Cleaning and sanitation of heavy equipment for pathogens and weeds

California Invasive Pest Council
October 27, 2020

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Special thanks to Mike Jones and Just Rent It! for the assistance and equipment.

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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

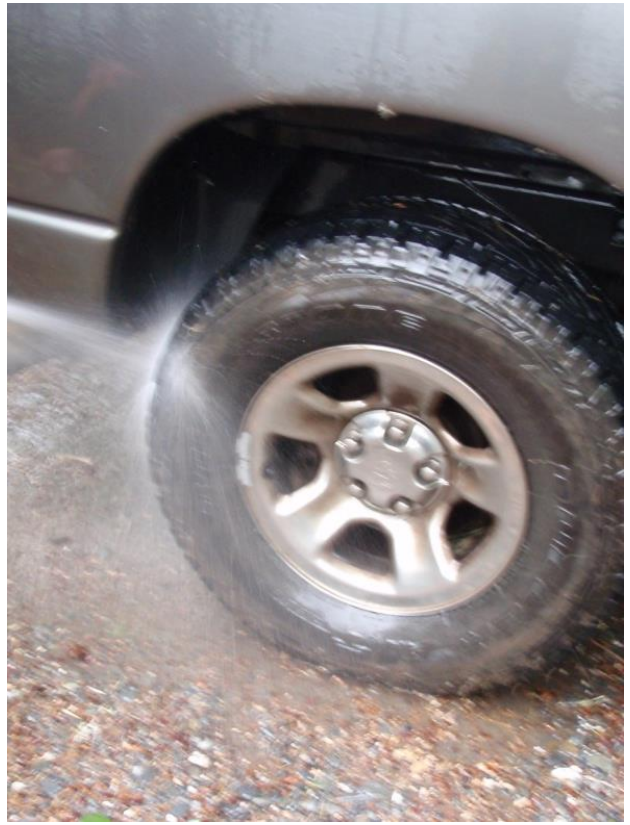
Portable Washing Stations Require

- 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^{o} 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

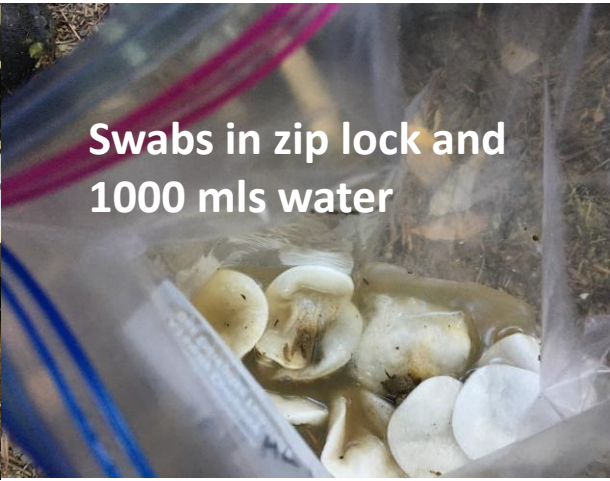
✓air compressor plus peracetic acid (an oxidizer)

✓air compressor + a pressure washing

Methods

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 3 -7, 2019

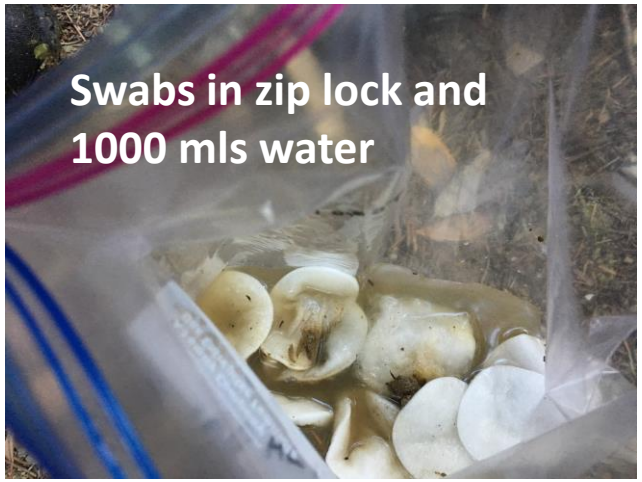
All treatment methods removed most of the soil adhered to the equipment

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

Swab result

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

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

Control soil results

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)***

Part 2: 2019 sanitation research lab inoculated soil

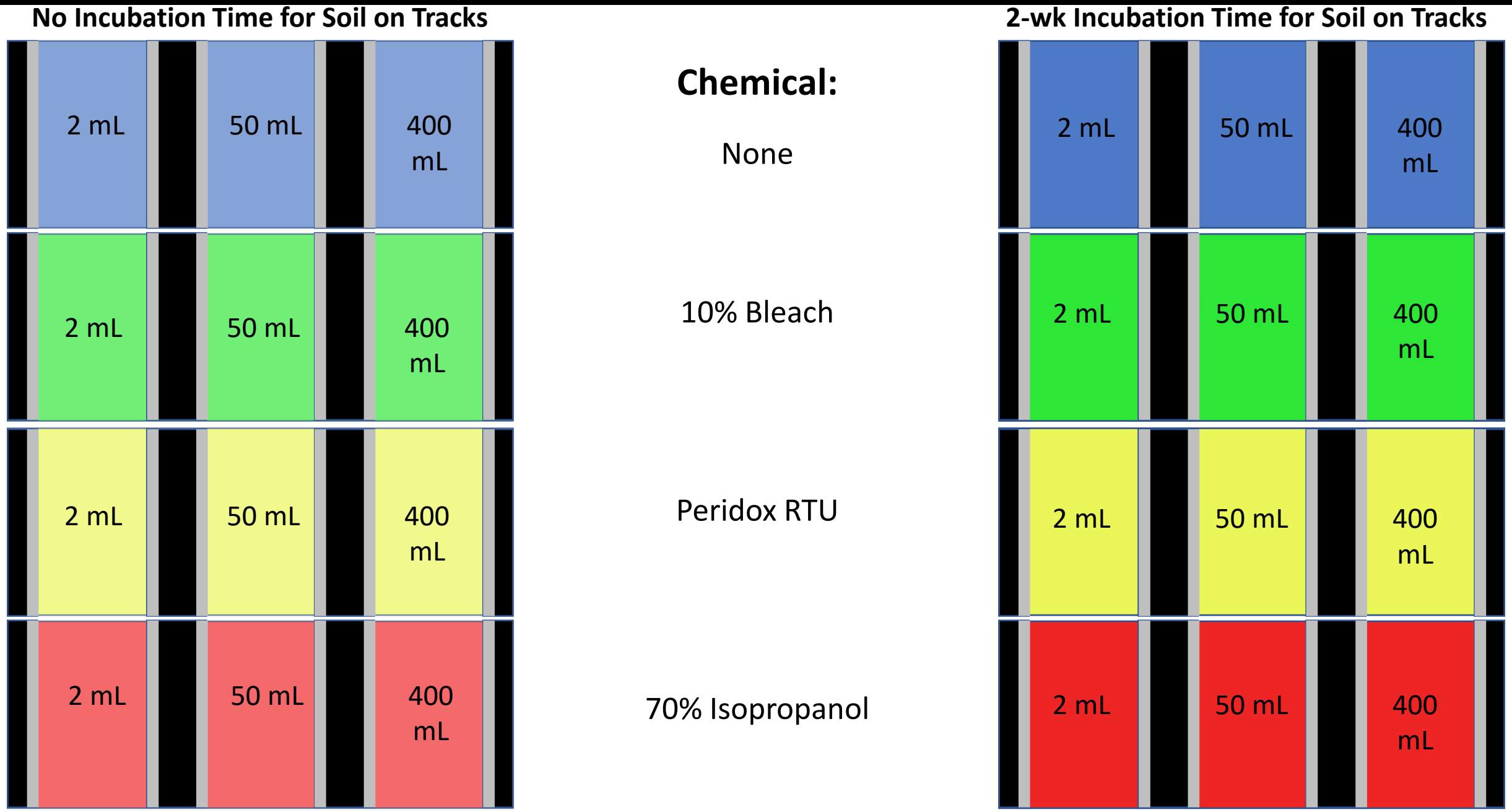
Fall 2019 lab study in constructed metal tracks

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



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**

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

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

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?**