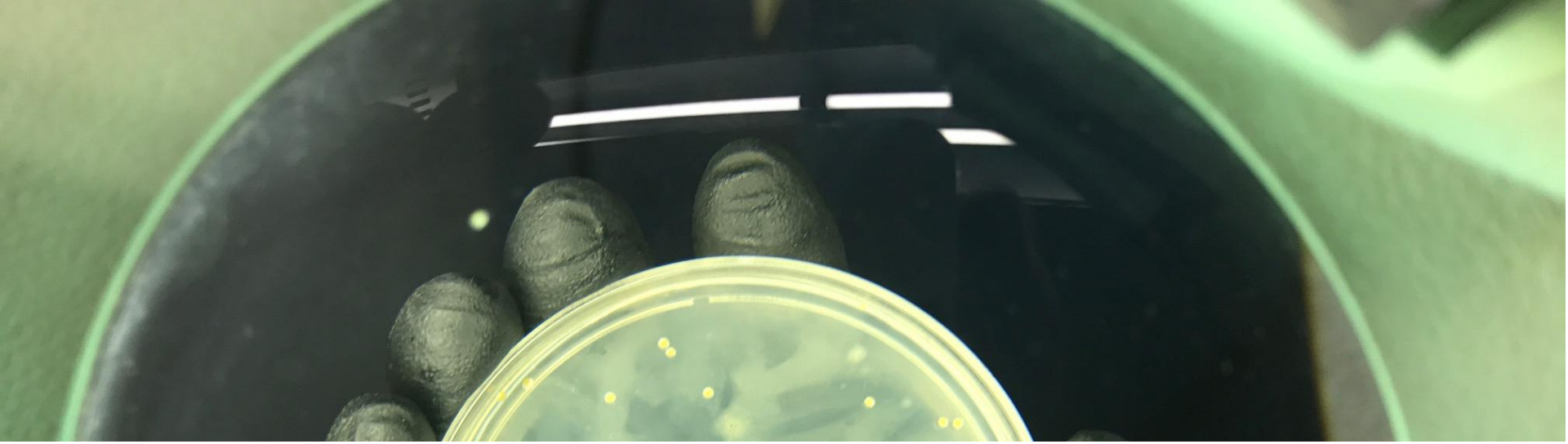
Sample Location Dataset



Interpolated Dataset

Take Away Messages:

- Map results will differ depending on how data is treated, type of modeling used, and the scale used
- Choosing the right metric for measuring model performance is important

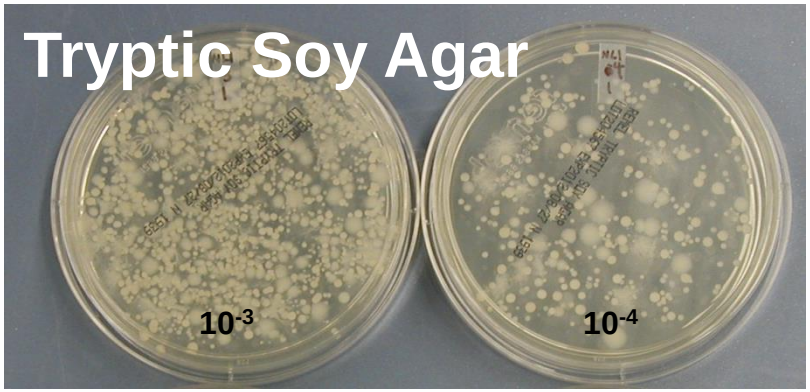


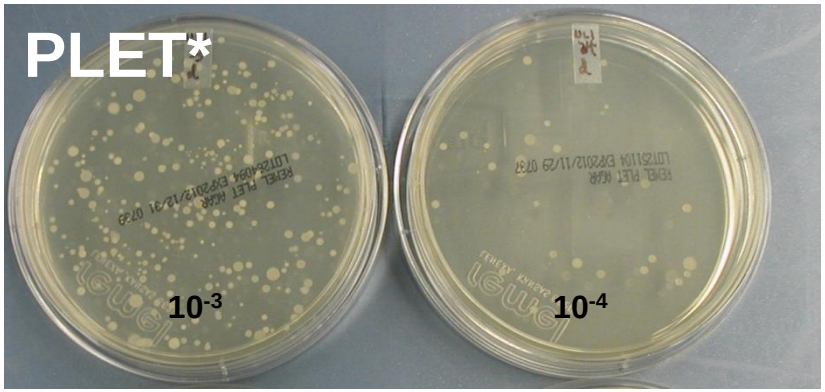
Efforts to Develop Soil Processing and Analysis Methods for *B. anthracis* in Soil Samples

Issues Detecting Spores with Culture

- Culture is typically considered the “gold standard.”
  - However, use of culture with nonsterile soil is difficult due to presence of background microorganisms in the soil.

Nonsterile loam soil with no *B. anthracis* spores added shows growth in selective media of background organisms





* *polymyxin-lysozyme-EDTA-thallous* acetate

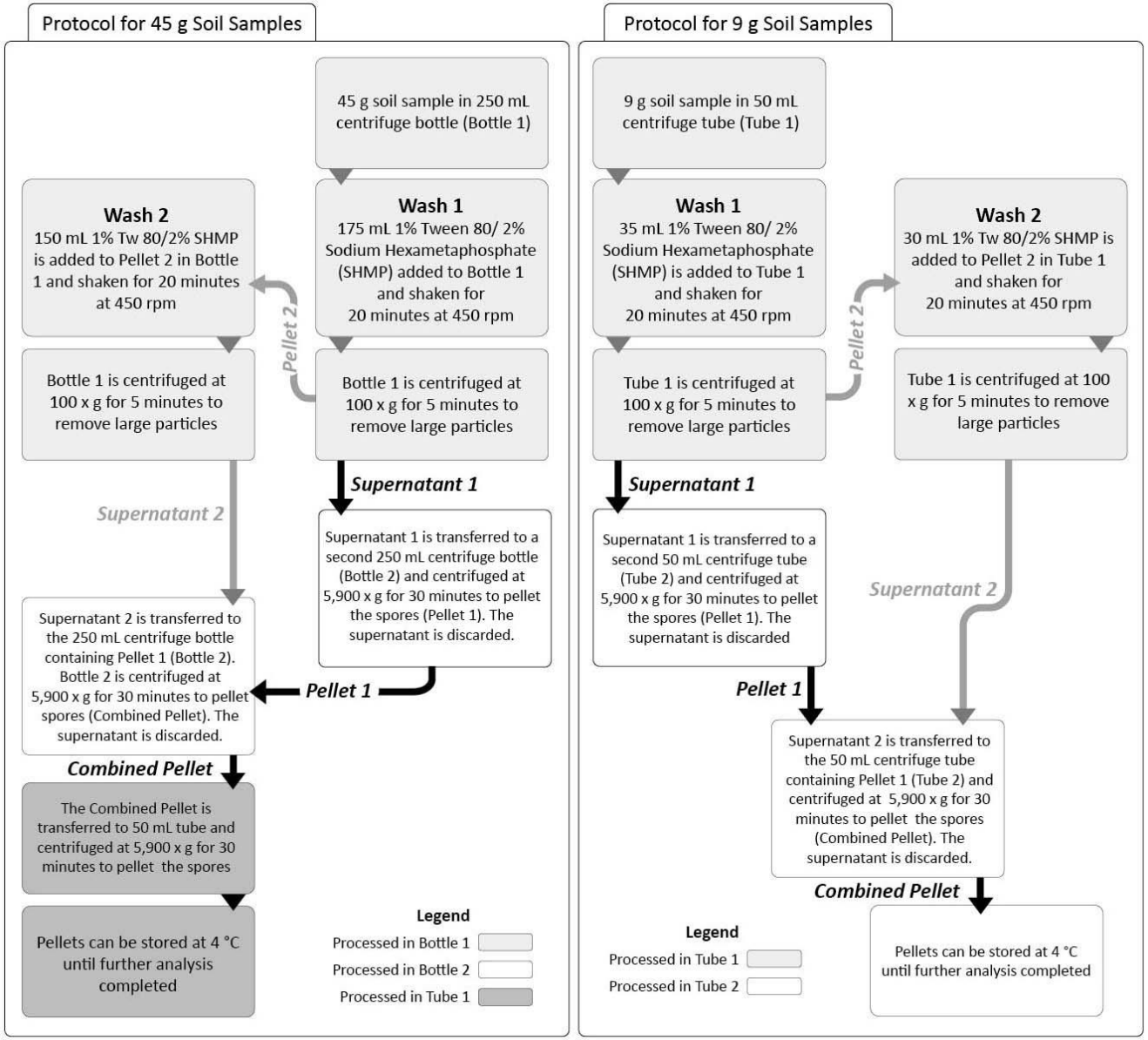
Processing Soil Samples

- PCR can be used to detect low numbers of spore DNA.
  - Sample processing is needed to remove debris, chemical components, and biological impurities that can interfere with detection.

- *Bacillus* colony forming units (CFU) recovered from soil spike on TSA spread-plate
  - Note the carryover of soil particulate matter that could inhibit PCR

Development of an Optimized Processing Protocol

Efforts began in May 2012 to optimize extraction of *B. anthracis* spores from soil



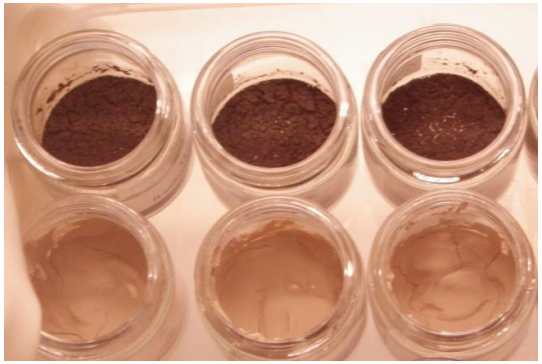
Loam, Sand, and Clay Soils

Enrichment of Soil Samples

- Enrichment of samples might help improve detection of low number of viable spores in the presence of non-target organisms
- Enrichment PCR brings advantages from both culture and PCR techniques to bear on analytical challenge
- Samples processed at Time= 0 and Time = 24 hours to assess viability

Preliminary Study with Enrichment

- We compared the optimized processing protocol (followed by use of culture) to an enrichment-PCR protocol
- Wanted to determine if lower number of spores can be detected with enrichment-PCR
- The experiments utilized:
  - 9g of sterile soil
  - Spikes of 1350, 675, 225, 45 and 4.5 per 9g of soil



Optimized Process/Culture Compared to Enrichment-PCR Study Results

- The optimized protocol/culture could only detect spores in samples spiked with at least 225 spores/9g soil for loam and sand and 45 spores/9g soil in clay soils or higher.
- The enrichment-PCR assay was able to detect spores at all spike concentrations including the lower level spikes (4.5 spores/9 g soil).
- The enrichment-PCR assay has the potential to be more sensitive method for detecting spores in soil samples than the optimized processing protocol

Influence of Clays on Spore Germination

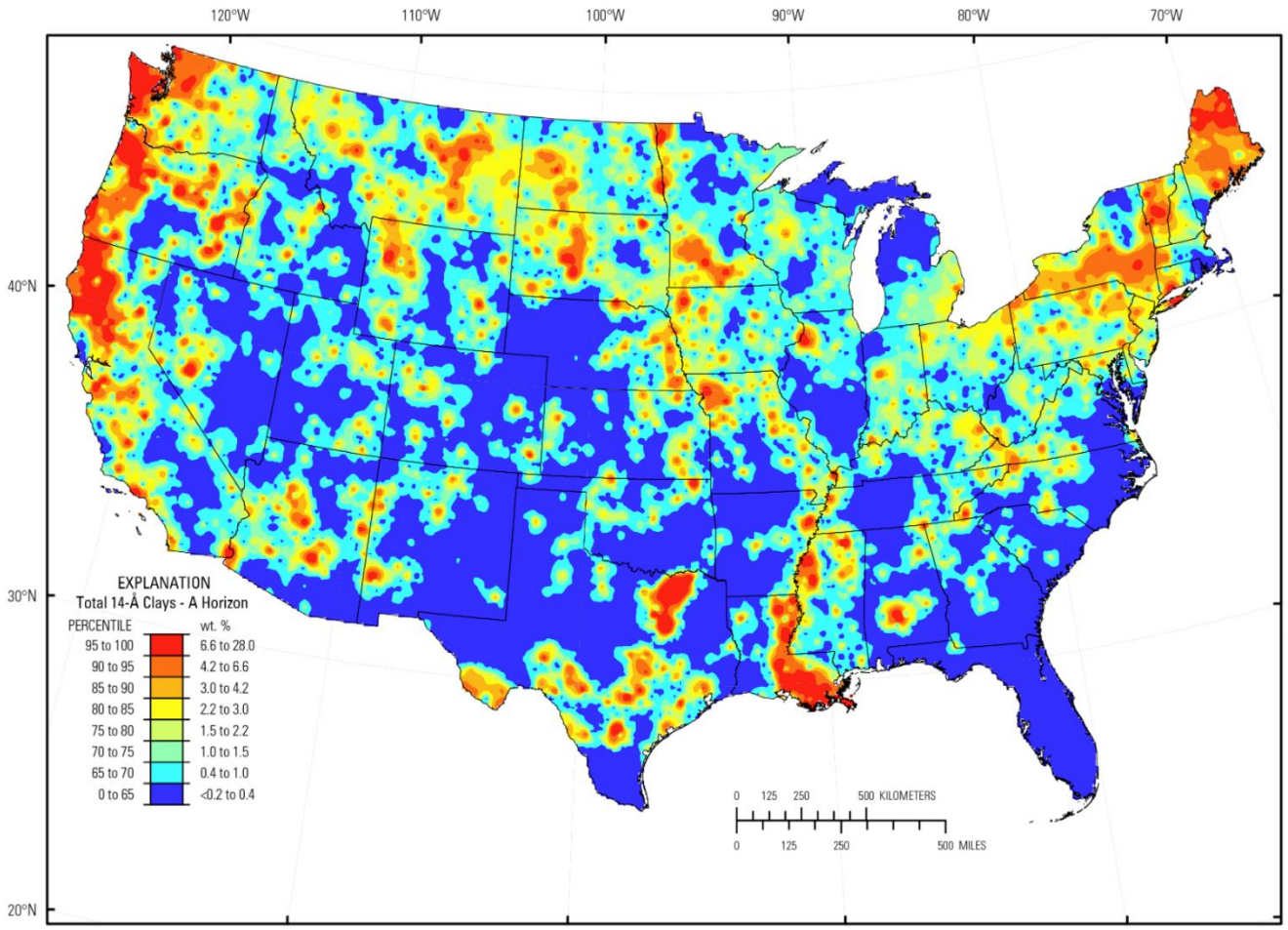
- Clay soil CFU percent recoveries were greater than 100% of the spike, indicating a potential influence of clay in spore germination.

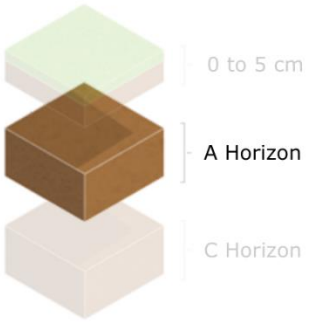
- To assess the influence of clays on spore germination:
  - Sterile pure sand and clay and sand:clay soil mixtures were spiked with concentrations of spores as previously described.

- The CFU assay detected an average of 11 CFU/9 grams of soil for pure sand through a stepped increase of 14 CFU/9 grams of soil for pure clay.

Geochemistry of Soils

14Å CLAY





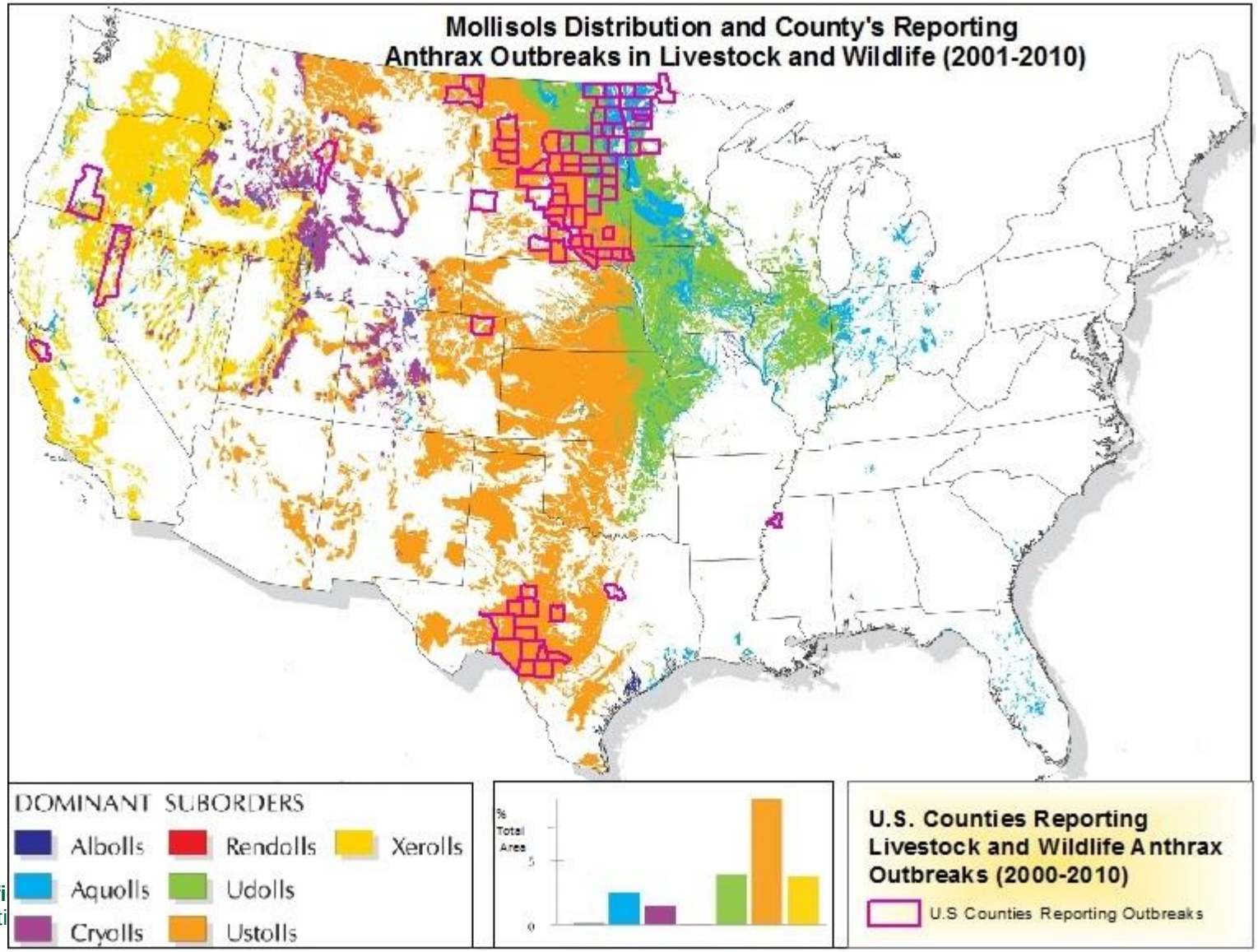
STATISTICS

DOWNLOAD KML
(OPENS WITH GOOGLE EARTH)

DOWNLOAD PDF

DOWNLOAD TIFF

Soil Distribution and Outbreaks



Spore Germination Project

- Evaluate conditions that cause spores to become dormant or remain viable in the soil using soil microcosms.
- Study the migration of spores in the subsurface using soil columns and chambers.
- Will be useful for:
  - Predicting conditions where exposure to natural or intentionally released *B. anthracis* might occur for long periods of time.
  - Aiding responders in decontamination decisions regarding contaminated soil.
- Currently in the initial stages of research.

Lessons Learned and Gaps

- Currently no collection procedure for sub-surface soil or soil containing vegetation
- Analysis of soil for presence of *B. anthracis* is extremely difficult:
  - Culture can't be used with native soils and current processing protocols are showing interference with PCR
- Re-analysis of USGS soil samples using improved processing and analysis methods may help identify additional locations where *B. anthracis* could be located
- When mapping *B. anthracis* presence data, the scale, treatment of the data, and appropriate model metrics are important for accurate map interpretation
- Determination of factors that affect spore germination will help inform decontamination decisions

Disclaimer

- USGS collaborated with the EPA, through its Office of Research and Development under EPA IA# DW14-95774801, DW14-92404401, and DW 14-92486401 on the analysis of samples for the presence of *Bacillus* and *B. anthracis*, GIS mapping efforts, sample collection procedure, and spore germination project.
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