Pest Risk Analysis for the establishment of a new infestation of phylloxera in Australia as a result of harvest processes

1.0 Purpose

The purpose of this Pest Risk Analysis (PRA) is to use the information available in the literature and other sources to estimate the risk for spreading grape phylloxera *Daktulosphaira vitifoliae* (Fitch) from a phylloxera infested vineyard to an uninfested vineyard and establishing an infestation in that vineyard. The information derived from the PRA will be used to review the risks identified with the movement of various grape products and, where appropriate and justified, consider modifications to the National Phylloxera Management Protocol.

This document is based on an original pest risk analysis prepared by Bob Paton, NSW Department of Agriculture. This revision has been prepared by the National Phylloxera Technical Reference Group on behalf of the National Vine Health Steering Committee, and funded by the GWRDC. It is intended to support the National Phylloxera Management Protocol and to inform decisions regarding future phylloxera research priorities.

2.0 Scope

The PRA will be restricted to the processes involved in grape harvest and processing. The following risk vectors for moving phylloxera will be considered:

- Fresh winegrapes;
- Red grape must;
- Unfiltered white grape juice;
- Pre-fermentation marc;
- Vineyard equipment associated with harvest of fruit: mechanical harvesters, grape bins and trucks.

Propagation material (cuttings and rootlings) is specifically excluded from the PRA, as movement of these products interstate is already prohibited or subject to strict controls. However, it should be noted that the movement of propagation material (particularly rootlings) from a vineyard with an undetected infestation would almost certainly result in an infestation in the receiving vineyard if the material were not disinfested by hot water treatment. This underlines the importance of ongoing vigilance in early detection and implementation of protocols for areas believed to be free of phylloxera, where hygiene requirements for movement of propagation material are removed.

Table grapes are also excluded. The transfer of these is not normally to a winery or vineyard, but rather to a market in a city or suburban area. Sulphur pads are routinely used in the transport of table grapes, which is an effective disinfestation against phylloxera. With these provisos, the movement of table grapes is not considered to be a significant risk for the spread of phylloxera between Australian vineyards.

Vineyard equipment or machinery apart from harvest equipment is not included in the scope of this PRA. However, it does warrant separate consideration, as there is theoretically a risk that phylloxera (including eggs) could be transferred via mud collected on the machinery.

This document applies generally to transfer of grapes and grape products between an infested vineyard (whether this is in a declared phylloxera infested zone, or has not been detected) and an uninfested winery/vineyard. Section 6.8 considers how the risk might vary according to specific characteristics of the vineyards involved, transport distance, weather conditions etc.

It is important to note that the risk assessment incorporates the risk of establishing an infestation in a *vineyard* in the destination region (or en route) – not just the risk of viable phylloxera being transported to a *winery* in that region.

3.0 Definitions and Abbreviations

The definitions of terms used in this document are consistent with those in the glossary of terms in the National Phylloxera Management Protocol. The risk vector definitions for the PRA are reproduced below.

Fresh or unfiltered juice is the liquid fraction from must, excluding skins, seeds, and other large solids, but may contain some suspended solids.

Filtered juice is juice that has been filtered or otherwise processed to achieve a maximum particle size of 50 microns. Centrifugation and cold settling are acceptable alternatives to filtration for the purposes of this definition provided that the same outcome is achieved.

Grape product includes any product made from winegrapes – ie grapes grown for making into wine. Note that table grapes and dried fruit are not included in the ambit of this document.

Must is the total product of crushing grape berries, includes juice skins, seeds, pulp and possibly some stems and leaves.

Pre-fermentation marc is the solids residue from pressing of must, containing skins, seeds and possibly stems. For the purposes of this document, pre-fermentation marc is any marc that has not completed at least four days in the fermentation process – whether or not it contains any residual sugar ("sweet marc").

4.0 References

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5.0 Background: biology and behaviour of phylloxera

In south-east Australia grape phylloxera predominantly exist on the roots of European grapevines (*Vitis vinifera*) throughout the year, where they form characteristic galls on fibrous and storage roots due to their feeding.

Phylloxera overwinters as first instar nymphs (crawlers) under the bark of roots. When the vines begin to grow in spring the crawlers begin feeding, reaching the adult stage by November when they begin to lay eggs asexually. There can be several generations during summer, prior to nymphs entering dormancy during May or June.

The dispersive stages are the crawlers and winged alates, the most important of which are the crawlers. Crawlers migrate from the infested roots in search of uninfested roots. Some move through cracks in the soil, or along root channels, to reach uninfested roots, while others move over the ground, re-entering the soil through cracks. The absence of these cracks (airspaces) in sandy soils is believed to restrict the insect's ability to spread. This is thought to be the reason for the relative resistance that susceptible vines have when growing on these kinds of soils. Crawlers also climb up the vine trunk and onto the foliage and grapes, from where they can be blown by wind over distances of several metres, to other vines.

Winged phylloxera insects can develop from the root populations and emerge during mid summer. These are the precursor to a sexual reproductive cycle that has been described in the literature. However, the sexual cycle is extremely uncommon in Australia (9). Therefore these winged alates are not considered a risk for starting a new infestation, as they do not seem to be able to reproduce.

According to overseas literature, leaf galls occur when the crawlers from root populations or the offspring of a sexually reproducing female begin feeding on the leaf of a resistant rootstock vine or sucker. Research in Australian conditions (4) suggests that the establishment of leaf galling populations does not in fact appear to be by the offspring of a sexual cycle. Leaf feeding gives rise to a gall that encloses the insect. Within the gall the nymph develops to an adult and lays eggs, which in turn hatch into leaf-feeding nymphs, producing more galls. The foliage of the European *Vitis vinifera* species grown for winegrapes in Australia is resistant to the leaf galling stage of phylloxera. However, some of the American vine hybrids used as rootstocks because of the relative resistance of their roots to phylloxera and nematodes, and some ornamental vine species, are susceptible to leaf galls, particularly during seasons of high summer rainfall. Although theoretically this provides further potential for spread of phylloxera, it could only occur where rootstock suckers are present both in the infested vineyard and in the destination vineyard (which is unlikely, as most grapevines in Australia except in known infested regions are on their own roots). Phylloxera distribution studies in Australia indicate that insects from leaf galls rarely establish on roots, and indeed that leaf galls occur only rarely and are geographically restricted – even in vineyards with abundant rootstock suckers for leaf gall formation (1).

Root-galling crawlers are therefore considered to be the only significant stage involved in the spread of phylloxera in Australian vineyards. This spread can occur either naturally (including crawling of the insect from vine to vine, and wind-blown), or human-assisted – on grapevine material and grape products, in soil associated with infested roots (eg carried on footwear or vehicle tyres), or carried by harvesting machinery, other equipment or tools. It is not considered likely that eggs of phylloxera root populations would be picked up as part of the harvesting process, as these remain underground. Even if some were collected on a grape harvester, the chances are negligible that they could hatch at exactly the right instant to escape into a vineyard and find a host (see section 6.6).

Research indicates that natural movement is important for localised spread within the vineyard of around 20m annually (9). However, natural movement is restricted to contiguous vines. In one area in Victoria, a buffer of 2km from an infested vineyard was sufficient to protect other vineyards from infestation for a number of years (1). The risk of moving crawlers by human-assisted means is the focus of this analysis.

6.0 Pest Risk Analysis

6.1 Overview

Estimating the risk associated with moving phylloxera during vintage involves:

1. Estimating the initial population in the canopy and therefore the likely number of insects that would be transferred into a specified grape load;
2. Calculating the number of individuals from that original sample that would be expected to survive through a number of stages to reach their destination, using estimates of survival percentages derived from experimental research or informed opinion.

The four scenarios considered are:

1. Contamination via a 25t truckload of infested grapes
2. Contamination via a 20,000L tanker load of must or unclarified juice
3. Contamination via a 5t residue of pre-fermentation marc
4. Contamination via a piece of vineyard equipment moving directly from an infested to an uninfested vineyard.

For fresh winegrapes, the stages involved are:

- harvest
- transport to the destination winery as fruit
- establishment in a vineyard

For must or juice, the stages involved are:

- harvest
- crushing, pumping and chilling (red varieties)
- pressing, pumping and chilling (white varieties)
- transport to the destination winery as product
- establishment in a vineyard

For pre-fermentation marc, the stages involved are:

- harvest
- transport to the destination winery (as fruit)
- crushing or pressing and removal of solids (pre-fermentation marc)

Then either:

- establishment in a vineyard associated with the destination winery through accidental contamination

Or:

- transport of the marc to a vineyard elsewhere to use as mulch
- establishment in a vineyard

For vineyard equipment, the stages involved are:

- harvest
- transport to another vineyard as grapevine material
- establishment in a vineyard

Each of the stages is analysed in the following sections and a survival percentage assigned, based on research work and other considerations. Any assumptions made are also stated. The summary table attached as appendix 1 gives estimated numbers of insects at each stage for each scenario, using the survival percentages and estimated starting numbers given below.

6.2 Initial population

Crawlers are at their most active both above and below ground during the months of December – March. Therefore harvest time in Australia is a particularly high risk period for spreading phylloxera because the insects are more likely to be moving up into the canopy. Crawlers are delicate insects that cannot survive high temperatures and low humidities. Survival has been found to be very low at a relative humidity less than 50% combined with a temperature over 25 degrees C (4). Crawler survival will be greatest under cool humid conditions, which may not always apply at harvest. However, because harvest is carried out wherever possible at cooler temperatures (eg at night) for the sake of the fruit, optimal conditions are assumed in this analysis.

The foliar population of crawlers in infested vineyards in the period from February to April has been estimated as three individuals per 20 bunches and one crawler per 200 leaves (10). Bunch size varies from around 60g to 200g (depending mainly on region and variety – and also the level of phylloxera infestation of the vineyard). Assuming an average weight of 100g per bunch, the number of bunches in one tonne is 10,000, and the estimated number of phylloxera insects is 10000/20 x 3 = 1500. In addition, there are estimated to be around two leaves per bunch (60 shoots per vine x 4 leaves per shoot divided by 120 bunches per vine) passing through a mechanical harvester. Therefore the additional number of crawlers in one tonne of harvested grapes is estimated to be 2 leaves x 10,000 bunches, divided by 200 (the number of leaves to every one crawler) = 100.

On the basis of the above calculations, it is assumed that the maximum number of crawlers that could be collected during harvesting **of a 100% infested vineyard** is 1600 per tonne. Therefore in a 25 tonne load of grapes (equivalent to about 20,000L of juice) there is assumed to be a maximum of 40,000 individual insects.

In fact, the load population would be a proportion of this, depending on the level of infestation in the vineyard/s and the proportion of fruit from each vineyard that make up the load. Potentially, the number in a 25 tonne load could vary from 0 to 40,000.

These initial population figures are derived from research work in a cool climate vineyard in reasonably cool and wet daytime conditions, with a virulent – ie high population – phylloxera genotype. They are likely to represent a worst-case scenario. There is insufficient research evidence available comparing populations over a number of seasons and in drier, warmer conditions, and to compare day-time with night-time populations, although one study (1) found crawlers to be most abundant between the hours of 9am to 9pm, with numbers substantially lower (84% reduced) between 9pm and 9am. The latter is generally the time when most harvesting takes place.

In the case of contaminated vineyard equipment, the initial population is derived from the amount of grapevine material that is likely to be collected by the equipment during harvest – not counting the fruit load itself. The quantities used in this analysis are:

- mechanical harvester: 20kg
- grape truck (tray): 5kg
- grape bin (outside): 100g (0.1kg)

Therefore the number of crawlers on the equipment is estimated to be:

- mechanical harvester: 32 (1600 x 20/1000)
- grape truck: 8
- grape bin (outside): 0.2

6.3 Harvest

6.3.1 Mechanical harvesting

Mechanical harvesting is a relatively vigorous operation which is likely to dislodge some of the crawlers from within the bunches, or from the surface of leaves and stems. Some would be shaken onto the ground, while it is likely that many would be blown away by the force of air from the fans. However, they are very small relative to the grapevine material, and could adhere to the sticky surface of the grape bunches. There is insufficient research evidence on the collection of crawlers from the canopy during mechanical harvesting; therefore a conservative estimate of **75% capture** is used.

Research work is not available on survival of phylloxera through mechanical harvesting. However, it is highly likely that a substantial proportion of those that were captured would be killed through the operation of the equipment – which is likely to be very traumatic. Given that survival of phylloxera crawlers after pressing is 53% (see 6.4.2), a conservative estimate of **50% survival** for mechanical harvesting is used.

This gives a combined estimate of **37.5% capture and survival** after mechanical harvesting.

6.3.2 Hand harvesting

The capture and survival of crawlers on fruit that is hand picked is likely to be higher than for that which is mechanically harvested, given the much gentler nature of the process, and the absence of fans to blow the crawlers off the grapes.

However, hand-picking accounts for less than 10% of harvesting across Australia, and is mostly associated with small vineyards with their own wineries (rather than large quantities of fruit being transported between regions as fruit or must/juice). Mechanical harvesting is therefore the focus of this analysis. For comparison, a combined estimate of **60% capture and survival** after hand-picking is assumed.

6.4 Processing

6.4.1 Crushing

Under normal operations, trucks are filled progressively as grapes are augured into the crush and split between rotating plates. The must, which comprises the juice and skins, is pumped directly from a receiving tank to the truck by means of a centrifugal pump.

Research work (10) using a mechanical crusher/destemmer have shown a maximum of 38% survival, with 4% coming through on the stems and the rest on the unfermented must. Therefore an estimate of **34% survival for must** is made.

6.4.2 Pressing

Red must generally proceeds directly from crushing to fermentation. White must is usually pressed after crushing. The press is a cylinder in which the exterior surface has screens. Pressure is applied by means of an inflatable membrane within the press, which pushes the fruit to the exterior. Various pressures can be applied which will result in different fractions of juice varying in composition and amount of solids.

As pressure is increased it is likely that insect mortality will also rise through the effects of the hydraulic pressure and the compression of the solids that build up between the screens and the exterior surface of the membrane. These solids would also tend to act as a barrier to crawlers being extruded into the unclarified fraction and any insects that were caught would be dumped locally with the solids.

Research work (10) using a mechanical basket press has found a maximum of 83% survival. 73% of insects come through in the juice, and 10% in the pressed solids. Therefore an estimate of **73% survival** is used, for insects continuing on in the process of being transported to another winery. An estimate of **10% survival** is used for insects remaining in the solids residue – ie pre-fermentation marc.

6.5 Transport

6.5.1 Fruit

Once the fruit is picked, it is normally transported for processing in field bins. Under these conditions the survival of crawlers will depend on the temperature and duration of the journey. It is also possible that some insects could be blown off the top of the load, if it is uncovered, or injured by the movement of the load. The greater the distance travelled, the less the chance of survival for the insects. In general, however, attempts will be made to travel when the weather is cooler in order to protect the quality of the grapes, and where distances are large, the load may be covered; therefore optimum conditions for the phylloxera insects are assumed. Research studies have shown that up to 90% survival under simulated grape transport conditions is possible (11). Therefore an estimate of **90% survival** is used.

6.5.2 Must or juice

Normally red must being transported to another winery for further processing is pumped directly into a refrigerated or insulated tanker from a receiving tank, as the fruit is crushed. The tanker moves by a direct route to the receiving winery where the must is discharged. Survival of crawlers in must is a function of time and temperature of the must. It is assumed that, due to the agitation of the liquid caused by movement, the insects would be submerged rather than floating on the surface.

Research studies have indicated that survival of crawlers submerged in red must is 43% after six hours, 6% after 12 hours, and 2% after 24 hours (13). This is likely to be a conservative estimate (high) because the mechanical and fluid pressures during transport are also likely to reduce the survival. Most transport distances are likely to be less than 500km (approximately 6 hours travel time). Therefore an estimate of **43% survival** is used for red must.

Research studies indicate that crawlers survive better submerged in unclarified white grape juice than they do in red must. A recent study (11) found 80% - 90% survival after 24 hours. Therefore an estimate of **90% survival** is used for unclarified white juice.

6.5.3 Pre-fermentation marc

The only possibility for transport of pre-fermentation marc after processing, is where the source winery gives or sells the marc to a winery, vineyard or processing facility elsewhere (such as Tarac in the Barossa Valley). Survival of phylloxera in pre-fermentation marc has not been studied; however, as there is no food source and the marc is a heavy, manure-like substance, it is assumed that phylloxera would not survive for more than 48 hours – even in ideal conditions (moist, low temperatures), which are not likely to occur over a 48 hour period during vintage. Therefore survival during (or prior to) transport is assumed to be zero, unless the marc is moved within 48 hours. Assuming this to be the case, an estimate of **50% survival** is used (although this is really an arbitrary figure, as it would in fact depend entirely on the conditions and the travel time involved).

6.5.4 Vineyard equipment

Mechanical harvesters

Harvesters moving between regions are normally subjected to thorough cleaning and heat disinfestation before commencing work in a new location. However, this is not always the case where the first region is not a known phylloxera infested region, or where movement is within the same region or state. For the purpose of this risk analysis, it is assumed that heat disinfestation is not carried out. Even then, it is unlikely that a harvester would not be cleaned after finishing a shift, because the machine operates better, does not develop a smell and looks better to the customer when it is clean. Whether or not the harvester is cleaned, evidence suggests that it is not possible to remove all grapevine material from the internal spaces of the machine. These spaces are likely to be sheltered, but there is not likely to be a food source available to the phylloxera (unless root fragments were included in the material). It is assumed that 90% of the grapevine material – and therefore the initial population of insects – is removed from the harvester by cleaning, leaving a remainder of 10% of grapevine material, and that the survival of the remaining insects is **90%** (for up to a week). Therefore a combined estimate of **9% survival** (0.1 x 0.9) is used.

Grape bins
Once grapes have been unloaded from a grape bin, the bin is loaded back onto the truck (or, less often, onto another truck) and taken back to the vineyard or to another vineyard for reloading. The survival of any crawlers in or on the outside of the bin would depend on grape material being left behind, and on the conditions (temperature and humidity) at the time. It is also possible that crawlers could have been picked up directly while the bin was in the source vineyard – either on the underside where the bin was left on the ground for filling (less common with machine harvesting), or on the sides where the bin was carried down the vine rows behind the harvester. Most bins are squirted out but the outsides and undersides are not washed before being reloaded. Therefore there may be some small amount of grapevine material and, in particular, juice, stuck to the outside of the bin, that phylloxera insects could adhere to. Assuming conditions to be suitable during transport, survival of insects in this material could be high. An estimate of **90% survival** is used. NB Any bins that are hot-water dipped would have a **0% survival** for phylloxera.

Grape trucks
Grape trucks are likely to carry spilled grapes and grapevine material on the tray. Survival of phylloxera in these conditions would depend on the temperature and humidity, and the distance travelled. Assuming conditions to be favourable, an estimate of **90% survival** is used.

6.6 Establishment in a vineyard

The establishment of an infestation in a vineyard near the receiving winery requires that one or more viable (live) crawlers can escape from the grape truck, tanker or processing equipment, reach a vineyard, find a food source and then survive long enough to lay and hatch eggs.

Assuming that one or more viable insects escaped through spillage or other means, the probability of this occurring would be a combination of:

- probability of escape through handling or spillage (including avoiding being cleaned up)
- probability of finding a host
- probability of infesting the roots
- probability of surviving to reproduce.

These probabilities are assessed separately for crawlers in fruit, in must/juice and pre-fermentation marc and in machinery. It should be noted that in all cases, ideal conditions for survival are assumed (ie temperature below 20 degrees and relative humidity above 50%). This is in fact not likely to occur in a typical Australian vintage, unless all stages of the process could be completed at night, and/or during an unseasonally cool, wet period. Therefore, these estimates are “worst-case” estimates. It has been shown (12) that crawlers do not survive at temperatures at or above 25 degrees, when the relative humidity is below 50%. These conditions would constitute an unusually mild day during February – March in most grapegrowing regions.

6.6.1 Fruit

P1 Probability of escaping and avoiding decontamination
With imported infested fresh grapes, a number of opportunities would be available during storage and handling to allow escape of crawlers. Escape would require crawling or being blown or jolted out of the truck and reaching the ground, or spillage of bunches of grapes onto the ground during unloading. Spillage is not likely to be more than 1% of the grape load, while escaping from the surface is only available to those crawlers positioned near the top of the load (perhaps 10% of the total population). A “middle ground” estimate of **5% escaping** is used.

Typically, grape unloading would not be on a hard stand, and spilled grapes, stems etc would not be cleaned up immediately. Therefore there is a relatively high probability of crawlers escaping decontamination once they reached the ground. As a worst-case scenario, it is assumed there is no reduction in numbers due to decontamination (clean-up).

P2 Probability of finding host

A number of factors might favour crawlers carried on fruit compared with those arriving in must, or grape juice. These would include:

- greater opportunity for crawlers to be windblown
- increased opportunity to be carried on clothing or other items
- individual insects relatively unaffected by transport conditions

However, the probability of a crawler finding a grapevine depends primarily on the proximity of grapevines to the receival point for grape trucks. Research suggests that natural spread *within a vineyard* is limited to around 20m per year (9). A crawler is not likely to be able to crawl more than 5m across dirt (this is an assumption that needs to be confirmed by research). It is therefore assumed that if the winery does not have an adjacent vineyard within 10m of the grape truck receival point, then the probability of a crawler finding a host is zero (0). If there is a vineyard within that range, then the probability of a crawler finding a host is conservatively estimated at **10%.**

P3 Probability of infesting the roots

Once a viable insect has reached a grapevine, the probability of it infesting the roots would be relatively high. It would now be undisturbed and have a food source available. Its success in reaching the roots is likely to depend on the soil moisture content, air temperature and humidity, and a combination of the depth of the rootzone and the soil type. It will also depend on the condition of the insect, after its arduous journey from the infested vineyard. An estimate of **20% infestation** is used.

P4 Probability of surviving to reproduce

Since individuals would have to be relatively vital and robust to have reached the roots, it can be assumed that the battle is now all but over. Therefore an estimate of **50% survival** is used.

PX Combined probability of establishing in a vineyard

The combined probability of establishing in a vineyard is a function of the four elements described above. Therefore the combined risk is 0.05 x 0.1 x 0.2 x 0.5 = 0.0005 (0.05% or 1 in 2000).

6.6.2 Must or juice

PI Probability of escaping and avoiding decontamination

Discharge of tankers is normally done on hard stand areas, where the product is pumped directly from the tanker into fermentation tanks for processing. Any spillage is washed into the wineries' wastewater system. In some wineries, a closed sewerage system is used for treatment of wastewater. However, in other wineries the wastewater may be processed by other methods, such as settling ponds, which may pose more risk for incidentally spreading the pest. The biggest risk exists where wastewater is disposed of directly onto adjacent vineyards.

Results from laboratory trials indicate that phylloxera will not survive fermentation of red varieties. No phylloxera survived for more than 15 hours in fermenting Merlot grapes (13). Since fermentation takes several days there will be no survival of crawlers in post-fermentation marc when it is removed.

Clarified white grape juice is pumped into tanks for fermentation but unclarified juice would require time for sedimentation of solids. This sediment might contain crawlers but after 24 hours it is unlikely that any would be alive - laboratory trials indicate that after 24 hours immersion in clear grape juice about 13% of crawlers survived (13).

After allowing settlement, the sediment is filtered through a rotary drum vacuum filter, which traps the sediment, including any surviving phylloxera insects. If this fraction is not filtered for 36 hours then no survival of crawlers is likely. However if filtration is done earlier, some surviving crawlers may become incorporated in the diatomaceous earth that is used in the process. Phylloxera survival in this spent material is not known but it is likely to be low, as would be the aphid's chance of escaping from this fine-grained dense material.

Therefore the only chance of crawlers escaping is through spillage prior to processing *and* either avoiding clean-up, or entering a waste water pathway that puts untreated water directly onto a vineyard. If the latter possibility is excluded, this probability is considered to be very low. Assuming that *every* tanker had a 200L spillage (which would be very unusual, in fact), that crawlers were distributed evenly throughout the tanker and that all crawlers in the spilled fraction survived and escaped clean-up, then the proportion escaping would be 1%. The estimate used is **0.5% escaping** (ie 50% of those spilled)**.**

P2 Probability of finding host

Individuals that escape would face substantial barriers in finding a host. Their most likely means of reaching a host would be on footwear associated with grape skins of the must, or carried through the waste water system into evaporation ponds next to a vineyard. The insects are also likely to have been significantly affected by their ordeal, reducing their ability to travel, even if deposited on cool, moist soil next to a vineyard.

As in the case of crawlers in a grape truck, the probability of a crawler reaching a grapevine depends primarily on the proximity of grapevines to the receival point for tankers. Once again, it can be assumed that if the winery does not have an adjacent vineyard within 10m of the tanker receival point, then the probability of finding a host is 0. If there is a vineyard within that range, then the probability of a crawler finding a host is estimated at **1%** for crawlers from must or juice, given their additional disadvantages**.**

P3 Probability of infesting the roots

As for fruit, once a viable insect has reached a grapevine, the probability of it infesting the roots would be relatively high. It would now be undisturbed and have a food source available. Its success in reaching the roots is likely to depend on the soil moisture content, air temperature and humidity, and a combination of the depth of the root zone and the soil type. Because the condition of survivors is likely to relatively poor, however, an estimate of **10% infestation** is used.

P4 Probability of surviving to reproduce

Since individuals would have to be relatively vital and robust to have reached the roots, it can be assumed that the battle is now all but over. Therefore an estimate of **50% survival to reproduce** is used.

PX Combined probability of establishing in a vineyard

The combined probability of establishing in a vineyard is a function of the four elements described above. Therefore the combined risk is 0.005 x 0.01 x 0.1 x 0.5 = 0.000025 (1 in 40,000).

6.6.3 Pre-fermentation marc

P1 Probability of escaping

The combined probability of escaping and finding a host is included in P2 below.

P2 Probability of finding host

There are three possibilities for a crawler buried in pre-fermentation marc finding a grapevine: either to crawl out of a load of marc deposited near a vineyard after processing, to be picked up accidentally on the tyres of a vehicle and driven into a vineyard, or to be picked up and deposited directly onto a vineyard where the marc is being used as a mulch. In the first case, the probability is extremely low. The insects would have to crawl out of a dense, hard-packed material and then traverse the distance to the nearest vineyard, without being cleaned up – with the only possible assistance being that of some passing footwear. If there is no vineyard within 10m, then this probability is considered to be negligible. If there is a vineyard within 10m, then the probability is estimated to be **0.1% escaping and finding a host**.

Alternatively, a truck could drive over an area where the marc has been deposited, pick up some material containing live phylloxera on its tyres, and drive into a vineyard with it. The probability of a truck picking up material in this way is relatively high if the marc is left lying around in or near the grape receival area of the winery. (Note: wet weather would greatly increase the chances of marc sticking to the tyres.) Such a truck is extremely likely to be driving straight back to a nearby vineyard to collect more grapes. Therefore the probability of "escaping" is relatively high – particularly in wet conditions. However, some insects would be dropped off on the road, killed by the frictional heat or squashed by the rotations of the tyres. The amount of marc that could be collected on the tyres is estimated as 1kg, which is 0.02% of a 5t load of pre-fermentation marc. There is no research work on the survival of phylloxera in mud on truck tyres, therefore a conservative (high) estimate of **0.01% escaping and finding a host** is used – which assumes that half of the insects picked up in the truck tyres will make it alive to a vineyard.

The other possibility exists where the winery's practice is to deposit pre-fermentation marc directly onto a vineyard. This is not common industry practice, particularly among the large wine companies, which account for over 80% of the national grape crush. Even if it were done, the insect would have to be deposited on the vineyard within about 48 hours, as it would not have a food source within the marc. *A conditional probability* of **10% escaping and finding a host** is used, for situations where marc is directly deposited onto a vineyard within 48 hours.

P3 Probability of infesting the roots
Once a viable insect has reached a grapevine, the probability of it infesting the roots would be relatively high. It would now be undisturbed and have a food source available. Its success in reaching the roots is likely to depend on the soil moisture content, air temperature and humidity, and a combination of the depth of the rootzone and the soil type. An estimate of **20% infestation** is used.

P4 Probability of surviving to reproduce
Since individuals would have to be relatively vital and robust to have reached the roots, it can be assumed that the battle is now all but over. Therefore an estimate of **50% survival** is used.

PX Combined probability of establishing in a vineyard
The combined probability of establishing in a vineyard is a function of the four elements described above. Therefore the combined risk for the different transport options is:

- moving to an adjacent vineyard: 0.001 x 0.1 x 0.5 = 0.0005 (0.05% or 1 in 2,000)
- being carried by a truck to a vineyard: 0.0001 x 0.2 x 0.5 = 0.00005 (0.005% or 1 in 20,000)
- direct deposit on a vineyard: 0.1 x 0.2 x 0.5 = 0.02 (2% or 1 in 50)

For the purpose of estimating a risk level, the first option is selected because either of the first and second options is much more likely to occur than direct deposit onto a vineyard, and the probability of establishment via the second option is negligible.

6.6.4 Vineyard machinery

PI Probability of escaping and avoiding decontamination

Harvesters
If phylloxera were transported to another vineyard on a mechanical harvester, it has been assumed in this analysis that it has not been subject to any effective clean-up or decontamination process (see 6.5.4 above). The chances of it escaping voluntarily are not high; however, as part of the normal operation of the harvester, it might be discharged along with other grapevine material caught in the recesses of the machine. Given that these are likely to be hard-to-access areas of the machine (because otherwise the insects would have been dislodged during cleaning), the chances of the material being dislodged during a subsequent operation are not very high. An estimate of **10% escaping** is used.

Grape bins
In the case of a grape bin, phylloxera insects caught in the bottom of the bin are not likely to be discharged back into the vineyard. Insects stuck on the outside of the bin might fall off on the way to the vineyard, or might remain stuck and not fall off in the next vineyard. An estimate of **10% escaping** is used.

Trucks
In the case of a grape truck, any insects caught in grapevine material in the tray or other external parts of the truck could be dropped into the next vineyard. It is likely that some would have been dropped in transit to the winery or en route to the next vineyard, while some would remain stuck to the truck. The greater the distance travelled, the lower the chance of survival. An estimate of **10% escaping** into the destination vineyard is used. NB If the trucks are loaded more than 10m away from vines, the risk of depositing material onto the vineyard is reduced to zero.

P2 Probability of finding host
Individuals that escape from vineyard equipment or a grape truck would find themselves deposited close to a vine (closer in the case of a harvester than a truck, which would not drive down the rows). The only factor reducing their chance of finding a host would be any injury they suffered during the process. An estimate of **80% probability of finding a host** is used.

P3 Probability of infesting the roots
As for fruit, once a viable insect has reached a grapevine, the probability of it infesting the roots would be relatively high. It would now be undisturbed and have a food source available. Its success in reaching the roots is likely to depend on the soil moisture content, air temperature and humidity, and a combination of the depth of the rootzone and the soil type. Because the condition of survivors is likely to relatively good, an estimate of **20% infestation** is used.

P4 Probability of surviving to reproduce
Since individuals would have to be relatively vital and robust to have reached the roots, it can be assumed that the battle is now all but over. Therefore an estimate of **50% survival to reproduce** is used.

PX Combined probability of establishing in a vineyard
The combined probability of establishing in a vineyard is a function of the four elements described above. Therefore the combined risk for each type of vineyard equipment is:

- Harvesters: 0.1 x 0.8 x 0.2 x 0.5 = 0.008 (0.8% or 1 in 125)
- Grape bins: 0.1 x 0.8 x 0.2 x 0.5 = 0.008 (0.8% or 1 in 125)
- Grape trucks: 0.10 x 0.8 x 0.2 x 0.5 = 0.008 (08% or 1 in 125)

6.7 Pest risk analysis – the combined risk

The combined risk of a phylloxera infestation arising from each of the above pathways is calculated by multiplying together the probability of survival for each stage. To determine the number of insects likely to start an infestation, the starting population of phylloxera under each scenario is multiplied by the probability of survival.

Using the estimates quoted in the previous sections, the table attached as appendix 1 gives estimates of the population of crawlers that could infest a vineyard in each 25 tonne load of fruit, tanker load (20,000L) of red must or white unclarified juice, 5t load of pre-fermentation marc or piece of harvest equipment contaminated with grapevine material. Alternatives are given for:

- A 100% infested vineyard
- A 25% infested vineyard

Note: in fact it is not realistic to presume that movement of grape products would occur without protocols from a 100% infested vineyard – particularly for harvesters, fresh grapes and pre-fermentation marc – *because this would be a known infested vineyard*. Assuming the infestation is undetected, even a 25% infestation is a very high estimate to use. Therefore these figures are likely to over-state the risk quite substantially. However, this analysis presents a “worst case scenario” which is valuable to help establish upper limits for risk, to enable comparisons to be made between different risk vectors, and to identify critical points where the risk is greatest.

In summary, the risk analysis shows that the risk of an infestation occurring if the source vineyard is **100% infested** is approximately:

- At least one in every truckload for fresh grapes
- One in 4 - 9 truckloads for pre-fermentation marc
- One in 14 tanker loads for fresh or unclarified juice
- One in 20 tanker loads for must
- One in 46 trucks.
- One in 116 harvesters
- One in 1850 grape bins

For the 25% infested source vineyard, the risk of an infestation occurring is approximately:

- At least one in every truckload for fresh grapes
- One in 20 truckloads for pre-fermentation marc
- One in 53 tanker loads for fresh or unclarified juice
- One in 81 tanker loads for must
- One in 185 trucks.
- One in 463 harvesters
- One in 7400 grape bins

6.8 Pest risk analysis – situational variations

The above calculations give a generic estimate of risk for moving grape products or machinery between a hypothetical infested vineyard and a winery/vineyard within 500km. In reality, the risk would vary depending on the particular situation. The main variables that would affect the level of risk are (most important contributors highlighted in bold):

Regional/stable factors:

1. **The likelihood or level of infestation in the source vineyard**
2. The distance between the two vineyards (ie time taken to transport the product)
3. The climate in each vineyard (and in-between)
4. The biotype of phylloxera involved

Individual/unstable factors:

1. **The weather during the transfer (temperature, humidity, rainfall)**
2. Harvesting practice: mechanical or hand-harvesting
3. **The proximity of the destination winery to a vineyard**
4. The waste water treatment system at the destination winery
5. Traffic systems at the destination winery – opportunity for trucks to pick up mud from other truck tyres or grape products
6. Disinfestation practices – eg cleaning bins, disinfesting harvesters etc.
7. The soil type at the destination vineyard
8. Management practices – eg use of (and variety of) phylloxera resistant rootstocks in the destination vineyard; use of pre-fermentation marc as mulch

6.8.1 Influence of weather and climate

Research evidence clearly indicates that the risk of spreading phylloxera would be greater in wet, cool conditions. This is because such conditions would:

- Increase the initial population of insects
- Increase the survival of insects during transport, and over time
- Increase the likelihood of trucks picking up mud on tyres etc.

In addition, wet conditions may increase time pressures on everyone involved in getting grapes off the vines, safely processed and into storage tanks – resulting in less care being taken over hygiene and other practices.

It is not useful to attempt to quantify the differences in risk between a wet and a dry vintage, because there would still be too many variables. This analysis has adopted a starting population figure derived from research work in a cool season, and has assumed generally ideal conditions for survival of phylloxera, and therefore it is likely that the overall level of risk calculated is at the higher end of the spectrum. A typically hot, dry vintage would greatly reduce the risk of an infestation, even with harvesting being conducted mainly at night.

6.8.2 Individual practices

This analysis has clearly identified certain practices that can substantially increase or decrease the risk of a particular winery / vineyard being infested. The practices or conditions that would most significantly increase the likelihood of an infestation are:

- Vineyard within 10m of winery receival area (or where marc is left)
- Winery wastewater running directly onto vines
- Deposit of pre-fermentation marc from fruit from another vineyard directly onto vineyard
- Allowing exiting trucks to drive over grape residue – including marc, spilled fruit
- Trucks moving between vineyards without cleaning (hosing off trays)
- Harvesters moving between vineyards without heat disinfestation
- Grape bins not being hosed down on the outside

6.9 Pest risk analysis – additional pathways not considered

There are other minor pathways associated with vintage whereby a phylloxera infestation could theoretically occur, that have not been considered in this analysis because the risk is considered to be non-material. These are:

1. Stems and stalks after crushing
2. Must or juice being spilt before fermenting or filtration
3. Phylloxera eggs from mud caught in harvester or truck and then deposited direct to next vineyard
4. Fresh winegrapes spilt at destination winery – driven over by a truck and taken to a vineyard
5. Crawlers blown off grape load or spilt in accident in transit – infest vineyard adjacent to road
6. Must or juice spilt in transit to destination winery – infest vineyard adjacent to road

7.0 Conclusions

The aim of this pest risk analysis has been to analyse movements of risk vectors associated with vintage, and assess the inherent risk in each of causing a phylloxera infestation. It should be remembered that the model represents a worst-case scenario, having erred at each stage on the conservative (higher risk) side, and adopted the upper limits of risk wherever a range was indicated.

7.1 Implications for protocols

While the absolute risks are impossible to verify, the general outcome appears consistent with anecdotal and research evidence, and with the assumptions underlying the current industry protocols for preventing the spread of phylloxera. A comparison of the risks for the different vectors provides important information that should help to allocate resources and direct efforts regarding protocols and regulations. In particular:

- Fresh or unclarified white juice is more of a risk than red must
- Trucks are a significantly higher risk than harvesters or grape bins
- Pre-fermentation marc and fresh grapes present the highest level of risk – provided that the winery is within 10m of the destination vineyard, and assuming that no protocols or prohibitions operate (ie the infestation must be undetected).

Compared with the results of the earlier pest risk analysis, the risk associated with movement of red must has been estimated to be substantially higher. This is because the estimate of the initial population of phylloxera in the canopy has been significantly increased. However, the comparative risk of infestation from import of must or unclarified juice compared with fresh grapes is similar to that found in the earlier analysis.

7.2 General messages for awareness-raising activities

General messages for awareness-raising activities include:

- Distance of the nearest vineyard from a receiving winery is the most important variable. If there is no vineyard within 10m, the risk of contamination from fresh grapes, must or juice is effectively zero. NB This does not apply to contamination via trucks, harvesters or grape bins.
- Pre-fermentation marc should never be directly deposited onto a vineyard.
- Waste water should not be deposited directly onto a vineyard.
- Grape bins should be hosed down or hot-dipped between deliveries.
- Trucks should be loaded away from vines (at least 10m) and stay on hard surfaces wherever possible.
- Trucks should be hosed down before leaving the winery.
- Grapevine material – including spilt grapes, stems and stalks, pre-fermentation marc – should not be left in the path of trucks entering or leaving the winery. Hard stand receival areas, one-way traffic systems and hose-down facilities all contribute to reducing the risk of contamination.
- Wet, cool periods during vintage are highest risk times for contamination to occur.
- The greater the distance travelled between source vineyard or winery (in the case of must or unfiltered juice) and destination winery, the less the risk of infestation – particularly where conditions are warm and low humidity.

7.3 Research gaps identified

In the context of this pest risk analysis, some research gaps have been identified, where there is insufficient evidence to underpin estimates of survival percentages used. The main ones are:

- More information on foliar populations – particularly daytime vs night-time variations in populations; populations in warmer, drier regions; population variation between seasons.
- Survival of phylloxera in pre-fermentation marc.
- Analysis of likelihood of survival on truck tyres.
- Probability of a live phylloxera crawler infesting a vine if placed on the surface, and surviving to reproduce and establish a population.

Apart from survival in pre-fermentation marc, the other issues are central to the pest risk analysis, and would have broader application to the further development of protocols and assessment of risk in different regions. However, although research in these areas would improve the accuracy of the estimates used in this document, it is unlikely to make any material difference to the conclusions of the analysis. Given the large number of unknowns, variables and estimates, it is not possible to quantify the risk of a phylloxera infestation along a given pathway – only to provide a general indication of relative and absolute risk.

Appendix 1 Calculation of risk of phylloxera establishment along different pathways

100% infested vineyard

Products		Fresh grapes	Must	Juice	Pre-fermentation marc 1	Pre-fermentation marc 2	Grape bin	Truck	Harvester
	Initial quantity of grapes (t)	25	25	25	25	25	0.1kg	5kg	20kg
	Quantity of processed product	25t	20,000L	20,000L	5t	5t	0.1kg	5kg	20kg
	Initial population of crawlers	40,000	40,000	40,000	40,000	40,000	0.2	8	32
Stages	Harvest	0.375	0.375	0.375	0.375	0.375	0.375	0.375	0.375
	Transport as fruit	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.09
	Crushing		0.34	0.34	0.34	0.34			
	Pressing			0.73	0.1	0.1			
	Transport as product		0.43	0.9		0.5			
	Establishment	0.000500	0.000025	0.000025	0.000500	0.000500	0.008000	0.008000	0.008000
	Final population	6.7500	0.0493	0.0754	0.2295	0.1148	0.0005	0.0216	0.0086
	Number of units to achieve an infestation	Less than one truckload	20 tanker loads	14 tanker loads	4 tanker loads	9 tanker loads	1850 bins	46 trucks	116 harvesters

25% infested vineyard

Products		Fresh grapes	Must	Juice	Pre-fermentation marc 1	Pre-fermentation marc 2	Grape bin	Truck	Harvester
	Initial quantity of grapes (t)	25	25	25	25	25	0.1kg	5kg	20kg
	Quantity of processed product	25t	20,000L	20,000L	5t	5t	0.1kg	5kg	20kg
	Initial population at 25% infestation	10,000	10,000	10,000	10,000	10,000	0.05	2	8
Stages	Harvest	0.375	0.375	0.375	0.375	0.375	0.375	0.375	0.375
	Transport as fruit	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.09
	Crushing		0.34	0.34	0.34	0.34			
	Pressing			0.73	0.1	0.1			
	Transport as product		0.43	0.9		0.5			
	Establishment	0.0005	0.000025	0.000025	0.000500	0.000500	0.008000	0.008000	0.008000
	Final population	1.6875	0.0123	0.0188	0.0574	0.0287	0.0001	0.0054	0.0022
	Number of units to achieve an infestation	Less than one truckload	81 tanker loads	53 tanker loads	17 tanker loads	35 tanker loads	7400 bins	185 trucks	463 harvesters