APPENDIX 1

Surveillance of grape phylloxera in vineyards – Root inspection

1. What is Grape Phylloxera?

Grape phylloxera is an aphid-like insect that feeds on roots of vines.

1.1 Biology and life cycle of grape phylloxera

First-instar nymphs (called crawlers) pass the winter season (overwinters) on grapevine roots. In early spring, the crawlers undergo four moults (called intermediates), before developing into adults (Figure 1). Adults are approximately 1mm in length and 0.5mm in width (Kingston et al., 2007). In spring and summer, the adults lay oblong yellow eggs, nearly twice as long as wide (approx. 0.3mm in length and 0.15mm in width) (Kingston et al. 2007). Eggs hatch into crawlers (approx. 0.5mm), which are similar to adults but smaller in size. Adults may also develop into the winged form (called alates) but there is no evidence that the alates complete a sexual reproduction cycle in Australia.

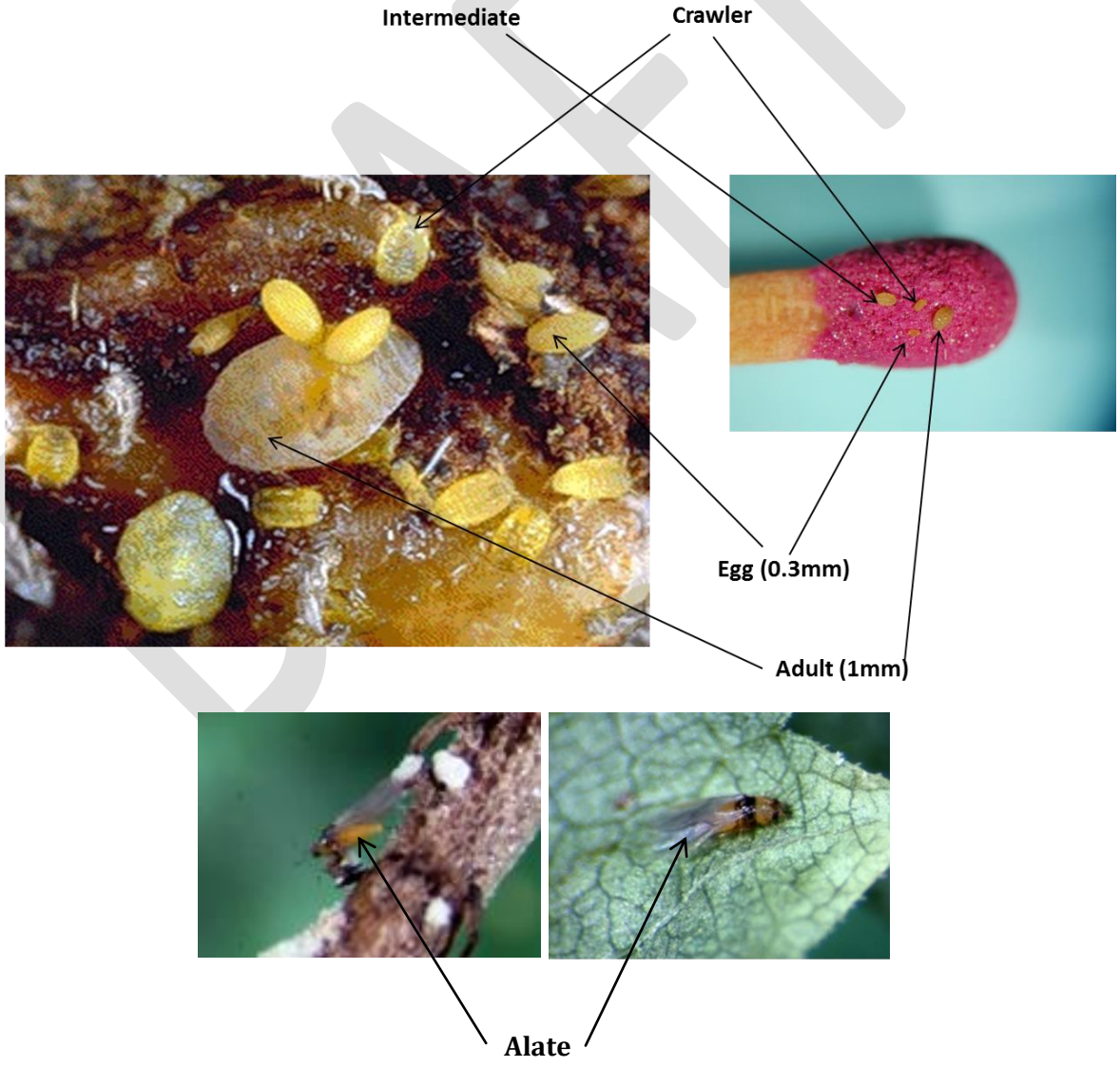


Figure 1. Phylloxera developmental stages

Following hatching, crawlers move under the ground and along roots to establish new feeding sites. All phylloxera developmental stages remain attached to the same feeding site in the entire life cycle (De Klerk 1974, Granett et al. 2003). In Australia, adults undergo asexual reproduction. Each individual lays 3-6 eggs per day and the reproductive period lasts between one and two months, depending on temperature (Granett et al., 1983; Buchanan 1990).

1.2 Recognising damage on vines due to grape phylloxera

Phylloxera induces galls (also called nodosities) by feeding on non- lignified young feeder roots (Figure 2). On old lignified storage roots, phylloxera populations are found as clusters (Figure 3). Root galling phylloxera is the predominant cycle in Australia (Powell et al. 2013).

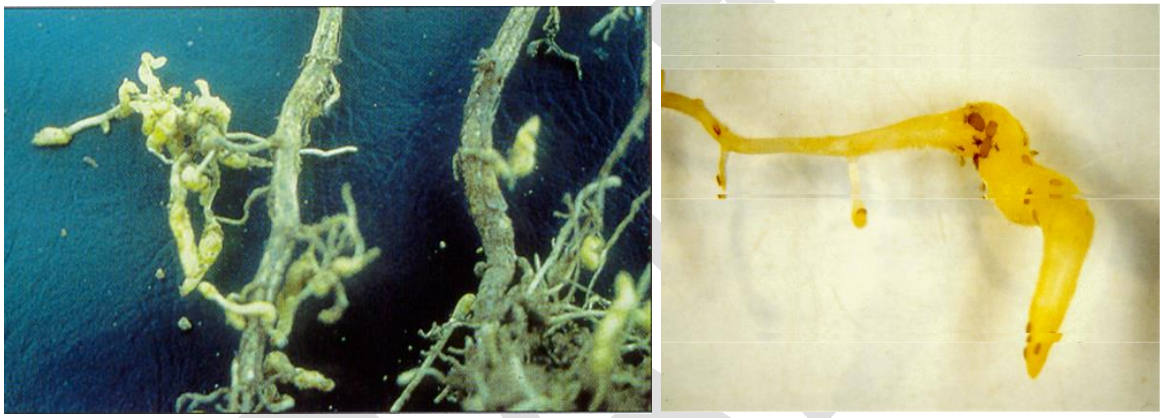


Figure 2. Phylloxera-induced galls on roots (left) and insects at feeding sites on a fresh gall.

Figure 3. Phylloxera eggs, intermediates and adults in clusters on a gall in a non-lignified root (left) and on old lignified storage roots (right)

Phylloxera may induce galls on vine leaves of American *Vitis* species or hybrids (Figure 4). The insects live inside the leaf galls where they reproduce (lay eggs). Leaf galling phylloxera is rare but has been observed in North East Victoria.

Figure 4: Leaf galls on grape vines due to phylloxera

1.3 Symptoms of vines infested by grape phylloxera

Initial infestations of vines by grape phylloxera appear as a few weak spots in a vineyard. Classic visual symptoms on vines due to phylloxera damage include, yellowing of leaves, stunted growth, reduced grape bunch sizes, strong weed or grass growth at the base of the vines and/or presence of dead vines.

Figure 5. A block showing a weak spot in a vineyard (above). Yellowing of leaves, weed undergrowth and stunted growth due to phylloxera (below).

Eighty-three phylloxera genetic strains (biotypes) exist in Australia (Umina et al. 2007). Two root galling biotypes, namely, G1 and G4, predominate and have been described as 'superclones' (Umina et al., 2007). These two biotypes are more virulent and display classic phylloxera damage in vines (Powell et al. 2003, Herbert et al. 2006).

1.4 Surveillance of phylloxera by digging

Digging at the base of the vines, collecting and inspecting roots for presence of phylloxera is the standard method used during scouting surveys (surveillance) and also, sampling. The method is a destructive technique that involves digging out roots with a shovel (Figure 6) and examining phylloxera on galls in young non-lignified roots (see Figure 2) and clusters of phylloxera eggs, intermediates and adults on old lignified storage roots (See Figure 3).

Figure 6. Digging at the base of the vines (left) to uncover galls (right) with phylloxera at feeding sites. Phylloxera colonies and nodosities appear as yellow or cream.

The dig method is based on visual vine stress symptoms and is suitable for screening a wide area and large numbers of blocks and vineyards. The dig method is suitable at pinpointing weak spots in a vineyard and for predicting the presence of highly virulent strains (e.g G1 and G4) as roots have numerous large galls and high numbers of insects present in all stages (egg, intermediates and adult) (Herbert et al., 2006).

2 Materials required

Description	Items
General	Reference material -The digging protocol -Phylloxera pictures Hand lens Notebook and pen GPS units for recording property location Camera Mobile phone
Digging	Shovel Trowel Secateurs Screw driver to remove mud attached on footwear
Collecting samples	Snaplock bags for storage of samples during transport Sampling sheets for recording details of dug samples Eskies or equivalent containers for sample storage and transport
Disinfestation	Disposable coveralls Tie garbage bags Spray bottle containing 80% ethanol Cleaning kit (4% sodium hypochlorite - household bleach, scrubbing brushes) 20 litres oblong or rectangular wash basin Hand soap and detergent Mortein insect spray to disinfest hats and other clothing Latex, nitrile gloves Paper towel
OHS	First aid and snake bite kit Sunscreen Drinking water (approx. eight litres) Broad brimmed hat Fly net (optional) Latex, nitrile gloves

2.1 Digging method for surveys/surveillance/scouting of phylloxera in suspect vines (where Phylloxera has not been detected before).

i. Take a GPS reading of the survey block location. A 3-Dimensional reading is recommended (longitude, latitude and elevation).
ii. Record the vineyard name and location, row and panel numbers as well as the sampling date.
iii. Observe both sides of the vines for phylloxera symptoms e.g areas of poor and stunted growth, weed undergrowth and yellowing of leaves (See Figure 5).
iv. Dig at the target vine within a radius of 600 mm from the trunk or near irrigation drippers to expose the young non-lignified roots (See Figure 6) as well as old lignified roots (Figure 7) or dying roots.
v. Check for yellow galls on non-lignified roots and a yellowing colouration on older and dying roots.

vi. Use a trowel to pick up galls and insects that may be on the soil at the digging area (Figure 8).

vii. If possible, follow the root out and sample further from the base of the vine.

Figure 7. Galls on non-lignifed roots (left), phylloxera on old lignified roots (right).

Figure 8. Use a trowel to pick up galls and insects from the soil.

viii. Sever galls and roots with a pair of secateurs.

ix. Use a 10xmagnification hand lens to carefully examine the galls and roots for all phylloxera life stages (eggs, crawlers intermediates and adults). Phylloxera appear yellow on lignified roots (Figure 7) and on galls and (Figure 9). Insects may appear small, brown and shrivelled in late summer. On roots, phylloxera can appear flattened on or just under the bark of lignified mature roots.

Note: An egg measures approx. 0.3mm and adult 1mm in length (Refer to section 1.2 and Figure 1).

Figure 9. Phylloxera colonies on galls

2.2 Sample identification

It is possible that phylloxera may not be seen due to poor lighting conditions, personnel sight acuity, fatigue, inaccurate use of the magnifying hand lens or human error. It is, therefore, likely to discount a dug up sample when it is indeed positive (a false negative). On the other hand, a sample may appear to have insects but it is indeed negative (a false positive). For all suspect samples:

i. Collect roots and galls into a snaplock bag or place in sample bottle containing 80% ethanol.
ii. Clearly label, appropriately seal and transport samples in a foam esky for further identification.
iii. Record details of vineyard location, vineyard block, vine position and row number and a sketch plan and mark suspect vine and end of row with a flagging tape.
iv. Store samples in a cool environment and dispatch as soon as possible.
v. Obtain an **appropriate permit** from the nearest Plant Standards branch prior to collection of trap samples. A permit is not required if you are within the same PIZ at all times.
vi. Liaise with the biosecurity and agriculture services for transport processes of the sample to the reference entomologist at AGRIBIO, Bundoora, Victoria. A charge for sample identification applies.
vii. Samples can be transported by courier or express mail. However, note that Australia Post and courier regulations for transport requirements when using flammable liquids.
viii. Additional surveys may be necessary.

3 Disinfestation Procedures

Appropriate disinfestation procedures should always be followed when entering and leaving a vineyard. Permission to access a vineyard should also be sought before surveys. All equipment used and clothing worn on the property must be disinfested before leaving the vineyard. While in the vineyard, drive vehicles on designated roads.

3.1. Disinfestation of footwear, clothing and equipment.

i. Set up a disinfestation station on a suitable hard surface.

ii. Prepare 2% sodium hypochlorite to reach at least ¼ of a 20litres rectangular basin. To prepare a 2% sodium hypochlorite solution, mix equal quantities of water and household bleach (active ingredient 4% sodium hypochlorite).

Note

Bleach solution should be prepared fresh and only when it is required as chlorine readily breaks down, particularly in sunlight.

OH&S precautions should be applied when using household bleach. Compliance with labels and safety requirements should be observed. Use of gloves is highly recommended.

iii. Remove soil that may be attached to shovels, trowels and secateurs by scrubbing with a brush (Figure 10) and mud from shoes with a screw driver.

iv. Disinfest, footwear, the trowels and shovel by dipping in the bleach solution for a minimum of 60 seconds and allow them to dry (preferably on a black surface in the sun when conditions are favourable). Do not rinse the disinfested footwear and other equipment in water (Figure 11).

v. Spray any accessories or clothing such as fly nets, kneepads, hats and socks with a Mortein insecticide spray (a.i. 1.1g/kg Esbiothrin and 0.5g.kg Permethrin) (Figure 12). It is highly recommended that the spray comes into contact with the entire surface of the accessory/clothing. A hot wash cycle (hot cycle >50°C for at least 30 minutes of any clothing that may have come into contact with foliage and soils is preferred. Transport any clothing in sealed double bags if hot washing is used.

Figure 10. Use a brush to remove soil from shovels, trowels and secateurs in 2% sodium hypochlorite .

Figure 11. Dip footwear, shovels, trowels and secateurs in 2% sodium hypochlorite solution and dry on a black surface.

Figure 12. Disinfest the eye lenses with 80% ethanol and clothing with an insecticide spray.

3.2 Disinfestation of other equipment

i. Spray sample bags and other material that may have come in contact with soil during the digging surveys with 80% ethanol before placing them in the car. Ensure that all surfaces come into contact with the spray disinfectant.
ii. Avoid walking back and forth the disinfestation area during the cleaning process.
iii. Rinse hands in clean water. Place all waste in a garbage bag (double bagging is highly recommended) (Figure 13) and dispose appropriately.
iv. Before leaving the vineyard, hose down the tyres in a holding sump that drains away from the vineyard and roads (Figure 13). A hot water (above 45°C) pressure washer is recommended.


Figure 13. Place all waste in a garbage bag. Double bagging is highly recommended. Hose down the tyres with water before leaving the vineyard.

References

Buchanan, G. A. 1986. Dispersal of grape phylloxera within vineyards. The Australian Grapegrower and Winemaker **December**:24-25.

Buchanan, G. A. 1987. The distribution of grape phylloxera, *Daktulosphaira vitifolii* (Fitch), in central and north-eastern Victoria. Australian Journal of Experimental Agriculture **27**:591-595.

Buchanan, G. A. 1990. The Distribution, Biology and Control of Grape Phylloxera, *Daktulosphaira vitifolii* (Fitch), in Victoria. PhD. La Trobe University, Australia.

De Klerk, C. A. 1974. Biology of Phylloxera vitifoliae (Fitch) (Homoptera: Phylloxeridae) in South Africa. Pages 109-117.

Granett, J., A. Walker, and L. Kocsis. 2003. Grape phylloxera, damage, ecology, variability, and management. Pages 409-413 6th Slovenian Plant Protection Conference, Zrece, Slovenia.

Herbert, K. S., A. A. Hoffman, and K. S. Powell. 2006. Changes in grape phylloxera abundance in ungrafted vineyards. Journal of Economic Entomology **99**:1774-1783.

Kingston, K. B., P. D. Cooper, and K. S. Powell. 2007. Grape phylloxera external morphology observations under scanning electron microscopy. Acta Horticulturae **733**:107-114.

Powell, K. S., P. D. Cooper, and A. Forneck. 2013. Chapter Four - The Biology, Physiology and Host–Plant Interactions of Grape Phylloxera Daktulosphaira vitifoliae. Pages 159-218 *in* I. H. Scott N. Johnson and C. J. T. Ted, editors. Advances in Insect Physiology. Academic Press.

Powell, K. S., W. J. Slattery, J. Deretic, K. S. Herbert, and S. Hetherington. 2003. Influence of soil type and climate on the population dynamics of grapevine phylloxera in Australia. Acta Horticulturae **617**:33-37.

Umina, P. A., A. M. Corrie, K. S. Herbert, V. L. White, K. S. Powell, and A. A. Hoffmann. 2007. The use of DNA markers for pest management - clonal lineages and population biology of grape phylloxera. Acta Horticulturae **733**:183-195.

APPENDIX 2

Emergence traps for surveillance and detection of grape phylloxera in vineyards

1. Life stages of phylloxera and trapping

Phylloxera is an aphid-like insect that feeds on roots of vines causing deformations, gradual decline and eventual death. First-instar nymphs (called crawlers) undergo four moults (called intermediates) before developing into adults (Figure 1). Crawlers pass the winter season (overwintering) in an inactive stage on grapevine roots and then in early spring, adults reproduce asexually. An individual adult lays up to 200 eggs in her lifetime (Buchanan 1990, Grannett 2007). Eggs hatch into crawlers which move under the ground and along roots to establish new feeding sites. The reproductive period lasts between 1-2 months, with 3-5 generations in a year, depending on spring, summer and autumn temperatures (Granett and Timper 1987). Adults may also develop into the winged form (called alate) likely caused by overcrowding (Granett et al. 2001b), high soil moisture (Maillet 1957) or host plant quality (Dixon 1973). There is no evidence that the alates complete a sexual cycle in Australia.

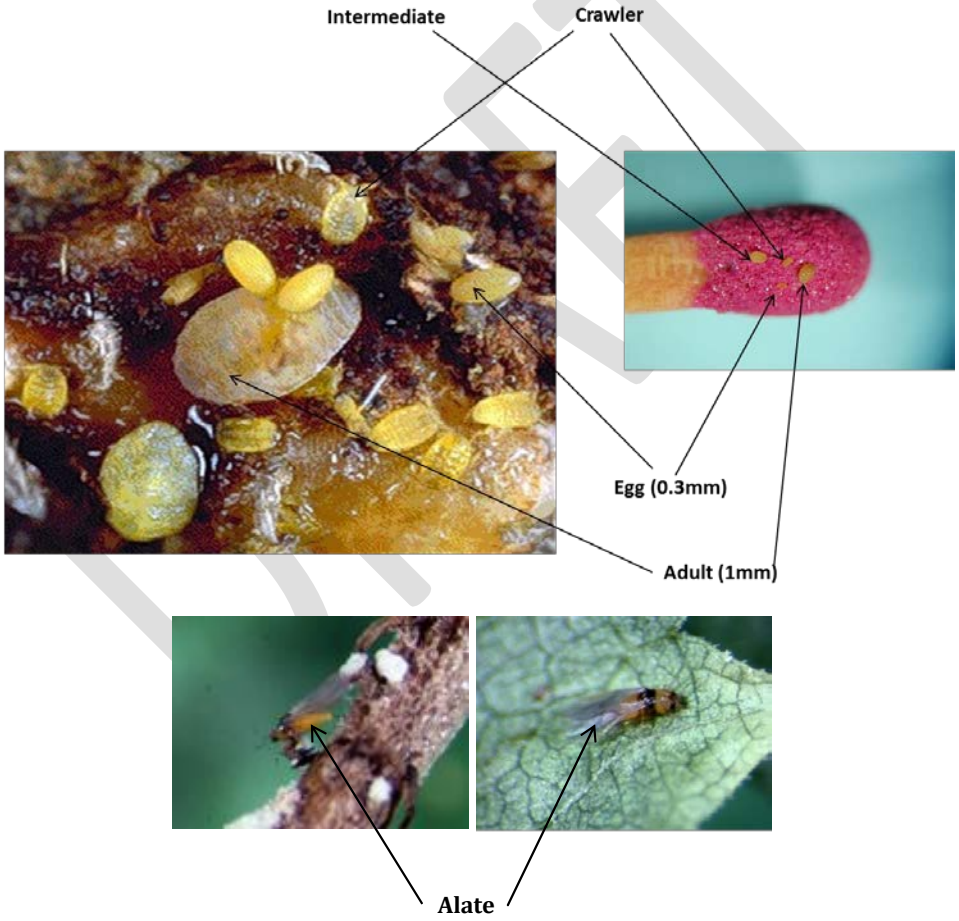


Figure 1. Phylloxera developmental stages

2. Symptoms of phylloxera damage on grapevines.
Symptoms of phylloxera infestation of roots first appear as a few weak spots in a vineyard. Classic visual symptoms due to damage and high infestation of roots are yellowing of leaves, stunted growth, reduced grape bunch sizes, strong weed undergrowth at the base of the vines and/or presence of dead vines.

Figure 2. Weak spots indicating phylloxera infestation (above). Late stage infestation, showing yellowing of leaves, weed undergrowth and stunted

3. Trapping as a phylloxera surveillance and detection method
The root-feeding phylloxera (called radicole) is found on vine roots all year round. Phylloxera emergence traps primarily target the crawler life stages and sometimes the alates. Crawlers are abundant in late summer to early autumn when temperatures are optimal for reproduction. Newly hatched crawlers move along roots to find suitable feeding sites. The crawlers also move through cracks to the soil surface and from there, can move up the vine stems to the foliage (King and Buchanan 1986, Herbert et al. 2006). Emergence traps catch the crawlers (and alates) as they move above the ground. Crawlers can survive for 7-21 days depending on temperature and water availability (Powell et al. 2013).

Trapping protocols involve selection of potential hot spots of phylloxera infestation, based on visual symptoms on grapevines. The optimal time to install traps is in the summer months when phylloxera populations reach peak abundance (December to February) (Buchanan 1990, Powell et al. 2003, Herbert et al. 2006). Emergence traps have an advantage over the widely used method of digging (root inspection) as they are relatively cheap and labour efficient, and installation does not require prior expertise in identifying phylloxera. Moreover, the trapping method uses a non-destructive technique with minimal damage to the vine roots.

Emergence traps are made from 4-5 litre translucent round plastic containers (21cm width X 18 depth) that have been wetted with water on the internal surface, and secured upside down to create an airtight seal that encourages a build-up of humidity (Figure 3). Metal tent pegs are used to secure the containers in place, and prevent damage or displacement of the traps by vineyard machinery, vineyard personnel and animals (Figure 4). Crawlers and alates moving above-ground enter the containers and are trapped in the condensate. The trapped insects can then be washed into a sample container or vial using a preservative such as ethanol (80% concentrate recommended) to enable DNA identification in the laboratory (Figures 5 & 6).

4. Recommended use of emergence traps

Surveillance (new detections)

Phylloxera is difficult to detect in a healthy vineyard and installing traps along the edges of vines that have shown consistent weakness over 2-3 summers could be useful for new detections. Emergence traps are usually placed on base of vines around weak spots in the vineyards (Section 2), throughout late spring, summer and autumn months (November to April). To optimise efficiency, traps are best placed on vines within a radius of 100 m around vines in weak spots (reference vines). This distance is the estimated distance that phylloxera can spread in a season (Buchanan 1990), In vineyards where phylloxera has not been previously reported, recommended trapping is one trap per panel, placed in adjacent rows surrounding a weak spot (Figure 7).

Trap samples should be sent to an entomologist for identification.

Monitoring phylloxera populations

Emergence traps are also used to monitor populations in vineyards where phylloxera is already present (Herbert et al. 2008). Monitoring may help growers contain the spread of phylloxera (e.g. between blocks), and assess the effectiveness of new rootstocks. Monitoring may include molecular analysis of the phylloxera genetic strain that is present in the vineyard, which may be useful to the grower for selecting rootstocks that have the best resistance to the local phylloxera strain (Powell and Krstic 2015).

Figure 3. Placement of traps in adjacent rows and panels of symptomatic and asymptomatic vines.

4. Disinfestation Procedures

Appropriate disinfestation procedures must always be followed when entering and leaving a vineyard. Permission to access a vineyard should also be sought before trap installation. All equipment used and clothing worn on the property during trap installation must be disinfested before leaving the vineyard. While in the vineyard, drive vehicles on designated roads.

5. Equipment required for installation and collection of emergence traps

	Items
General	• Protocols / guides for use of emergence traps • Notebook and pen • Mobile phone • Camera
Trap installation	• Translucent round plastic container (4 to 5 litres). • Claw hammer • 3 metal tent pegs (per trap) • Water (approx. 20 litres) • Permanent markers for labelling traps • Sampling sheets for recording details of the traps • GPS units for team leaders, for recording property location • Latex, nitrile gloves • Flagging tape
Trap sample collection	• Sample plastic containers (120ml) with a screw top lid (. • 500 ml squirt bottle containing 80% ethanol • Snaplock bags for storage of samples during transport • Foam coolers (eskies) or other appropriate containers for storage of samples • Latex, nitrile gloves
Disinfestation procedure	• Spray bottle containing 80% ethanol • Disposable coveralls • Cleaning kit. Household bleach (4% sodium hypochlorite), water, screw drivers, scrubbing brushes. • 20 litre rectangular wash basin (or similar) • Hand soap or suitable detergent • Insect spray (to disinfest hats and other clothing) • Tie garbage bags
OHS	• First aid kit and snake bite kit • Sunscreen • Drinking water • Hat • Fly net (optional) • Knee pads (optional) • Latex, nitrile gloves

3. Methods

Selecting blocks/sites for trap placement

Weak spots can be caused by phylloxera infestation, and are evident as yellow of leaves and stunted growth, weed undergrowth (See section 2).

i. To use emergence traps for new phylloxera detections, identify any weak patches in the block.
ii. Pick a vine (reference vine) in the middle of the weak patch. Select a vine on either side of the reference vine (sample row) to place traps.
iii. Pick three vines on both sides of the sample row (buffer rows) (Figure 4: Herbert et al., 2006).

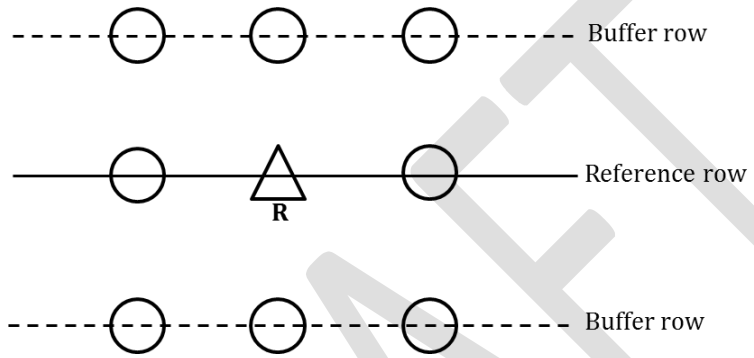


Figure 4. Trap placement for new detections. Place traps at at the reference vine (R) and individual vines in buffer and sample rows (represented in circles).

iv. To monitor phylloxera populations in a vineyard where phylloxera is known to exist, place traps spaced throughout the block, placing under any vine on every 3rd row and fifth panel (Figure 5). **Note.** Some blocks are set up with a restraining post in the first panel which have two or three vines and not representative of the rest of the panels in that row and block. Start counting panel 1 as the first panel which is representative of the whole block.

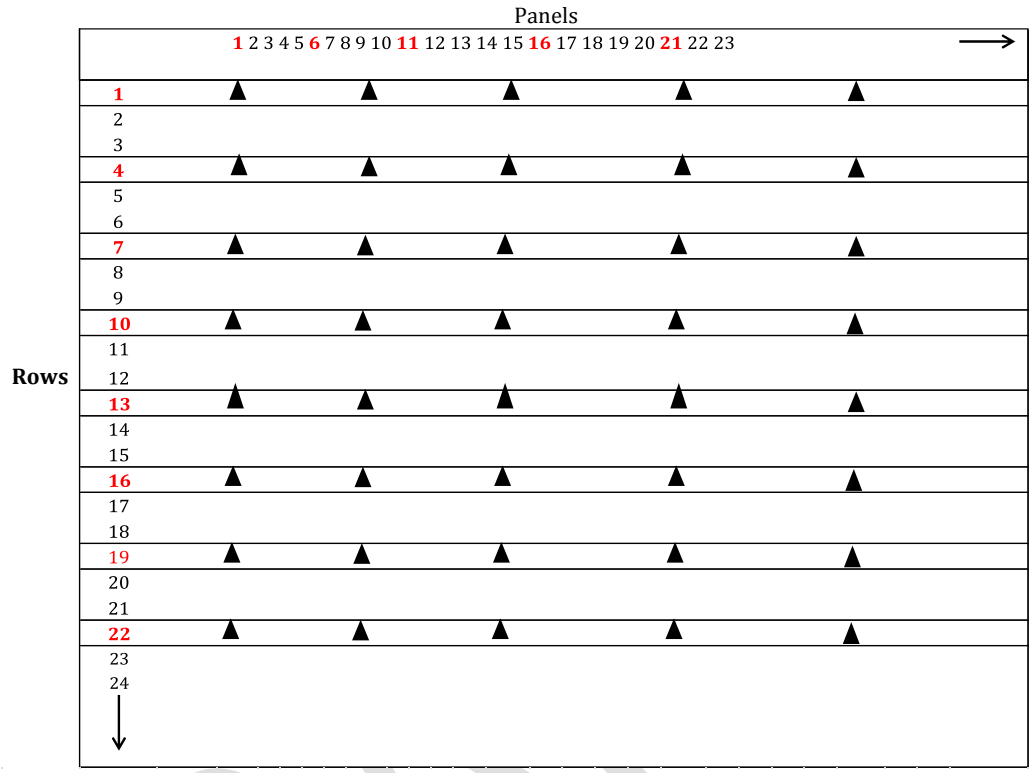


Figure 5. For monitoring phylloxera populations, place traps under vine in every third row at every 5th panel.

Trap Installation

i. Clear any mulch, debris or plant material adjacent to the trunk of the selected grapevine. Level the soil surface (e.g., with a claw hammer) (Figure 6).
ii. Rinse the emergence trap with a small volume of water (about 200 ml) leaving a thin film of moisture (Figure 7).
iii. Place the emergence trap (inverted) on the levelled ground at a distance of approximately 10 cm from the base of the vine (Figure 8).
iv. Secure the trap on the soil surface using three metal tent pegs (recommended to use a hammer). Ensure that the container edges are sealed so that condensation forms in the trap (this will usually occur within a 1-2 hour period) (Figures 9 & 10).
v. Record detailed notes including block, rows and panels numbers, vineyard location, and name and date the traps were installed.
vi. Collect trap samples after 2-4 weeks.

Figure 6. Clear mulch and debris and level the soil surface with a claw hammer

Figure 7. Rinse emergence trap with water, leaving the surface wet.

Figure 8. Place the trap on levelled ground (inverted_

Figure 9. Secure traps to the soil surface using three metal pegs

Figure 10. Condensation collects on the inner surface of trap within 1-2 hours. Crawlers and alates are trapped in the condensate as they move above ground.

3.3. Collection of trap samples

i. Label sample containers clearly, placing a printed label inside the container in addition to labelling the outside. Use a pencil (not an ink pen, as this will wash off with ethanol). Label details must show i) vineyard location ii) vineyard name iii) collection date iv) row number v) panel number and vi) vine number. It is highly recommended to label collecting containers before going to the field.
ii. Carefully remove pegs, ensuring that the condensate holds onto the inner surfaces of the container (Figure 11).
iii. Gently flip the trap.
iv. Rinse the container by squirting 80% ethanol on the sides, ensuring that all the inner surfaces are washed (Figure 12).
v. Empty contents into a sample container (Figure 13). Seal immediately, ensuring that the lid is tight to avoid spillage during transport (Figure 14).
vi. If re-installing the trap, rinse with water. Peg back into position.
vii. Place samples in an esky (or appropriate holding container). It is recommended that samples from one row are packed separately in a labelled snaplock bag during transport.

Figure 11. Carefully remove metal pegs and flip trap

Figure 12. To collect insect specimens, rinse inner surface with 80% ethanol.

Figure 13. Transfer insects to a sterile container.

Figure 14. Transport insects for identification in a sealed container .

3.4. Sample identification

Emergence traps collect a range of insects that may look similar to phylloxera to the untrained eye. **For accurate diagnostics of any trapped insects, it is essential to liaise with the a biosecurity officer.** Samples will be examined a trained entomologist, and will include both visual and molecular diagnostics if phylloxera is suspected.

a. Obtain an **appropriate permit** from the nearest Plant Standards branch prior to collection of trap samples. A permit is not required if you are within the same PIZ at all times.
b. Samples can be transported by courier or express mail. Note: Australia Post and courier regulations for transport requirements when using flammable liquids.

4 Disinfestation of footwear, equipment and clothing

a. Set up a disinfestation station on a suitable hard surface.
b. Prepare 2% sodium hypochlorite to fill at least a quarter of a 20 litre rectangular basin. To prepare a 2% sodium hypochlorite solution, mix equal quantities of water and household bleach (as bleach is 4 % sodium hypochlorite).
c. **Note:** Bleach solution needs to be prepared only when it is required, as chlorine readily breaks down (particularly in sunlight).
d. OH&S precautions should be applied when using household bleach. Compliance with labels and safety requirements should be observed. Use of latex nitrile gloves is highly recommended.
e. Remove any soil and plant debris from footwear and equipment using a scrubbing brush Dip footwear and hammer in 2% sodium hypochlorite for a minimum of 60 seconds. **Note:** Do not rinse disinfested footwear and other equipment in water (Figure 15).

Figure 15. Wash off mud and dip footwear and hammer in bleach for 60 seconds.

f. Spray any accessories or clothing such as fly nets, kneepads, hats and socks with Mortein insecticide spray (active ingredients 1.1g/kg Esbiothrin and 0.5g.kg Permethrin) (Figure 16). It is highly recommended that the spray comes into contact with the entire surface of the accessory/clothing. Wash clothing that may have come into contact with foliage and soils on a hot wash cycle (over 50°C) for at least 30 minutes. Transport any clothing in sealed double bags for washing.

g. Disinfest sample bags, unused pegs (that may have come into contact with soil), containers, with 80% ethanol (using a squirt bottle), and other material that may have come in contact with soil during the trap installation process with 80% ethanol. Ensure that all surfaces come into contact with the ethanol before placing them in the car (Figure 16).
h. Disinfest equipment with 80% ethanol (using squirt bottle) before packing for transport(Figure 17). Disinfest the brush in bleach solution or ethanol before placing it in the car.
i. Rinse hands in clean water. Upon exiting the vineyard, coveralls must be removed and placed in a plastic bag for disinfestation and/or disposal. Double bagging is highly recommended.
   Avoid returning to the disinfestation area during the cleaning process.
j. Hose down vehicle tyres at a holding sump before leaving the vineyard (as water drains away from the vineyard and roads) (Figure 18). Preferably, use a hot washer and / or pressure washer.

Figure 16. Spray clothing with an insecticide spray and double bag all waste for disposal.

Figure 17. Disinfest containers used during transport and sample bags with 80% ethanol

Figure 18. Hose down the vehicle and tyres in a holding sump before leaving the vineyard

5. References

Buchanan, G. A. 1990. The Distribution, Biology and Control of Grape Phylloxera, *Daktulosphaira vitifolii* (Fitch), in Victoria. PhD. La Trobe University, Australia.

Granett, J., and P. Timper. 1987. Demography of grape phylloxera, *Daktulosphaira vitifoliae* (Homophtera: Phylloxerideae), at different temperatures. Journal of Economic Entomology **80**:327-329.

Grannett, J. 2007. BIOLOGY AND INTEGRATED PEST MANAGEMENT OF GRAPE PHYLLOXERA. University of California, Davis.

Herbert, K. S., A. A. Hoffman, and K. S. Powell. 2006. Changes in grape phylloxera abundance in ungrafted vineyards. Journal of Economic Entomology **99**:1774-1783.

Herbert, K. S., K. S. Powell, A. McKay, D. Hartley, Herdina, K. Ophel-Keller, M. Schiffer, and A. Hoffmann. 2008. Developing and Testing a Diagnostic Probe for Grape Phylloxera Applicable to Soil Samples. Journal of Economic Entomology **101**:1934-1943.

King, P. D., and G. A. Buchanan. 1986. The dispersal of phylloxera crawlers and spread of phylloxera infestations in New Zealand and Australian vineyards. American Journal of Enology and Viticulture **37**:26-33.

Powell, K. S., P. D. Cooper, and A. Forneck. 2013. Chapter Four - The Biology, Physiology and Host–Plant Interactions of Grape Phylloxera Daktulosphaira vitifoliae. Pages 159-218 *in* I. H. Scott N. Johnson and C. J. T. Ted, editors. Advances in Insect Physiology. Academic Press.

Powell, K. S., and M. Krstic. 2015. Phylloxera: Rootstock tolerance and resistance to different genetic strains of phylloxera Wine and Viticulture Journal **30**:48-51.

Powell, K. S., W. J. Slattery, J. Deretic, K. Herbert, and S. Hetherington. 2003. Influence of soil type and climate on the population dynamics of grapevine phylloxera in Australia. Acta Horticulturae **617**:33-41.

APPENDIX 3

DRAFT PROTOCOL: DNA METHOD

Standard soil sampling and handling protocol for phylloxera DNA analysis by qPCR

Resources

- 14mm diameter x 100mm long AccuCore soil corer
- Screwdriver
- Sample bags, courier bags and box or other container for triple bagging
- Bar-coded labels (supplied by laboratory)
- Cable ties
- All items required to implement farm gate hygiene practices

---

Farm gate hygiene practices

If you are entering someone else's vineyard to collect samples with their permission, it is important that you familiarise yourself with farm gate hygiene practices that you must implement prior to entry and on exit of a vineyard. For current practices, please refer to www.vinehealth.com.au.

Where to take a sample in your vineyard

- Adopt a grid sampling method in the vineyard, sampling a vine every 3rd row / 5th panel. This frequency equates to approximately 40 vines per hectare with standard 2.75m row spacing x 1.8m vine spacing.
- Collect one soil core per vine and place 5 consecutive soil cores in a single sample bag.
- You will therefore have approximately 8 labelled sample bags with 5 cores in each per hectare of vineyard.

Note: in addition to the above samples, soil cores from visibly weak vines should also be collected. These samples should be collected in a separate bag.

Prior to collecting samples contact the diagnostic laboratory to advise that you would like to submit samples. The laboratory will let you know what quarantine permits are required. Pre-printed labels can also be supplied by the laboratory.

How to take a soil sample

1. Ensure that you have all the materials ready to undertake the sampling.
2. Label all sample bags (pre-printed labels are available from the diagnostic laboratory).
3. Stand facing the vine. Place the tip of the corer on the surface of the soil as close to the trunk as possible (within 10cm from the vine trunk).
4. Push the corer into the soil – twisting slightly as you go if you hit vine roots. *If you hit a large root that you are unable to get through when inserting the corer, you may have to remove the corer and take a sample in a different position around the vine trunk.*
5. Twist the corer clockwise 180° and then back anticlockwise 180°.

6. Remove the corer from the soil, twisting as you go to ensure that the soil remains in the corer cavity (especially important in sandier soils). *Ensure your core completely fills the corer cavity with soil.*
7. Place the tip of the corer into a plastic sample bag labelled with the correct details.
8. Carefully use a screwdriver to scrape all soil from the corer cavity into the sample bag.
   Note – the sides of the corer cavity are sharp, complete this step carefully to avoid cutting fingers or piercing the sample bag with the screwdriver.
9. Repeat steps 3 – 8 to collect core samples from the next 4 vines. Therefore, you will have 5 cores samples composited in the sample bag.

How to package samples ready for dispatch to the laboratory

10. Use a cable tie to tightly seal the sample bag containing the 5 cores.
11. Once you have collected all soil samples, place each composite soil sample bag in an individual courier bag
12. Roll up lengthways to close, removing as much air as possible. Seal well.
13. Place all sealed courier bags in a foam box to insulate and seal well. Important to ensure that temperature of samples remains at or below 20°C during transport to the laboratory.
14. Prior to sending samples, complete all quarantine documentation and affix to box.
15. Dispatch samples to laboratory via express courier (ensure tracking of parcel) and notify laboratory. Ensure that samples are not in transit over the weekend.

Step 1 - Items that you will need for soil collection:

- Soil corer
- Sample bag
- Sample label (pre printed from laboratory)
- Screwdriver

Plus foam box for transport to diagnostic laboratory and any documentation required by state plant quarantine standards for the state in which the diagnostic laboratory is located.

Step 2 - Label all sample bags.

Step 3 - Place the tip of the soil corer within 10cm of the base of the vine trunk to be sampled.

Step 4 - Push the corer into the soil – twisting slightly as you go if you hit vine roots. If you hit a large root that you are unable to get through when inserting the corer, you may have to remove the corer and take a sample in a different position around the vine trunk.

Step 5 - Twist the corer clockwise 180° and then back anticlockwise 180°.

Step 6 - Remove the corer from the soil, twisting as you go to ensure that the soil remains in the corer cavity (especially important in sandier soils).

Step 7 - Place the tip of the corer into a plastic sample bag labelled with the correct details.

Step 8 - Carefully use a screwdriver to scrape all soil from the corer cavity into the sample bag.

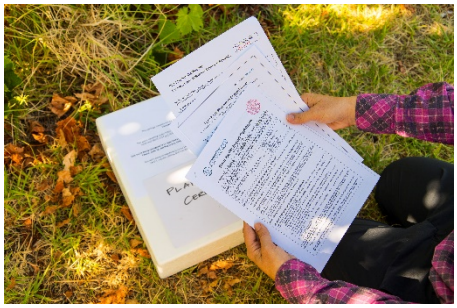
Step 9 - **Repeat steps 3 – 8 to collect core samples from the next 4 vines. Therefore, you will have 5 cores samples composited in the sample bag.**

Step 10 - Use a cable tie to tightly seal the sample bag containing the 5 cores.

Step 11 - Once you have collected all soil samples, place each composite soil sample bag in an individual courier bag.

Step 12 - Roll up lengthways to close, removing as much air as possible. Seal well.

Step 13 - Place all sealed courier bags in a foam box to insulate and seal well.



Step 14 - Prior to sending samples, complete all quarantine documentation and affix to box.

Step 15 - Dispatch samples to laboratory via express courier (ensure tracking of parcel) and notify laboratory. Ensure that samples are not in transit over the weekend.

APPENDIX 4

Import risk analysis on the movement of vineyard soil samples from a Phylloxera Infested Zone into South Australia for diagnostics

Phylloxera adults, nymphs and eggs. Photo courtesy Agriculture Victoria (Rutherglen).

Author: Vinehealth Australia

Date: February 2018

Table of Contents

Summary

This Pest Risk Analysis (PRA) assesses the risk of causing an incursion of Phylloxera (*Daktulosphaira vitifoliae* Fitch) in South Australia through the importation for diagnostic testing, of non-disinfested soil samples from interstate Phylloxera Infested Zones.

The report recommends that the importation of soil samples into South Australia for diagnostic testing, from all commercial winegrowing production areas of Australia can be permitted with negligible risk of contributing to a phylloxera infestation in South Australia.

This report identifies that current risk management measures, commercial practices and quarantine standards are sufficient in managing risk associated with the importation of non-disinfested soil samples from all Australian winegrowing regions, into South Australia, irrespective of the phylloxera status of these regions, to achieve Australia's ALOP.

Glossary of terms

Term or acronym	Definition
Resistant	Used to describe the ability of the vine to maintain performance in the presence of the pest compared with more susceptible varieties. Phylloxera resistant rootstock are those on which phylloxera cannot develop to the adult stage so there is no egg production and no gall production.
Tolerant	Used to describe the ability of the vine to maintain performance despite supporting a phylloxera population.
ABS	Australian Bureau of Statistics
ALOP	Appropriate Level of Protection
CA12	Certification Assurance 12 – Laboratory Accreditation for Importation of Grapevine, Soil and Plant Diagnostic Material from Biosecurity South Australia
CABI	Centre for Agriculture and Bioscience International
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAWR	Department of Agriculture and Water Resources
DNA	Deoxyribonucleic Acid
EE	Entry x Establishment (likelihood)
EPPO	European and Mediterranean Plant Protection Organisation
EES	Entry x Establishment x Spread (likelihood)
FAO	Food and Agriculture Organization of the United Nations
ISPM	International Standard for Phytosanitary Measures
MDC	Molecular Diagnostic Centre at SARDI
PBCRC	Plant Biosecurity Cooperative Research Centre
PEZ	Phylloxera Exclusion Zone
PGIBSA	Acronym of the former Phylloxera and Grape Industry Board of South Australia, which is now known as Vinehealth Australia
PHC	Plant Health Certificate
PIRSA	Primary Industries and Regions South Australia
PIZ	Phylloxera Infested Zone
PRA	Pest Risk Analysis
PRZ	Phylloxera Restricted Zone
qPCR	Quantitative Polymerase Chain Reaction
RFID	Radio-Frequency Identification
SA	South Australia
SARDI	South Australian Research and Development Institute

1. Introduction

1.1 Australia's biosecurity policy framework

Australia's biosecurity policies aim to protect Australia against the risks that may arise from exotic pests entering, establishing and spreading in Australia, thereby threatening Australia's unique flora and fauna, as well as those agricultural industries that are relatively free from serious pests (DAWR, 2016).

The risk analysis process is an important part of Australia's biosecurity policies. It enables formal consideration of risks that could be associated with proposals to import new products into Australia, or between parts of Australia. If the risks are found to exceed Australia's overarching appropriate level of protection (ALOP), risk management measures are proposed to reduce the risks to an acceptable level. If it is not possible to reduce the risks to an acceptable level, then no trade will be allowed.

In the context of this PRA, trade is construed to include the movement of samples for diagnostic testing.

1.2 This import risk analysis

1.2.1 Background

Phylloxera is a devastating pest of grapevines worldwide. There have been over 500 genotypes of the pest documented worldwide, of which Australia is known to have 83 (Umina et al., 2007) (Powell & Korosi, 2014). At present, these genotypes are reported to be confined to parts of Victoria and New South Wales, thereby deeming the insect both exotic and endemic at the same time. Under a PBCRC-funded project 2061 'On farm DNA surveillance for grapegrowers', managed by lead agency, Vinehealth Australia (previously The Phylloxera and Grape Industry Board of South Australia), a sensitive, accurate and rapid method of detecting phylloxera DNA via qPCR has been developed by the South Australian Research and Development Institute (SARDI) at their Molecular Diagnostics Centre (MDC) at the Waite Campus.

This laboratory is a nationally and internationally recognised high throughput molecular diagnostics facility that has been routinely processing up to 35,000 samples per year. The facility is a designated QC2 quarantine facility, with additional South Australian CA 12 laboratory biosecurity accreditation. It is proposed that this laboratory be the single source of diagnostics for phylloxera in Australia using this qPCR method.

Currently, the SARDI MDC receives unsterilized samples (including soil) for disease testing, originating from intrastate, interstate and overseas. Other laboratories at the Waite campus including those at CSIRO Land and Water receive similar material, as do other laboratories in South Australia such as the Centre for Assessment and Remediation at the Mawson Lakes Campus of University of South Australia which receives samples from Bangladesh. More locally, unsterilized Branched Broomrape material has also been brought from the Broomrape Quarantine area near Murray Bridge to the Waite campus for testing. All the above entries of material are currently permitted. Permits are granted because the material

is destined for a listed quarantine laboratory. To date, no adverse consequences from these arrangements have been recorded.

1.2.2 Scope

The scope of this risk analysis is to analyse the biosecurity risks that are associated with the importation of non-disinfested soil samples collected from vineyards in all winegrowing regions in Australia, but importantly from those inside Phylloxera Infested Zones, for commercial diagnostic testing in South Australia, which has a phylloxera-free status.

1.2.3 Existing policy

The Commonwealth Government is responsible for regulating the movement of plants and plant products into and out of Australia. However, state and territory governments are responsible for plant health controls within their individual jurisdiction (DAWR, 2016) and these are documented in Plant Quarantine Standards or equivalent.

In Australia, phylloxera is managed in accordance with the National Phylloxera Management Protocol, which allows for the delineation of grape growing regions by phylloxera status. Phylloxera Exclusion Zones (PEZ) are areas that have been surveyed and found free of phylloxera, Phylloxera Risk Zones (PRZ) are areas that have not been surveyed for phylloxera and are of unknown status, and Phylloxera Infested Zones (PIZ) are areas that are known to be within 5 km of a phylloxera infested property (NVHSC, 2009).

According to Condition 8A of South Australia's Plant Quarantine Standard (PIRSA, 2015), vineyard soil samples destined for diagnostic testing in South Australia from a PIZ are required to be accompanied by a permit from the source state acknowledging the samples are being moved out of a PIZ, a Plant Health Certificate documenting proof of origin and the disinfestation procedure used, and an Import Permit from South Australia' Chief Inspector. In addition, *"wherever possible, diagnostic procedures should be carried out within a PIZ, before the sample is moved to another region for testing. Diagnostic samples to be removed from a PIZ for analysis must undergo one of the disinfestation procedures listed (in Condition 8A) before they can enter South Australia."*

Some laboratories in South Australia, including the SARDI MDC, have been accredited by PIRSA (Biosecurity SA) under CA12 accreditation, which allows for the import of state biosecurity risk material under controlled conditions. This accreditation is contingent to the MDC (i) maintaining its DAWR biosecurity accreditation and (ii) operating in strict accordance with the CA12 procedures. The procedures cover the importation, security, receival, storage, handling and disposal of material for diagnostic or research purposes that have an entry requirement under the South Australian Plant Quarantine Standard. The CA12 accreditation is renewed annually following a compliance audit, which reviews procedures and management and ensures that the MDC is operating in accordance with the conditions of the accreditation.

The following applies to samples with a biosecurity risk (these include soil samples received for phylloxera testing whether they come from a PIZ, PEZ or PRZ):

- Samples are delivered to the MDC in biosecurity-approved packaging (double bags plus outer packaging) accompanied by the appropriate import permit (international biosecurity samples) or by the SARDI CA12 certificate of accreditation and a copy of the CA12 diagnostic sample declaration form or equivalent (interstate biosecurity samples). All samples listed on the declaration form need to be accounted for. If the samples are coming from a PIZ they also need to be accompanied by a permit issued by the appropriate authority before leaving the PIZ;
- Upon receival, samples, including outer packaging, are transferred to the processing lab through a pass, with interlocking doors;
- Personal protective equipment is disposed of immediately after handling biosecurity risk material in the biosecurity-approved bins within the facility; and
- DNA extraction waste from biosecurity risk samples is double-bagged, autoclaved and disposed of as biological waste.

For facilities such as SARDI with CA12 accreditation under the Plant Health Act 2009, the "*certificate of accreditation fulfils the requirement for an annual Plant Health Import Certificate for grapevine diagnostic material from a Phylloxera Infested Zone (PIZ), a Phylloxera Risk Zine (PRZ) or Phylloxera Exclusion Zone (PEZ)*".

1.2.4 Next steps

Once this report has been reviewed and upon acceptance by stakeholders, amendments to South Australia's Plant Quarantine Standard are suggested to improve the clarity and therefore interpretation of the wording in Condition 8A.

This risk assessment also raises the issue of movement of genotypes (strains) of phylloxera within zones known to contain phylloxera. Documented management of biosecurity risk for phylloxera at a regional and potentially even a vineyard level has not been formally undertaken, but must be considered as the next step to minimising movement of phylloxera within currently infested zones.

2. Method for pest risk analysis

This section sets outlines the method used for the pest risk analysis (PRA) in this report. Vinehealth Australia has conducted this PRA in accordance with the International Standards for Phytosanitary Measures (ISPMs), including ISPM 2: *Framework for pest risk analysis* (FAO, Framework for pest risk analysis, 2007) and ISPM 11: *Pest risk analysis for quarantine pests* (FAO, 2013).

A PRA evaluates biological and other scientific and economic evidence to determine whether a pest should be regulated and the strength of any phytosanitary measures to be taken against it. Two major components of quarantine risk are evaluated in this PRA and then combined to give an overall estimate of risk:

(i) probability of the pest phylloxera entering, establishing and spreading in South Australia from imports into the state; and

(ii) consequences should this happen.

Quarantine risk is evaluated taking into account existing commercial practices, incorporating phytosanitary measures currently practiced.

This PRA is conducted in three steps: initiation, pest risk assessment and pest risk management.

2.1 Stage 1: Initiation

Initiation identifies the pest(s) and pathway(s) that are of quarantine concern and should be considered for risk analysis in relation to the identified PRA area.

Identification of a new pathway that presents a potential pest hazard has initiated this PRA - being the importation of non-disinfested soil samples collected from vineyards inside Phylloxera Infested Zones, for commercial diagnostic testing in South Australia.

The 'PRA area' is defined as South Australia, which has area freedom status from phylloxera.

Where appropriate, previous qualitative risk assessments undertaken by the Department of Agriculture and Water Resources (previously Biosecurity Australia): *Final import risk analysis report for table grapes from the People's Republic of China* (Biosecurity Australia, 2011) and *Draft report for the non-regulated analysis of existing policy for table grapes from Sonora, Mexico* (DAWR, 2016) have been taken into consideration when developing the new policy.

2.2 Stage 2: Pest risk assessment

In this PRA, pest risk assessment has been divided into the following interrelated processes:

2.2.1 Pest categorisation

Pest categorisation identifies which of the pests with the potential to be on the commodity are quarantine pests (a pest of potential economic importance to an area where the pest has not yet been detected) for Australia and require pest risk assessments.

This pest risk analysis is confined to a single pest as follows:

- Identity

Scientific name: *Daktulosphaira vitifoliae* Fitch, 1856

Synonyms: *Phylloxera vastatrix* Planchon, 1868
Viteus vitifoliae Fitch 1867
Viteus vitifolii Fitch 1867

Common names: Grape phylloxera
Grapevine phylloxera
Phylloxera
Filoxera
Vine louse
Reblaus
Vine fretter

- Host range

Vitis species (grapevines and ornamental vines)

- Symptoms (plant parts affected):

Phylloxera symptoms include slow and stunted shoot growth, with gradual decrease in vigour, leaf yellowing and ultimately, failure of budburst. Leaf yellowing is a common early symptom of the pest and will normally be seen in two to three neighbouring vines – usually, but not always within the same row. Phylloxera tends to spread out from the roots of the vine where it was first introduced, causing an 'oil spot' pattern of symptomatic vines, which increases progressively in size as more vines become infested (Vinehealth Australia, 2017). Phylloxera feed on the roots of grapevines, resulting in distinctive nodules or tuberosities. Depending on the phylloxera genotype (strain), leaf galls may occur on the leaves of suckers of rootstocks.

Grapevines grafted to phylloxera tolerant rootstocks may show signs of phylloxera on the roots but visual symptoms in the canopy do not occur, which makes detection difficult. Grafted vines can sustain populations of phylloxera, which can spread to ungrafted vines.

Crop losses range from no noticeable impact, to almost total crop loss. The infestation rate and yield decline is significantly related to vine variety, phylloxera strain, seasonal temperatures and moisture levels. Vines planted on own roots rather than onto phylloxera resistant rootstock are most at risk to succumbing to phylloxera. South Australia only has approximately 26% of area under vine planted to rootstocks (Vinehealth Australia, 2016).

- Geographic distribution:

As per (Powell, 2017),

> "Grape phylloxera is native to the North-East Rockies in North America. It has worldwide distribution covering most major grape-growing regions. It was first reported in Europe in 1863 and subsequently recorded in all major grape-growing regions of Europe. Grape phylloxera gained economic pest status in the late nineteenth century when it spread to Europe causing significant damage to highly susceptible ungrafted European grapevine *Vitis vinifera* L. Considerable economic and social hardship occurred (Banerjee *et al*., 2007) initially in France, then Western Europe where it spread to over 2.5 million hectares of grapevines (Bournier, 1976). It then spread to Australia, Russia, New Zealand, China, South Africa and South America (Crovetti and Rossi, 1987; Rossi, 1988) after it was inadvertently transferred on imported *Vitis* planting material (Buchanan, 1990; Mayet, 1890). However, in some countries, including China (Du *et al*., 2011), Australia (Powell, 2008), Russia (Frolov and David'yan, 2009) and Armenia (ARD, 2007), its distribution appears restricted only to some grape-growing regions. More recent detections include the Yarra Valley, in Australia in 2006; the Ararat Valley, Armenia in 2009 (V. Keushguerian, pers. comm.) and Hunan, Shaanxi and Liaoning provinces of China in 2006–2007 (Du *et al*., 2011). The reported areas of absence include Chile, Cyprus, Denmark, Estonia, Finland, Ireland, Latvia, Lithuania, Norway, Sweden and The Netherlands. However, in some of these regions surveys to confirm the absence of phylloxera are unclear (EFSA, 2014)."

- Distribution in Australia:

Phylloxera was first discovered in Australia near Geelong in 1877 and was detected in New South Wales in 1884 at Camden and further infestations were subsequently found nearby. Phylloxera was first found in Queensland at Enoggera, Brisbane, in 1910 and has not been detected in that state since the 1960s. Victoria and New South Wales are the only Australian states currently known to have phylloxera (Vinehealth Australia, 2017).

- Biology:

Grape phylloxera is an insect that lives and feeds on grapevines. The phylloxera lifecycle involves egg, crawler (nymph) and adult stages. Phylloxera can occur in both above-ground and below-ground forms; however, the above-ground form is rare in Australia. The below-ground damage to vine roots is the predominant cause of vine death. In Australia, 83 strains (genotypes or xenotypes) have been characterised (Umina et al., 2007) (Powell & Korosi, 2014). These strains are known to display different levels of virulence and therefore the risk of spread of these strains will differ in practice.

- Movement:

Movement of phylloxera is considered to be mainly by crawlers. These may be associated with grapevine material, grape products, vineyard or winery equipment and machinery, and clothing and footwear – the movement from which can lead to unlimited spread if no control measures are practiced.

Crawlers can also naturally spread from vine to vine by crawling along the soil surface or crawling from root to root. They may also be carried by wind, with spread in strong winds of up to 25 metres (Powell, 2000). Natural spread occurs at a rate of 100-200 metres per year within a vineyard (King and Buchanan, 1986).

To prevent the spread of phylloxera from infested areas, each state has legislation (laws) and associated regulations, which restrict or prohibit the movement of "phylloxera risk vectors". These include grapevine material, grape products, soil, diagnostic samples and machinery or equipment used in vineyards (PIRSA, 2015). For diagnostic samples, material may be sent to a registered (testing) premises[1] that meets specified conditions (e.g. holds South Australian CA 12 Laboratory Biosecurity accreditation).

- Management tools:

In Australia, movement of phylloxera risk vectors are undertaken in accordance with state plant quarantine standards (or equivalent), all of which are underpinned by the National Phylloxera Management Protocol, which allows for the delineation of grape growing regions by phylloxera status. These quarantine movement regulations aim to minimise the spread of phylloxera. There are three management zones - a Phylloxera Infested Zone (PIZ) which is known to have (or had) vineyards infested with phylloxera, a Phylloxera Risk Zone (PRZ) which has unknown phylloxera status (but never detected), and a Phylloxera Exclusion Zone (PEZ) which is recognised as being free of phylloxera.

[1] Registered Premises - The importer nominates a premise for the receipt of the imported produce. Upon arrival the importer will ensure that the consignment remains securely packaged and isolated by one metre from other produce and arrange for an inspection by a Biosecurity SA Plant Health Inspector prior to the release of the produce

There are no proven chemical methods to control the phylloxera insect. Little information on biological control is available. The only proven cultural method to ensure vine productivity in the presence of phylloxera is to replant infested vineyards with tolerant rootstock. Best practice farm-gate hygiene is also a vital management tool growers employ to prevent the spread of phylloxera to non-infested vineyards.

2.2.2 Assessment of the probability of entry, establishment and spread

Details of how to assess the 'probability of entry', 'probability of establishment' and 'probability of spread' of a pest are given in ISPM 11 (FAO, 2013). The use of the term 'probability' is limited to the direct quotation of ISPM definitions. In qualitative PRAs such as this one, the term 'likelihood' is the descriptor used for estimates of likelihood of entry, establishment and spread. Methodology used in this PRA is consistent with that of the DAWR, outlined in recent table grape assessments (DAWR, 2016) (Biosecurity Australia, 2011).

A summary of the process is given below, including a description of the qualitative methodology used in this risk analysis.

Likelihoods are assigned to each step of entry, establishment and spread using six descriptors as outlined in Table 1 below, along with their definitions and indicative probabilities.

Table 1 Nomenclature of quantitative likelihoods (Biosecurity Australia, 2011) (DAWR, 2016)

Likelihood	Descriptive definition	Indicative probability range
High	The event would be very likely to occur	>0.7→1
Moderate	The event would occur with an even probability	>0.3→0.7
Low	The event would be unlikely to occur	>0.05→0.3
Very low	The event would be very unlikely to occur	>0.001→0.05
Extremely low	The event would be extremely unlikely to occur	>0.000001→0.001
Negligible	The event would almost certainly not occur	0→0.000001

2.2.2.1 Likelihood of entry

The likelihood of entry for PRAs, describes the likelihood that a quarantine pest will enter Australia as a result of trade in a given commodity, be distributed in a viable state in the PRA area and subsequently be transferred to a host. It is based on pathway scenarios depicting necessary steps in the sourcing of the commodity for export, its processing, transport and storage, its use in Australia and the generation and disposal of waste. In particular, the ability of the pest to survive is considered for each of these various stages.

This PRA determines the likelihood of entry of phylloxera into South Australia, focussing on collection of soil samples from vineyards in Phylloxera Infested Zones. Likelihoods of entry estimates are based on the use of existing practices by grapegrowers in sample collection, packaging and transport from the exporting quarantine areas outside South Australia, including adherence to current state quarantine protocols, as well as sample receival, processing and disposal at the SARDI DNA diagnostics laboratory inside South Australia. The likelihood is assessed qualitatively for individual steps and then combined.

One factor affecting the likelihood of entry is the volume and duration of trade. Here, the likelihood of entry has been estimated on the basis of one year's trade, with the knowledge that the SARDI diagnostics laboratory has the capability of diagnosing more than 500 soil samples in a day. It is assumed that as the overall volume of trade (soil samples sent for diagnostics) increases, the overall likelihood of phylloxera entering SA will increase up to a point at which known phylloxera areas have been verified and then potentially decrease or remain at a constant thereafter, as sampling strategies move from 'verification of area freedom status' to 'maintenance' status.

For the purposes of considering the likelihood of entry, it is divided into two components:

(a) The *likelihood of importation* – which is the likelihood that a pest will arrive in Australia when a given commodity is imported. For this PRA, it means the likelihood that phylloxera will arrive in South Australia with non-disinfested soil samples potentially infested with phylloxera. Factors considered in the likelihood of importation include:

- distribution and incidence of phylloxera in the source area (PIZs);
- incidence of phylloxera likely to be associated with a consignment;
- the life-stages of phylloxera in the soil and relative vulnerability of these life-stages during transport or storage;
- volume and frequency of movement of the soil samples into SA;
- seasonal timing of movement;
- pest management, cultural and commercial procedures applied in the source area (PIZs);
- speed of transport and conditions of storage compared with the duration of the phylloxera lifecycle; and
- commercial procedures (e.g. cooling, heating) applied to consignments during packaging, transport and storage.

Figure 1 Phylloxera adults, crawlers and eggs. (Agriculture Victoria – Rutherglen).

Phylloxera insects are very small (adults 1mm long) – and the crawlers are much smaller (Figure 1). This means that only a small amount of soil could carry an adult or crawler. Analyses of soil coming from potentially infested areas in a recent study had approximately 1-2 phylloxera per 200g of soil (Vinehealth Australia, unpublished). Considering the soil samples in question are to be analysed specifically to quantify phylloxera presence, they will be collected from vineyards of both known and unknown phylloxera status. The field sampling protocol for the DNA diagnostic method has been tested year-round, with positive results, even though phylloxera numbers are highest during summer. The SARDI diagnostics laboratory has the capability of testing approximately 500 soil samples per day using the qPCR technique. Research by Powell (unpublished) has shown phylloxera are able to survive both hot and cold temperatures, thus very likely to survive from the vineyard, through consignment to the laboratory.

It is important to acknowledge that during sample collection there are three main vector types which also could potentially contribute to phylloxera arriving in South Australia. However, considering existing commercial practices, the likelihood of importation of these vectors is considered 'negligible' as they are not part of the commodity being imported.

(i) Clothing and footwear

Clothing and footwear both have the potential as vectors for phylloxera crawlers. This PRA, however, assumes no personal carriage of samples from winegrowing areas outside SA into the SARDI diagnostics laboratory, thus negating this vector.

(i) Vehicle

Vehicles have the potential to carry soil. However, assuming there is no personal carriage of samples from winegrowing areas outside SA into the SARDI diagnostics laboratory, this risk factor is considered negated.

(ii) Field equipment including soil corer or other digging device

Field equipment is considered to include notebooks, washing devices, spare bags etc. which could be placed on the soil or other contaminated surfaces, potentially acting as vectors for phylloxera. Assuming there is no personal carriage of samples from winegrowing areas outside SA into the SARDI diagnostics laboratory, this risk factor is negated.

In addition, field equipment includes soil corers, screw drivers and other digging devices used to collect the soil samples. Commercially, it is expected that this equipment could be used between vineyards in the same management zone within a state. The recommended sampling procedure denotes that each digging device must be disinfested between blocks within the same vineyard and between vineyards, as well as at the end of the day. According to Condition 7A of the South Australian Plant Quarantine Standard (PIRSA, 2015), '*grape equipment used in vineyards must be clean and free of plant residues and soil on arrival in South Australia'* and accompanied by a Plant Health Certificate (PHC). Assuming there is no personal carriage of samples from winegrowing areas outside SA into the SARDI diagnostics laboratory, including of the equipment used in the field sampling process, this risk factor is considered negated. The requirement of a PHC though is also considered to contribute to a 'negligible' rating for likelihood of importation as if the equipment was brought into South Australia, it would need to be sighted by a Biosecurity Officer of the importing state and certified as clean.

Although soil samples are envisaged to be collected from all grapegrowing regions in Australia, and sent to the SARDI diagnostics laboratory for analysis, **the likelihood of soil samples collected from vineyards inside a PIZ to arrive carrying phylloxera adults and crawlers is rated as 'high' according to Table 1.**

(b) The *likelihood of distribution* – which is the likelihood that the pest will be distributed, as a result of the processing, sale or disposal of the commodity, in the PRA area and subsequently transferred to a susceptible part of a host. For this PRA, it means the likelihood of phylloxera transferring to a susceptible host, due to both transport of the soil samples between vineyards outside of South Australia and the laboratory in South

Australia and sample receival, processing and disposal by SARDI's DNA diagnostics laboratories. Factors considered in the likelihood of distribution include:

- whether the imported infested soil is to be sent to a few or many destination points in South Australia for testing;
- commercial procedures (e.g. heating, cooling, disinfestation, packaging) applied to consignments during transport;
- dispersal mechanisms of the pest, including vectors, to allow movement from the pathway to a host;
- proximity of entry, transit and destination points to susceptible vine hosts;
- time of year at which import takes place;
- intended use of the soil; and
- risks from by-products and waste.

The likelihoods of an infestation arising via transport and taking into account packaging of the samples, receival, processing and disposal as well as intentional distribution, are discussed below. This PRA considers the risk likelihood from the perspective of an individual grower packaging up the soil samples using a kit provided and according to the supplied protocol, and using a courier for transport to the SARDI diagnostics laboratory that receives and processes the samples under CA12 laboratory certification. Each of these factors are assigned an individual qualitative likelihood of distribution rating according to Table 1 and these ratings are combined to provide an overall likelihood of distribution rating.

i. Transport of samples from the field to the laboratory

Transport of the soil samples in this PRA is considered to be performed by an external transport provider, as the scenario with the highest likelihood of distribution. Personal transport by road or by plane are two other transportation methods available, but considered less likely to be adopted by growers in grapegrowing regions outside of South Australia, when submitting samples for analysis to a South Australian laboratory.

Transport of soil samples by an external provider to South Australia's SARDI diagnostic laboratory may have several unwanted possibilities:

- Samples are mislaid at the external transport provider's depot;
    - This would not increase the likelihood of distribution if the external transport provider was local to the sampling area and the samples were contained within the depot.
- Samples are taken to an incorrect address or the samples were delivered to the laboratory but not given to a responsible person (e.g. samples left at the door after hours);
- Samples are mislaid beyond the external transport provider's depot; and
- External transport provider's vehicle is involved in a mishap (accident, stolen or car-jacked).

The above events are considered only to present possibility of harm, if:

- Soil samples become deposited adjacent to grapevines in a non-phylloxera infested area;
- This area has vines on own roots or a vulnerable rootstock – an estimate of this probability can be obtained by considering the proportion of the highway from Victoria to South Australia where there are vines in close proximity;
- The stout cold box is sufficiently damaged to open, exposing the double-bagged soil samples, which then need to be pierced or opened, spilling the soil contents; and
- There is potential for the outer surface of the cold box to harbour soil or phylloxera crawlers. However, the recommended sampling procedure is to place the double-bagged soil samples into a sturdy cold box away from the vineyard in an office or equivalent, so the base of the cold box does not come into contact with the soil surface.

Considering the above, the likelihood of distribution of phylloxera from transport of soil samples into South Australia is considered 'Negligible' according to Table 1.

ii. Intentional distribution

There is a possibility that during the transport of soil samples into South Australia to the SARDI diagnostic laboratory, or during sample receival, processing or disposal stages, that intentional distribution of contaminated soil could occur. Considering the sample protocol requires biohazard stickers to be attached to the outside of the stout cold box and the occurrence of education and awareness programs surrounding the potential damage a phylloxera infestation could cause to the South Australian wine industry, relevant to employees in the industry, **the likelihood of distribution of phylloxera via intentional means is considered 'Negligible' according to Table 1.**

iii. Sample receival

Samples are delivered to the MDC in biosecurity-approved packaging (double bags plus outer packaging) accompanied by the appropriate import permit (international biosecurity samples) or by the SARDI CA12 certificate of accreditation and a copy of the CA12 diagnostic sample declaration form or equivalent (interstate biosecurity samples). All samples listed on the declaration form must be accounted for and recorded. If the samples are coming from a PIZ they also need to be accompanied by a permit issued by the appropriate authority before leaving the PIZ.

Upon receival, samples, including outer packaging, are transferred to the processing lab through a pass, with interlocking doors. Personal protective equipment worn by laboratory staff is disposed of immediately after handling the soil samples, and placed along with all packaging, in the biosecurity-approved bins within the facility. These bins are removed from the premises by a DAWR-approved transporter and waste is incinerated at a DAWR-approved facility.

The recommended soil sampling procedure includes a Chain of Custody process, whereby upon receival of the soil samples at the SARDI diagnostic laboratory, confirmation in writing will be provided to the grower of the number of samples received and a report on the packaging and sample integrity.

For sample receival to contribute to the distribution of phylloxera, soil samples would have to be mislaid and placed within close proximity to grapevines as per explanation for 'Transport of samples from the field to the laboratory'. **As the SARDI diagnostics laboratory operates under CA12 laboratory biosecurity accreditation, the likelihood of sample receival contributing to phylloxera distribution is considered 'Negligible' according to Table 1.**

iv. Sample processing

As a quarantine laboratory, the SARDI diagnostic laboratory is required to undertake rigorous procedural assessments as part of maintenance of its biosecurity accreditation; notwithstanding those pertaining to sample processing. Upon receival, soil samples are transferred to the processing lab, dried overnight at 40°C in a dehydrating oven (effectively killing any phylloxera present), then destructively processed prior to DNA extraction. Soil samples infested with phylloxera would have to find their way from the Waite laboratory approximately 120 metres to the Waite Vineyard for any potential infestation to occur. **The likelihood of sample processing contributing to phylloxera distribution is considered 'Negligible' according to Table 1.**

v. Disposal of waste during sample receival and processing

As a quarantine laboratory, the SARDI MDC is required to undertake rigorous procedural assessments as part of maintenance of its biosecurity accreditation; notwithstanding those pertaining to waste disposal. Through the sample receival and processing stage, irrespective of whether samples have come from a PIZ, PRZ or PEZ, there are three stages of waste disposal:

a) When the soil samples enter the building, they are transferred to the processing laboratory through a pass with interlocking doors. The packaging is then removed and the soil is placed into drying trays and dried at 40°C. The waste from this step is the sample packaging and personal protective equipment worn by laboratory staff. This step involves the greatest risk of phylloxera distribution as there is potential for the samples to contain live phylloxera crawlers. The waste from this step is disposed of in red biosecurity-approved bins within the facility, removed from the premised by a DAWR-approved transporter and waste is incinerated at high heat at a DAWR-approved facility.

b) Once the soil is dried, the DNA extraction process is undertaken. Any soil remaining after the extraction process is placed into 44-gallon drums, removed from the premises by DAWR-approved transporters and deep buried at a DAWR-approved facility. This waste removal step involves minimal risk of distributing phylloxera as the soil has already undergone heat treatment and therefore no live phylloxera remain.

c) The actual qPCR testing is the last stage of sample processing. From this step, any waste is double-bagged, autoclaved and disposed of as biological waste. This step provides nil risk to distributing phylloxera and the soil has already been dried and then has been autoclaved using high-pressure saturated steam with temperatures over 100°C, again sterilising the soil.

Considering the three stages of waste removal, the likelihood of sample disposal contributing to phylloxera distribution is considered 'Extremely low' according to Table 1.

Determining overall likelihood of distribution

The determination of likelihood of distribution of phylloxera in South Australia from transport, intentional distribution, sample receival, processing and disposal is calculated as follows:

Likelihood of distribution = 1 – (1 - Probability of transport) x (1 – Probability of intentional distribution) x (1 – Probability of sample receival) x (1 – Probability of sample processing) x (1 – Probability of waste disposal)

∴ Likelihood of distribution = 1 – (1 – Negligible) x (1 – Negligible) x (1 – Negligible) x (1 – Negligible) x (1 – Extremely low)

∴ Likelihood of distribution = 1 – (1 – 0.0000005) x (1 – 0.0000005) x (1 – 0.0000005) x (1 – 0.0000005) x (1 – 0.0005005)

∴ **Likelihood of distribution = 0.000502499, which according to Table 1 gives a rating of 'Extremely low'**

2.2.2.2 Likelihood of establishment

Establishment is defined as the 'perpetuation for the foreseeable future, of a pest within an area after entry' (FAO, 2015). This process is based on comparative assessment of factors that operate in the pest source area (potentially a PIZ in this PRA) and the pest risk area (South Australia), as relevant to the ability of the pest (phylloxera) to survive and reproduce, considering:

- availability of vines hosts (own rooted vines) and vectors;
- suitability of the environment;
- reproductive strategy and potential for adaptation;
- minimum population needed for establishment; and
- cultural practices and control measures.

Categorisation of likelihood also uses Table 1.

If soil infected with phylloxera was distributed in close proximity to vines on the Waite campus, **the likelihood of establishment of phylloxera has to be taken as almost certain and using Table 1 is rated as 'high'.** This rating is given due to:

- the susceptibility of the *Vitis* vines (as the only host) in the Waite vineyard to phylloxera as only approximately 15% of the vines are planted to (phylloxera tolerant) rootstocks;
- that phylloxera can survive under a wide range of temperature conditions during transport (Powell, personal communication) and can survive in all climates where grapevines are grown (CABI-EPPO, 1997); and
- that only a single phylloxera can infest a vineyard.

2.2.2.3 Likelihood of spread

Spread is defined as 'the expansion of the geographical distribution of a pest within an area' (FAO, 2015). The likelihood of spread considers the factors relevant to the movement of the pest, after establishment on a host plant or plants, to other susceptible host plants of the same or different species in other areas. This process is based on a comparative assessment of biological information from the source area and the pest risk area as this relates to the ability of the pest to disperse, considering:

- suitability of the natural and/or managed environment for natural spread of phylloxera;
- presence of natural barriers;
- potential for movement of phylloxera by vectors in the Waite vineyard; and
- potential natural enemies of phylloxera in the Waite vineyard.

The rate of spread of phylloxera should also be considered. Phylloxera infestations have the capability of moving up to 100m per season in a vineyard (www.vinehealth.com.au, 2016), this movement is dependent on many factors, including but not limited to those below:

- natural spread due to wind;
- presence of winged phase of phylloxera as part of the lifecycle - dependent on the genotype (strain) present;
- the natural virulence of the genotype present;
- presence of natural barriers where no vines are present;
- method of initial vector transmission;
- abundance of vineyard traffic from machinery with the potential to spread the phylloxera down vine rows; and
- presence of own rooted vines in a vineyard block.

Categorisation of likelihood of spread also uses Table 1 and is independent of the mode of entry into South Australia.

If soil infected with phylloxera was distributed in close proximity to vines on the Waite campus and subsequently phylloxera established in this vineyard, **the likelihood of spread of phylloxera from this point of establishment has to be taken as almost certain and using Table 1 is rated as 'high'.** This rating is given due to:

- the susceptibility of the vines to phylloxera infestations as only approximately 15% of the vineyard is planted to rootstocks (and phylloxera can survive on grafted vines);
- lack of natural enemies;
- the potential for natural movement within the vineyard from wind; and
- movement both within the vineyard and off the vineyard via other vectors including students, staff, machinery, clothing, footwear and soil.

Combining likelihoods

The likelihoods of entry, establishment and spread are now combined using the tabular matrix shown in Table 2. The likelihood of entry P [entry] is determined by combining the likelihood that the pest will be imported into the phylloxera free area of SA, P [importation], and the likelihood that phylloxera will be distributed within this area, P [distribution]. This matrix is then used to combine the likelihoods of entry P [entry] and establishment P [establishment]. The result is then combined with the likelihood of spread P [spread] to determine the overall likelihood of entry, establishment and spread P [EES], as follows:

P [importation] x P [distribution] = P [entry]

P [entry] x P [establishment] = P [EE]

P [EE] x P [spread] = P [EES]

Table 2 A matrix of 'rules' for combining qualitative likelihoods (Biosecurity Australia, 2011) (DAWR, 2016)

	High	**Moderate**	**Low**	**Very low**	**Extremely low**	**Negligible**
High	High	Moderate	Low	Very low	Extremely low	Negligible
Moderate		Low	Low	Very low	Extremely low	Negligible
Low			Very low	Very low	Extremely low	Negligible
Very low				Extremely low	Extremely low	Negligible
Extremely low					Negligible	Negligible
Negligible						Negligible

The likelihood of entry, establishment and spread of phylloxera in South Australia is summarised in Table 3 below.

Table 3 Likelihood of entry, establishment and spread for each distribution pathway.

Entry			**Establishment**	**P [EE]**	**Spread**	**P [EES]**
Importation	**Distribution**	**P [entry]**				
High	Extremely Low	Extremely Low	High	Extremely Low	High	Extremely Low

2.2.3 Assessment of potential consequences

The objective of the consequence assessment is to provide a structured and transparent analysis of the likely consequences if the pests or disease agents were to enter, establish and spread in Australia. For this PRA, we are considering consequences for winegrape production in South Australia, if phylloxera were to enter, establish and spread at the geographic state level (given the current pest free nature of the entire state). This can also be recognised as *Regional level* estimation. Pest effects with the potential to cause harm have been considered, and may be described as direct (effect on plant life or health, and other environmental effects) or indirect (effect on control, eradication, domestic and international trade, environment and communities) and may occur in economic, environmental and social contexts.

When considering the extent of a consequence of a disease, it is important to consider the persistence of its effects. In general, where the effect is prolonged, the consequences are considered to be greater (Biosecurity Australia, 2001)

For this regional level consequence estimation of harm, the magnitude of the potential impact is described using four categories, defined as:

- An 'Indiscernible' impact is an impact on vine mortality and grape production not likely to be noticeable from normal day-to-day variation;
- An impact of 'Minor significance' is not expected to threaten economic viability, but would lead to a minor increase in vine mortality or a minor decrease in grape production. For non-commercial factors, the impact is not expected to threaten the 'intrinsic' value of the South Australian wine industry – though the value would be considered as 'disturbed'. Effects would generally be reversible;
- A 'Significant' impact would threaten the economic viability of grape production through a moderate increase in vine mortality, or a moderate decrease in grape production. For non-commercial factors, the intrinsic 'value' of the criterion would be considered as significantly diminished or threatened. Effects may not be reversible; and
- An impact of Major significance' would threaten economic viability through a large increase in vine mortality, or a large decrease in grape production. For non-commercial factors, the intrinsic 'value' of the criterion would be considered as severely or irreversibly damaged.

Table 4 Assessment of potential impact at a regional level

Criterion	**Estimate and rationale**
Direct	
Plant life or health	Impact score E – Significant at the regional level Phylloxera only causes direct harm to grapes (Vitis spp.). It can form galls on the roots and leaves of susceptible plants with root feeding allowing entry of fungi into the roots leading to the decline of the plants (Granett, J. et al., 2001). Most infestations of phylloxera render vineyards uneconomic. The presence of phylloxera in previously uninfected areas will (in most cases) result in control measures that require the complete removal of infested vines and their replacement with grapevines grown on phylloxera tolerant rootstock (PGIBSA, 2003). Herbert et al. (unpublished) has indicated that one infestation in Rutherglen, Victoria on *Vitis vinifera* has been present for over 40 years without presenting visible symptoms or causing yield loss. The reason for this is unknown. The phylloxera genotype (strain) present will also have an impact on the level of damage in the vineyard (Herbert et al., unpublished). However, the assumption used in this PRA is that vines in infested vineyards will need to be replaced with resistant rootstock over time to maintain yield and quality specifications. The proportion of winegrape area in South Australia planted to grafted rootstock was only 26% (www.vinehealth.com.au, 2016), therefore the vast majority of planted area of winegrapes are highly susceptible to phylloxera.

Criterion	Estimate and rationale
Direct	
Other environmental effects	*Impact score A – Indiscernible at the regional level* There are no known direct consequences of phylloxera on other aspects of the environment. It is assumed that infested vines will either die and/or be pulled out, and in commercial operations, replanted with resistant rootstock (Biosecurity Australia, 2011).
Indirect	
Eradication, control, etc.	*Impact score E – Significant at the regional level* There is no proven chemical method to eradicate phylloxera on roots of ungrafted *V. vinifera* grapevines (Loch, A. and Slack, J., 2007). A pesticide treatment will not eradicate phylloxera populations; the chemical cannot easily penetrate the heavy soils that this pest prefers. Also, effectiveness of a treatment is difficult to evaluate because although many phylloxera may be killed, populations may rebound rapidly and resume feeding on the vines (University of California Agriculture and Natural Resources, 2015). Little information on biological control of grape phylloxera is available; environmental and root conditions are more important than natural enemies (University of California Agriculture and Natural Resources, 2015). In 2007, approximately 80% of Australia's commercial winegrapes were reported to be ungrafted *Vitis Vinifera,* susceptible to phylloxera (Trethowan, C.J. and Powell, K.S., 2007). From a South Australian perspective nearly ten years on, 74% of winegrapes are planted on own roots (Vinehealth Australia, 2016). With the lack of available chemical or biological controls for phylloxera, the only cultural control measure available to growers is to replant infested vineyards with resistant rootstock. The cost of grafted material alone is 3-5 times that of own rooted vine material, notwithstanding costs of vine removal, ground preparation, planting, trellising, additional water and nutrition. In addition to vine material costs of replanting a vineyard post phylloxera infection, other secondary management costs are likely to be incurred by the grower, which may include, but are not limited to additional machinery, heightened farm-gate hygiene practices (including cleaning and disinfestation), people management, logistics. Loss of brand value and company reputation, as well as visitor offerings, may be affected.
Domestic trade	*Impact score E – Significant at the regional level* The presence of phylloxera in commercial winegrape production areas results in movement restrictions of grapes and grape products (including vineyard soil and plant material) out of infested areas (DEDJTR, 2016) (NSW Dept. Ag., 2016). These restrictions require winegrapes to be processed inside a Phylloxera Infested Zone only. If processing infrastructure is unavailable at the time of an outbreak, and/or of the required specifications for winemaking style to maintain brand requirements, this is likely to lead to a loss of domestic trade, at least initially, until processing capacity (of the desired quality) becomes available. A recent report (Wine Australia, 2016) indicates that 313 million litres of Australian wine worth $3.2 billion is sold domestically per annum.

Criterion	Estimate and rationale
Indirect	
International trade	*Impact score D – Minor significance at the regional level* A recent report (Wine Australia, 2016) indicates that Australia exports 738 million litres of wine annually to 119 countries, valued at $2.1 billion. In 2013, 53% of wine producers nationally exported wine (Export 61, 2013). In 2014/15, South Australia's winegrape crush was 46% of the total crush (ABS, 2015). The introduction of phylloxera into winegrowing areas of South Australia is therefore expected to have the greatest impact nationally on both domestic and international trade. Movement restrictions outlined above are also relevant with regards to international trade. However, as with domestic trade, there are no quarantine restrictions on movement of wine within Australia or internationally, due to the processed nature of wine as a product.
Environmental and social	*Impact score D – Minor significance at the regional level* Grapevines are grown in domestic gardens for both food and amenity value as shade or ornamental features. Infested grapevines would need to be removed and the garden may lose some of its amenity value (Biosecurity Australia, 2011). With over 8,500 employees employed in grapegrowing and winemaking, the wine sector in South Australia is the most significant (ABS, 2011). As a result, a phylloxera outbreak in South Australia (a current phylloxera exclusion zone) is expected to affect South Australia socially. There could be a potential for job losses if businesses discontinued, however once replanting programs are carried out and additional farm-gate hygiene practices are required to be adhered to, labour requirements are forecast to be higher to service the industry.

If Phylloxera were to enter, establish and spread at the South Australian state level, **the magnitude of overall potential consequence from both direct and indirect harms can be described as 'significant'.** This description is predominantly assigned due to:

- the susceptibility of the vines to phylloxera infestations given the general lack of planting to rootstocks across the state;
- the potential for natural movement within vineyards;
- lack of natural enemies;
- the magnitude of vectors moving between vineyards in the state;
- the potential economic damage to business and brand reputation; and
- moderate decrease in production and potential social implications.

After obtaining an overall measure of consequence for the Regional level assessment, a qualitative rating of the consequences of entry, establishment and spread must be determined. Using Table 5 we can obtain an impact score of 'E' equivalent to the overall potential consequence of 'significant' and then use the impact score of 'E' to equate to a **qualitative rating of 'moderate'** according to a predefined set of rules outlined in Table 6.

Table 5. Decision rules for determining the consequence impact score based on the magnitude of consequences at the Regional geographic scale (DAWR, 2016).

			Geographic Scale			
			Local	**District**	**Regional**	**National**
		Description	An aggregate of households or enterprises (a rural community, a town or a local government area)	A geographically or geopolitically associated collection of aggregates (generally a recognised section of a state or territory)	A geographically or geopolitically associated collection of districts in a geographic area (generally a state or territory, although there may be exceptions with larger states such as Western Australia)	Australia wide
Magnitude	**Indiscernible**	Pest impact on vine mortality and grape production unlikely to be noticeable from day-to-day variation	A	A	A	A
	Minor Significance	Not expected to threaten economic viability, but would lead to a minor increase in vine mortality or minor decrease in grape production. Effects would generally be reversible.	B	C	D	E
	Significant	Expected to threaten the economic viability of production through a moderate increase in vine mortality, or a moderate decrease in grape production. Effects may not be reversible.	C	D	E	F
	Major Significance	Expected to threaten the economic viability through a large increase in vine mortality, or a large decrease in grape production. Expected to severely or irreversibly damage the intrinsic 'value' of non-commercial criteria.	D	E	F	G

Table 6. Decision rules for determining the overall consequence rating (DAWR, 2016).

	Impact Scores for consequences of direct and indirect criteria	***Overall consequence rating***
1	*Any criterion has an impact of 'G'; or more than one criterion has an impact of 'F'; or a single criterion has an impact of 'F' and each remaining criterion an 'E'*	*Extreme*
2	*A single criterion has an impact of 'F'; or all criteria have an impact of 'E'*	*High*
3	*One or more criteria have an impact of 'E'; or all criteria have an impact of 'D'*	*Moderate*
4	*One or more criteria have an impact of 'D'; or all criteria have an impact of 'C'*	*Low*
5	*One or more criteria have an impact of 'C'; or all criteria have an impact of 'B'*	*Very low*
6	*One or more but not all criteria have an impact of 'B', and all remaining criteria have an impact of 'A'*	*Negligible*

2.2.4 Unrestricted risk estimate

Unrestricted risk is the result of combining the probability of entry, establishment and spread with the estimate of consequences. Probabilities and consequences are combined using the risk estimation matrix in Table 7. When interpreting the risk estimation matrix, note the descriptors for each axis are similar (e.g. low, moderate, high) but the vertical axis refers to likelihood and the horizontal axis refers to consequences, and the matrix is not symmetrical.

For this PRA, considering the likelihood of entry, establishment and spread of phylloxera and the consequences of this, the overall unrestricted risk for this PRA given current management practices and legislation in place is rated as 'Negligible.'

Table 7. Risk estimation matrix (Biosecurity Australia, 2011) (DAWR, 2016)

Likelihood of entry, establishment or spread	**High likelihood**	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
	Moderate	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
	Low	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk	High risk
	Very low	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk
	Extremely low	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk
	Negligible likelihood	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk
		Negligible impact	**Very Low**	**Low**	**Moderate**	**High**	**Extreme impact**
		Consequences of entry, establishment and spread					

2.3 Stage 3: Pest risk management

Pest risk management describes the process of identifying and implementing phytosanitary measures to manage risks to achieve Australia's ALOP, while ensuring that any negative effects on trade are minimised.

The conclusions from pest risk assessment are used to decide whether risk management is required and if so, the appropriate measures to be used. Where the unrestricted risk estimate exceeds Australia's ALOP, risk management measures are required to reduce this risk to a very low level. The guiding principle for risk management is to manage risk to achieve Australia's ALOP. The effectiveness of any proposed phytosanitary measure (or combination of measures) is evaluated, using the same approach as used to evaluate the unrestricted risk, to ensure it reduces the restricted risk for the relevant pest or pests to meet Australia's ALOP (Biosecurity Australia, 2011).

Details on the identification and selection of appropriate risk management options are described in ISPM 11 (FAO, 2013), where it is noted that the choice of measures should be based on their effectiveness in reducing the probability of entry of the pest.

Examples given of measures commonly applied to traded commodities include:

- options for consignments – e.g., inspection or testing for freedom from pests, prohibition of parts of the host, a pre-entry or post-entry quarantine system, specified conditions on preparation of the consignment, specified treatment of the consignment, restrictions on end-use, distribution and periods of entry of the commodity;
- options preventing or reducing infestation in the crop – e.g., treatment of the crop, restriction on the composition of a consignment so it is composed of plants belonging to resistant or less susceptible species, harvesting of plants at a certain age or specified time of the year, production in a certification scheme;
- options ensuring that the area, place or site of production or crop is free from the pest – e.g., pest-free area, pest-free place of production or pest-free production site;
- options for other types of pathways – e.g., consider natural spread, measures for human travellers and their baggage, cleaning or disinfestation of contaminated machinery;
- options within the importing country – e.g., surveillance and eradication programs; and
- prohibition of commodities – if no satisfactory measure can be found.

2.3.1 Unrestricted pathways in this PRA

Despite the overall 'negligible' rating for unrestricted risk for this PRA, there is one individual pathway considered unrestricted, with a risk rating above Australia's ALOP of 'very low'. This pathway, was rated as a 'high likelihood' of soil samples collected from vineyards inside a PIZ to arrive carrying phylloxera adults and crawlers.

The risk of bringing phylloxera into South Australia from moving soil samples which are likely to contain phylloxera in some situations, could be mitigated by either of the following two options:

1. Not removing samples from the vineyard. This does in fact happen with the dig primary surveillance method of verifying the presence of phylloxera. Currently there is no field-based DNA diagnostic analysis for phylloxera, however, in future it may be possible to produce a DNA-based field test kit which could then negate the use for soil samples to leave the vineyard of origin; or
2. Carrying out the qPCR analysis in each state so samples are not required to be sent across state borders. The qPCR DNA diagnostic analysis is proposed to be conducted commercially through the SARDI MDC at the Waite campus in Adelaide, South Australia as the single service provider. However, through commercial arrangements, the analysis could potentially be conducted in multiple state-based quarantine laboratories, negating the need for soil samples to be sent outside a state; particularly from a PIZ into a PEZ as this PRA is assessing. There is still the risk of moving samples within designated phylloxera zones to consider alongside this option, however. Vinehealth Australia considers that if the DNA analysis is to be conducted in South Australia alone, the risk of an incursion resulting from this analysis is lowered by having a single laboratory in the state conducting the analysis as opposed to multiple laboratories.

2.3.2 Other recommended management practices

Assuming the SARDI MDC will remain as the sole provider of the qPCR analysis, at least at the beginning of the commercial operation, the risk pathway identified in section 2.3.1 still holds for this PRA. There are, however, numerous management practices that when adopted by relevant stakeholders along the supply chain, will ensure the risk of a phylloxera outbreak in South Australia as a result of the diagnostic analysis being undertaken inside the state, remains very unlikely:

- Vinehealth Australia to implement a communication strategy to ensure grower awareness of the DNA method including requirement to strictly follow the soil sampling protocol;
- Vinehealth Australia to implement a communication and engagement strategy with external transport providers prior to the commercialisation of the DNA surveillance method being approved, to ensure risks involved with carriage of the soil samples identified in the PRA are fully understood are samples are treated with special care;
- SARDI MDC to supply sampling kit to growers to ensure sample packaging utilised is consistent and fit-for-purpose;
- field sampling protocol to be included with the sampling kit to ensure, (i) sample collection is carried out appropriately to facilitate detections where phylloxera is present, (ii) samples are correctly packaged to ensure phylloxera or infected soil is not carried on the outside of the packaging, and (iii) samples are correctly sealed and packaged to ensure integrity during transport to the laboratory including the placement of biohazard stickers and a sticker with a contact number to call if a sample is found or received inadvertently;
- growers to explicitly follow a provided field sampling protocol to ensure tools used to collect the soil samples are appropriately disinfested to minimise phylloxera spread;
- SARDI MDC to maintain CA12 accreditation to ensure procedures relating to importation, security, receival, storage, handling and disposal are undertaken in accordance with requirements of the CA12, to minimise escape of phylloxera.; and
- Vinehealth Australia in conjunction with SARDI to investigate the addition of real-time tracking mechanisms such as RFID tags as part of the sampling kit.

2.3.3 Recommended changes to Condition 8A in South Australian Plant Quarantine Standard (PQS)

Condition 8A – Grapevine diagnostic samples and vineyard soils of the SA PQS outlines requirements for importing diagnostic samples including vineyard soils into South Australia. Two changes are recommended to the current version of Condition 8A (Figure 2), both as a result of this PRA, and in general to improve clarity and therefore understanding and adherence to requirements by growers:

1. An amendment to allow diagnostic samples to be imported into SA (for the strict purpose of phylloxera testing using the DNA method at the SARDI MDC) without undergoing prior approved disinfestation procedures, as per the allowance stated in the National Phylloxera Management Protocol in Procedure C, which states, "*Sample material MUST undergo one of the disinfestation procedures listed below, within the originating PIZ or PRZ region, before the sample is moved to another region for testing. ONLY IF it is not possible to disinfest the material without compromising the integrity of the sample or the validity of the diagnostic procedure, or if there are no disinfestation*

facilities available in the region of origin, then movement may be allowed without disinfestation direct to the diagnostic laboratory, under strict conditions of security."

2. An amendment to improve clarity around samples coming in from PIZ region (section 3) to a CA12 Biosecurity SA Accredited Laboratory, as for samples imported from a PRZ region stated in section 2 of Condition 8A.

Condition 8A – Grapevine Diagnostic Samples and Vineyard Soils

All grapevine diagnostic samples and vineyard soil samples for analysis in South Australia may only be handled in a laboratory that is accredited or approved by Biosecurity SA for this purpose. Grapevine diagnostic samples and vineyard soils require **prior written approval** (Import Certificate) from the Chief Inspector, Biosecurity SA before they can enter the State.

Accredited laboratories must document and maintain agreed procedures for the secure handling and disposal of grapevine diagnostic samples and vineyard soils from interstate sources and specific conditions, approved by the Chief Inspector, will be applied depending upon the perceived risk associated with samples from the three key grape phylloxera zones (see below).

The following requirements apply to samples from specified areas:

1. Grapevine material and vineyard soil as diagnostic samples from a **Phylloxera Exclusion Zone (PEZ)** region can enter South Australia provided they are:

   1.1. Securely packaged transport - ie double ziploc/sealed bag for each sample and in a cooler box (or similar hard structure), which is then placed into an overnight courier bag, express post pack or similar for transport or personal carriage; and
   1.2. Accompanied by Plant Health Certificate indicating the origin of the sample(s) and a copy of an Import Certificate from the Chief Inspector.

Proof: Accompanied by a Plant Health Certificate and Import Certificate from Chief Inspector.

2. Grapevine material and vineyard soil going to a CA12 Biosecurity SA Accredited Laboratory as diagnostic samples from a **Phylloxera Risk Zone (PRZ)** region can enter South Australia provided they are:

   2.1. Issued with permit for the movement out of the PRZ by the Chief Plant Health Officer, Victoria (Victorian PRZ regions only) or by the Principal Director Biosecurity or Director Compliance Operations, NSW (NSW PRZ regions only) or their equivalents;

   2.2. Treated using one of the approved disinfestation procedures (see below);

   2.3. Accompanied by a Plant Health Certificate indicating both the treatment process and the origin of the sample(s); and an Import Certificate from the Chief Inspector

Proof: Accompanied by a Plant Health Certificate and Import Certificate from Chief Inspector.

3. Grapevine material and vineyard soil as diagnostic samples from a **Phylloxera Infested Zone (PIZ)** region can **only** enter South Australia provided they are:

   3.1. Issued with a permit for the movement out of the PIZ by the Chief Plant Health Officer, Victoria (Victorian PIZ regions only) or by the Principal Director Biosecurity or Director Compliance Operations, NSW (NSW PIZ regions only) or their equivalents; and

   3.2. Must be handled in accordance with one of the following **approved disinfestation** procedures prior to entering South Australia;

   - Freezing to -18ºC for 24 hours and packed in dry ice for transport
   - Freezing and transfer under liquid nitrogen at -196ºC
   - Freeze Drying
   - Oven drying at 45ºC for a minimum of 2 hours
   - Hot water treatment @ 54ºC ± 1ºC for 5 minutes
   - Fixative - devitalisation using formalin/acetic acid, gluteraldehyde, or 70% ethanol
   - Gamma irradiation at 50 grays in an approved facility

- (For juice): placed in a sealed, unbreakable vessel.

And

3.3. Accompanied by a Plant Health Certificate indicating both the treatment process and the origin of the sample(s); and an Import Certificate from the Chief Inspector.

Proof: Accompanied by a Plant Health Certificate and Import Certificate from Chief Inspector.

Note: *Wherever possible, diagnostic procedures should be carried out within the PIZ, before the sample is moved to another region for testing.*

Note: *For non-grapevine plant samples refer to Condition 6 and for non-vineyard soil samples refer to Condition 20 for specific requirements.*

For Fact Sheets and information on regulated pests go to following web page;
http://www.pir.sa.gov.au/biosecurity/plant_health/exotic_plant_pest_emergency_response

Figure 2. Condition 8A – South Australian Plant Quarantine Standard version 13.

Conclusion

For this Pest Risk Analysis on the movement of vineyard soil samples from a Phylloxera Infested Zone into South Australia for diagnostics at the SARDI Molecular Diagnostics Centre, considering the likelihood of entry, establishment and spread of phylloxera and the consequences of this, the overall unrestricted risk given current management practices and legislation in place is rated as 'Negligible.' This risk rating meets Australia's ALOP.

With this finding, the report recommends that the importation of soil samples into South Australia for diagnostic testing, presents negligible risk of contributing to a phylloxera infestation in South Australia.

This finding does however rely on maintenance of 'current' risk management measures and those proposed as part of rolling out the DNA method commercially (including compliance with quarantine standards and changes to wording of Condition 8A in the SA PQS), and that these measures are sufficient in managing risk associated with the importation of non-disinfested soil samples from all Australian winegrowing regions into South Australia, irrespective of the phylloxera status of these regions.

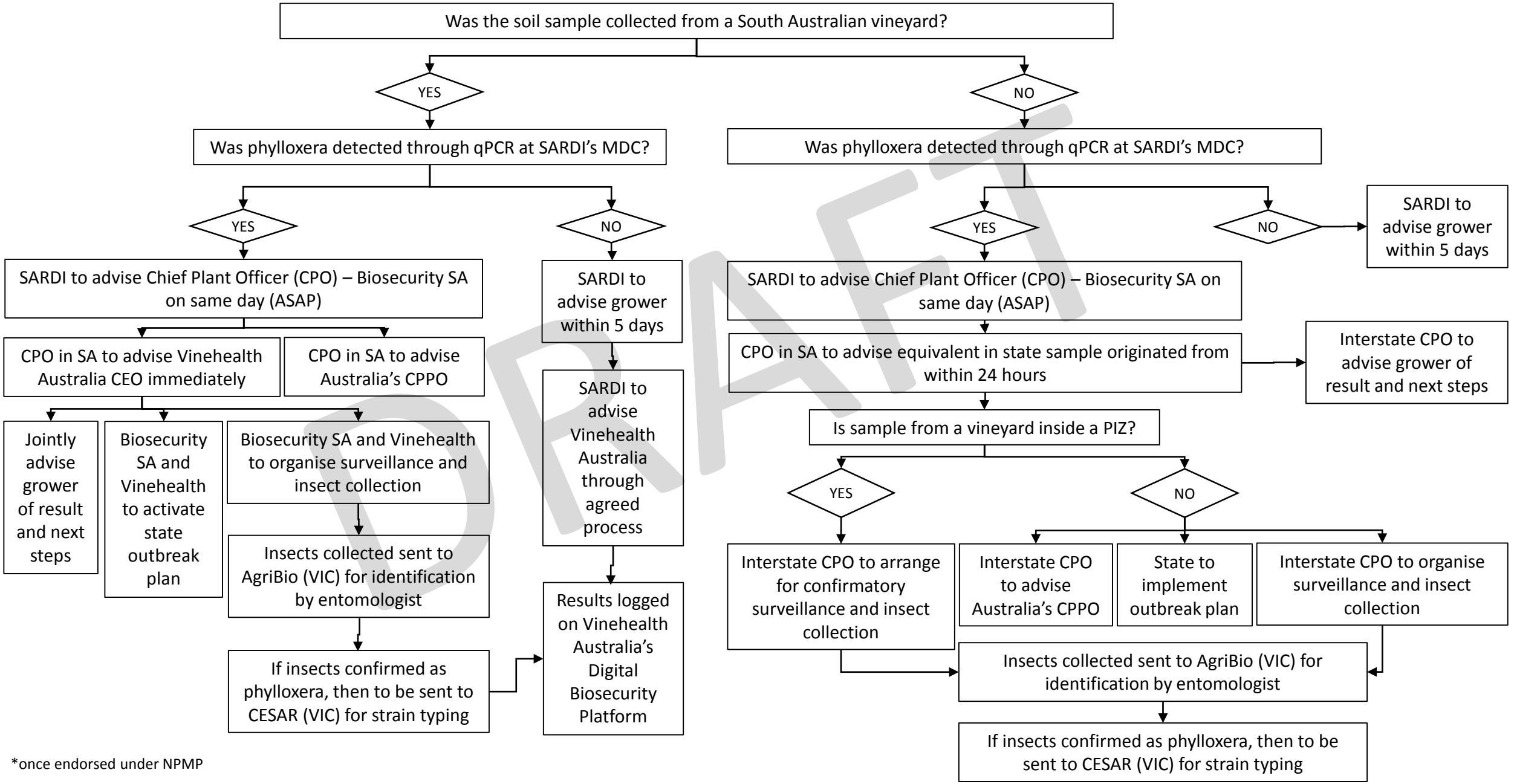
References

1. Australian Bureau of Statistics (ABS) (2011). Census of Population and Housing.
2. Australian Bureau of Statistics (ABS) (2015). Grape Crush by State – Tonnes (2014/15). ABS Catalogue No. 1329.0.
3. Biosecurity Australia (2001). Guidelines for Import Risk Analysis – Draft.
4. Biosecurity Australia (2011). Final import risk analysis report for table grapes from the People's Republic of China.
5. CABI-EPPO (1997). Datasheets on quarantine pests: *Viteus vitifoliae*. European and Mediterranean Plant Protection Organisation. http://www.eppo.org/
6. Department of Agriculture and Water Resources (DAWR) (2016). Draft report for the non-regulated analysis of existing policy for table grapes from Sonora, Mexico.
7. Department of Economic Development, Jobs, Transport and Resources (DEDJTR) (2016). Plant Quarantine Manual Version 26.3.
8. Export 61 (2013). Australian Wine Exports 2012-2013. http://www.export61.com.au/wine-exports
9. Food and Agriculture Organisation (FAO) (2007). *Framework for pest risk analysis. IPSM 2.*
10. Food and Agriculture Organisation (FAO) (2013). *Pest risk analysis for quarantine pests. IPSM 11.*
11. Food and Agriculture Organisation (FAO) (2015). *International Standards for Phytosanitary Measures. ISPM 5: Glossary of phytosanitary terms*.
12. Granett J., Walker M.A., Kocsis L., Omer A.D. (2001). Biology and management of grape phylloxera. *Annual Review of Entomology* 46: 387-412.
13. Herbert, K.S., Unina, P.A., Mitrovski, P.J., Powell, K.S., Viduka, K. and Hoffmann, A.A. Clone lineages of grape phylloxera differ in their performance on *Vitis Vinifera*. Bulletin of Entomological Research. Unpublished: 1-4.
14. King P.D. and Buchanan G.A. (1986). The dispersal of phylloxera crawlers and spread of phylloxera infestations in New Zealand and Australian vineyards. *American Journal of Enology and Viticulture.* 37: 26–33.
15. Loch A. and Slack, J. (2007). Grape phylloxera: the world's worst grapevine pest. Primefacts: Profitable and Sustainable Primary Industries 553: 1-4.
16. National Vine Health Steering Committee (NVHSC) (2009). National Phylloxera Management Protocol.
17. NSW Department of Primary Industries. (2016). Plant Quarantine Manual for NSW. Version 1.0.
18. PGIBSA. (2003). A guide to grape phylloxera in Australia. Phylloxera and Grape Industry Board of South Australia, Australia.
19. PGIBSA. (2010). Phylloxera in the Yarra Valley: A case study. Adelaide: Phylloxera and Grape Industry Board of South Australia.
20. Powell, K.S. (2000). Management of grape phylloxera in South-east Australia Phase I and II. GWRDC Final Project Report, p 17.
21. Powell, K. S. and Korosi, G. A. (2014). 'Taking the strain' – selecting the right rootstock to protect against endemic phylloxera strains. *Acta Horticulturae*. (ISHS) 1045:99-107.
22. Powell, K. (2017). Risks and Management of Endemic and Exotic Phylloxera. Final Report to Australian Grape & Wine Authority.
23. Primary Industries and Resources South Australia (PIRSA) (2015). Plant Quarantine Standard South Australia. Version 11.1.
24. Trethowan, C.J. and Powell, K.S. (2007). Rootstock-Phylloxera interactions under Australian field conditions. ISHS *Acta Horticulturae* 733: 115-122.

25. Umina P.A., Corrie A.M., Herbert K.S., White V.L., Powell K.S. and Hoffmann A.A. (2007). The use of DNA markers for pest management: clonal lineages and population biology of Grape phylloxera. *Acta Horticulturae* (ISHS) 733: 183-195.
26. University of California Agriculture and Natural Resources (2015). UC Pest Management Guidelines – Grape phylloxera. http://ipm.ucanr.edu/PMG/r302300811.html
27. Vinehealth Australia (2017). Vigilance required in Phylloxera fight. *Australian & New Zealand Grapegrower and Winemaker* Feb 2018 p34-38.
28. Vinehealth Australia (2016). 2015-2016 Annual Report. http://www.vinehealth.com.au/media/VHA-2015-16-Annual-Report.pdf
29. Wine Australia (2016). Sector by the numbers report. April 2016.

APPENDIX 5

Communicating results from phylloxera DNA analysis at SARDI MDC



*once endorsed under NPMP