Cleaning and sanitation of heavy equipment for pathogens and weeds

California Invasive Pest Council
October 27, 2020

Yana Valachovic
Brendan Twieg
David McLean
Madeline Lueck
Christopher Lee

Special thanks to Mike Jones and Just Rent It! for the assistance and equipment.
Heavy equipment cleaning for weeds, pests, and pathogens

When, where, and how?

- Projects in remote locations
- Rental companies
- Fire demobilization
- Road crews
- Cleaning is not always required and the trigger are not consistent across and within organizations
- Assumption is if the cleaning works for a pathogen, it should work for a pest or weed seed.
- Present results of a three-part study
Tracked equipment collects soils and debris

LOG LOADER: CAN HARBOUR OVER 500 LITERS OF SOIL/DEBRIS
Portable Washing Stations Require

- Flat ground
- Clean water source
- System to capture and dispose of dirty water
BMPs for working in SOD infested areas

BMPs for Sudden Oak Death

➢ Based upon *Port Orford Cedar Disease* research and other practical approaches to cleaning

➢ Clean soil and debris off personal equipment, machines, and vehicles

➢ Sanitize boots with Lysol, ethanol, 10% bleach
Pilot study (2012)

- **100% (n=22)** pathogen recovery rate from soil/debris samples from **heavy equipment** (3 dates with 400-ml samples)
  - 40% (n=15) pathogen recovery from residue after cleaning and incubation with **water** (3 dates with < 2 ml soil)
  - 20% (n=15) pathogen recovery from residue after cleaning and incubation with **10% bleach** (3 dates with < 2 ml soil)
- **67% (n=6)** recovery from **boot treads** (1 sample date)
- **0% (n=16)** recovery from debris on **chainsaws** (from cotton swabs)
How can we clean pathogens from heavy equipment?

What is the most effective and inexpensive cleaning method?

- Air compressor
- Power washing
- Hotsy (180° F water) pressure washer *(later dropped this treatment)*
- Peracetic acid or peroxide *(not registered for SOD)*
Part 1: 2019 sanitation research field trial

- Established 5 separate study sites that had California bay laurel trees that were positively-tested for *P. ramorum* (May 2019)

- June 2019 these infested bay trees were cut and dropped on to the ground

- A skidsteer and dozer drove over the cut material and native soil to fill the tracks of the equipment in separate replicates.

- Dozer tested first- one week before skidsteer.
Methods

• A top or bottom of each track was randomly assigned a cleaning treatment (producing 4 study regions on each piece of equipment).

• From each replicate, all adherent soil from each of three track segments was taken for control samples prior to cleaning, to determine initial presence of propagules.

• Each of 4 tracks was assigned a treatment:
  ✓ air compressor
  ✓ pressure washer
  ✓ air compressor plus peracetic acid (an oxidizer)
  ✓ air compressor + a pressure washing
Methods
Residual soil was collected from 3 separate tracks within each treatment region using cotton swabs.

The cotton swabs were placed in zip lock bags with 1000 mls of distilled water.

In the lab, 24 six mm sized Rhododendron leaf disks were suspended in the zip lock bags with the solution of distilled water, soil, and cotton swabs.

After one week, the leaf disks were collected, surface sterilized, and plated on PARP using standards culture techniques for *P. ramorum*.

The control soil collections received an equivalent process with a 500-ml subsample of the collected soil.
Results- Dozer
Field trial was from June 3 - 7, 2019
All treatment methods removed most of the soil adhered to the equipment

Swab results
Swabs were baited with Rhododendron leaf disks within 1 week (JBCD through JBFD); disks were immediately surface sterilized
Plated within 9 days after sterilization, checked regularly
➢ No swabs positive for P. ramorum regardless of treatment

Control soil results
Soil samples were baited ~ 6 weeks after collection, in 500 mL volumes (with water added to saturation and ~ 1 cm above for baits to sit upon in cotton mesh sachets); 3-day incubation
16 out of 72 total samples positive (22%)
One location (JBCD) negative for all 12 samples
Other locations ranged from 1/12 to 8/12 control samples positive
  • Number of Rhododendron discs positive ranged from 1 to 18 (mean 6.1) for samples with P. ramorum detected
➢ Control soils were difficult to detect P. ramorum (22% detection rate)
## Results – Skid steer

**Field trial June 12-13, 2019**
The skid steer’s rubber tracks were harder to clean, 1-3 ml of material remained on each track

<table>
<thead>
<tr>
<th>Swab result</th>
<th>Control soil results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment swabs</strong> baited after 1-2 weeks; left in samples for 1 week before removal and immediate surface sterilization</td>
<td><strong>Control soil samples</strong> baited ~ 6 weeks after collection, in 500 mL volumes (with water added to saturation and ~ 1 cm above for baits to sit upon in cotton mesh sachets); 3-day incubation</td>
</tr>
<tr>
<td>Plated within 9 days after sterilization, checked regularly</td>
<td>2 out of 24 total samples positive (8.3%)</td>
</tr>
<tr>
<td>One positive from air-only treatment, from same location as only 2 positive control samples from skid steer trial</td>
<td>Only one location positive (one control sample from each of left and right tracks)</td>
</tr>
<tr>
<td>➢ <strong>One single swab sample positive for P. ramorum</strong></td>
<td>One sample had 5 discs positive (same side as swab sample); other had only 1 disc positive</td>
</tr>
<tr>
<td></td>
<td>➢ <strong>Control soils were difficult to detect P. ramorum (8% detection rate)</strong></td>
</tr>
</tbody>
</table>
Part 2: 2019 sanitation research lab inoculated soil

Fall 2019 lab study in constructed metal tracks
Soil spiked with known concentrations of *P. ramorum* zoospores

**Study Variables**
- 3 amounts of soil (mLs)
  - 2
  - 50
  - 400
- 4 chemical sanitizers
  - 10% Bleach
  - 70% Isopropanol
  - Peridox RTU (peracetic acid)
  - Water only
- 2 soil sources
- 2 incubation time (immediate and 2-week)
- 3 replications
# One replicate made from same batch of artificially infested soil

<table>
<thead>
<tr>
<th>No Incubation Time for Soil on Tracks</th>
<th>2-wk Incubation Time for Soil on Tracks</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 mL</td>
<td>2 mL</td>
</tr>
<tr>
<td>50 mL</td>
<td>50 mL</td>
</tr>
<tr>
<td>400 mL</td>
<td>400 mL</td>
</tr>
<tr>
<td>10% Bleach</td>
<td>10% Bleach</td>
</tr>
<tr>
<td>Peridox RTU</td>
<td>Peridox RTU</td>
</tr>
<tr>
<td>70% Isopropanol</td>
<td>70% Isopropanol</td>
</tr>
</tbody>
</table>

**Chemical:**
- None
- 10% Bleach
- Peridox RTU
- 70% Isopropanol
### Soil Amount vs Treatment

<table>
<thead>
<tr>
<th>Soil Amount</th>
<th>Treatment</th>
<th>Soil 1 – Lacks Creek</th>
<th>Soil 2 – Redwood Valley</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 ml</td>
<td>No treatment - control</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>2 ml</td>
<td>10 % Bleach</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>2 ml</td>
<td>70% Isopropanol</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>2 ml</td>
<td>Peridox RTU</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>50 ml</td>
<td>No treatment - control</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>50 ml</td>
<td>10 % Bleach</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>50 ml</td>
<td>70% Isopropanol</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>50 ml</td>
<td>Peridox RTU</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>400 ml</td>
<td>No treatment - control</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>400 ml</td>
<td>10 % Bleach</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>400 ml</td>
<td>70% Isopropanol</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>400 ml</td>
<td>Peridox RTU</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

#### Best sanitizers:
- **No incubation**
  - Isopropanol
  - Peridox RTU
- **Two-week incubation**
  - Isopropanol
  - Peridox RTU (effect only for 400 ml sample)

---

- Chemical added to soil amounts via spray bottle: 1 ml to 2-ml samples; 5 ml to 50-ml samples; 40 ml to 400-ml samples. Allowed to sit in the soil for 10 minutes prior to adding distilled water for baiting.
- 400-ml samples, 1.5 liters of water was added, while to 2-ml and 50-ml samples, 500 ml was added.
- Zoospores added 7,500 per ml of soil.
- 90 4-ml plugs of the source culture plugs used to generate sporangia were also blended in water and added to the inoculation mixture; these contained chlamydospores.
- Experiment replicated following a two-week incubation treatment (results not shown)
### Part 3: Water treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Positive Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment – control</td>
<td>2 of 3</td>
</tr>
<tr>
<td>0.1% Bleach</td>
<td>0 of 3</td>
</tr>
<tr>
<td>0.7% Isopropanol</td>
<td>3 of 3</td>
</tr>
<tr>
<td>Peridox RTU 1:100 dilution</td>
<td>0 of 3</td>
</tr>
</tbody>
</table>

#### Effective sanitizers
- 1% bleach
- Peridox RTU

#### Methods
- Chlorox bleach is EPA registered for a 1:1,000 dilution in water to kill *P. ramorum* in drafted water (5-min incubation). This was 1/100 of the concentration used in the soil experiment, so we diluted the other chemicals by the same amount (1:100).
- Zoospore concentration was around 700 per ml, and each chemical was tested in a 1-liter volume.
- **10 minutes exposure** before removing aliquots for dilution into the baiting liquid.
- From the 1-liter volume for each chemical, 3 subsamples each were taken: 50 ml of the 1-liter mix was combined into 450 ml for each baiting container (i.e. each was diluted 1:10 further from it’s original 1:100 dilution).
Conclusions - soil removal

• Cleaning is time consuming
• Equipment clearly collects soil and vegetation when soils are wet
• In drier soil conditions it may be more difficult to infest heavy equipment
• Previous pilot study was during the wet season
• All treatments were effective at removal
• Air compressor was the easiest to use at 120 PSI with a modified wand
• Not wise to put water near expensive electronics
Conclusions - sterilization of soils and water

• 10% bleach was not more effective than the control (EPA registered) with soils, but was effective with water
• Mixed results with isopropanol
• Peracetic acid was effective. It is used in the food industry. It is corrosive and requires a post-treatment rinse.
• Is any detection of *P. ramorum* acceptable after cleaning? What is our standard?
• When do we require cleaning?