‘INTEGRATING NEW SCIENCE ON GRAPE PHYLLOXERA INTO PRACTICE’

2018

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Background

In 2009, the National Phylloxera Management Protocol (NPMP) was published by the National Vine Health Steering Committee as a national approach to reducing the risk of spread of grape phylloxera. This document was considered to provide a technically justified basis from which legislation and regulations for the movement of phylloxera risk vectors could be developed by each state and territory government, and to which the regulations could be aligned, creating a consistent set of requirements across Australia. In addition, the procedures in the NPMP could be used as best practice guidelines for the viticultural industry from which to develop regional, company-wide or individual protocols to prevent the spread of phylloxera.

The NPMP has not been updated since 2009, but recognition of the need for a review has been agreed upon by industry and state regulators. As part of this formal review, a greater consideration of the efficacy of disinfestation treatments on phylloxera strains is imperative. There have been several hundred strains of grape phylloxera documented worldwide, of which Australia is known to have 83 endemic strains [8], [7]. When the NPMP was first created, however, knowledge about endemic phylloxera strains was limited. Recent research has focused on testing the ability of seven key endemic grape phylloxera strains to withstand the different disinfestation methods outlined on the NPMP. Results revealed differences in strain susceptibility to different disinfestation methods.

Several phylloxera research projects conducted since 2009, have focused specially on scientifically validating the range of disinfestation treatments currently recommended by the NPMP to reduce the risk of spread of grape phylloxera. These validations to date, however, have only been undertaken in laboratory conditions rather than under field conditions. This presents a tradeoff between external and internal validity. In the laboratory, experiments have been performed under controlled conditions to ensure repeatability and to limit additional variables impacting on results. However, artificiality of laboratory settings can present problems in then applying the findings to field situations. Factors such as human behavior, presence of contaminants such as soil and plant material, size and density of machinery and equipment being disinfested and the size of the treatment vessels, each may influence the efficacy of the disinfestation treatments under field conditions. This paper makes recommendations of the use of various disinfestation treatments, taking into account field conditions.

Research projects included the April 2012 DPI 08/01 *'The Three R's – Rootstock, Resistance and Resilience to Grape Phylloxera'* [5] and the June 2017 DEP 1301 '*Risks and Management of Endemic and Exotic Phylloxera*' [4]. Whilst not all validations have included the use of all key endemic phylloxera strain groups, it is vital that knowledge identified through this research is reflected in practice at the three key levels around the movement of phylloxera risk vectors:

(i) Updated National Phylloxera Management Protocol

(ii) State and territory legislation and regulations

(iii) Best-practice guidelines for industry and regulators

Issue

Quarantine regulations of multiple states, currently specify disinfestation procedures as part of entry requirements, that do not cause 100% mortality of the key endemic strains of grape phylloxera.

Phylloxera disinfestation procedure review

Vinehealth Australia has undertaken a review of the completed research on the effectiveness of a selection of disinfestation procedures against grape phylloxera strains, as presented in Appendices 1-5 for the different phylloxera risk vectors. **This research has indicated that not all current disinfestation procedures are 100% effective against known key strains of endemic grape phylloxera and that some tested disinfestation procedures are not safe to undertake from a health and safety perspective. Accordingly, disinfestation procedures must now be updated as a priority for both regulators and industry.**

This review has also identified an opportunity to modify some time and temperature specifications of some disinfestation procedures to improve the turnaround time of undertaking these procedures which is often in the peak of vintage, without compromising efficacy.

Vinehealth Australia coordinated a meeting on 8th October 2018 with regulators from DEDJTR and NSW DPI and phylloxera researchers, to review this latest science together and come to consensus about which disinfestation protocols needed modification in state quarantine regulations in the short term and specifications of the proposed changes. Each state then agreed to review these specifications with local industry.

Recommended changes to disinfestation protocols

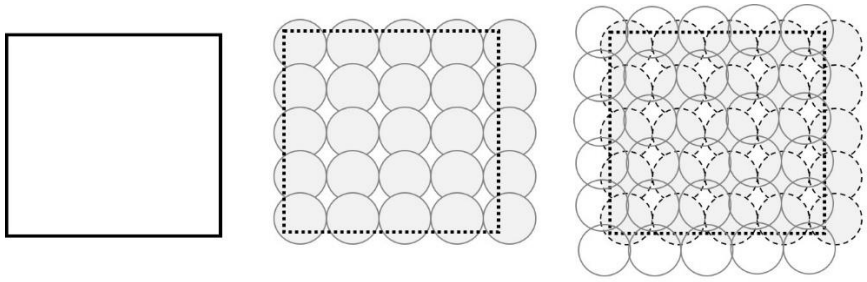
The following changes are recommended to state regulations on movement of phylloxera risk vectors and summarised in Table 1:

a. Dry heat treatment of machinery and equipment to be changed from “EITHER 45°C for 75 minutes (1¼ hours) OR 40°C for 120 minutes (2 hours)” to “EITHER 45°C for 90 minutes (1½ hours) OR 40°C for 180 minutes (3 hours)”

b. Hot water treatment of machinery and equipment to be changed from “2 minutes at 70˚C” to “1½ minutes at 60˚C”

c. Steam to be available as a valid disinfestation treatment option only of equipment where it can be applied efficiently – e.g. small hand tools, with process as follows:

i. "Steam applied must be 100˚C or above.
ii. Steam must contact all surfaces of the hand tools at a distance of approximately 10cm.
iii. The spray coverage at this distance must be held for at least 10 seconds on each portion of the item. Refer to diagram below for step-wise application instructions.
iv. Steam will only be accepted as a valid disinfestation treatment when applied in the presence of the Biosecurity Officer issuing the Plant Health Certificate."



1

Start with equipment cleaned of all soil and plant material

2

Apply the steam at a distance of 10cm from the equipment to ensure the steam is at least 100˚C upon surface contact. Apply the spray cones in a regular pattern across the equipment surface, holding each sprayed cone for a minimum of 10 seconds.

3

Continue with a second offset layer of the sprayed cone pattern to fill in the gaps to ensure each segment of the equipment surface is sprayed with steam for a minimum of 10 seconds. Repeat on all sides of the equipment.

d. Freezing as a disinfestation treatment of diagnostic samples to be changed from "freezing to -18˚C for 24 hours and packed in dry ice for transport", to "freezing and then being held at -18˚C for 12 hours and packed in dry ice or with an ice pack for transport"

e. Disinfestation of grape juice as a diagnostic sample [SA procedure only] to be changed from "sealed in an unbreakable vessel" to "EITHER

i. Filter, centrifuge or cold-settle to ensure remaining particles are less than 50 microns in size; or
ii. Freezing and then being held at -18˚C for 12 hours and packed in dry ice or with an ice pack for transport; and
iii. Seal in an unbreakable vessel prior to sending"

Given the research of the disinfestation procedures was not undertaken in commercial (field) conditions, as part of the recommendations, a degree of buffering around time and/or temperature specifications has been proposed for the disinfestation procedures as a means to ensure efficacy in practice.

Table 1. Recommended disinfestation treatments and method of application to be adopted by state regulators and industry as part of preventing the importation and spread of grape phylloxera.

Risk vector	Disinfestation treatment	Reference Appendix	Recommended method	Description of change compared to current NPMP	Relevant to:[1]		
					NPMP	PQS	FG
Vineyard visitors – footwear and small hand tools	Chlorine (2% active sodium hypochlorite)	1	i. Scrub free of soil and plant material with water wash. ii. Mix a 2% sodium hypochlorite solution in a tub in sufficient volume to cover the top of the footwear or hand tools. If using a 4% sodium hypochlorite product, mix 1-part water to 1 part product. iii. Completely immerse footwear or hand tools for a minimum of 60 seconds. Do not rinse with water after immersion.	Increase in time from 30 to 60 seconds and removal of water rinse after immersion	Exit from and within all PMZs	Small hand tools from PIZ or PRZ	●
	Methylated spirits (95% ethyl alcohol)		Note: - *Whilst research has shown methylated spirits to be an effective disinfectant, flammability concerns of the product negate its recommendation for field use.*				

[1] NPMP = National Phylloxera Management Protocol; PQM = State Plant Quarantine Standard or equivalent; FG = Farm-gate hygiene procedures

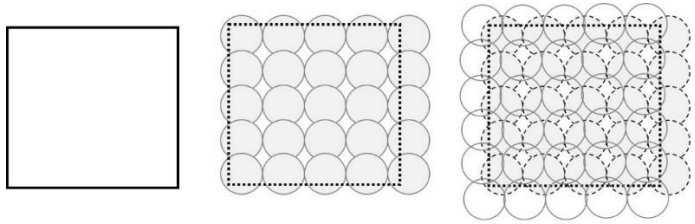
Vineyard visitors – clothing	Hot water and dry heat	1	A. For a vineyard in a PEZ: 1. If your operational visitors have either not visited vineyards in the three weeks prior and walked down vine rows or near vines, or only visited vineyards in a PEZ, provide them alternative footwear (including gum boots or work boots), or sturdy shoe covers, or ensure footwear disinfestation is undertaken on entry and exit of vine rows. 2. If your operational visitors have visited vineyards in a PRZ, PIZ or overseas wine region in the three weeks prior and walked down vine rows or near vines: a. If the operational visitors are wearing the same clothing (including hats) they wore in the PRZ, PIZ or overseas wine region, they must be denied access to your vine rows, even if they wore a disposable suit in the vineyard they recently visited. b. If the operational visitors have changed their clothing (including hats) and shoes that they wore in the PRZ, PIZ or overseas wine region, provide them alternative footwear, or sturdy shoe covers, or ensure footwear disinfestation is undertaken on entry and exit of vine rows. c. If the operational visitors have changed their clothing (including hats) but not shoes that they wore in the PRZ, PIZ or overseas wine region: i. If the shoes were disinfested prior to entering your property, provide alternative footwear or ensure footwear disinfestation is undertaken on entry and exit of vine rows. ii. If the shoes were not disinfested prior to entering your property, deny access to your vine rows and advise the operational visitors that footwear presents a risk and must be disinfested prior to coming onto your property and away from vineyards.	Deny access to visitors to a PEZ vineyard if wearing the same clothing worn in a PIZ or PRZ vineyard in the 21 days prior	Movt of visitors out of a PIZ or PRZ into a PEZ		●

			B. For a vineyard in a PRZ/PIZ: 3. If your operational visitors have either not visited another vineyard in the three weeks prior and walked down vine rows or near vines, or only visited vineyards in a PEZ, provide them alternative footwear, or ensure footwear disinfestation is undertaken on entry and exit of vine rows. And, provide a disposable chemical suit, or alternative clothing that remains on your vineyard. 4. If your operational visitors have visited another vineyard in a PRZ, PIZ or overseas wine region in the three weeks prior, and walked down vine rows or near vines: a. If the visitor is not wearing the same clothes (including hats) or shoes that they wore in the PRZ, PIR or overseas wine region, they must wear a disposable chemical suit or change into alternative clothing that remains on your vineyard. And, wear alternative footwear or undertake footwear disinfestation on entry and exit of vine rows. b. If the visitor is wearing the same shoes that they wore in the PRZ, PIR or overseas wine region, and these shoes have not been disinfested according to the Footwear and Small Hand Tool Disinfestation protocol (http://vinehealth.com.au/wp-content/uploads/2016/01/Vinehealth-Footwear-and-Small-Hand-Tools-Disinfestation-Protocol-White-A3.pdf) prior to entering your property, deny these operational visitors access to your vine rows. Explain that their footwear presents a risk and must be disinfested according to the Footwear and Small Hand Tool Disinfestation protocol prior to coming onto your property and away from vines. c. If the visitor is wearing the same shoes that they wore in the PRZ, PIZ or overseas wine region, and these shoes have been disinfested according to the Footwear and Small Hand Tool Disinfestation protocol prior to entering your property, provide alternative footwear or ensure footwear disinfestation is				

Risk vector	Disinfestation treatment	Reference Appendix	Recommended method	Description of change compared to current NPMP	Relevant to:[1]		
					NPMP	PQS	FG
			undertaken again on entry and exit of vine rows. Conditional entry at this stage though is subject to the state of the clothing worn. If the operational visitors are also wearing the same clothing (including hats) they wore in the PRZ, PIZ or overseas wine region, they must be denied access, even if they wore a disposable suit in the vineyard they recently visited. Only options for controlled entry are for visitors to change into their own clean clothes and then wear a disposable chemical suit, or to change into alternative clothing that remains on your vineyard.				
Vineyard visitors – clothing	Hot water and dry heat	1	For any disposable clothing worn, remove carefully prior to leaving the vineyard on which it is worn. These are single use clothing items. Collect and double-bag in sealed garbage bags for disposal. Even if a visitor has worn a disposable chemical suit in the vineyard, there is a risk that this suit can rip, and pests can be transferred from the outside of the suit to clothing beneath during removal of the suit if not done carefully. All clothes worn under a disposable chemical suit must be sealed in a bag prior to leaving the vineyard. The clothes must be hot washed on the highest temperature setting (54˚C or above using the longest wash cycle setting as clothes will not always remain immersed in the water if using a front loader), followed by tumble-drying also on the highest heat setting. Hot washing of clothes must be completed by visitors moving between Phylloxera Management Zones and between vineyards within a PIZ or PRZ.	Additional movement scenarios and appropriate clothing for each	Movt of visitors out of a PIZ, PRZ, or PEZ into a PIZ or PRZ		●

Risk vector	Disinfestation treatment	Reference Appendix	Recommended method	Description of change compared to current NPMP	Relevant to:[1]		
					NPMP	PQS	FG
Vineyard visitors – clothing (hats)	Mortein Fast Knockdown Multi-Insect Killer (1.1g/kg Esbiothrin and 0.5g/kg Permethrin)	1	Note: - *Whilst research has shown Mortein Fast Knockdown Multi-Insect Killer to be an effective disinfectant for hats, OH&S concerns of the product negate its recommendation for field use.*				

Risk vector	Disinfestation treatment	Reference Appendix	Recommended method	Description of change compared to current NPMP	Relevant to:[1]		
					NPMP	PQS	FG
Machinery and equipment	Hot water	2	i. Fully immerse the equipment in water. ii. Once the water temperature has stabilised at 60°C or higher, hold the machinery or equipment in the water for at least 90 seconds (1½ minutes). Note: - *Despite 50˚C for 1 minute showing 100% mortality of all key grape phylloxera strains, water temperature and time buffers have been incorporated into the recommendation for the following reasons:* *1. Lack of an incremental dose-response relationship close to the time/temperature specifications found to be effective in the research.* *2. When considering dropping time and temperature by such a large amount based on the research compared to current regulations, it would be reasonable to take a cautious approach, knowing that even small time and temperature reductions will be valuable for industry during the height of vintage. It was therefore decided that a compromise between the research and current regulations would be appropriate.* *3. The research was carried out on first instars only and therefore the effect of hot water treatment on adult phylloxera or eggs is currently unknown.* 4. *Assumed level of hot water treatment vessel sensor inaccuracy.* 5. *Machinery and equipment that is not fully clean of soil and plant material prior to the disinfestation treatment, could increase time to ensure 100% mortality of grape phylloxera, where clods of soil or leaf material remain.* 6. *No scientific papers found to validate requirement for 70˚C hot water treatment of machinery and equipment against grape phylloxera, but considered to have originated to treat fungal spores.*	Decrease in time from 2 minutes to 1½ minutes and decrease in temperature from 70˚C to 60˚C	Exit from a PIZ or PRZ	●	

Risk vector	Disinfestation treatment	Reference Appendix	Recommended method	Description of change compared to current NPMP	Relevant to:[1]		
					NPMP	PQS	FG
Machinery and equipment	Steam	2	Note: - *Whilst steam has proven to be an effective disinfestation treatment under laboratory conditions, application to ensure efficacy is highly unlikely in the field, except when used on small equipment.* *The following method is recommended only for small hand tools (including technical equipment):* *i. Steam applied must be 100˚C or above.* *ii. Steam must contact all surfaces of the hand tools at a distance of approximately 10cm.* *iii. The spray coverage at this distance must be held for at least 10 seconds on each portion of the item. Refer to diagram below for step-wise application instructions.* *iv. Steam will only be accepted as a valid disinfestation treatment when applied in the presence of the Biosecurity Officer issuing the Plant Health Certificate.*  1 Start with equipment cleaned of all soil and plant material 2 Apply the steam at a distance of 10cm from the equipment to ensure the steam is at least 100˚C upon surface contact. Apply the spray cones in a regular pattern across the equipment surface, holding each sprayed cone for a minimum of 10 seconds. 3 Continue with a second offset layer of the sprayed cone pattern to fill in the gaps to ensure each segment of the equipment surface is sprayed with steam for a minimum of 10 seconds. Repeat on all sides of the equipment.	Removal as a valid disinfestation treatment for all but small hand tools (including technical equipment)	●	●	

Machinery and equipment	Dry heat	2	i) Place the machinery and equipment in a suitable room, shed or container that can be heated up to the required temperature; and ii) Apply temperature probes to the machinery and equipment and measure the surface temperature and preferably some deeper parts of the machinery and equipment; and iii) Heat up the room until the probes indicate the required temperature has been reached and hold the machinery and equipment at the required temperature for the required time: EITHER 45°C for 90 minutes (1½ hours) OR 40°C for 180 minutes (3 hours). *Under laboratory conditions, dry heat treatment carried out at 40˚C required an additional 15 minutes above current regulations - from 120 to 135 minutes, to achieve 100% mortality of first instars of strains G20 and G30, which were not completely killed at 120 minutes. At 45˚C, 100% mortality of first instars of 7 phylloxera strains was achieved at 75 minutes.* *Including a time buffer is a key component to ensuring the efficacy of dry heat sterilisation in the field for machinery and equipment. Machinery and grape harvesters in particular, are considered particularly high risk vectors for grape phylloxera. They are inherent difficult to clean free of all soil and plant material, are commonly moved between vineyards, and are operated at peak times in the season when phylloxera venture out of the soil and into the vine canopy, increasing the chance that they can pick up and spread the insect.* *Note that prolonging dry heat treatment by the same time duration will not have equivalent effect at 45˚C as compared to 40˚C, and therefore a longer time buffer is required at the lower temperature.* *The new recommendation for dry heat treatment undertaken at 40˚C is to hold the item being treated at 40˚C for 3 hours.* *This recommendation with an inbuilt time buffer is proposed for the following additional reasons:*	Increase in time from 75 to 90 minutes for 45°C treatment and from 120 to 180 minutes for 40°C treatment ***Under laboratory conditions, heat treatment at 40˚C for 120 minutes did not cause 100% mortality of key endemic grape phylloxera strains***	●	●	

Risk vector	Disinfestation treatment	Reference Appendix	Recommended method	Description of change compared to current NPMP	Relevant to:[1]		
					NPMP	PQS	FG
			• The recent research on dry heat was only undertaken at set oven temperatures for set durations (refer review of research section below), not as a study to determine incremental dose-response relationships. Under laboratory conditions, 100% mortality of first instar grape phylloxera was declared to have been achieved at 40˚C by 135 minutes, but in reality the science is unclear as to whether this complete mortality was only achieved at the 134th minute mark, and thereby if the proposed duration of 135 minutes at 40˚C were adopted, there would be no inbuilt time buffer to account for infield variables. The same reasoning then applies to the 45˚C treatment, whereby if 100% mortality was only achieved at 74th minute mark, there would be no inbuilt time buffer. *• The possibility that different phylloxera life stages vary in resilience to dry heat. Research on dry heat has only been carried out on first instar grape phylloxera, not the larger adult phylloxera or egg stage with a thicker outer wall.* *• Machinery and equipment that is not fully clean of all soil and plant material prior to the disinfestation treatment, could increase the time to ensure 100% mortality of grape phylloxera, where clods of soil or leaf material remain.* *• Potential for heat shed temperature sensor inaccuracy, given current lack of operation of heat sheds under a certification scheme.*				

Risk vector	Disinfestation treatment	Reference Appendix	Recommended method	Description of change compared to current NPMP	Relevant to:[1]		
					NPMP	PQS	FG
Grapevine cuttings and rootlings	Hot water	3	i. Completely immerse and maintain bundles at a minimum of 50°C ± 1°C for 30 minutes OR 54°C ± 1°C for 5 minutes. ii. A minimum of three (3) sensors must be used for each hot water treatment vessel. One sensor should be located at a depth of 100mm from the base of the vessel, another at 100mm from the surface and the other inserted into the centre of the load mass. **Note: this research has not been published in a peer reviewed at the time of writing this paper.** Note: *- despite 45˚C for 30 minutes and 50˚C for 5 minutes showing 100% mortality of all key grape phylloxera strains under laboratory conditions, recommendation is to retain current higher temperature specifications of 50˚C ± 1°C and 54˚C ± 1°C respectively. This recommendation is proposed for the following reasons:* *1. Absence of peer review of this research to date.* *2. Lack of an incremental dose-response relationship close to the time/temperature specifications found to be effective in the research.*	No change	●	●	

Risk vector	Disinfestation treatment	Reference Appendix	Recommended method	Description of change compared to current NPMP	Relevant to:[1]		
					NPMP	PQS	FG
Diagnostic samples	Grape juice	4	For juice samples, either: i. Filter, centrifuge or cold-settle to ensure remaining particles are less than 50 microns in size; or ii. Freezing and then being held at -18˚C for 12 hours and packed in dry ice or with an ice pack for transport; and iii. Seal in an unbreakable vessel prior to sending Note: - *Placing juice in an unbreakable vessel itself is not considered a valid disinfestation treatment as no vessel is completely unbreakable. Proposed treatment is to treat juice by either freezing or as per current filtered juice specifications for commercial loads.*	Treat to meet filtered juice specifications OR freeze as unfiltered juice	●	●	
Diagnostic samples	Freezing	4	Freezing and then being held at -18˚C for 12 hours and packed in dry ice or with an ice pack for transport. Note: - *this recommendation is proposed in lieu of peer review paper in preparation. Despite -20˚C for 12 hours showing 100% mortality of all key grape phylloxera strains under laboratory conditions, recommendation is for -18˚C for 12 hours to align with standard freezer temperature. Tested temperature of -20˚C is expected to be as effective as -18˚C over the 12-hour duration.* *Despite time and temperate buffers being proposed for dry heat treatment and hot water treatment of machinery and equipment, diagnostic samples given their small volume and packaging, are considered lower risk for moving and spreading phylloxera by comparison. Building in time and temperature buffers are therefore considered unnecessary.*	Decrease in time from 24 to 12 hours	●	●	

Risk vector	Disinfestation treatment	Reference Appendix	Recommended method	Description of change compared to current NPMP	Relevant to:[1]		
					NPMP	PQS	FG
Diagnostic samples	Hot water	3	i. Completely immerse and maintain bundles at a minimum of 50°C ± 1°C for 30 minutes OR 54°C ± 1°C for 5 minutes. ii. A minimum of three (3) sensors must be used for each hot water treatment vessel. One sensor should be located at a depth of 100mm from the base of the vessel, another at 100mm from the surface and the other inserted into the centre of the load mass.	No change, but ensure both time/ temperature options available for diagnostic samples	●	●	
Diagnostic samples	Oven drying	2	Oven drying at 45˚C for a minimum of 120 minutes. Note: *Probes must be used with large samples to ensure middle of sample has reached the required temperature for the required time. Bulky samples must be spread out on trays prior to placing in oven to increase surface area exposed to the heat.*	No change but addition of note	●	●	
Diagnostic samples	70% ethanol	4	Retain current treatment	No change	●	●	
Diagnostic samples	Formalin/ acetic acid	4	Retain current treatment	No change	●	●	
Diagnostic samples	Freeze drying	4	Retain current treatment	No change	●	●	
Diagnostic samples	Freezing and transfer under liquid nitrogen at -196˚C	4	Retain current treatment	No change	●	●	

Appendix 1

Risk vector: Vineyard visitors (footwear and small hand tools)

Current procedure in NPMP		Research undertaken		Is current disinfestation procedure in NPMP adequate?	Proposed disinfestation procedure based on research findings
Procedure number/ title	**Disinfestation procedure**	**Research treatments**	**Summary findings**		
H. Movement of vineyard visitors out of a PIZ or PRZ	Dilute chlorine with water in a tub to give a 2% active sodium hypochlorite concentration and dip and scrub boots in the freshly prepared solution for a minimum of 30 seconds. Rinse thoroughly in clean water after immersion. Wash and disinfect snips, small tools etc. with 2% active sodium hypochlorite solution.	Trial 1 • Mean survival of first instars from six phylloxera strains: G1, G4, G7, G19, G20, G30 • Three treatment times: 30, 40, 60 seconds • Water control • Four sodium hypochlorite treatments: 2% active, 2% active + water rinse, 3% active + water rinse, 4% active + water rinse • 5 replicates per treatment	• Differences found in strain susceptibility to disinfestation treatments. • Minimal strain mortality in water only treatment for all three treatment times. • As sodium hypochlorite concentration was increased in conjunction with a water rinse, almost all strains still survived irrespective of the sodium hypochlorite concentration. • 'No rinse' treatments showed significantly higher mortality rates across all phylloxera strains, irrespective of the length of the treatment. • With a 2% sodium hypochlorite solution and no rinse thereafter, a 60 second treatment was required to demonstrate 100% mortality across all strains. This was not achieved when treatment duration was either 30 or 40 seconds in length. • References [4], [3]	No	i) Scrub free of soil and plant material with water wash. ii) Mix a 2% sodium hypochlorite solution in a tub in sufficient volume to cover the top of the footwear or hand tools. If using a 4% sodium hypochlorite product, mix 1-part water to 1-part product. iii) Completely immerse footwear or hand tools for a minimum of 60 seconds. Do not rinse with water after immersion.
		Trial 2 • Mean survival of first instars of G38 phylloxera strain only • Three sodium hypochlorite treatments: 2% active for 30 seconds; 2% active for 60 seconds; 2% active for 30 seconds + water rinse • 50 replicates per treatment	• For strain G38, 100% mortality was achieved for each of the three treatments. • Reference [4]		

Risk vector: Vineyard visitors (footwear and small hand tools) continued

Current procedure in NPMP		Research undertaken		Is current disinfestation procedure in NPMP adequate?	Proposed disinfestation procedure based on research findings
Procedure number/ title	**Disinfestation procedure**	**Research treatments**	**Summary findings**		
H. Movement of vineyard visitors out of a PIZ or PRZ	Dilute chlorine with water in a tub to give a 2% active sodium hypochlorite concentration and dip and scrub boots in the freshly prepared solution for a minimum of 30 seconds. Rinse thoroughly in clean water after immersion. Wash and disinfect snips, small tools etc. with 2% active sodium hypochlorite solution. No current recommended disinfestation protocol for footwear and small tools other than use of sodium hypochlorite.	Trial 1 • Mean survival of first instars of six phylloxera strains: G1, G4, G7, G19, G20, G30 • Six products compared to sodium hypochlorite: Biopest (815 g/L paraffinic oil), ethanol (ethyl alcohol), methylated spirits (ethyl alcohol), Phytoclean (100g/L benzalkonium chloride), Pulse penetrant (1020g/L olydimethyl siloxane) and Virkon (49.8% potassium peroxymonosulfate) • Ethanol used at 80% strength and methylated spirits undiluted. Other products used at recommended rates. • Two durations with 30 second water rinse thereafter: 30, 60 seconds • 5 replicates per treatment	• Time of exposure to treatments influenced survival with a high survival recorded at 30 seconds compared to 60 seconds immersion. • Treatments with the recommended dilution using Biopest, Pulse Penetrant, Phytoclean and Virkon followed by a water rinse or without the water rinse did not achieve 100% first instar mortality across the six phylloxera strains. • Of the six chemical products tested, only undiluted methylated spirits applied for 30 seconds or 60 seconds with or without a 30 second water rinse thereafter was effective in achieving 100% mortality across all the six genetic strains. • 80% ethanol applied for 60 seconds with or without a 30 second water rinse thereafter (concentration unstated) was 100% effective against G1, G4, G7 and G20 phylloxera strains but not against G19 and G30. • Reference [4] **Note: this research has not been published in a peer reviewed journal at the time of writing this paper.**	Currently no procedure for chemical disinfestation other than for sodium hypochlorite. Whilst validation is new, this is not a formal recommendation.	Whilst undiluted methylated spirits, was demonstrated as an effective disinfestation treatment under laboratory conditions when used for 30 seconds for footwear and small hand tools, it is not recommended for field use due to its high flammability.
		Trial 2 • Mean survival of first instars of G38 phylloxera strain only • Two treatments: Undiluted methylated spirits; undiluted ethanol • Two durations: 30, 60 seconds • 50 replicates per treatment	• For strain G38, only methylated spirits at 30 seconds and 60 seconds was effective in achieving 100% mortality. • Reference [4]		

Risk vector: Vineyard visitors (clothing)

Current procedure in NPMP		Research undertaken		Is current disinfestation procedure in NPMP adequate?	Proposed disinfestation procedure based on research findings
Procedure number/ title	**Disinfestation procedure**	**Research treatments**	**Summary findings**		
H. Movement of vineyard visitors out of a PIZ or PRZ	Access to actual vine rows should be limited as much as possible. Authorised entry may be granted under controlled conditions: • Vineyard workers wear disposable, dedicated or cleaned clothing (eg overalls) for each vineyard. • Change, wash or discard (if disposable) clothing before entering next vineyard.	Trial 1 Desktop analysis of 12 common domestic washing machine brands used to examine the potential effectiveness of both warm or hot water treatment through washing using a standard domestic washing machine.	• Domestic washing machine temperatures and cycle length are variable, with no set standard temperatures or cycle lengths for 'hot' or 'warm' - therefore difficult to standardise in an experimental setting. • Warm cycles ranged from 32-53˚C and hot cycles from 54˚C and above. • Washing temperatures were compared to data from hot water disinfestation protocols, indicating that phylloxera first instars from six endemic strains are likely to survive a warm water treatment but not a hot water treatment wash [4]. • Reference [4]	No	For a vineyard in a PEZ [9]: 1. If your operational visitors have either not visited vineyards in the three weeks prior and walked down vine rows or near vines, or only visited vineyards in a PEZ, provide them alternative footwear (including gum boots or work boots), or sturdy shoe covers, or ensure footwear disinfestation is undertaken on entry and exit of vine rows. 2. If your operational visitors have visited vineyards in a PRZ, PIZ or overseas wine region in the three weeks prior and walked down vine rows or near vines: a. If the operational visitors are wearing the same clothing (including hats) they wore in the PRZ, PIZ or overseas wine region, they must be denied access to your vine rows, even if they wore a disposable suit in the vineyard they recently visited. b. If the operational visitors have changed their clothing (including hats) and shoes that they wore in the PRZ, PIZ or overseas wine region, provide them alternative footwear, or sturdy shoe covers, or ensure footwear disinfestation is undertaken on entry and exit of vine rows. c. If the operational visitors have changed their clothing (including hats) but not shoes that they wore in the PRZ, PIZ or overseas wine region: i. If the shoes were disinfested prior to entering your property, provide alternative footwear or ensure

					footwear disinfestation is undertaken on entry and exit of vine rows. ii. If the shoes were not disinfested prior to entering your property, deny access to your vine rows and advise the operational visitors that footwear presents a risk and must be disinfested prior to coming onto your property and away from vineyards. For a vineyard in a PRZ/PIZ [9]: 3. If your operational visitors have either not visited another vineyard in the three weeks prior and walked down vine rows or near vines, or only visited vineyards in a PEZ, provide them alternative footwear, or ensure footwear disinfestation is undertaken on entry and exit of vine rows. And, provide a disposable chemical suit, or alternative clothing that remains on your vineyard. 4. If your operational visitors have visited another vineyard in a PRZ, PIZ or overseas wine region in the three weeks prior, and walked down vine rows or near vines: d. If the visitor is not wearing the same clothes (including hats) or shoes that they wore in the PRZ, PIR or overseas wine region, they must wear a disposable chemical suit or change into alternative clothing that remains on your vineyard. And, wear alternative footwear or undertake footwear disinfestation on entry and exit of vine rows. e. If the visitor is wearing the same shoes that they wore in the PRZ, PIR or overseas wine region, and these shoes have not been disinfested according to the Footwear and Small Hand Tool Disinfestation protocol (http://vinehealth.com.au/wp-content/uploads/2016/01/Vinehealth-

					Footwear-and-Small-Hand-Tools-Disinfestation-Protocol-White-A3.pdf) prior to entering your property, deny these operational visitors access to your vine rows. Explain that their footwear presents a risk and must be disinfested according to the Footwear and Small Hand Tool Disinfestation protocol prior to coming onto your property and away from vines. f. If the visitor is wearing the same shoes that they wore in the PRZ, PIZ or overseas wine region, and these shoes have been disinfested according to the Footwear and Small Hand Tool Disinfestation protocol prior to entering your property, provide alternative footwear or ensure footwear disinfestation is undertaken again on entry and exit of vine rows. Conditional entry at this stage though is subject to the state of the clothing worn. If the operational visitors are also wearing the same clothing (including hats) they wore in the PRZ, PIZ or overseas wine region, they must be denied access, even if they wore a disposable suit in the vineyard they recently visited. Only options for controlled entry are for visitors to change into their own clean clothes and then wear a disposable chemical suit, or to change into alternative clothing that remains on your vineyard. For any disposable clothing worn, remove carefully prior to leaving the vineyard on which it is worn. These are single use clothing items. Collect and double-bag in sealed garbage bags for disposal. Even if a visitor has worn a disposable chemical suit in the vineyard, there is a risk that this suit can rip, and

					pests can be transferred from the outside of the suit to clothing beneath during removal of the suit if not done carefully. All clothes worn under a disposable chemical suit must be sealed in a bag prior to leaving the vineyard. The clothes must be hot washed on the highest temperature setting (54˚C or above using the longest wash cycle setting as clothes will not always remain immersed in the water if using a front loader [4]), followed by tumble-drying also on the highest heat setting. Hot washing of clothes must be completed by visitors moving between Phylloxera Management Zones and between vineyards within a PIZ or PRZ [9].
		Trial 2 • Mean survival of first instars of six phylloxera strains: G1, G4, G7, G19, G20, G30 • One treatment for disinfestation of hats: Mortein Fast Knockdown Multi-Insect Killer (1.1g/kg Esbiothrin and 0.5g/kg Permethrin) • Spray distance from hat: 8cm • Spray duration: 5 seconds per sprayed spot • Treatment replicated 5 times	5. When first instar phylloxera were subjected to a five second insecticide spray treatment, 100% mortality was achieved across the seven genetic strains [4]. 6. Reference [4]	Currently no procedure for chemical disinfestation of hats. This is not a formal recommendation.	Whilst Mortein Fast Knockdown Multi-Insect Killer was demonstrated as an effective disinfestation treatment for hats under laboratory conditions, it is not recommended for field use for OH&S reasons; as no effects on hair were tested.
		Trial 3 • G38 phylloxera strain only (life stage not disclosed) • Mean survival using Mortein Fast Knockdown Multi-Insect Killer sprayed for 5 seconds (distance not disclosed)			

Appendix 2

Risk vector: Machinery and equipment

Current procedure in NPMP		Research undertaken		Is current disinfestation procedure in NPMP adequate?	Proposed disinfestation procedure based on research findings
Risk vector Procedure number/ title	**Disinfestation procedure**	**Research method/treatments**	**Summary findings**		
G. Movement of vineyard equipment out of a PIZ or PRZ	HOT WATER Fully immerse the item in water at 70˚C minimum, and hold in water for at least 2 minutes after it has reached 70˚C.	1st instars placed into small vial – 2.5cm wide x 5.5cm high with 25-micron mesh at top and bottom to allow water in. Vials placed into a water bath. Trial 1 • Mean survival of first instars pf six phylloxera strains: G1, G4, G7, G19, G20, G30 • Six water temperatures: 22 (room temperature), 40, 45, 50, 60, 70˚C • Two treatment durations: 60, 120 seconds • 5 replicates per treatment	• None of the first instars survived treatments when subjected to hot water at 50, 60 and 70˚C for 60 and 120 seconds across the six genetic strains. • When subjected to hot water treatment at 40 and 45˚C for 60 and 120 seconds, a proportion of first instars across the six phylloxera genetic strains survived treatments; at room temperature (22˚C) there was 99% survival of instars across all strains. • References [4], [2]	Yes, but current temperature and time specifications can be decreased	i. Fully immerse the equipment in water. ii. Once the water temperature has stabilised at 60°C or higher, hold the machinery or equipment in the water for at least 90 seconds (1½ minutes).
		Trial 2 • Mean survival of first instars of G38 phylloxera strain only • One water temperature: 50˚C • One treatment duration: 60 seconds • 50 replicates	• For strain G38, 50˚C for 60 seconds was effective in achieving 100% mortality. • Reference [4]		

Risk vector: Machinery and equipment continued

Current procedure in NPMP		Research undertaken		Is current disinfestation procedure in NPMP adequate?	Proposed disinfestation procedure based on research findings
Risk vector Procedure number/ title	**Disinfestation procedure**	**Research treatments**	**Summary findings**		
G. Movement of vineyard equipment out of a PIZ or PRZ	STEAM Steam applied must be above 100˚C as indicated by a jet of clear invisible steam between steam outlet and the visible condensate cloud. Steam must contact all surfaces until the surface is left dry not wet with condensate.	Trial 1 • Mean survival of first instars of six phylloxera strains: G1, G4, G7, G19, G20, G30 • Three treatment times: 10, 20, 30 seconds • Two distances from which steam was projected: 8, 24 cm • For G1 only, also projection of steam at 92cm for 10, 20, 30 seconds • 5 replicates per treatment	• All steam treatments resulted in 100% mortality of first instars tested. • After 10 seconds, projection of steam at 8cm resulted in a steam temperature of 102˚C; projection of steam at 24cm resulted in a steam temperature of 98˚C, and projection of steam at 92cm resulted in steam temperature of 90˚C. • No survival was observed when steam was projected from a distance of 24cm for 20 and 30 seconds, across the six genetic strains, and at 92cm for G1 phylloxera. • Although phylloxera mortality was not impacted by distance from which the steam was projected in this trial, the greater the distance that steam was projected from the target, the faster the temperature declined and theoretically this could reduce efficacy of the treatment. The results suggest that steam application should be close to the surface being disinfested to ensure maximum efficacy. • References [4], [2]	No	Application of steam as a disinfestation treatment to ensure efficacy is highly unlikely in the field, except for if applied to small equipment, as each section of the item must be treated for the required time until the entire surface has been disinfested. Steam is therefore recommended to be removed as a valid disinfestation treatment for grape phylloxera for all purposes other than for small hand tools (including technical equipment). i. Steam applied must be 100˚C or above. ii. Steam must contact all surfaces of the hand tools at a distance of approximately 10cm. iii. The spray coverage at this distance must be held for at least 10 seconds on each portion of the item. Refer to diagram below for step-wise application instructions. iv. Steam will only be accepted as a valid disinfestation treatment when applied in the presence of the Biosecurity Officer issuing the Plant Health Certificate. **Distance of 10cm expected to be more accurately judged in the field than 8cm*
		Trial 2 • Mean survival of first instars of G38 phylloxera strain only • One treatment distance: 8cm • One treatment time: 10 seconds • 50 replicates	• For strain G38, steam applied for 10 seconds at a distance of 8cm was effective in achieving 100% mortality. • Reference [4]		

Risk vector: Machinery and equipment continued

Current procedure in NPMP		Research undertaken		Is current disinfestation procedure in NPMP adequate?	Proposed disinfestation procedure based on research findings
Risk vector Procedure number/ title	**Disinfestation procedure**	**Research method/treatments**	**Summary findings**		
G. Movement of vineyard equipment out of a PIZ or PRZ	DRY HEAT Hold in the hot room for a minimum of EITHER 75 minutes after the machinery has reached 45˚C OR two hours after the machine has reached 40˚C	Trial 1 1st instars placed into small vial – 2.5cm wide x 5.5cm high which was placed into a sealed plastic cylinder partially filled with magnesium chloride to create a 30% relative humidity environment. Cylinder placed on one of three shelves in an oven. • Mean survival of six first instar phylloxera strains: G1, G4, G7, G19, G20, G30 • Three oven temperatures: 35, 40, 45˚C • Three treatment times: 75, 90, 120 minutes • 5 replicates per treatment	• Temperature thresholds differ between endemic phylloxera strains, particularly at 35 and 40˚C, over a range of time durations from 75 mins to 120 mins. • A temperature of 35˚C for up to 2 hours is ineffective as a disinfestation treatment against all six phylloxera strains tested. • Heat treatment at 40˚C for 120 minutes was effective against G1, G4, G7 and G19, but not G20 and G30. Upper thermal limit at 40°C for G20 and G30 was reached after 135 minutes. • Heat treatment at 45°C for 75 minutes resulted in 100% mortality against the six endemic strains. • References [4], [1]	No, in particular, heat treatment at 40˚C	i. Place the machinery and equipment in a suitable room, shed or container that can be heated up to the required temperature; and ii. Apply temperature probes to the machinery and equipment and measure the surface temperature and preferably some deeper parts of the posts (e.g. if bundled); and iii. Heat up the room until the probes indicate the required temperature has been reached and hold the machinery and equipment at the required temperature for the required time: EITHER 45°C for 90 minutes (1½ hours) OR 40°C for 190 minutes (3 hours).
		Trial 2 • Mean survival of first instars of G38 phylloxera strain only • Two treatments: 40˚C for 120 minutes, 45˚C for 75 minutes • 50 replicates per treatment	• For strain G38, both 40˚C for 120 minutes and 45˚C for 75 minutes treatments were effective in achieving 100% mortality. • Reference [4]		

Appendix 3

Risk vector: Grapevine cuttings and rootlings

Current procedure in NPMP		Research undertaken		Is current disinfestation procedure in NPMP adequate?	Proposed disinfestation procedure based on research findings
Procedure number/ title	**Disinfestation procedure**	**Research treatments**	**Summary findings**		
B. Movement of grapevine cuttings and rootlings from a PRZ or PEZ vineyard or nursery into a PEZ	HOT WATER Cuttings / rootlings must be hot water treated immediately prior to dispatch as follows: EITHER at 50˚C +/- 1˚C for 30 minutes OR at 54˚C +/- 1˚C for 5 minutes.	Trial 1 • Mean survival of first instars of six phylloxera strains: G1, G4, G7, G19, G20, G30 • Four water temperatures: 40, 45, 50, 54˚C • Two treatment times: 5, 30 minutes • 3 replicates per treatment • Following the hot water treatments, the bundles were immediately immersed in cold water for 30 seconds to stop the heat reaction	• Current hot water treatment recommendations of either 54°C for 5 minutes or 50°C for 30 minutes were effective, with 100% mortality observed against all six strains tested. • Hot water treatment at 40˚C was ineffective at both durations, resulting in over 80% survival of the first instars, continued development to adult stage and egg laying for all six phylloxera strains. • Hot water treatment at 45˚C for 5 minutes, saw 100% mortality of only strains G1, G4 and G7. Increasing the duration to 30 minutes at this temperature resulted in 100% mortality across all six phylloxera strains. • Hot water treatment at 50˚C for 5 minutes resulted in 100% mortality of all strains tested. • Reference [4].	Yes	i. Completely immerse and maintain bundles at a minimum of 50°C ± 1°C for 30 minute OR 54°C ± 1°C for 5 minutes. ii. A minimum of three (3) sensors must be used for each hot water treatment vessel. One sensor should be located at a depth of 100mm from the base of the vessel, another at 100mm from the surface and the other inserted into the centre of the load mass. NOTE: *Some plant material may be damaged by hot water treatment. A trial treatment is recommended unless the response of the plant material to this treatment is known.*
		Trial 2 • Mean survival of first instars of phylloxera strain G38 only • Two water temperature/time treatments: 50˚C for 30 minutes; 54˚C for 5 minutes • 30 replicates per treatment • Following the hot water treatments, the bundles were immediately immersed in cold water for 30 seconds to stop the heat reaction	• For strain G38, both treatments trialed were effective in achieving 100% mortality. • Reference [4] **Note: this research has not been published in a peer reviewed journal at the time of writing this paper.**		

Appendix 4

Risk vector: Diagnostic samples

Current procedure in NPMP		Research undertaken		Is current disinfestation procedure in NPMP adequate?	Proposed disinfestation procedure based on research findings
Procedure number/ title	**Disinfestation procedure**	**Research treatments**	**Summary findings**		
C. Movement of diagnostic samples from a PIZ or PRZ into a PRZ or PEZ	Diagnostic samples are required to be disinfested by ONE of the following procedures: a. Freezing to -18˚C for 24 hours, pack in dry ice for transport b. Freezing and transfer under liquid nitrogen at -196˚C c. Freeze drying d. Oven drying at 45˚C for a minimum of 2 hours (probes should be used with large samples to ensure middle has reached the required temperature). e. Sealed, unbreakable vessel (for juice samples) f. Hot water treatment at 50˚C ± 1°C for 30 OR mins 54˚C ± 1°C for 5 mins g. Fixative – devitalisation using formalin/acetic acid, gluteraldehyde, 70% ethanol or similar	(a) Freezing to -18˚C for 24 hours, pack in dry ice for transport Trial 1 • Mean survival of first instars and eggs of six phylloxera strains: G1, G4, G7, G19, G20, G30 • Three temperatures: -20, 4 and 22˚C (control) • Three treatment times: 6, 12, 24 hours • 5 replicates per treatment Trial 2 • Mean survival of first instars and eggs of G38 phylloxera strain • Two temperatures: -20, 4˚C • Three treatment times: 6, 12, 24 hours • 50 replicates per treatment except for first instars subjected to 4˚C for six hours for which there were 30 replicates.	• Survival of first instars reduced as temperature decreased and time of exposure increased. • In study 1, results validated the disinfestation recommendation of -20˚C for an exposure period of 24 hours, with 100% mortality achieved for first instars across the six genetic strains, but also showed no survival at -20˚C for 12 hours. No egg hatching occurred when eggs were subjected to 12 or 24 hours of -20˚C temperature. At 4˚C, irrespective of the treatment time, there was first instar and egg survival. • In study 2, results validated the disinfestation recommendation of -20˚C for an exposure period of 24 hours, with 100% mortality achieved for G38 first instars, but also showed no survival at -20˚C for 12 hours. No egg hatching occurred when eggs were subjected to 12 or 24 hours of -20˚C temperature. At 4˚C, irrespective of the treatment time, there was first instar and egg survival. • Reference [4]. **Note: this research has not been published in a peer reviewed journal at the time of writing this paper, but an article is in preparation.**	Yes, but current time specification can be decreased	Freezing of diagnostic samples to be carried out by freezing and then holding samples at -18˚C for 12 hours and packed in dry ice or with an ice pack for transport NOTE: *this recommendation is proposed in lieu of peer review paper in preparation. Despite -20˚C for 12 hours showing 100% mortality of all key grape phylloxera strains under laboratory conditions, recommendation is for -18˚C for 12 hours to align with standard freezer temperature. Tested temperature of -20˚C is expected to be as effective as -18˚C over the 12-hour duration.*

		(b) Freezing and transfer under liquid nitrogen at -196˚C	N/A	N/A	Retain current procedure
		(c)Freeze drying	N/A	N/A	Retain current procedure
		(d) Oven drying at 45˚C for a minimum of 2 hours (probes should be used with large samples to ensure middle has reached the required temperature).	Refer Appendix 2	Yes	Oven drying at 45˚C for a minimum of 90 minutes (1½ hours). Note: *Probes must be used with large samples to ensure middle of sample has reached the required temperature for the required time. Bulky samples must be spread out on trays prior to placing in oven to increase surface area exposed to the heat.*
		(e) Sealed, unbreakable vessel (for juice samples). No research undertaken.	• Current specification is not a disinfestation procedure per se. For juice to be able to move unrestricted between Phylloxera Management Zones, it needs to be classified as 'filtered juice'. Unfiltered juice is required to be processed through a filter that removes all particles larger than 50 microns. Centrifugation and cold settling are accepted alternatives to filtration for the purposes of this definition provided that the same outcomes are achieved.	No	For juice samples, either: i. Filter, centrifuge or cold-settle to ensure remaining particles are less than 50 microns in size; or ii. Freeze and then hold at -18ºC for 12 hours and packed in dry ice or with an ice pack for transport; and iii. Seal in an unbreakable vessel prior to sending

		(f) Hot water treatment at 50˚C ± 1°C for 30 mins OR 54˚C ± 1°C for 5 mins.	Refer Appendix 3	Yes	i. Completely immerse and maintain bundles at a minimum of 50˚C ± 1°C for 30 mins OR 54˚C ± 1°C for 5 mins. ii. A minimum of three (3) sensors must be used for each hot water treatment vessel. One sensor should be located at a depth of 100mm from the base of the vessel, another at 100mm from the surface and the other inserted into the centre of the load mass. NOTE: *Some plant material may be damaged by hot water treatment. A trial treatment is recommended unless the response of the plant material to this treatment is known.*
		(g) Fixative – devitalisation using formalin/acetic acid, gluteraldehyde, 70% ethanol or similar	Refer Appendix 1. 80% ethanol for 30 or 60 seconds with or without a water rinse did not result in 100% mortality of all key phylloxera strains. It could be expected that survival of phylloxera strains in 70% ethanol may be higher than in 80% ethanol. However, for use as a fixative, survival of phylloxera strains on diagnostic samples placed into 70% ethanol for an extended period of time during transport to a laboratory remains untested.	Yes, further work required.	Retain current procedure

Appendix 5

Risk vector: Must and unfiltered juice

Current procedure in NPMP		Research undertaken		Is current disinfestation procedure in NPMP adequate?	Proposed disinfestation procedure based on research findings
Procedure number/ title	**Disinfestation procedure**	**Research treatments**	**Summary findings**		
D. Movement of must or (unfiltered) juice from a PIZ or PRZ into a PEZ	Unfiltered juice is required to be filtered or otherwise processed to achieve a maximum particle size of 50 microns.	The impact of white juice, pH, baumé, sulphur dioxide and cold temperature on phylloxera mortality has either never before been examined or only conducted under limited conditions. Current disinfestation procedure has only been evaluated on red juice. • Mean survival of one phylloxera strain for all experiments: G4, • Chardonnay grapes from vintage 2009 and 2010 used. • Experiment 1 Two pH levels: 3.0, 3.7; baumé of 11.7˚; standard temperature of 5˚C; water controls with pH 3.0 and pH unadjusted; 10 replicates per treatment • Experiment 2 Two baumé levels: 11.1˚, 14.1˚; standard pH of 3.4; water control with pH 6.7, glucose/fructose controls at 11˚ baumé and 14˚ baumé; 10 replicates per treatment • Experiment 3 Three temperature levels: 2, 5, 10˚C; standard pH of 3.4; standard baumé of 11.1˚; water control with pH 3.4; glucose/ fructose control of 11˚ baumé; 10 replicates per treatment	• Experiment 1: G4 strain in 5˚C white juice survived for up to 5 days at pH 3.7 and up to 7 days at pH 3.0, compared with survival of at least 8-10 days in the water controls. • Experiment 2: Glucose/fructose control reduced survival of G4 to a maximum of 7 days compared to 10 days in the water control. Some phylloxera still survived for up to 7 days in both baumé treatments in white juice at 5˚C and there was no significant difference (p>0.001) in phylloxera survival with the different baumé treatments. • Experiment 3: Overall, as temperature decreased from 10˚C to 2˚C, phylloxera mortality increased. At 10˚C, phylloxera survived for up to 21 days in water with pH adjusted to 3.4, at 5˚C, survival in water had reduced to 10 days [6]. In white juice and for the glucose/fructose control at 10˚C, 100% mortality of strain G4 was achieved at 10 days and 8 days respectively. Similar survival trends were noted for white juice and glucose/fructose at 5˚C [6]. At 2˚C in white juice, 100% phylloxera mortality of the G4 strain was achieved by day 3. However, there was still some phylloxera survival at day 7 in the water and glucose/fructose controls [6]. • Experiment 4: At 10˚C, some phylloxera still survived after 8 days in the water + sulphur control, but in water alone, over 30% of the phylloxera survived up to 9 days [6]. In white juice at 10˚C, phylloxera survived for 6 days when sulphur was added, compared to 7 days with no sulphur. At 2˚C, there was 100% mortality in the water control with added sulphur within 4 days, whereas in the absence	Yes. Further work is required using more strains than just G4 before alternate disinfestation treatments to 50-micron filtration for white juice can be specified.	Unfiltered juice is required to be processed (filtered, centrifuged, cold settled or other) to a 50-micron filtration rating (or tighter). NOTE: – *this research showed that at 10˚C, phylloxera survived for up to 21 days in water. This survival is important to take into consideration for vector movement of phylloxera.*

		• Experiment 4 Sulphur dioxide at 20 ppm; two temperatures of 2, 10˚C; standard pH of 3.4; standard baumé of 11.5˚; no sulphur dioxide control; 10 replicates per treatment	of sulphur, phylloxera survival in water was at least 7 days. In white juice at 2˚C, 100% mortality was achieved at 2 days whether sulphur was present or absent. • Phylloxera was shown to survive in water for over 10 days at 10˚C. Overall acidity and baumé, within the ranges tested, had minimal impact on phylloxera survival. However white juice, sulphur dioxide and cold temperature did increase mortality of the G4 strain. • References [5], [6]		

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