

## 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.

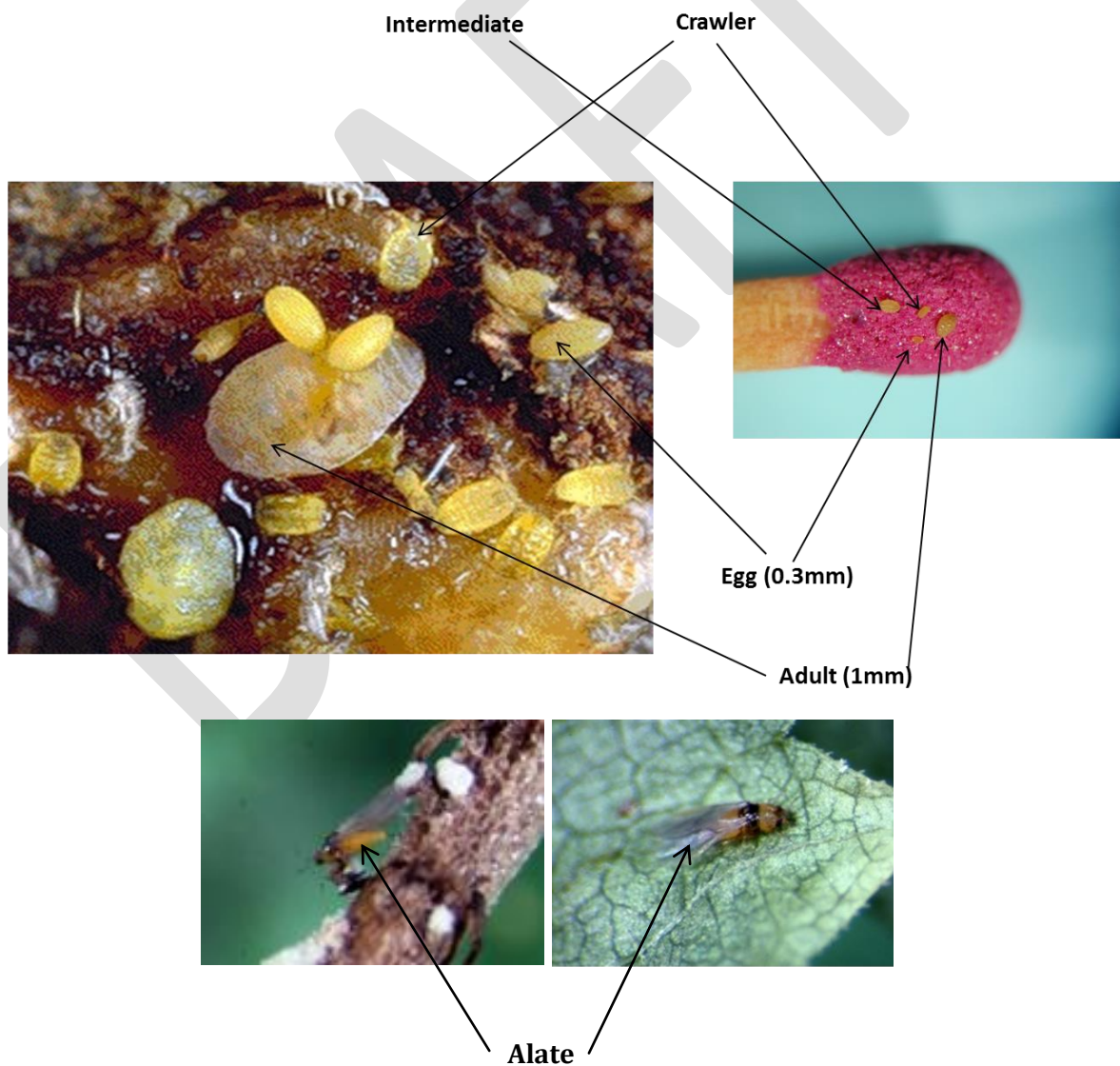


Figure 1. Phylloxera developmental stages

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
<b>General</b>	Reference material -The digging protocol -Phylloxera pictures Hand lens Notebook and pen GPS units for recording property location Camera Mobile phone
<b>Digging</b>	Shovel Trowel Secateurs Screw driver to remove mud attached on footwear
<b>Collecting samples</b>	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
<b>Disinfestation</b>	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
<b>OHS</b>	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-lignified 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  $\frac{1}{4}$  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  $>50^{\circ}\text{C}$  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. Disinfect 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.**

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