

# Appendix 1

## Project Outputs aligned against five key themes

Outputs		Key Objectives					
		Improved quarantine	New and improved tools for early detection	Improved rootstock selection	Re-evaluating biocontrol options	Phylloxera Biology	Industry reference group
<b>2017-18</b>							
a	Recommendations provided to key biosecurity stakeholders and committees on NPMP procedures for disinfestation of footwear. Information disseminated to industry through at least one extension mechanism.	x					
b	Collection of phylloxera -samples from the King Valley region (NE Victoria) for genotype (strain) mapping. Collection of in-field G38 samples across different rootstocks from the NE VIC PIZ.		x				
<b>2018-19</b>							
a	Data set characterising detectable volatiles from phylloxera/infested grapevines.		x				
b	Desktop study on biocontrol options.  Survey/analysis on sniffer dog feasibility to inform stop/go point.		x		x		
c	Recommendations to relevant groups on the use of a grower friendly, passive and integrated phylloxera surveillance system.	x					
d	Industry Reference Group						x
e	Recommendations to key biosecurity stakeholders and committees on updated disinfestation procedures in the NPMP.	x					
<b>2019-20</b>							
a	Data set for trials evaluating sniffer dogs for phylloxera detection.		x				

b	Data set from comprehensive odour profiling of phylloxera-infested grapevines. Data set evaluating electronic noses for phylloxera surveillance		x					
c	Results of phylloxera genotyping study in the King Valley. Results of G38 biotype mapping in a mixed rootstock vineyard.			x				
d	Updated genotype-specific phylloxera-rootstock recommendations for industry.			x				
e	Industry feedback on progress and direction of project.							x
<b>2020-21</b>								
a	Thermal survival profile for selected endemic strains of phylloxera					x		
b	New distribution maps for phylloxera strains in King Valley. Recommendations for further strain mapping across the state. Recommendations for region-specific rootstocks 'resistant' to G38.			x				
c	Industry publication on improved disinfestation techniques for endemic grape phylloxera. At least one industry publication / website update / media release on other outputs of the project (e.g. e-nose, sniffer dogs, biotyping).	x						x
d	Industry feedback on progress and direction of project.							x
e	Final Report.							x

## Appendix 2

### Part B- looking for key identifying compounds in the odour profiles of infested and uninfested plants using gas chromatography-mass spectrometry (GC-MS).

#### METHODS for Volatiles sampling and GC-MS analysis

##### a) Volatiles sampling

A branchlet of each plant comprising 3-4 fully expanded leaves was selected and enveloped in an oven bag sealed around the stem using a cable tie. A tube connected to a laboratory pump blowing charcoal-purified air at 800 ml. min<sup>-1</sup> was inserted via a small incision on one side of the oven bag and sealed with a cable tie. Another tube equipped with an adsorbent filter packed with 200 mg of Porapak Q (Sigma Aldrich, Castle Hill, Australia) was secured in a similar way on the other side of the bag, pulling air inside the bag and trapping entrained volatiles on the adsorbent at 700 ml. min<sup>-1</sup> (see Figure 7.9A). Leaf volatiles were collected for 16-18 hours. At the end of the collections, the oven bags were removed, and volatiles were eluted from the filters using 2 mL of high purity dichloromethane. Liquid volatile extracts were stored in GC vials at -80°C until analysis. Prior to analysis, extracts were condensed to 1 ml via solvent evaporation under a gentle stream of nitrogen following the addition of 250 ng of nonyl acetate, used as an internal standard.

##### b) GC-MS analysis

Leaf volatiles were analysed using a Agilent 7890B gas chromatograph coupled with a 5977B mass spectrometer equipped with an apolar column (HP-5MS, 30 m × 0.25 mm × 0.25 µm). Helium was used as carrier gas in constant flow mode (1.3 ml.min<sup>-1</sup>). Injections of 2 µl aliquots of samples were performed in splitless mode at 250°C using a 7650 ALS liquid autosampler. Initial oven temperature was 40°C maintained for 2 min, subsequently increased at 10°C.min<sup>-1</sup> to 220°C and increased again to a final temperature of 280°C, held for a minute (total run time = 23 min). The temperature of the transfer line was set at 150°C. Ionization was performed in EI mode (70 eV) and the scan range set between 35 and 550.

Root volatiles were extracted using an Agilent CombiPAL autosampler equipped with a SPME fibre (DVB/CAR/PDMS, Supelco). SPME vials were heated at 60°C under agitation (250 rpm) for 5 min before volatiles were extracted by exposing the fibre inside the vial for 20 min, at 60°C. After extraction, the SPME fibre was directly injected in an Agilent 7890A Gas Chromatograph coupled with an Agilent 7000 tandem triple quadrupole mass spectrometer. Injection was performed at 270°C in splitless mode for 1 min. Better separation was this time achieved using a polar column (Supelcowax 10, 30 m × 0.32 mm × 0.25 µm). GC runs were performed in constant flow mode (1.2 ml.min<sup>-1</sup>) using Helium as carrier gas. Initial oven temperature was set at 40°C held for 2 min, then increased at 10°C.min<sup>-1</sup> to 270°C held for 2 min. The temperature of the

transfer line was set at 280°C. Other mass spectrometer settings were similar to those used for leaf volatiles analysis.

Chromatographic peaks and mass spectra were extracted using the peak alignment and deconvolution tool built in the eRah package in R (Domingo-Almenara et al. 2016). Compound were identified by comparisons of their mass spectra with a NIST14 mass spectral library and their retention indices calculated using a n-alkanes standard solution, were compared with those available in the literature.

#### c) Chemical data analysis and statistics

Leaf volatiles emissions rates ( $\text{ng}\cdot\text{hr}^{-1}$ ) were calculated by comparing peak areas to the peak area of the internal standard. However, peak areas were directly used to analyse root volatiles data (as the use of internal standard is not recommended with SPME). First, peak areas were adjusted to areas/g of sample to account for the different quantities of root material used between samples. Then, corrected peak areas were transformed using the probabilistic normalization method (Noonan et al. 2018) to account for the variation caused by the use of different SPME fibres for volatiles extractions between samples (two SPME fibres broke during volatiles extraction). Normalised data were then used in multivariate analysis. NMDS (Non-Metric Dimensional Scaling) plots using a Bray-Curtis dissimilarity matrix (different from the Euclidean distances used with the e-nose) were generated to visualize differences between different odour profiles. In addition, ANOSIM; a non-parametric analysis statistical test commonly used to analyse similarities was performed (using the same dissimilarity matrix) focusing on G4-infested and Shiraz grapevine variety – for which replication was high enough to obtain robust results. ANOSIM results are presented under the form of a p value (test for difference between chemical profiles) and a r value that indicates how different the profiles are. SIMPER (Similarity Percentage analysis) was used to determine the contribution of different volatiles to the dissimilarity between odour profiles.

## RESULTS

### Statistical analysis – volatiles data

**Table 1. SIMPER results describing the contribution of different compounds to the dissimilarity between leaf odours of G4 infested and healthy plants at 6 weeks post-infestation.**

Compound ID	Contrib. %	Cumul. %	Relative amount (% ± se)		Rel rates ng/hr	
			Healthy	infested	Healthy	infested
<b>1103P – resistant</b>						
α-Farnesene	50.59	50.59	53.2 ± 4.0	46.8 ± 7.4	3330	752
α-Ocimene	19.19	69.78	19.1 ± 2.1	12.8 ± 2.6	1190	211
Indole	6.521	76.3	6.1 ± 2.1	2.1 ± 0.6	343	40
Cosmene	2.61	78.91	2.3 ± 0.4	0.5 ± 0.2	155	9.01
(Z)-3-Hexenyl acetate	2.537	81.45	1.6 ± 0.9	16.7 ± 5.6	48.9	123
<b>Ruggeri 140 – tolerant</b>						
α-Farnesene	46.62	46.62	48.8 ± 15.5	45.7 ± 3.6	3060	284
α-Ocimene	20.29	66.91	19.9 ± 5.4	8.7 ± 2.5	1290	59.8
Indole	5.887	72.8	5.3 ± 4.5	1.4 ± 0.4	423	10.2
Cosmene	2.308	75.11	2.1 ± 1.1	0.2 ± 0.1	160	1.64
Phenylethyl Alcohol	2.016	77.12	2.2 ± 5.4	1.5 ± 0.6	49.4	9.67
(Z)-3-Hexenyl acetate	1.708	78.83	1.2 ± 1.3	15.1 ± 3.9	80.9	85.2
(E)-2-Hexenal	1.435	80.26	1.4 ± 3.0	2.6 ± 2.2	36.6	21.6
<b>Vitis vinifera (Shiraz) – susceptible</b>						
α-Farnesene	39.02	39.02	42.5 ± 4.4	23.7 ± 4.7	1310	93.3
α-Ocimene	17.22	56.24	17.2 ± 1.4	12.6 ± 4.1	659	54.6
Phenylacetaldehyde	8.407	64.65	8.7 ± 0.7	7.3 ± 1.8	318	31.1
(Z)-3-Hexenyl acetate	8.183	72.83	3.4 ± 1.5	24.7 ± 5.5	124	68.8
Indole	3.714	76.54	0.5 ± 0.2	0.7 ± 0.3	208	4.49
Cosmene	2.112	78.65	1.8 ± 0.6	0.2 ± 0.0	112	0.82
Phenylethyl Alcohol	1.839	80.49	2.3 ± 1.4	4.1 ± 1.3	19.9	17.3

**Table 2. SIMPER results describing the contribution of different compounds to the dissimilarity between root odours of G4 infested and healthy plants, 12 weeks post infestation.**

Compound ID	Contrib. %	Cumul. %	Relative amount (% ± se)		Mean peak areas (×10 <sup>6</sup> )	
			Healthy	infested	Healthy	infested
<b>1103P – resistant</b>						
Benzaldehyde	40.83	40.83	38.2 ± 5.4	16.7 ± 2.0	197	15.8
(E)-2-Hexenal	9.348	50.18	9.1 ± 2.1	11.3 ± 3.8	32.9	12.6
2-Methylbutanoic anhydride †	4.411	54.59	5.2 ± 1.3	4.2 ± 0.9	15.6	3.84
Myrtenol	4.142	58.73	4.9 ± 1.7	17.3 ± 3.8	9.03	14.7
Phenylacetaldehyde	3.437	62.17	3.7 ± 0.5	2.7 ± 0.3	15.6	2.49
Benzyl alcohol	3.356	65.53	2.9 ± 1.4	3.9 ± 1.6	12.5	4.17
Hexanal	3.234	68.76	2.8 ± 0.6	4.0 ± 1.9	10.3	4.78
Phenylethyl Alcohol	2.41	71.17	2.9 ± 1.0	7.0 ± 1.2	7.53	7.13
Anisole	2.196	73.37	2.0 ± 0.6	0.3 ± 0.1	6.12	0.317
3,7-Dimethyl-3,6-octadienal †	2.145	75.51	2.3 ± 0.7	2.2 ± 0.2	6.97	2.02
2-methoxy-3-(2-methylpropyl)-Pyrazine	1.992	77.5	1.6 ± 0.3	1.1 ± 0.2	6.42	1.47
2-Heptanone	1.576	79.08	1.5 ± 0.6	0.3 ± 0.1	3.13	0.203
Methyl salicylate	1.563	80.64	1.0 ± 0.4	5.0 ± 1.1	2.35	4.25
<b>Ruggeri 140 – tolerant</b>						
Myrtenol	10.05	10.05	12.4 ± 1.6	8.5 ± 2.0	10.6	7.66
Benzaldehyde	9.336	19.39	11.9 ± 2.5	15.5 ± 2.6	10	11.9
Borneol	7.297	26.69	5.4 ± 1.2	5.0 ± 2.3	4.75	5.13
3-Ethyl-4-methylpentan-1-ol	6.533	33.22	9.0 ± 2.1	4.6 ± 1.6	6.93	3.29
2-Methylbutanoic anhydride †	6.43	39.65	9.2 ± 1.4	6.8 ± 1.3	7.91	5.57
3,7-Dimethyl-3,6-octadienal †	6.422	46.07	7.2 ± 1.5	5.4 ± 1.5	6.57	4.22
Methyl salicylate	6.015	52.09	1.0 ± 0.4	6.4 ± 1.4	0.856	5.06
Phenylacetaldehyde	4.705	56.79	2.7 ± 0.4	6.9 ± 1.5	2.16	5.56
2-phenoxyethanol	4.668	61.46	4.8 ± 1.4	0.9 ± 0.3	3.9	0.63
(E)-2-Hexenal	4.554	66.01	3.6 ± 1.2	6.0 ± 1.1	3.06	4.66
2-Pentyl-furan	3.21	69.22	4.4 ± 0.5	6.2 ± 1.1	3.74	4.6
Hexanoic acid	3.197	72.42	2.3 ± 0.5	5.0 ± 1.0	1.83	4.04
α-Citrylidene ethanol †	3.002	75.42	5.7 ± 0.9	4.4 ± 1.0	4.58	3.26
(E)-2-Hexen-1-ol	2.728	78.15	0.7 ± 0.3	3.0 ± 1.1	0.475	2.49
3-Octanone	2.462	80.61	2.2 ± 1.0	1.0 ± 0.3	2.04	0.744
<b>Vitis vinifera (Shiraz) – susceptible</b>						
Phenylethyl alcohol	9.038	9.038	3.4 ± 0.7	9.1 ± 1.3	8.78	24.7
Benzyl alcohol	8.926	17.96	8.9 ± 1.5	11.2 ± 1.5	21.6	30.3
Borneol	8.567	26.53	13.1 ± 2.3	7.5 ± 1.4	33.1	19.4
Myrtenol	8.177	34.71	17.8 ± 2.3	17.4 ± 1.2	44.8	45.2
trans-Geraniol	6.283	40.99	1.2 ± 0.4	5.6 ± 0.9	3.56	15.3
exo-Norbornyl alcohol †	4.351	45.34	0.3 ± 0.1	0.1 ± 0.0	2.92	11.1
2-methoxy-3-(1-methylethyl)-Pyrazine	3.923	49.27	5.1 ± 0.9	3.9 ± 1.1	13.2	9.68
p-Cymen-7-ol	3.702	52.97	2.1 ± 0.4	4.8 ± 0.5	5.57	12.2

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3,7,7-trimethyl-1,3,5- Cycloheptatriene†	2.489	55.46	2.3 ± 0.7	2.0 ± 0.9	5.21	4.68
Benzaldehyde	2.435	57.89	8.4 ± 4.0	2.9 ± 0.4	10.2	7.8
Phenylacetaldehyde	1.828	59.72	2.4 ± 0.4	2.8 ± 0.4	4.67	7.4
β-Myrcene	1.812	61.53	0.9 ± 0.3	1.4 ± 0.5	1.98	3.7

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† indicate tentative compound identification (lacking confirmation using retention indices)

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## Appendix 3

### Phylloxera surveillance methods

A review of emergence traps and root inspection methods for in-field detection of grapevine phylloxera



Economic Development,  
Jobs, Transport  
and Resources

AGRICULTURE VICTORIA



## Appendix 4

### New Detection Tools: LAMP

**Table 1. Amplified DNA by LAMP for samples from the bucket traps in the first season of 2018-19**

18S primers	Phylloxera specific primers	Trap number
Negative	Negative	PR#111; PR#127; PR#129B; PR#131 SL#148; SL#152-153; SL#155; SL#158; SL#160
Positive	Negative	PR#112-124; PR#126; PR#128-129; PR#130; PR#131-134 SL#135-147; SL#149-151; SL#154; SL#156-157; SL#159
	Positive	PR#125

**Table 2A. Amplification time and annealing temperature on LAMP assay for phylloxera-positive plots in the Vineyard-PR in 2020.**

Phylloxera positive plot ID* <sup>1</sup>	LAMP assay	
	Amplification time (minutes)	Annealing temp (°C)
PR#022	26.24	79.46
PR#023	11.53	80.11
PR#025	11.47	80.13
PR#030	14.72	80.11
PR#034	12.72	80.11
PR#054	11.52	79.95
PR#066	13.63	79.63
PR#067	17.02	79.15
PR#074	15.48	79.65
PR#085	11.36	79.81
PR#088	23.02	79.00
PR#097	15.95	79.65
PR#165	17.50	79.63
Mean	15.55	79.72
Standard error (S.E.)	1.23	0.10

\*<sup>1</sup>Thirteen plots were detected as phylloxera positive within 150 experimental plots (8.7%).

**Table 2B. Amplification time and annealing temperature on LAMP assay for phylloxera-positive plots in the Vineyard-SL in 2020.**

Phylloxera positive plot ID* <sup>2</sup>	LAMP assay	
	Amplification time (minutes)	Annealing temp (°C)
SL#286	11.43	79.80

SL#304	12.53	79.97
SL#312	17.02	79.13
Mean	13.66	79.63
Standard error (S.E.)	1.71	0.26

\*<sup>2</sup>Three plots were detected as phylloxera positive within 150 experimental plots (2.0%).

## Appendix 5

In-field screening of G38 across multiple rootstocks.

Randomised rootstock vines across four blocks in the North East Victoria study site. Highlighted vines were monitored for phylloxera infestation (first instars and alates) using emergence traps

B4	101-14	SORI	SORI	126AA KOBER
	6A TELEKI	8B TELEKI	126AA KOBER	OWN ROOTS
	R88	6A TELEKI	8B TELEKI	SCWARZMANN
	SO4	3309C	OWN ROOTS	1103P
	1103P	R88	SO4	SORI
	8B TELEKI	126AA KOBER	1103P	SO4
	SORI	101-14	R88	8B TELEKI
	OWN ROOTS	OWN ROOTS	3309C	101-14
	SCWARZMANN	SCWARZMANN	101-14	R88
	126AA KOBER	SO4	6A TELEKI	6A TELEKI
	3309C	1103P	SCWARZMANN	3309C
	B3	1103P	126AA KOBER	SO4
3309C		R88	6A TELEKI	3309C
SO4		SCWARZMANN	SORI	8B TELEKI
SORI		3309C	SCWARZMANN	1103P
126AA KOBER		SO4	3309C	101-14
R88		OWN ROOTS	R88	SCWARZMANN
6A TELEKI		1103P	OWN ROOTS	126AA KOBER
8B TELEKI		6A TELEKI	126AA KOBER	SO4
101-14		8B TELEKI	101-14	6A TELEKI
SCWARZMANN		SORI	1103P	R88
OWN ROOTS		101-14	8B TELEKI	SORI
B2		6A TELEKI	SO4	R88
	3309C	3309C	SO4	1103P
	SORI	6A TELEKI	1103P	R88
	SCWARZMANN	8B TELEKI	3309C	6A TELEKI
	8B TELEKI	SCWARZMANN	6A TELEKI	SCWARZMANN
	SO4	126AA KOBER	OWN ROOTS	SO4
	126AA KOBER	101-14	101-14	SORI
	1103P	R88	SORI	101-14
	R88	SORI	126AA KOBER	3309C
	OWN ROOTS	OWN ROOTS	8B TELEKI	126AA KOBER
	101-14	1103P	SCWARZMANN	OWN ROOTS
	B1	R88	R88	SORI
126AA KOBER		8B TELEKI	SCWARZMANN	SCWARZMANN
6A TELEKI		101-14	101-14	OWN ROOTS
SORI		OWN ROOTS	126AA KOBER	6A TELEKI
3309C		SORI	OWN ROOTS	3309C
SCWARZMANN		6A TELEKI	8B TELEKI	SO4
8B TELEKI		126AA KOBER	R88	R88
1103P		3309C	1103P	1103P
OWN ROOTS		1103P	6A TELEKI	8B TELEKI
SO4		SO4	3309C	126AA KOBER
101-14		SCWARZMANN	SO4	101-14
ROW 95                      ROW 96                      ROW 97                      ROW 98				
		TRACK		

## Appendix 6

### Methods – Screening of rootstocks *in potted vines*

#### Infesting roots of potted vines with phylloxera

20 eggs of a particular phylloxera strains were collected from cultures maintained in the laboratory . The eggs were placed on strips of filter paper before infesting rootstock vines.

To infest vines, the entire plant was removed from the pot, and healthy lignified fibrous roots cleaned of soil. A mass of roots, each approximately 3 cm in length and 1cm wide was selected. A filter paper containing 20 eggs were placed face down onto the root mass and a small amount of potting mix gently placed on top. A muslin cloth was placed around the mound of potting mix, the filter paper with eggs and the roots, and the ends of the cloth carefully tied with a cable tie to enclose the insects, roots and soil into a pocket (Figure 1a). To stop phylloxera from escaping, a sticky gum-based insect barrier, *Tanglefoot*, was applied along the edges of the cloth, the vine repotted and topped up with potting mix and perlite. A further layer of Tanglefoot was applied around the base of stem and rim of pot to avoid cross contamination between replicate vines. At commencement of the trials, vines were fertilised with 3.5 g Osmocote™ and 500 ml Thrive™ per potted vine. Each vine was drip irrigated daily for two minutes. Eight replicate vines for both 5C Teleki and *V. vinifera* (control treatment) were used for each phylloxera strain. Vines were randomised in glasshouses according to the specified experimental design (Figure 1b). Temperatures in the glasshouses were set at 22°C (minimum 20°C and maximum 24°C) and monitored using Gemini Tinytag Ultra™ dataloggers (Hastings Data Loggers, Port Macquarie, New South Wales).



Figure 1. Infestation of potted vines a) (pocket of roots infested with phylloxera and b) set up of infested vines in glasshouses.

## Appendix 7

### Phylloxera genetic Diversity in the King Valley

Table 1. Phylloxera samples collected during the King Valley survey.

Vineyard	Location	Rootstock	No. of vines	No. of phylloxera
MIL-1	Milawa	1202	28	47
	Milawa	Swartzmman	10	19
	Milawa	Swartzmman	28	49
	Milawa	Swartzmman	30	33
	Total			148
CHE-2	Cheshunt	Swartzmman	13	29
	Cheshunt	Unknown1	13	27
	Cheshunt	Swartzmman	28	53
	Total			109
WHL-1	Whitland	Swartzmman	30	59
	Whitland	Swartzmman/Riparia Grigio	26	48
	Total			107
CHE-1	Cheshunt	Swartzmman	17	33
	Total			33
WHF-2	Whitfield	Swartzmman	30	48
	Total			48
WHF-1	Whitfield	Pulsen 10114	3	9
	Whitfield	Pulsen 10114	27	52
	Whitfield	Swartzmman	6	10
	Whitfield	Swartzmman	13	25
	Total			96
CHE-2P	Cheshunt	Swartzmman	30	60
	Cheshunt	Unknown2	30	63
	Total			123
EDI-1	Edi Upper	Swartzmman	30	61
	Total			61

Table 2. Genotypes on rootstocks

Rootstock	Genotype	Count of Genotype	Overall frequency of genotype (%)	Rootstock frequency of genotype (%)
1202	G04	24	3.31	51.06
	G20	22	3.03	46.81
	G109	1	0.14	2.13
	Total	47		
Pulsen 10114	G91	5	0.69	8.20
	G92	27	3.72	44.26
	G94	15	2.07	24.59
	G100	3	0.41	4.92
	G101	1	0.14	1.64
	G102	3	0.41	4.92
	G103	3	0.41	4.92
	G104	4	0.55	6.56
	Total	61		
Swartzmann	G04	6	0.83	1.25
	G20	116	16.00	24.22
	G35	49	6.76	10.23
	G41	6	0.83	1.25
	G84	12	1.66	2.51
	G85	1	0.14	0.21
	G86	8	1.10	1.67
	G87	1	0.14	0.21
	G88	5	0.69	1.04
	G89	2	0.28	0.42
	G90	1	0.14	0.21
	G91	16	2.21	3.34
	G92	61	8.41	12.73
	G93	3	0.41	0.63
	G94	28	3.86	5.85
	G95	16	2.21	3.34
	G96	2	0.28	0.42
	G99	41	5.66	8.56
	G101	1	0.14	0.21
	G102	1	0.14	0.21
	G104	57	7.86	11.90
	G105	37	5.10	7.72
	G106	6	0.83	1.25
G107	2	0.28	0.42	
G108	1	0.14	0.21	
Total	479			
Swartzmann/Riparia Grigio	G20	48	6.62	100.00
	Total	48		
Unknown1 (CHE-2)	G94	13	1.79	48.15

	G95	7	0.97	25.93
	G96	1	0.14	3.70
	G97	2	0.28	7.41
	G98	1	0.14	3.70
	G99	2	0.28	7.41
	G114	1	0.14	3.70
	Total	27		
Unknown2 (CHE-2P)	G84	1	0.14	1.59
	G88	15	2.07	23.81
	G90	1	0.14	1.59
	G92	2	0.28	3.17
	G94	3	0.41	4.76
	G99	17	2.34	26.98
	G101	7	0.97	11.11
	G104	6	0.83	9.52
	G107	3	0.41	4.76
	G110	1	0.14	1.59
	G111	3	0.41	4.76
	G112	2	0.28	3.17
	G113	1	0.14	1.59
	G115	1	0.14	1.59
	Total	63		

## Appendix 8

Products screened against phylloxera - availability, cost relative to bleach, practicality and effectiveness as a disinfestation treatment. Highlighted in blue is the recommended disinfestation procedure for footwear and hand-held tools.

Product name; concentration used; <i>active ingredient</i>	Source	OHS; Practicality	Cost per 5 litre footbath (AUD) <sup>a</sup> .	Evidence for management of pathogens and insect pests	Effectiveness / phylloxera genetic strains tested
<b>Household Bleach<sup>d</sup></b> ; 2% v/v; <i>Sodium hypochlorite</i>	Supermarket (IGA)	Damage to skin, irritant; Easy to prepare.	4.25	Phylloxera, mosquitoes.	E/All
<b>Dettol</b> ; 5% & 2.5% v/v; <i>Chloroxyleneol</i>	Supermarket (IGA)	Damage to skin, irritant; Easy to prepare.	4.25 & 2.13	Midges, spiders, mosquitoes.	NE at 2.5%; E at 5%/All
<b>Methylated spirit<sup>d</sup></b> ; 95% v/v; <i>Ethyl alcohol</i>	Supermarket (IGA)	Highly flammable; Easy to prepare.	20	Scales, mealy bug.	E/All
<b>Destainex<sup>b</sup></b> ; 2% w/v; <i>Sodium carbonate percarbonate, bicarbonate; Propylene glycol</i>	Test samples	Non- hazardous, non-dangerous, odourless; Easy to prepare.	0.72	Surface cleaner. No evidence against insect pests.	NE/ G4, G20
<b>Enviro-san<sup>b</sup></b> ; 1% & 0.4% v/v; <i>Dodecylbenzenesulfoni c and lactic acid &lt;10%</i>	Test samples	Causes severe burns and eye damage; Easy to prepare.	0.60 & 0.24	Surface cleaner. No evidence against insect pests.	NE/ G4, G20, G38
<b>Vinclean<sup>b</sup></b> ; 1% v/v; <i>Sodium carbonate&gt;60%, Disodium metasilicate 18-30%</i>	Test samples	Non- hazardous, non-dangerous; Easy to prepare.	0.51	Surface cleaner. No evidence against insect pests.	NE/ G4
<b>Linvasan<sup>b</sup></b> ; 1% w/v; <i>demineralised water, tartaric acid FG, hydrogen peroxide proprietary chelatant- stabiliser</i>	Test samples	Corrosive, irritant to eyes and skin; Easy to prepare.	0.31	Surface cleaner. No evidence against insect pests.	NE/ G4
<b>White Vinegar</b> ; As is; 5-20% <i>Acetic acid</i>	Supermarket (IGA)	Hazardous, irritant; Easy to prepare.	2.79	Surface cleaner. No evidence against insect pests.	NE/ G1, G4



<b>Mineral Turpentine<sup>c</sup></b> ; As is; <i>Hydrocarbon Liquid</i>	Hardware stores (Bunnings)	Highly corrosive, irritant; Easy to prepare.	18.75	Paint thinner. No evidence available against insect pests.	E/G4
<b>Epsom salts</b> ; 1.5% w/v; Magnesium sulfate	Supermarket (IGA)	Causes nausea, headache when taken by mouth.	0.62	Pest deterrent	NE/ G4
<b>Common salt</b> ; 0.5% w/v & 3.5%; <i>Sodium Chloride</i>	Lab supplies Sigma	Irritant to skin.	2.83 & 19.81	Green vegetable bug ( <i>Nezara viridula</i> ) in beans and cotton.	NE/ G4
<b>Coffee</b> ; 10% w/v; <i>Caffeine</i>	Supermarket (IGA)	Non- hazardous.	23.35	Deterrent of garden pests.	NE/ G4
<b>Cayenne pepper</b> ; 1%; <i>Capsaicin</i>	Supermarket (IGA)	Causes eye and skin irritation.	4.3	Deterrent of garden pests.	NE/ G4
<b>Borax</b> ; 1%; <i>Disodium tetraborate decahydrate</i> 999g/kg	Hardware store (Bunnings)	Non- hazardous.	0.41	Cleaning agent. Deterrent of cockroaches, ants.	NE/ G4
<b>Coca cola</b> ; As is; <i>Sugar, caffeine, phosphoric acid</i>	Supermarket (IGA)	Non- hazardous; Easy to prepare.	25	Deterrent of rice pests.	NE/ G4
<b>Steri-maX Biocide</b> ; 1%v/v; 120g/L <i>DDAC Didecyl dimethyl ammonium chloride</i>	Test sample (Promotion sample)	Irritant to skin; Easy to prepare.	0.9	Disinfectant in poultry sheds and equipment against bacteria, fungal spores and viruses.	NE/ G4
<b>FarmFluid<sup>c</sup></b> ; 1%; Tar acid 419.2g/L, Acetic acid 324.9g/L, Cresylic Acid 52.4g/L	Test sample (Promotion sample)	Corrosive, causes allergic disorders, irritant.	1.28	Disinfectant of farm equipment.	E/ G4
<b>Klorsept 87</b> ; 1 tablet: 25L H <sub>2</sub> O; <i>Sodium dichloroisocyanurate</i> 500g/kg and available chlorine 32.3%w/w	Test sample (Promotion sample)	Irritant, hazardous to environment	2.01	Bacteria, fungi, spores and viruses.	NE/ G4

<b>Klorkleen</b> ; 1 tablet:5L H <sub>2</sub> O; <i>Sodium dichloroisocyanurate, Adipic acid and Acid Yellow 23</i>	Test sample (Promotion sample)	Irritant, hazardous to environment.	0.81	Bacteria, fungi, spores and viruses.	NE/ G4
<b>Virkon<sup>d</sup></b> ; 1% (w/v); 49.8% <i>potassium peroxymonosulfate</i>	Chemist (Medshop)	Low toxicity; Easy to prepare.	1.38	Viruses, bacteria, fungi, Avian influenza, salmonella and campylobacter	NE/ G1, G4, G19, G20, G30
<b>Phytoclean<sup>d</sup></b> ; 10% (v/v); 100g/L <i>benzalkonium chloride</i>	AgNVet	Damaging to eyes, irritant, allergen.	8.48	Sanitiser and disinfectant of various plant pathogens.	NE/ G1, G4, G19, G20, G30
<b>Pulse Penetrant<sup>d</sup></b> ; 0.2% (v/v); 1020g/L <i>olydimethyl siloxane</i>	AgNVet	Irritant if inhaled, hazardous.	0.71	Mealy bugs, leafhoppers, psyllids.	NE/ G1, G4, G19, G20, G30
<b>Biopest<sup>d</sup></b> ; 1% (v/v); 815 g/L <i>paraffinic oil</i>	Test sample (Promotion sample)	Irritant to skin.	0.25	Aphids and mites.	NE/ All
<b>Ethanol<sup>d</sup></b> ; 80% (v/v); <i>absolute alcohol</i>	Wilmar Bioethanol	Flammable, irritant to eyes.	5.03	Green peach aphid <i>Myzus persicae</i> Sulzer.	NE/ All
Teracep	-	Toxic	Not applicable		
Bytrol; 0.5%; <i>Polyhexmethylene hydrochloride, dimethylammonium chloride, benzalkonium chloride</i>	-	Toxic	-	Used in orchards; Footbath for plant pathogens	NT
Kiwi Lustre; 1%; <i>Phosphate lactic acid.</i>	-	Toxic	-	Used in orchards; Footbath for plant pathogens	NT
Citrox; 1%; <i>Citrus pulp extract, water, citric acid.</i>	-	Toxic	-	Used in orchards; Footbath for plant pathogens	NT

<sup>a</sup>Prices inc GST as at May 2020

<sup>b</sup>Industry recommendation

<sup>c</sup> Discontinued due to damaging and hazardous effects to staff and vials after testing with G4 phylloxera

<sup>d</sup>Data used for comparing product effectiveness were derived from similar studies under project DED1301 (Powell, 2017). **E**=Effective, **NE**=Not Effective; **NT**=Not tested. **ALL** means six phylloxera strains were tested (G1, G4, G19, G20, G30 and G38) unless specified.

# Appendix 9

## Feasibility of biological control of grape phylloxera

### Biocontrol options for grape phylloxera in Australia

Agriculture Victoria Research Technical Report

## **Appendix 10**

### **Terms of Reference Industry Reference Group**

**Wine  
Australia  
for  
Australian  
Wine**

**Project DED 1701**

**Integrated Management of Established Grapevine Phylloxera**

**Project Industry Reference Group**

**Terms of Reference**

## Industry Reference Group Meeting

Location: Agriculture Victoria (Attwood)  
475 Mickleham Rd, Attwood, Vic, 3049  
Friday 8 October 2019, 8:30 AM to 12:30 PM (Vic time)

### AGENDA

Chair: Inca Lee

8:30	Welcome, apologies, introduction	Inca Lee
8:45 – 9:45	Project overview Project tracking and results to date (including discussion)	Paul Cunningham/ Catherine Clarke
9:45-10:05	<b>Morning tea</b>	
10:05	Project tracking and results to date (including discussion) (cont.)	Paul Cunningham/ Catherine Clarke
10:35	Variation (sniffer dogs) and new LAMP component	Paul Cunningham
10:45	Communications update and engagement	Paul Cunningham/ Catherine Clarke
11:00	Discussion on priorities for next phase research (2020-2023)	All
11:45	Any other business Next meeting	Inca Lee
12:00	<b>Lunch and depart</b>	

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#### Attendees:

Paul Cunningham	Agriculture Victoria
Catherine Clarke	Agriculture Victoria
Daniel Mansell	Agriculture Victoria
Nick Secomb	Biosecurity SA
Mark Krstic	Australian Wine Research Institute
Andy Clarke	The Dirt Dude
Damien Sheehan	Mount Langi Ghiran
Brett McClen	Brown Brothers
Sharon Harvey	Wine Australia
Ben Harris	Treasury Wine Estates
Harley Smith	CSIRO
Anna Hooper	Australian Grape & Wine
Suzanne McLoughlin	Vinehealth Australia
Inca Lee	Vinehealth Australia

**INTEGRATED MANAGEMENT OF ESTABLISHED  
GRAPEVINE PHYLLOXERA**

**Industry Reference Group Meeting**

Location: via Zoom  
Wednesday 17 June 2020, 9:30 to 11:00 AM (SA time)

**AGENDA**

Chair: Inca Lee

9:30	Welcome, apologies, introduction	Inca Lee
9:35 – 9:55	Update on research since last Industry Reference Group meeting	Paul Cunningham/ Catherine Clarke
9:55 – 10.55	Discussion on next phase of work	All
10:55 – 11.00	Other business / close	Inca Lee

# Appendix 11

Recommendations for the NPMP review

**Review of Disinfestation Protocols in the National  
Phylloxera Management Protocol**

AGRICULTURE VICTORIA