Heat treatment protocol to kill *Phytophthora agathidicida* in soil, plant material, and on inanimate objects.

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Role	Name	Date submitted
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Approval

Name	Role	Signature/ Date	Endorsement
J. Walsh, Ministry for Primary Industries	**Approve / Note** the contents of this document	04/08/2020	Yes

Associated documents

Document name	Link
Hygiene procedures for Kauri Dieback	https://www.kauridieback.co.nz/how-to-guides/
Land disturbance activities around kauri	
Tree removal and pruning of kauri	
Vehicle and heavy machinery hygiene	
Landfill disposal of contaminated material	
Propagation and Planting of Kauri	

Glossary

Terminology	Meaning
Carbon to Nitrogen (C:N) Ratio	The ratio of the weight of organic carbon (C) to that of total nitrogen (N) in an organic material.
Core Temperature	The temperature at the core of the target of the heat treatment, or an acceptable substitute.
Disinfectant	Sterigene at 2%
Exposure Period	The amount of time, in one continuous block, that the item to be heat treated must be exposed to targeted temperature over the exposure period specified.
Kauri dieback	Name of the disease that causes dieback on kauri caused by the pathogen *Phytophthora agathidicida*
Moisture Content	The fraction or percentage of a substrate comprised of water. Moisture content equals the weight of the water portion divided by the total weight (water plus dry matter portion).
Oospore	The 'resting' or 'hibernation' spore of *Phytophthora agathidicida*
PA	*Phytophthora agathidicida*
Phytophthora agathidicida	Microscopic fungus-like organism that is the primary cause of kauri dieback disease in kauri.
Propagule	Microscopic life stage (like seeds) whose role is to progress the propagation of an organism to the next stage in their life cycle.
Turning	A composting operation which mixes and agitates material in a windrow pile or vessel.

Disclaimer

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1.0 Purpose

To provide heat treatment guidelines for pathway management on items that have the potential to be contaminated or infected with the pathogen *Phytophthora agathidicida*, and subsequently spread through human- assisted conveyances.

Guidelines provide advice on the elimination of the pathogen below non-detectable levels.

A safety margin to minimise any residual risk that may remain post- heat treatment has been incorporated into the protocols. This is to manage the level of scientific uncertainty around current knowledge and understanding into the efficacy of heat treatment on *P. agathidicida*.

These guidelines should form part of a layered approach to reducing risk that starts with good hygiene practices as outlined in the associated best-practice guidelines.

2.0 Background

Phytophthora species are not thermophilic, in that they don't thrive when exposed to relatively high temperatures between 41 and 122 °C. Indeed Bellgard et al., (2013) found that the cardinal temperature for optimal growth of *P. agathidicida* mycelia was 22°C, with no growth occurring at 30°C.

It has been shown that Phytophthora species can be killed by exposure to high temperatures for a sufficient length of time (Widmer, 2010; Ramsfield et al. 2010; Lobet & Siverio, 1995; Barbercheck & Broembsen, 1986; Noble & Roberts 2004: Swain et al. 2005; Wichuk, Tewari & McCartney, 2011). However it is recognised that certain life stages of Phytophthora, are more difficult to kill than others.

Oospores are the most persistent propagule type produced by *Phytophthora* spp. and are the main survival propagule in soil, bark and root material for many species including *P. agathidicida*. Oospores are characterized by a thick wall, resistance to desiccation and ability to enter a dormant phase which is more resistant to degradation under conditions of increased heat and external stressors (Williams, 2015).

Resistant, hardy exospore structures often comprise of proteinaceous structures. Lippman et al. (1974) has found that *Phytophthora sojae* oospores have a high proportion of protein relative to its other life cycle stages. Exposure to temperatures breaks down intracellular proteins causing failure of cells in critical tissue, thereby killing the organism.

Research has found that oospores of *P. agathidicida*, when exposed to temperatures above their thermal limit, are deactivated or killed (Dick & Kimberley, 2013; Williams, 2015 & Horner *et. al*., 2019).

A critical assessment of this research (Ashcroft, 2020) concluded the following minimum temperature-time protocols were deemed to be appropriate to deactivate *P. agathidicida* oospores in soil, potting mix and certain types of vegetation material.

- *50 degrees for 24 hours; and*
- *40 degrees for 14 days*.*

The minimum treatment period **<u>must be continuously</u>** at or above the required temperature.

From an operational perspective, the desired temperature-time combination can be achieved in a number of different ways. These are:

- Soil solarisation.
- Composting.
- Direct heat (e.g., insulated or non-insulated containers heated by electricity, natural gas or fire).

**Please note, the minimum temperature-time protocol for composting differs to align with the existing NZ standard for composting (NZS, 2005).*

3.0 Assumptions & Constraints

3.1 Specific research has not been undertaken to determine the efficacy of solarisation and composting on *P. agathidicida* oospores. These guidelines are based on current research into the efficacy of temperature against *P. agathidicida* in non-composting and non-solarisation systems as well as overseas research into the efficacy of using heat treatment against other Phytophthora species using solarisation and composting.

3.2 A safety margin has been built into the temperature – time protocols to further reduce the likelihood of surviving *P. agathidicida* propagules post-treatment.

3.3 At present there is no known detection assay that can provide an absolute guarantee that compost is free from *P. agathidicida*, as a result these guidelines provide advice on the elimination of the pathogen below non-detectable levels.

3.4 The success of composting in eliminating pathogens is not solely a result of the heating process, but also depends on the many and complex microbial interactions that may occur, as well as other parameters such as moisture content. There is little information on the independent effect of composting feedstocks on the eradication of plant pathogens.

3.5 The reliability of data will depend on the accuracy and calibration of the temperature monitoring equipment.

4.0 Before you begin

4.1 These guidelines have been developed to provide written advice on the use of heat treatment.

4.2 The guidelines are not policy but should be considered by planners, land managers and contractors when planning any operations.

4.3 Contact your local regional council, unitary authority or Department of Conservation if there are local policy or regulatory constraints e.g. commercial-scale composting operations generally require approval to operate from regional councils, and in some cases, territorial authorities (city or district councils).

4.4 The guide provides what is considered best practice based on the current information and uses risk management principles to reduce the likelihood of spread of PA during operations.

4.5 These guidelines should form part of a layered approach to reducing risk. The Best Practice Guidelines listed under ‘Associated documents’ should be read in conjunction with these guidelines, prior to undertaking any on-site operations.

4.6 For soil-contaminated equipment, removal of soil and then spraying with sterigene is the preferred hygiene method. If this cannot be achieved, then heat treatment is advised.

5.0 Heat Treatment Protocols

Vector Risk	Treatment Tool	Minimum Temp. (◦C)	Minimum Time	Moisture Content	Operational Considerations
Soil or potting mix (any volume)	Kiln, oven or heat chamber	≥50◦C	≥24 h	• 20-30% before treatment • If this cannot be measured, then soil must be moist to touch before treatment.	1. Target treatment must be reached throughout the entire profile. The temperature should be measured in the coldest spot e.g. at the core of the media. 2. A closed system is advised so moisture is not lost during heat treatment.
Soil or potting mix (less than 10kg)	Solarisation	≥50◦C	≥24 h	• Soil must be moist to touch before treatment	1. Use of a solar oven is advised and constructed in accordance to Section 6. 2. Small amounts of soil can be solarized in bags, pots, or plastic buckets. 3. Target treatment must be reached throughout the entire profile. The temperature should be measured in the coldest spot e.g. at the core of the soil/potting mix. 4. Ensure the solar oven is closed where possible to prevent moisture loss.
		≥40◦C	≥14 days		
Equipment (incl. hand tools, footwear, containers, misc. equipment).	Kiln, oven or heat chamber	≥50◦C	≥24 h	• >10 ml of free water (in an open heat proof container) before treatment.	1. Only heat treat, if removal of soil cannot be undertaken. Soil contaminates to be removed for destruction by incineration or deep burial at an appropriate landfill 2. Not suitable for vehicles and complex machinery.

	Solarisation	≥50◦C ≥40◦C	≥24 h ≥14 days	• >10 ml of free water (in an open heat proof container) should be included with the equipment before treatment.	1. Only solarise, if removal of soil cannot be undertaken. Soil contaminates to be removed for destruction by incineration or deep burial at an appropriate landfill. 2. Use of a solar oven is advised and constructed in accordance to Section 6. 3. Target treatment must be reached throughout the entire profile. The temperature should be measured in the coldest spot e.g. at the core of the soil/potting mix. 4. Ensure the solar oven is enclosed where possible to prevent moisture lost.
Plant material	Composting*	≥55◦C	≥15 days	• 25% (minimum)	1. Minimum 5 turnings 2. Target treatment must be reached throughout the entire profile. The temperature should be measured in the coldest spot e.g. at the edge.
	Kiln, oven or heat chamber	≥50◦C	≥24 h	• 20-30% before treatment. • If this cannot be measured, then >10 ml of free water (in an open heat proof container).	1. Target treatment must be reached throughout the entire profile. The temperature should be measured in the coldest spot e.g. at the core.

* New Zealand Standard 4454:2005 – Composts, Soil Conditioners and Mulches

6.0 Solarisation

6.1 Background

Solarisation is using solar heat to increase temperature via the greenhouse effect under clear plastic or glass.

These guidelines are intended to be used to produce lethal temperatures for items such as hand held tools (shovels, spades), footwear and small amounts of soil or potting media (<10kg).

Solarisation is useful in remote field situations where other mitigation measures may not be available.

6.2 What can be treated?

- Small amounts of soil, potting mix (<10kg) in plastic pots (not ceramic pots).
- Soil contaminated items such as tools, footwear, small equipment, containers, pots etc.

6.3 What cannot be treated?

- Large volumes of soil.
- In situ application of ground soil.
- Vegetation (refer to composting guidelines).
- Large and complex machinery items such as bulldozers, vehicles etc.

6.4 Protocol

Temperature*	Time	Water content
≥50°C	≥24 hours	• *Soil (<10kg):* Soil is moist to touch • *Small equipment:* >10mls free water
≥40°C	≥14 days	

*The minimum treatment period **<u>must be continuously</u>** at or above the required temperature.

6.5 Operational Guidelines

- Solarisation is an alternative treatment where other mitigation measures **cannot occur** (e.g. removal of soil and spraying with sterigene for small soil contaminated equipment). Solarisation can also be used as a secondary treatment if a precautionary approach is required.
- Small amounts of soil and small items can be solarized in bags, pots, or plastic buckets.
- Solarisation will be most effective during the hottest weeks of the year when ambient temperatures are optimal and there are long periods of direct sunlight (non-cloudy) and little to no wind (Stapleton et al. 2019).

- Little to no wind is important, as wind moving across the plastic will rapidly dissipate the trapped heat, additionally strong winds may lift or tear the plastic.
- Temperature increases more easily if there is no slope or if the slope is north-facing (Elmore et al. 1997).
- Identifying locations where the monthly mean maximum air temperatures and mean number of days that are close to the lethal temperature threshold for PA will increase the likelihood of suitable temperatures necessary for solarisation to be successful. A useful guide to determine whether your site could be suitable, is by referring to the following climate maps https://niwa.co.nz/climate/daily-climate-maps.
- Where cooler temperatures are experienced, consideration should be given to undertake solarisation in greenhouse and nursery containerized solarisation units. These are likely to be more effective in cooler climates than field solarisation (Elmore, et al. 1997).
- Care must be taken to avoid contamination of potting media after heat treatment. Heat treated material should only be transferred into sanitized containers using sanitized tools by workers with clean gloves following phytosanitary working practices.

6.6 Solar ovens

- Solar ovens are recommended as the mechanism to use for solarisation due to its cost effectiveness to purchase or build and operate; its portability and hence its use in remote areas; and the ability for the oven to increase temperature to sufficient levels without the use of electricity, gas or fire.
- Some solar ovens used for cooking have reflective sheets to direct solar energy to the area being heat treated. Some of these ovens can reach temperatures >100°C.
- A solar oven can be easily constructed to optimise solar radiation to produce heat (Figure 1).

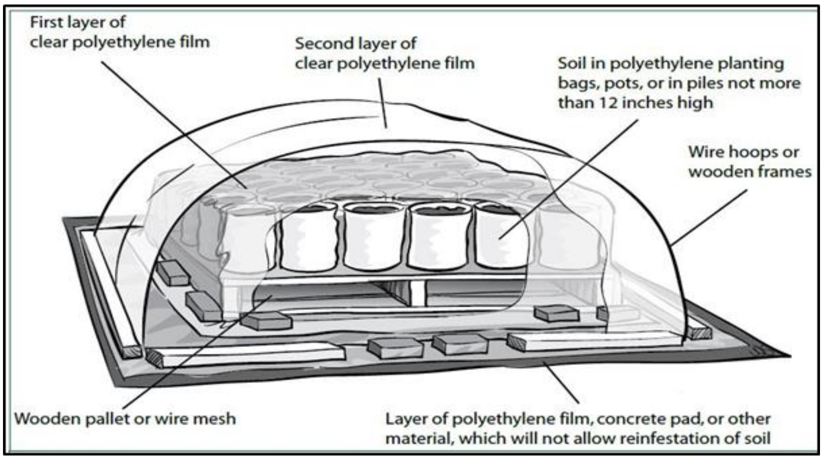

Figure 1: Solar oven construction (Source: Stapleton, et al.1997).

6.7 Solar oven design considerations

1. A solid base is covered with a layer of insulation (e.g., a foam insulation panel or polystyrene base) to reduce heat loss.

2. Heating will be more efficient and uniform if hot air can circulate beneath and around the item being treated, hence it is recommended that the item to be heat treated is placed on a structure like a wooden pallet or mesh stand that allows circulation from all sides.

3. A double layer of plastic film separated by an air gap between layers is placed over the item.
   - A double layer of plastic helps to reduce heat loss and acts as added insulation which can increase temperatures by up to 10°C compared to single layer (Elmore et al. 1997; Stapleton, et al.1997).
   - Clear plastic bottles or PVC pipes can be used to create an air space between the layers (Stapleton, et. al.1997). A 15 cm air space between the two layers is recommended (Duff, 2003).
   - Metal frames can be used to support the internal and external layers of plastic

4. Use transparent clear thin plastic:
   - Use clear polyethylene plastic. Clear plastic is more effective than black plastic as the heating rays of the sun will pass though the plastic whereas black plastic absorbs and deflects heat rather than trapping it (Stapleton *et. al*, 2019).
   - Use thin plastic. Thinner plastics (~1 to 2 mil) are more effective than thicker plastics (> 2 mil) in generating more heat. Note: 1 mil = 0.025mm. However if wind is an issue use a thicker plastic (for the outer layer) as it is studier (Stapleton, J.J & DeVay, 1986; Elmore *et.al*. 1997; Stapleton *et. al*, 2019).
   - The use of clear thermal anticondensate greenhouse film for solarisation of potting media should be considered (Anon, 2016).
   - Plastic that is UV absorbent should last indefinitely while plastics that are not may become brittle over time (Duff, 2003).

5. If holes or tears do occur in the plastic they should be patched with clear patching tape.

6. Reflective panels should be considered to direct solar heat towards the area being heat treated. These panels should be positioned and angled in such a way to optimise solar radiation.

6.8 Temperature-Time monitoring

For small volumes (<10kg) of soil or potting mix:

- Time duration should start when the coolest part of the media reaches the target temperature and that at least those minimum temperatures are maintained during the recommended time period. The coolest part may be at the centre or near the edges. You may need to place several temperature probes in the media to determine this.
- The use of temperature data loggers is preferred, as they are portable and capable of autonomously recording temperature over a defined period of time. The digital data can then be retrieved, viewed and evaluated in the field in real-time after it has been recorded.
- The media should be moist (e.g. wet to touch) prior to heating.
- Soil should be piled no more than 15-25 cm deep and the containers not more than this in diameter to facilitate heating (Anon, 2016).

For small items with soil residue:

- Temperatures should be monitored where the items are being heat treated (i.e. inside the area covered by the first layer of plastic). The use of temperature data loggers is preferred, as they are portable and capable of autonomously recording temperature over a defined period of time. The digital data can then be retrieved, viewed and evaluated in the field in real-time after it has been recorded.
- >10 ml of free water should be included in a container with the equipment.

7.0 Composting

7.1 Background

Compositing is organic waste being biologically degraded by micro-organisms to humus-like material.

Composting of plant waste infected with Phytophthora spp. has been found to be an effective management option if well managed, to ensure temperature – time requirements are met.

7.2 Who this guideline is for?

Commercial-scale composting operators, who follow the New Zealand Standard 4454:2005 – Composts, Soil Conditioners and Mulches, should only be used.

Time and temperature need to be directly controlled and measured to ensure that all of the plant material being composted achieves the target temperature during the minimum time period recommended. Survival of Phytophthora species can result from failures in the treatment operating process or of inadequate methods of treatment.

This guideline is not to be used for back-yard or small scale composting, as the treatment requirements stipulated in this guideline are unlikely to be met. Use of in vessel and static composting systems is not advised due to the lack of turning and cooler zones occurring in the compost below the recommended temperatures (Ormsby, 2017).

7.3 Protocol

Temperature	Exposure Time	Water content (Min)	Turnings (Min)
≥55◦C	≥15 days	25%	Five

The minimum treatment period **must be continuously** at or above the required temperature.

7.4 Operational Guidelines

1. All treated material should be stored in such a way as to avoid any contact with untreated materials e.g. direct contact, or contact through run off water, wind, machinery, tools, storage containers etc.

2. The outer surface of the compost should be insulated to ensure surface cooling is sufficiently reduced.

3. Composting facilities should be managed in such a way to ensure a thermophilic temperature range and a high level of biological activity.

4. Carbon: nitrogen ratio should be between 15:1 to 30:1.

5. Continuous monitoring and control of moisture content is necessary for optimal operations.

6. The compost should be well mixed, have optimal structure and hence optimum air space for circulation.

7. Avoid excess compaction in the centre, which reduces air and promotes lower temperatures.

8. All material must be exposed to the above described time-temperature conditions, including corners and surface areas where it is generally cooler.

9. The minimum temperature – time should be continuous and not averaged over a long period.

10. To ensure that the whole compost mass is exposed to the desired temperature-time, turning of the compost should be at a minimum of 5 turnings and designed to turn the oldest composted materials prior to the newest (raw) compost. If the compost is not turned regularly then the entire compost profile (particularly the edges) will not reach the desired temperatures.

11. Monitoring should be done in a way that is representative, with temperature loggers having the ability to record temperature continuously and automatically for each treatment period.

12. Each facility should record the quantity/description/date of treatment and batch or reference number for trace back. In addition, assurances that the requirements are satisfied (e.g. treatment certificate).

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