Sanitation of Heavy Equipment in Sudden Oak Death Infested Forests

California Forest Pest Council
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450J
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450J
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LT

Heavy equipment: potential SOD vector

California bay laurel stumps

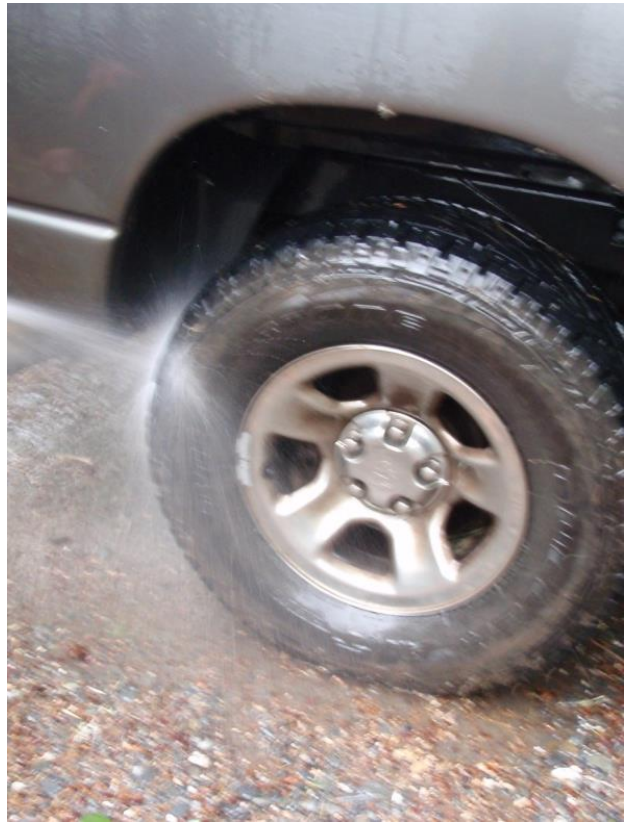
Equipment is working where trees have been fallen

Tracked equipment collects infested soils and debris

BMPs for working in SOD infested areas

BMPs for SOD

- Based upon *P. lateralis* research and other practical approaches to cleaning
- Clean soil and debris off personal equipment, machines, and vehicles
- Sanitize boots with Lysol, ethanol, 10% bleach



Redwood Valley 2012 pilot study

- 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 heavy equipment?

What is the best (effective and inexpensive) method for cleaning?

- Air compressor
- Power washing
- Hotsy (180^{o} water) pressure washer (later dropped this treatment)
- Peracetic acid or peroxide

2019 sanitation research (part 1)

- 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

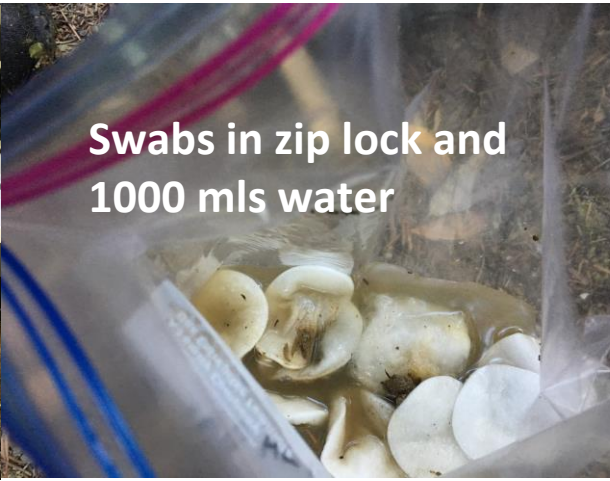
✓air compressor plus peracetic acid (an oxidizer)

✓air compressor + a pressure washing

Methods

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 trail was from June 3rd -7th

Treatment swabs 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) samples 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

- For skid steer, bays were fallen 6-10 days prior to trial (i.e., no extra trees fallen after initial effort)
- Field trial June 12th - 13th

Treatment swabs baited after 1-2 weeks; left in samples for 1 week before removal and immediate surface sterilization

- Plated within 9 days after sterilization, checked regularly

➢ ***One single swab sample positive for P. ramorum***

- From air-only treatment, from same location as only 2 positive control samples from skid steer trial

Control (soil) samples 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

- 2 out of 24 total samples positive (8.3%)
- Only one location positive (one control sample from each of left and right tracks)
  - One sample had 5 discs positive (same side as swab sample); other had only 1 disc positive

➢ ***Control soils were difficult to detect P. ramorum (8% detection rate)***

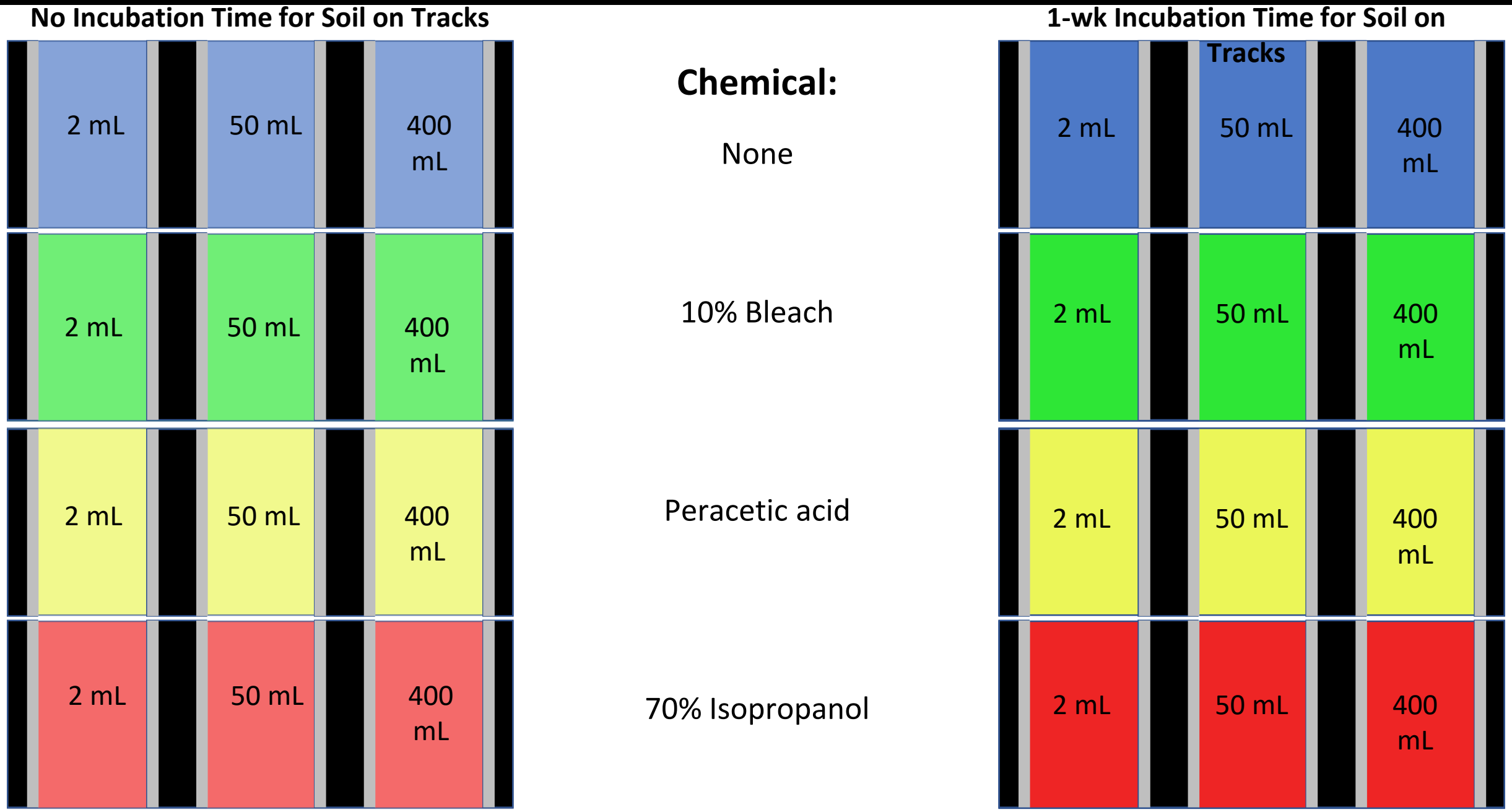
2019 sanitation research (part 2)

Fall 2019 lab study in constructed metal tracks

Soil spiked with known concentrations of *P. ramorum* zoospores

- ➢Chemical sanitizers
  - Bleach
  - Isopropanol
  - peracetic acid)
- ➢3 amounts of soil
  - pre-cleaning state (or track full of soil)
  - post-cleaning state
    - 50 ml
    - 2 ml of soil left

One replicate made from same batch of artificially infested soil



Soil Amount	*Treatment*	*Soil 1 – Lacks Creek*	*Soil 2 – Redwood Valley*
2 ml	*No treatment - control*	**Positive**	**Positive**
2 ml	*10 % Bleach*	**Positive**	**Positive**
2 ml	*70% Isopropanol*	Negative	**Positive**
2 ml	*Peridox RTU*	Negative	Negative
50 ml	*No treatment - control*	**Positive**	**Positive**
50 ml	*10 % Bleach*	**Positive**	**Positive**
50 ml	*70% Isopropanol*	Negative	**Positive**
50 ml	*Peridox RTU*	Negative	Negative
400 ml	*No treatment - control*	**Positive**	**Positive**
400 ml	*10 % Bleach*	**Positive**	**Positive**
400 ml	*70% Isopropanol*	Negative	Negative
400 ml	*Peridox RTU*	Negative	Negative

Effective sanitizers
No incubation time
- Isopropanol
- Peracetic acid

- 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.

Zoospores and sporangia in water treatment

Treatment	Number of Positive Samples (out of 3)
No treatment – control	**2**
0.1% Bleach	0
0.7% Isopropanol	**3**
Peridox RTU 1:100 dilution	0

Effective sanitizers

- 1% bleach
- Peracetic acid

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).

Interim conclusions…

- While we wait for the final lab study results….
- Cleaning is time consuming
- Is any detection of *P. ramorum* acceptable after cleaning? What is our standard?
- Do we understand pathways and risks of transmission?

Interim conclusions

- In slightly drier soil conditions it may be more difficult to infest heavy equipment
- Previous pilot study was during the wet season
- All treatments were effective
- Air compressor was the easiest to use at 120 PSI with a modified wand
- Not wise to put water near expensive electronics

Background weather conditions – Eel River Camp data station

- From October 1st, 2018 to the beginning of the study, 62.85 inches of rain fell
- From March 1st, 2019 to the beginning of the study, 16.4 inches fell
  - Avg. daily temp max. 60.8, 68.3, and 73 for March, April, and May, respectively
- Most of the 4.58 inches of rain that fell in May, 2019, occurred between the 15th and 21st of May
  - Daily temp maximum ranged from 57-64 degrees Fahrenheit on those days.
- An additional 1/20th inch fell May 25th through 26th, with daily maximum 62 and 67 degrees Fahrenheit, respectively
- No further rain fell during the study
- For the week prior to the study (dozer portion), daily maximum ranged from 70 to 90 degrees F (avg. max. 78.9 deg)
- During the dozer trials, the max daily temp ranged from 65 to 84 deg F (avg. max. 75.6)
- For the 4 days in between dozer and skid steer trials, max daily temp between 79 and 102 deg F (avg. max. 93.5)
- Daily temp max. during 2 days of skid steer trials 96 and 89 deg F