

Procedures in the current National Phylloxera Management Protocols (NPMP) were scientifically validated through a series of replicated experiments. Protocols that were addressed appear below in **bold** text. Procedures highlighted in **red font** were deemed **ineffective** on the basis that they did not achieve 100% first instar mortality.

In light of these results and further experiments to ascertain phylloxera mortality for the disinfestation treatments referred to in this document, recommendations for changes in the protocols are provided in **blue font**. Procedures and modification in **blue font and bolded** are not in the current NPMP and are **recommended** for the specified treatments.

MOVEMENT OF GRAPEVINE CUTTINGS AND ROOTLINGS FROM A PIZ OR PRZ VINEYARD OR NURSERY INTO A PEZ (PROCEDURE B).

Disinfestation procedure

Cuttings / rootlings must be hot water treated immediately prior to dispatch as follows:

EITHER at 50°C +/- 1°C for 30 min OR at 54°C +/- 1°C for 5 min (Effective)

The hot water treatment procedure MUST ensure that ALL material reaches the required temperature for the specified time.

Outcome

No change required

MOVEMENT OF DIAGNOSTIC SAMPLES FROM A PIZ OR PRZ INTO A PRZ OR PEZ (PROCEDURE C)

Disinfestation procedure

Any ONE of the following procedures:

a. Freezing to -18°C for 24 hr, pack in dry ice for transport (effective)

b. Freezing and transfer under liquid nitrogen at -196°C

c. Freeze drying

d. Oven drying at 45°C for a minimum of 2 hr (effective)

e. Sealed, unbreakable vessel (for juice samples)

f. Hot water treatment at 50°C for 30 min or 54°C +/- 1°C for 5 min (effective)

g. Fixative – devitalisation using formalin/acetic acid, gluteraldehyde, **70% ethanol** or similar (**ineffective**)

Outcomes, *suggested modifications* and an **additional procedure**

No change to procedures “a, d and f”.

For procedure “g”, first instars survived and developed to fecund adults after immersion in 70% ethanol for 30 and 60 sec. It can be assumed that first instars would not survive beyond 60 sec in the fixative. However, first instars move quickly and there is a risk that they can crawl out of the tube i) during the process of transferring into the holding containers ii) if they do not submerge in ethanol e.g. get stuck at the top of the lid and iii) if the containers are not correctly sealed during processing and transport.

We therefore recommend that procedure” g” be changed to

g. Fixative – devitalisation using formalin/acetic acid, gluteraldehyde, 95% ethanol or similar (formalin/acetic acid, gluteraldehyde OR similar have not been validated and the committee can decide if they need to be in the protocol)

- i. Suitably fill sample containers with ethanol such that insects are fully submerged and come into contact the liquid.*
- ii. Close containers while processing samples and seal correctly during transport to ensure that insects do not crawl out.*

For grape diagnostic samples as used in smoke taint analysis, we recommend “h” as an additional procedure;

h. Homogenise not more than 100 g of grape samples at either) i) 10 sec x 10000 rpm, ii) 20 sec x 5000 rpm or iii) 30 sec x 2000 rpm (rpm =rate per min)

MOVEMENT OF VINEYARD EQUIPMENT OUT OF A PIZ OR PRZ (Procedure G)

Disinfestation procedures (one of the following methods)

A. Steam (effective)

i) Steam applied must be above 100°C as indicated by a jet of clear invisible steam between steam outlet and the visible condensate cloud.

ii) Steam must contact all surfaces until the surface is left dry, not wet with condensate.

B. Hot water (effective)

i) Fully immerse the item in water at 70°C minimum and hold in water for at least 2 minutes at that temperature.

C. Dry heat

i) Place the item in a suitable room, shed or container that can be heated up to the required temperature (see below)

ii) Apply temperature probes to the item, and measure the surface temperature and preferably some deeper parts of the equipment

iii) Heat the room until the probes indicate that the machine has reached the required temperature (see below)

iv) Hold in the hot room for a minimum of either:

- i. 75 minutes after the machinery has reached 45°C (effective)
- ii. OR two (2) hours after the machine has reached 40°C (ineffective)

A. Steam.

Steam temperature drops the further away it is from the target. We therefore recommend adding note “iii” and “iv” as follows

- iii. **Steam must be applied at the target for at least 10 sec**
- iv. **Steam must be applied from not more than 1 m**

B. Hot Water.

Fully immersing first instars in water at 50°C minimum for at least 1 min after the water temperature has reached 50°C achieves 100% mortality across diverse genetic strains. There is, therefore, potential to modify the existing protocol accordingly. We recommend changing the procedure to

- i. **Fully immerse the item in water at 50°C (after water temperature has reached 50°C) and hold item for at least 1 min**

C. Dry Heat.

iv. No change is required to the procedure using 75 min after the machinery has reached 45°C. For 2 hours after the machinery has reached 40°C, first instars of two phylloxera genetic strains survived treatments. Increasing time to *2hr 15min after temperature reached 40°C achieved 100% mortality across diverse phylloxera strains.*

We therefore recommend changing the protocol to

- v. **Hold in the hot room for a minimum of either 75 minutes after the machinery has reached 45°C OR 2 hr 15 min after the machine has reached 40°C.**

MOVEMENT OF VINEYARD VISITORS OUT OF A PIZ OR PRZ (PROCEDURE H)

Disinfestation procedure

1. When leaving the vineyard, disinfest footwear as follows:
 - a. scrub boots with the scrubbing brush to remove mud –preferably in water and detergent.
 - b. dilute chlorine with water in a tub to give a 2% active sodium hypochlorite concentration (Effective), **dip and scrub boots in the freshly prepared solution for a minimum of 30 seconds (ineffective)**
 - c. **rinse thoroughly in clean water after immersion (ineffective).**
2. Wash and disinfect snips, small tools etc. with 2% active sodium hypochlorite solution.
3. Change, wash or discard (if disposable) clothing before entering next vineyard.

*Outcomes, suggested modifications and an **additional procedure:***

100% first instar mortality across diverse genetic strains was achieved by omitting the water-rinse after treatment in 2% NaOCl and by increasing time of immersion from 30 to 60s. In muddy conditions, boots are usually covered in mud and washing to remove soil particles and mud ensures that insects contact NaOCl. The water rinse step should be removed from the procedure.

We therefore recommend the following procedure for footwear and hand-held tools;

1. When leaving the vineyard, disinfect footwear as follows:
 - a. *In two tubs, dilute household bleach with water to give a 2% active sodium hypochlorite concentration.*
 - b. *Scrub mud off from boots with a brush in the freshly prepared solution in the first tub.*
 - c. *Dip boots in the freshly prepared solution in the second tub for a minimum of 60 sec.*
2. *Wash and disinfect snips, small tools etc. with 2% active sodium hypochlorite solution for a minimum of 60 sec.*
3. **Clothing**
 - a. *If using disposable overalls and boots, double bag and seal appropriately before entering next vineyard and leaving property.*
 - b. *If changing clothes for later washing, place in a double bag and seal appropriately during transport.*
 - c. *Wash clothes and hats in a hot wash cycle when temperature reaches EITHER 54°C for 5 min OR 50°C for 30 min.*