Government of South Australia
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South Australian Research and Development Institute

Australian Government
Grape and Wine Research and Development Corporation

Virus Tested Clones for National Nuclear Grapevine Collections

FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **SAR 03-05**

Principal Investigator: **Philip R. Nicholas**

Research Organisation: **South Australian Research and Development Institute**

Date: **December 2006**

Project title: Virus tested clones for national nuclear grapevine collections

GWRDC Project No: SAR 03/05

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Date: December 2006

Cover picture: Biological indexing test vine showing leafroll virus symptoms, which develop after inoculation of Cabernet Franc with a clone which has leafroll virus.

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Contents

1. Abstract

Over 300 clones were selected from the main grapevine genetic resource collections in Australia and screened for viruses, using RT-PCR and ELISA laboratory methods, then biologically indexed for viruses in the field. Clones that satisfied required virus status standards have been planted by the Australian Vine Improvement Association as nuclear clones in the Australian National Nuclear Grapevine Collection at Dareton, NSW. The clones will be made available under a licence agreement to vine improvement groups in Australia. A publication 'Grapevine clones used in Australia', which contains background information on the nuclear clones is available from the website www.sardi.sa.gov.au.

2. Executive summary

Many vineyards in Australia have vines with virus infections due to the use of infected planting material. Leafroll viruses are the most detrimental of these. They cause yield losses which vary from minor to more than 50% and affect grape quality by delaying maturity and reducing fruit colour in red varieties. Rugose wood viruses can affect vine vigour and yield and cause incompatibility and death of grafted vines. It is crucial that clones distributed in vine improvement schemes are from a virus tested source.

There are a number of genetic resource collections of grapevines in Australia – the largest of these are held by CSIRO and SARDI. However, when this project began, there were no nuclear collections. These are collections of clones certified free of important viruses, which are established as source material for vine improvement schemes. This project was seen as the first step in the establishment of such collections – the sanitary selection of clones.

Over 300 candidate clones in the project were selected from the main genetic resource collections in Australia. Preliminary screening of these clones for viruses was undertaken using RT-PCR and ELISA laboratory methods. The remaining clones were then further screened using biological indexing. This involved grafting them to indicators, growing them for several years in a field planting and observing them for virus symptoms.

The Australian Vine Improvement Association has established the Australian National Nuclear Grapevine Collection, with nuclear clones selected in the project, at the NSW DPI Research Station at Dareton. Protocols have been developed for the management of the collection and the clones will be made available under a licence agreement to vine improvement groups in Australia.

As part of this project a publication ‘Grapevine clones used in Australia’ has been produced, which contains more detailed information on the nuclear clones. It is available on the SARDI web site www.sardi.sa.gov.au. This publication will be of particular value in publicising the clones and will also be of interest to Australian vine improvement groups for its inclusion of the clones presently distributed in Australian vine improvement schemes. A companion ‘National Register of Grapevine Varieties and Clones’ has been produced which contains the clones held in genetic resource collections in Australia considered for inclusion in the project.

The Australian National Nuclear Grapevine Collection at Dareton is unique in Australia and industry should support the future maintenance and expansion of this collection, particularly in the funding of RT-PCR virus testing and biological indexing of additional clones.

3. Background

3.1 Grapevine collections

The recognition that Grapevine leafroll-associated virus 3 (GLRaV-3) was spreading in grapevine collections, which were the primary source of grapevine material in vine improvement schemes, gave an urgent stimulus for the development of a totally new system for the maintenance of germplasm in Australia. A study tour in 1998 by the author to most research institutes involved with vine improvement programs in USA, Canada, Germany, France, Switzerland and Italy revealed that two different types of grapevine germplasm collections are held in most countries:

- Genetic resource collections – these are maintained to retain genetic diversity of those varieties currently used commercially and to preserve varieties not presently used. The varieties held are also useful for ampelographical comparisons and for breeding. They typically contain a large number of clones – for example the largest collection, at Vassal (near Montpellier in France), has 3,200 varieties represented as 7,200 clones. Although many clones in these collections have viruses, such as leafroll, the virus status of clones is really not an issue.
- Nuclear collections of elite clones distributed in vine improvement schemes – these contain a much smaller number of clones which must be certified free of important viruses by biological indexing before they can be planted in the collection.

In Australia, the largest genetic resource collections are held by CSIRO at Merbein in Victoria and SARDI at Nuriootpa in South Australia. The SARDI collection is somewhat unique in that it contains many clones of the major winegrape varieties that have been identified over the previous 40 years of the SA Vine Improvement Program. There are also other collections held in Victoria, New South Wales, Western Australia, Tasmania and Queensland (Nicholas 2006a). A genetic resource collection is considered an unsatisfactory site to hold virus tested clones, as virus may be spread by an insect vector within it. When this project began, there were no nuclear collections in Australia and the project was seen as the first step in the establishment of such collections – the sanitary selection of clones. Sanitary selection involves the elimination of clones with detrimental viruses and other disease agents (Martelli 1999).

3.2 Grapevine viruses

The effect of virus diseases on grapevine yield and quality was reviewed by Walter and Martelli (1997) and Mannini (2003). Grapevine viruses and sanitary selection have recently been reviewed with reference to Australia (Nicholas 2004) and some information from this review follows as background for the project.

3.2.1 Leafroll viruses

Leafroll viruses are considered the most detrimental with reported yield losses varying from minor to more than 50%. Average yield loss has been estimated to be 20% in California (Goheen 1982). Leafroll viruses can also affect grape quality by causing delayed maturity, and reduced fruit colour of red varieties. Symptoms of the disease are displayed in autumn as a downward rolling of the margins of leaf blades and premature interveinal colouration (red with red varieties and yellow with white varieties). The most common leafroll-associated virus detected in diagnostic RT-PCR tests in Australia is GLRaV-3 followed by GLRaV-1 and GLRaV-9 (Habili and Symons 2000, Habili and Rowhani 2002). GLRaV-2, which is less common, is reported to be involved with the incompatibility of scions grafted onto 5BB Kober (Greif *et al.* 1995). Other leafroll viruses that are less common are GLRaV-4, which is found in the Sultana clones H4 and H5 (Habili *et al.* 1996), and GLRaV-5 found in a clone of Tempranillo.

3.2.2 Rugose wood

Rugose wood is a complex disease caused by viruses. It is characterised by symptoms on the woody cylinder (Figure 6-4) beneath the bark of the trunk. Symptoms are uncommon on ungrafted rootstocks and scions, but may appear following grafting. They include swelling above the graft union, with a marked difference in diameter between the scion and rootstock; and pits or grooves on the woody cylinder. Vine yield and vigour are often reduced. Budburst may be delayed and vines may decline or even die. Water stress in combination with rugose wood and leafroll viruses will increase the severity of symptoms.

Four different rugose wood disorders can be distinguished by biological indexing (Garau *et al.* 1997). These are Rupestris stem pitting, Kober stem grooving, corky bark and LN33 stem grooving. Rupestris stem pitting symptoms have been associated with the *Foveavirus* RSPaV, which is present in most Australian vineyards (Habili *et al.* 2006) – this may not be of major economic significance for vines on their own roots (Reynolds *et al.* 1997).

The phloem-limited *Vitivirus* Grapevine virus A (GVA) is the agent of Kober stem grooving (Garau *et al.* 1994, Chevalier *et al.* 1995). It can be symptomless in some varieties (Garau *et al.* 1990), but has been associated with Shiraz Disease in South Africa (Goszczynski and Jooste 2003) and Australia ((Habili and Randles 2004). Symptoms of Shiraz Disease include: delayed budburst; stunted growth; canes which never mature; and leaves with leafroll-virus-like symptoms that do not drop in winter.

Corky bark symptoms are characterised by atypical production of corky tissues above the graft union. The phloem-limited *Vitivirus* Grapevine virus B (GVB) has been associated with corky bark symptoms, but its presence is not always consistent with symptoms (Bonavia *et al.* 1996). There is a large sequence variation in the genome of GVB isolates (Shi *et al.* 2004). Although GVB has been detected in some Australian vineyards, corky bark is not known to occur in Australia (Whattam 2001).

3.2.3 Fleck

Grapevine fleck virus is common in Australia, but it is often unrecognised because varieties of *V. vinifera* and most rootstocks are symptomless. Translucent leaf spots are characteristic symptoms when Rupestris St George is used as the indicator. At least two variants of the virus exist (Shi *et al.* 2003) and they can be detected by a single RT-PCR assay. The detrimental effects of fleck have been described in a review by Walter and Martelli (1997). In particular, it can reduce graft take.

3.2.4 Fanleaf

Grapevine fanleaf virus is commonly found throughout the world, but not so in Australia (Habili *et al.* 2001), because the nematode vector *Xiphinema index* is confined to a small area near Rutherglen, Victoria. Fanleaf is very rarely seen in other regions in Australia. Symptoms of fanleaf include malformed fan-shaped leaves; canes with zigzag growth; foliage with chlorotic discolourations; fewer and smaller bunches; poor fruitset and shot berries; and greatly reduced yield.

3.2.5 Virus elimination

There are procedures which can be used for eliminating virus diseases from grapevines. In the past, heat treatment (HT) i.e. thermotherapy was the only method available (Nyland and Goheen 1969). Meristem tip culture was found to be a more successful method of removing leafroll virus (Savino *et al.* 1990). The procedure has been refined to the extent that the method is now considered to be reliable (Golino *et al.* 1998). Fragmented shoot apex culture (FSAC) has also been used in Australia to remove grapevine viruses (Barlass *et al.* 1982). This project has not involved a virus elimination program, but many of the clones tested have previously gone through HT or FSAC and this is reflected in the clonal names.

3.3 Testing for viruses

3.3.1 Disease detection by biological indexing

Historically biological indexing with woody indicators has been the method used in the sanitary selection of grapevine clones for vine improvement schemes. Indexing conducted in Australia involves grafting candidates and indicators together and the resultant vines are then grown for several years in the field to observe any virus symptoms. Therefore, this test detects the disease rather than the associated virus. The early indexing work in Australia was reported by Shanmuganathan and Fletcher (1980) and Cirami *et al.* (1988). Subsequent indexing in Australia was continued by G. Fletcher and by L. Krake from CSIRO (Krake *et al.* 1999). The biological indexing program for this project began in year 2000 and both G. Fletcher and L. Krake were initially closely involved including providing advice on the design and assessment procedures used. Indexing with woody indicators is still considered to be essential in sanitary selection.

3.3.2 Virus detection by laboratory methods

Laboratory methods are usually based on scrapings of the cortex of dormant (woody) cuttings (Habili and Randles 2002). Although green tissue can be sampled, timing is important as test reliability varies for different viruses at different times of the year. The use of ELISA

serological testing procedures for virus testing in grapevines has been reviewed by Boscia *et al.* (1997). ELISA tests are relatively cheap and easy to perform, provided high quality antiserum for the virus is available. The use of RT-PCR molecular methods has been reviewed by Minafra *et al.* (1997). As RT-PCR involves amplification and detection of a small part of the genome of the virus, this test is far more sensitive than ELISA. Test results from indexing and laboratory methods are complementary and their combined use provides a more reliable indication of virus status.

3.4 Project objectives

The project objectives were:

- To select elite clones free of important viruses by using three virus detection procedures, for all varieties and rootstocks likely to be used in future winegrape plantings in Australia
- To make these clones available for establishment in a new nuclear collection in an isolated location, to supply base material for vine improvement schemes
- To publish a catalogue for industry containing information on all clones held in the nuclear collection.

4. Project aims and performance targets

Outputs	Performance Target
1. Clones from existing collections screened for virus by RT-PCR and ELISA testing to supply elite selections for the establishment of a nuclear collection	More than 300 clones selected and material from these RT-PCR and ELISA tested (2000-01, 2001-02, 2002-2003)
2. Clones biologically indexed	Clones which have been screened free of virus by RT-PCR and ELISA tests, then biologically indexed (2001-02, 2002-2003, 2003-2004, 2004-2005, 2005-2006)
3. Establishment of new nuclear collection	Clones made available from project for planting in new collection (2004-05)
4. Publication of catalogue for industry containing information on clones held in the nuclear collection	Catalogue revised as more virus information becomes available (2003-2004, 2004-2005, 2005-2006)

5. Methods

5.1 Selection and screening of candidate clones

Over 300 candidate clones for the nuclear collection were initially selected in close consultation with the Australian Vine Improvement Association from existing clones in vine improvement schemes and other clones considered potentially useful from their known performance in trials. For each candidate, a vine was selected from the genetic resource collections held by SARDI at Nuriootpa in South Australia, VAMVIA at Irymple and CSIRO at Merbein in Victoria and all cuttings of the candidate were taken from this vine for the clone to undergo sanitary selection. This initially involved rapid screening of the clones for viruses primarily using RT-PCR, but also ELISA laboratory methods. In the cases where the vine selected had leafroll virus infection and it was suspected that this was from historically recent spread, tests on other vines of the clone from the same or different collections were used to try to obtain a vine free of leafroll.

Table 5-1 Virus detection methods and indexing indicators used for sanitary selection in the project

Virus diseases	RT-PCR tests for pathogen	ELISA tests for pathogen	Biological indexing indicator
Leafroll			
Grapevine leafroll	GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, GLRaV-5, GLRaV-9, GRSLaV	GLRaV-1, GLRaV-3	Cabernet Franc
Rugose wood complex			
Rupestris stem pitting	RSPaV1, RSPaV2		Rupestris St George
Kober stem grooving	GVA	GVA	5BB Kober
Grapevine corky-bark	GVB		LN33
LN33 stem grooving			LN33
Fleck			
Grapevine fleck virus	GFkV-A, GFkV-B	GFkV	Rupestris St George
Fanleaf			
Grapevine fanleaf virus	GFLV		Rupestris St George

5.2 RT-PCR and ELISA testing

RT-PCR and ELISA procedures used in the project involved taking scrapings of the cortex of dormant (woody) cutting and the tests that were conducted are given in Table 5-1. RT-PCR testing was conducted by Waite Diagnostics in Adelaide using the method of MacKenzie *et al.* (1997). ELISA testing was done by Crop Health Services at Knoxfield using tests supplied by Bioreba AG, Reinach, Switzerland according to the manufacturer's instructions. These tests resulted in the elimination of many clones with virus infection and the selection of specific clones, which appeared to be free of virus. These selected clones and some clones where RT-PCR results were inconclusive were then biologically indexed.

5.3 Biological indexing

Indexing of the clones was conducted at SARDI Loxton Research Centre. Indexing procedures have been reviewed by Martelli (1993) and Garau *et al.* (1997). A range of grafting methods can be used depending on the availability and diameter of indicator wood. Indexing has not been conducted on such a large scale before in Australia and the availability of indicator wood was the factor limiting the number of candidates, which could be grafted in each year and the method used. All available indicator wood grown by SARDI, DPI Victoria and CSIRO was RT-PCR tested. Some was rejected because of virus status and the remainder collected for use. Candidates were grafted to the four indicators given in Table 5-1.We used both chip budding and bench grafting methods:

- Chip budding – we propagated indicator vines in pots in winter, chip budded them with the candidate in summer and planted and trained up the vine in spring the following growing season.
- Bench grafting – we used combinations considered acceptable by Garau *et al.* (1997). For Rupestris St George and 5BB Kober we grafted the candidate onto the indicator with a bud being left under the graft on the indicator from which a shoot was trained up. For LN33 (due to the shortage of indicator material) and Cabernet Franc we grafted the indicator onto a candidate cutting. Grafting was done in winter and the vines generally planted in late spring.

The vines were planted at a spacing of 1.5 x 2.5 m and trellis and drip irrigation installed. For each grafted candidate/indicator combination, 3 replicate vines and an indicator control vine were planted. The planting area for the project covered 2.0 hectares. Test vines were observed over 3 growing seasons for leaf symptoms of leafroll virus (Figure 5-1), fleck and fanleaf viruses and cane symptoms of corky bark virus. When these observations were complete, the rugose wood test vines were dug (in May/June each year) and the trunks were boiled in water for one hour (as is done in an indexing program in Bari, Italy). The bark was then removed and the trunks inspected in the laboratory for symptoms of the rugose wood viruses to complete the indexing.

A virus reference collection was established at SARDI Loxton Research Centre as has been done in Europe (Greif and Walter 1997). Each year that grafting was conducted for indexing, clones with known viruses from the collection were grafted to indicators to establish positive controls, which were planted at the same time as candidate test vines to assist in identifying symptoms in the indexing.

Figure 5-1 Plot of vines in the biological indexing program showing a control vine followed by three vines infected with leafroll virus.

6. Results/discussion

6.1 RT-PCR and ELISA testing

RT-PCR testing of grapevine collections for viruses (Figure 6-1) was first conducted in Australia by SARDI at Nuriootpa in 1998 and was soon adopted by other states. Thus extensive additional RT-PCR data were available for use in the selection of clones, besides that obtained in the project. This enabled the appraisal of many of the clones over several years in collections in different states. Because of the occurrence of 'false negatives' in RT-PCR tests, the more data available the greater the confidence in assessing the true virus status of clones. Apart from quickly eliminating clones which have been long infected with viruses (Table 11-1), RT-PCR was also useful where vines of a particular clone had a historically recent infection with leafroll virus in one collection but not in another, allowing the selection of a disease free vine. In the initial years of the project, ELISA tests (Figure 6-2) were also considered in the selection of clones. However, greater use was made of RT-PCR data, because of the greater number of tests available from all sources and the greater number of viruses for which RT-PCR tests were available.

6.2 Biological indexing

In the project we had the opportunity to produce vines for indexing by different methods suggested by Garau *et al.* (1997). In an ideal situation the best way to produce these vines may be to chip bud inoculate one year old potted indicator vines. In our situation an early start to the project was required and one year old potted vines were not available. Also, although all available indicator wood in Australia was used, we had limitations on availability of the wood for the number of candidates to be grafted.

We consider we achieved good results in the indexing program, particularly as we inspected test vines for visual symptoms in the season of planting and in the following two growing seasons. Also rugose wood observations were made in the laboratory rather than in the field as in some other programs. Some observation we made were:

- Leafroll viruses – the degree of symptom expression for leafroll viruses (Figure 6-3) varied from year to year and for different viruses (we had positive controls of GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, GLRaV-5 and GLRaV-9 and found with these controls that GLRaV-4 and GLRaV-9 displayed weaker symptoms on Cabernet Franc).
- Rugose wood viruses – we found symptoms on Rupestris St George the indicator for Rupestris stem pitting (Figure 6-5) for about 80% of candidates. We also found symptoms of Kober stem grooving (Figure 6-4) on 5BB Kober for some candidates indicating the presence of GVA. We found no instance of symptoms on indicators for corky bark or LN33 stem grooving for any of the candidates.
- Fleck virus – it is particularly useful to use all detection methods ie RT-PCR and ELISA as well as indexing to confirm the presence of fleck virus.
- Fanleaf virus – we found no instance of fanleaf infection in any of the candidates by indexing (or by laboratory methods).

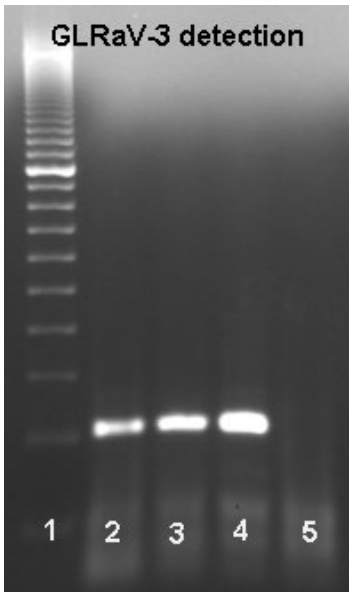

Figure 6-1 RT-PCR test showing in lane 1 DNA ruler, lanes 2 and 3 Grapevine leafroll-associated virus 3 positive samples, lane 4 positive control and lane 5 negative control – supplied by N Habili, Waite Diagnostics.

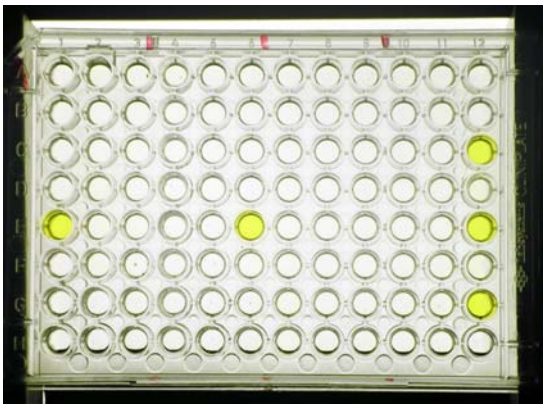

Figure 6-2 ELISA plate showing the positive reaction to Grapevine leafroll-associated virus 3 in yellow (5 of the 96 samples tested positive) – supplied by N Habili, Waite Diagnostics.

Figure 6-3 Biological indexing test vine showing leafroll virus symptoms, which develop after inoculation of Cabernet Franc with a clone which has leafroll virus.

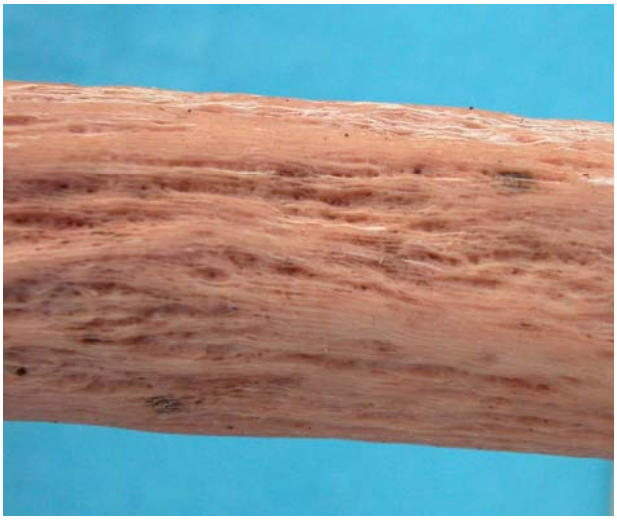

Figure 6-4 Biological indexing test showing Kober stem grooving symptoms, which develop after inoculation of 5BB Kober with a clone which has Grapevine virus A (GVA) seen after removal of bark.

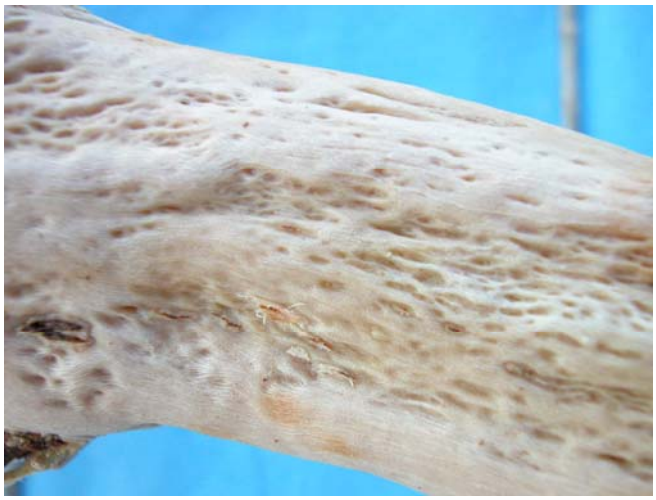

Figure 6-5 Biological indexing test showing Rupestris stem pitting symptoms, which develop after inoculation of Rupestris St George with a clone which has stem pitting seen after removal of bark.

6.3 Australian National Nuclear Grapevine Collection

The Australian Vine Improvement Association has recognised the unique quality of the virus tested clones selected in the project and in 2004 established the clones in a new Australian National Nuclear Grapevine Collection (Figure 6-6) at the NSW DPI Research Station at Dareton (Kerridge 2005, Nicholas 2006b). Four vines of each clone have been established at a spacing of 1.8m x 3.3m on a vertical shoot positioning trellis. A rabbit proof fence has been erected around the collection leaving enough space for the collection to be doubled in size.

Figure 6-6 Australian National Nuclear Grapevine Collection

The clones planted in the nuclear collection are given in Appendix 2 Table 10-1. The cuttings taken for the propagation of each clone were from a single vine and the RT-PCR tests in Table 10-1 were done on cuttings taken from this vine at the same time as the cuttings were taken for propagation. The indexing results in Table 10-1 were also from cuttings taken from the same vine. The ELISA tests in Table 10-1 were done on a composite sample taken in 2006 from each of the four vines of the clone planted in the nuclear collection. Viruses that spread are easily detectable by RT-PCR and ELISA and there was no evidence of any infection by virus during the propagation process. The vines growing in the collection have also been checked visually for leafroll virus each year.

In future, all vines in the nuclear collection will be periodically ELISA tested and checked visually each year to ensure that they remain free of viruses. The vines have all been checked by ampelographer George Kerridge for trueness to type.

Protocols have been developed in liaison with industry for the management of the nuclear collection (Anon. 2006). SARDI has prepared draft licence conditions for the further distribution of the clonal material to ensure its continued integrity and that its future health status is not compromised. The clones will be made available to vine improvement groups in Australia, provided there is agreement to these conditions.

Most of the clones in the nuclear collection of the varieties Cabernet Sauvignon, Chardonnay, Merlot, Riesling, Semillon and Shiraz have also been planted in fully replicated clonal trials at Monash in South Australia by the Riverland Vine Improvement Committee.

Additional clones on which indexing has commenced for this collection (and for which indexing needs to be continued beyond the project) are given in Table 6-1.

Table 6-1 Clones on which indexing is continuing

Variety	Clone	Variety	Clone
Wine grapes		Pinot Noir	115 FSAC
Aglianico	FPS 01	Pinot Noir	375 FSAC
Albarino	Galicia	Pinotage	FPS 01
Arinto	FSAC	Riesling	110-18
Chardonnay	277 FSAC	Riesling	198-25
Chardonnay	76 FSAC	Riesling	F8V13 FSAC
Chardonnay	95 FSAC	Sauvignon Blanc	Q97-20C
Chardonnay	96 FSAC	**Drying grapes**	
Chardonnay	G9V7 FSAC	Sultana	H5 2-2-255
Marsanne	Rutherglen	**Rootstocks**	
Merlot	FPS 18	110 Richter	Q554-01
Merlot	D3V14 FSAC	775 Paulsen	FPS 02
Nebbiolo	FPS 10	K51-32	FSAC
Pinot Noir	114 FSAC		

6.4 Catalogue

Information is available on clones distributed in many overseas schemes, including those in California (Bettiga *et al.* 2003), France (Boidron 1995) and Italy (Calo 2000). As part of the project the publication 'Grapevine clones used in Australia' has been produced, which contains background information on the clones planted in the nuclear collection (Nicholas 2006c). It is available on the SARDI web site www.sardi.sa.gov.au and is attached to this report. This catalogue will be of particular value in publicising the clones and will be of further interest to Australian vine improvement groups for its inclusion of the other clones distributed by vine improvement schemes in each Australian state. A companion to this has also been produced – the 'National Register of Grapevine Varieties and Clones' (Nicholas 2006a), which is a list of the clones held in genetic resource collections in Australia. (These were the clones considered for inclusion in the project). This register is available from the Australian Vine Improvement Association.

7. Outcomes/conclusions

The project objectives have been accomplished:

- Sanitary selection of a comprehensive collection of elite clones free of important viruses, from the major Australian genetic resource collections, has been achieved by eliminating those with debilitating viruses using RT-PCR, ELISA and biological indexing procedures. This has been achieved for the first time in Australia.
- The clones representing all major varieties and rootstocks likely to be used in future winegrape plantings in Australia have been established in the Australian National Nuclear Grapevine Collection at Dareton. Protocols have been established for the management of the nuclear collection and making the elite clones available to vine improvement groups in Australia.
- A catalogue has been produced which contains information on the clones planted in the nuclear collection.

The longer term outcomes will be that vineyards planted with propagation material sourced through vine improvement schemes distributing these clones will have superior health status and not be subject to reductions in yield and grape quality and stock/scion compatibility problems caused by viruses.

8. Recommendations

Further research in the area covered by this project is continuing in the GWRDC funded project ‘Development and validation of diagnostic protocols for the detection of endemic and exotic pathogens of grapevines’. Close liaison is being maintained with the leaders of this project Dr B. Rodoni and Dr F. Constable to ensure that there is continuity of information and findings between the two projects.

The Australian National Nuclear Grapevine Collection at Dareton is unique in Australia and industry should support the future maintenance and expansion of this collection, particularly in the funding of RT-PCR virus testing and biological indexing of additional clones from local and international sources to ensure Australian grapegrowers have access to the best possible planting material into the future.

9. Appendix 1: Communication

During the course of the project close liaison has been maintained with the Australian Vine Improvement Association concerning the establishment of the Australian National Nuclear Grapevine Collection. The catalogue produced will be used to publicise the results of the project. Publications related to the project are given below.

Constable F, Connellan J, Bass T, Nicholas P, Habili N, Rodoni B (2006) Genetic variation of *Grapevine virus A* and *Grapevine leafroll associated virus-3*. In '7th Australasian Plant Virology Workshop'. Rottnest Island, Western Australia.

Habili N, Farrokhi N, Lima MF, Nicholas P, Randles JW (2006) Distribution of Rupestris stem-pitting-associated virus variants in two Australian vineyards showing different symptoms. *Annals of Applied Biology* **148**, 91-96.

Nicholas PR (2004) Grapevine planting material. In 'Viticulture Volume 1 - Resources'. (Eds PR Dry, BG Coombe) pp. 189-195. (Winetitles: Adelaide).

Nicholas PR (2004) How clean is my planting material? In 'Proceedings of Conference Viticulture 2004 Growing our Future'. Mildura. (Department of Primary Industries Victoria).

Nicholas PR (2006) 'National Register of Grapevine Varieties and Clones.' (Australian Vine Improvement Association: Mildura, Victoria).

Nicholas PR (2006) Selection of clones for the Australian National Nuclear Grapevine Collection. *Australian and New Zealand Grapegrower and Winemaker* **508**, 32-35.

Nicholas PR (2006) 'Grapevine clones used in Australia.' (South Australian Research and Development Institute).

10. Appendix 2: Intellectual property

Table 10-1 Clones selected in the project which have been planted at the Australian National Nuclear Grapevine Collection at Dareton and virus tests on these clones – see Nicholas (2006c) for background information on these clones.

		RT-PCR														ELISA				Biological Indexing							
Variety	**Clone**	**GLRaV-1**	**GLRaV-2**	**GLRaV-3**	**GLRaV-4**	**GLRaV-5**	**GLRaV-9**	**RSPaV-1**	**RSPaV-2**	**GVA**	**GVB**	**GFkV-A**	**GFkV-B**	**GFLV**	**GRSLaV**	**GLRaV-1**	**GLRaV-3**	**GVA**	**GFkV**	**LR CAB F**	**LR LN33**	**FL RSG**	**FK RSG**	**RSP RSG**	**KSG 5BB**	**CB LN33**	**SG LN33**
Wine grapes																											
Albana	FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Arneis	CVT CN 15	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arneis	CVT CN 19	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbera	AT 84	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Barbera	CVT AT 424	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbera	F6V4	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Cabernet Franc	1334 Bord	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Cabernet Franc	C24-1	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cabernet Franc	C7V15	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Cabernet Franc	C7V15 FSAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Cabernet Sauvignon	125 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Cabernet Sauvignon	126	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cabernet Sauvignon	CW44	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Cabernet Sauvignon	FPS 12	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Cabernet Sauvignon	G9V3 FSAC CSIRO	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Cabernet Sauvignon	G9V3 FSAC DPIV	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Cabernet Sauvignon	LC10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Cabernet Sauvignon	LC14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Cabernet Sauvignon	LC84	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Cabernet Sauvignon	LC9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cabernet Sauvignon	LCR2V11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Cabernet Sauvignon	Q390-05	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

		RT-PCR														ELISA				Biological Indexing							
Variety	**Clone**	**GLRaV-1**	**GLRaV-2**	**GLRaV-3**	**GLRaV-4**	**GLRaV-5**	**GLRaV-9**	**RSPaV-1**	**RSPaV-2**	**GVA**	**GVB**	**GFkV-A**	**GFkV-B**	**GFLV**	**GRSLaV**	**GLRaV-1**	**GLRaV-3**	**GVA**	**GFkV**	**LR CAB F**	**LR LN33**	**FL RSG**	**FK RSG**	**RSP RSG**	**KSG 5BB**	**CB LN33**	**SG LN33**
Cabernet Sauvignon	R3V19E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Cabernet Sauvignon	R3V19E FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Canada Muscat	Vineland	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Chambourcin	Q106-35B	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Chardonnay	76	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Chardonnay	95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Chardonnay	G9V7	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Chardonnay	I10V1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Chardonnay	I10V3	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Chardonnay	I10V5 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Chardonnay	Q233-03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chardonnay	Q390-09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Chardonnay	Q661-04	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Chenin Blanc	C4V16	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Chenin Blanc	C4V16 FSAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Colombard	F13V7	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Colombard	F13V7 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Colombard	F13V8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Colombard	G3V1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Dolcetto	CN 69	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dolcetto	CVT AL 275	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dolcetto	SGW 1034 1-1	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Durif	H7V13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Fiano	FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Gamay	284	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Gamay	Beauj. H200A	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Gamay	BGW19 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-

		RT-PCR														ELISA				Biological Indexing							
Variety	**Clone**	**GLRaV-1**	**GLRaV-2**	**GLRaV-3**	**GLRaV-4**	**GLRaV-5**	**GLRaV-9**	**RSPaV-1**	**RSPaV-2**	**GVA**	**GVB**	**GFkV-A**	**GFkV-B**	**GFLV**	**GRSLaV**	**GLRaV-1**	**GLRaV-3**	**GVA**	**GFkV**	**LR CAB F**	**LR LN33**	**FL RSG**	**FK RSG**	**RSP RSG**	**KSG 5BB**	**CB LN33**	**SG LN33**
Gamay	RVC 12 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Graciano	WA6V6 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Grenache	1-248 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Grenache	38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lagrein	H9V7	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Lagrein	H9V9	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Malbec	1056 HT162	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Malbec	1056 FSAC	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Malbec	C6V11	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Malbec	E2V2	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Malbec	Kalimna 1	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Mataro	R2V13	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Mataro	R2V7	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Merlot	D3V7	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Merlot	Q45-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Merlot	RVC 13	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Merlot	FPS 08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Meunier	H10V5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Montepulciano	FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Muscadelle	32HT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Muscat Blanc	73-7	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Muscat Blanc	F3V14	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Nebbiolo	CVT CN 230	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Nebbiolo	K6V1	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Orange Muscat	C13V1	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Petit Verdot	G7V1	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Pinot Blanc	54	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

		RT-PCR														ELISA				Biological Indexing							
Variety	**Clone**	**GLRaV-1**	**GLRaV-2**	**GLRaV-3**	**GLRaV-4**	**GLRaV-5**	**GLRaV-9**	**RSPaV-1**	**RSPaV-2**	**GVA**	**GVB**	**GFkV-A**	**GFkV-B**	**GFLV**	**GRSLaV**	**GLRaV-1**	**GLRaV-3**	**GVA**	**GFkV**	**LR CAB F**	**LR LN33**	**FL RSG**	**FK RSG**	**RSP RSG**	**KSG 5BB**	**CB LN33**	**SG LN33**
Pinot Gris	D1V7	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Pinot Noir	18 Gm	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Pinot Noir	Cortaillod	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Pinot Noir	D2V5 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Pinot Noir	D2V6	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Pinot Noir	D5V12A	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Pinot Noir	G8V7	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Pinot Noir	H7V15	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Pinot Noir	Mariafeld	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Pinot Noir	MV6 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Riesling	156	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Riesling	237 Gm	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Riesling	356 Trier	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Riesling	68 Trier	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Riesling	810 Colmar H160A	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Riesling	812 Colmar	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Riesling	813 Colmar	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Riesling	D2V2	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Riesling	D2V3	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Riesling	E37 Trier	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Riesling	G9V15	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Riesling	I10V14	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Riesling	I10V15	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Roussanne	Vassal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Rubired	C5V14	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Ruby Cabernet	E5V4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Ruby Cabernet	E5V4 FSAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-

		RT-PCR														ELISA				Biological Indexing							
Variety	**Clone**	**GLRaV-1**	**GLRaV-2**	**GLRaV-3**	**GLRaV-4**	**GLRaV-5**	**GLRaV-9**	**RSPaV-1**	**RSPaV-2**	**GVA**	**GVB**	**GFkV-A**	**GFkV-B**	**GFLV**	**GRSLaV**	**GLRaV-1**	**GLRaV-3**	**GVA**	**GFkV**	**LR CAB F**	**LR LN33**	**FL RSG**	**FK RSG**	**RSP RSG**	**KSG 5BB**	**CB LN33**	**SG LN33**
Sangiovese	H6V9	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Saperavi	I11V10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sauvignon Blanc	5385 Bord H231A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Sauvignon Blanc	F7V7	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Sauvignon Blanc	H5V10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Sauvignon Blanc	H5V10 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Sauvignon Blanc	I4V9	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Semillon	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Semillon	143	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Semillon	3049	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Semillon	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Semillon	82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Semillon	D10V12	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Semillon	D10V12 FSAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Semillon	DA16162	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Semillon	F4V1	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Semillon	I11V14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Semillon	TO9081	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Shiraz	373	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Shiraz	1654	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Shiraz	2626	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Shiraz	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Shiraz	30	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shiraz	712	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Shiraz	ESA3021	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Shiraz	PT23 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Shiraz	R6V28W	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-

		RT-PCR														ELISA				Biological Indexing							
Variety	**Clone**	**GLRaV-1**	**GLRaV-2**	**GLRaV-3**	**GLRaV-4**	**GLRaV-5**	**GLRaV-9**	**RSPaV-1**	**RSPaV-2**	**GVA**	**GVB**	**GFkV-A**	**GFkV-B**	**GFLV**	**GRSLaV**	**GLRaV-1**	**GLRaV-3**	**GVA**	**GFkV**	**LR CAB F**	**LR LN33**	**FL RSG**	**FK RSG**	**RSP RSG**	**KSG 5BB**	**CB LN33**	**SG LN33**
Shiraz	SARDI 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Shiraz	SARDI 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Shiraz	SARDI 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Shiraz	SARDI 7	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Shiraz	SARDI 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Shiraz	SARDI 9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Shiraz	SARDI 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Tannat	H9V3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Tempranillo	D8V13	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Tinta Molle	F2V14	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Traminer	457 Colmar	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Traminer	C3V15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Traminer	H8V9	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Verdelho	Kosovich	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Verdelho	WA 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Vermentino	H62.1LN	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Viognier	642	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Zinfandel	C11V7	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Table grapes																											
Calmeria	H64-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Crimson Seedless	USDA	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Flame Seedless	K5V8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Muscat Hamburg	Irymple	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-

		RT-PCR														ELISA				Biological Indexing							
Variety	**Clone**	**GLRaV-1**	**GLRaV-2**	**GLRaV-3**	**GLRaV-4**	**GLRaV-5**	**GLRaV-9**	**RSPaV-1**	**RSPaV-2**	**GVA**	**GVB**	**GFkV-A**	**GFkV-B**	**GFLV**	**GRSLaV**	**GLRaV-1**	**GLRaV-3**	**GVA**	**GFkV**	**LR CAB F**	**LR LN33**	**FL RSG**	**FK RSG**	**RSP RSG**	**KSG 5BB**	**CB LN33**	**SG LN33**
Drying grapes																											
Carina	Merbein	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Muscat Gordo Blanco	138	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Muscat Gordo Blanco	173	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Muscat Gordo Blanco	LC3	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Zante Currant	F2V6 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Rootstocks																											
101-14	123-3HT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
101-14	2-4-84HT	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
101-14	2-5-84HT	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
1045 Paulsen	C8V4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
110 Richter	Requena	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
110 Richter	Requena FSAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1103 Paulsen	15VC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1103 Paulsen	200HT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1103 Paulsen	200HT FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
140 Ruggeri	18	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
225 Ruggeri	D4V10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5BB Kober	13-44-3 Gm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
5BB Kober	13-45-5 Gm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
5BB Kober	A10V19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
5BB Kober	A10V19 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
5BB Kober	A3V13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5BB Kober	A3V13 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
5C Teleki	6-4-22 Gm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
5C Teleki	A6V18 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

		RT-PCR														ELISA				Biological Indexing							
Variety	**Clone**	**GLRaV-1**	**GLRaV-2**	**GLRaV-3**	**GLRaV-4**	**GLRaV-5**	**GLRaV-9**	**RSPaV-1**	**RSPaV-2**	**GVA**	**GVB**	**GFkV-A**	**GFkV-B**	**GFLV**	**GRSLaV**	**GLRaV-1**	**GLRaV-3**	**GVA**	**GFkV**	**LR CAB F**	**LR LN33**	**FL RSG**	**FK RSG**	**RSP RSG**	**KSG 5BB**	**CB LN33**	**SG LN33**
779 Paulsen	C8V7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
99 Richter	2-10-285	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
99 Richter	2-9-285	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
99 Richter	2-9-285 FSAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dog Ridge	A6V8 FSAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Freedom	D11V1	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Freedom	D11V1 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
J17-48	D12V11	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
K51-32	D13V14	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Ramsey	A11V2 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Schwarzmann	WA5 FSAC	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
SO4	94 TC1	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Teleki C	8-285	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Virus and symptom abbreviations used

Abbreviation	Virus	Abbreviation	Virus	Abbreviation	Symptom
GLRaV-1	Grapevine leafroll-associated virus 1	GVA	Grapevine virus A	LR CAB F	Leafroll virus symptoms on Cabernet Franc
GLRaV-2	Grapevine leafroll-associated virus 2	GVB	Grapevine virus B	LR LN33	Leafroll virus symptoms on LN33
GLRaV-3	Grapevine leafroll-associated virus 3	GFkV-A	Grapevine fleck virus (strain A)	FL RSG	Fanleaf symptoms on Rupestris St George
GLRaV-4	Grapevine leafroll-associated virus 4	GFkV-B	Grapevine fleck virus (strain B)	FK RSG	Fleck symptoms on Rupestris St George
GLRaV-5	Grapevine leafroll-associated virus 5	GFLV	Grapevine fanleaf virus	RSP RSG	Rupestris stem pitting symptoms on Rupestris St George
GLRaV-9	Grapevine leafroll-associated virus 9	GRSLaV	Grapevine rootstock stem lesion associated-virus	KSG 5BB	Kober stem grooving symptoms on 5BB Kober
RSPaV-1	Rupestris stem pitting associated virus (strain 1)			CB LN33	Corky bark symptoms on LN33
RSPaV-2	Rupestris stem pitting associated virus (strain 2)			SG LN33	Stem grooving symptoms on LN33

11. Appendix 3: Clones with virus

Table 11-1 Clones eliminated from further use by using RT-PCR data (here have excluded data on the many clones where there was virus spread to some vines and not others ie where a virus free selection was still available for a clone)

		RT-PCR														Comments
Variety	**Clone**	**GLRaV-1**	**GLRaV-2**	**GLRaV-3**	**GLRaV-4**	**GLRaV-5**	**GLRaV-9**	**RSPaV-1**	**RSPaV-2**	**GVA**	**GVB**	**GFkV-A**	**GFkV-B**	**GFLV**	**GRSLaV**	
Wine grapes																
Brown Frontignac	BVRC 12	+	-	-	-			+	-	-	-	+	-	-		
Brown Frontignac	BVRC 16	+	-	-	-			+	-	-	-	+	-	-		
Brown Frontignac	LC 7	-	-	-	-			-	-	+	-	+	-	-		
Brown Frontignac	LC 8	-	-	-	-			-	-	+	-	+	-	-		
Cabernet Franc	Francese	-	-	+	-	-	-	+	-	-	-	-	-	-	-	
Cabernet Sauvignon	125	-	-	-	-	-	+	-	-	+	-	-	+	-	-	See also Habili and Rowhani (2002)
Cabernet Sauvignon	LC6	-	-	-	-	-	+	+	-	-	-	-	-	-	-	Biological indexing positive for leafroll
Chardonnay	13	-	-	+	-			+	-	-	-	-	-	-		
Chardonnay	84	-	-	-	-	-	-	+	-	-	-	+	-	-	-	
Chardonnay	277	-	-	-	-			+	+		-	+	-	-		
Chardonnay	Gin Gin	+	-	-	-	-	-	+	-	-	-	-	-	-	-	
Chardonnay	Pen 58	-	+	-	-	-	-	+	-	-	-	+	-	-	-	
Chardonnay	Q949-03	-	-	-	-			+	-	-	-	+	-	-		
Chenin Blanc	Steen	-	-	-	-	-	-	-	-	-	-	+	-	-	-	Biological indexing positive for fleck
Furmint	E2V11	-	-	+	-	-	-	-	-	-	-	-	-	-	-	Biological indexing positive for leafroll
Graciano	WA6V6	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
Grenache	139	-	-	-	-			+	-	+	-	-	-	-		
Grenache	139HT	-	-	-	-			+	-	+	-	-	-	-		
Grenache	2-248	-	-	-	-			+	-	+	-	-	-	-		
Grenache	LCE 11-1	+	-	-	-			-	-	+	-	-	-	-		
Grenache	LCE 11-2	-	-	-	-	-	+	-	-	-	-	-	-	-	-	Biological indexing positive for leafroll
Malbec	SGW 0539	+	-	-	-			+	-	+	-	-	-	-		

		RT-PCR														Comments
Variety	**Clone**	**GLRaV-1**	**GLRaV-2**	**GLRaV-3**	**GLRaV-4**	**GLRaV-5**	**GLRaV-9**	**RSPaV-1**	**RSPaV-2**	**GVA**	**GVB**	**GFkV-A**	**GFkV-B**	**GFLV**	**GRSLaV**	
Malbec	CW 14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	GLRaV-3 positive by RT-PCR (not all tests), best detected with specific primers (Constable, pers. com.)
Marsanne	NE Vic	-	-	-	-			+	-	+	-	-	-	-		
Merlot	D3V14	-	-	-	-			+	-	-	-	+	-	-		
Petit Verdot	H8V11	-	-	-	-			+	-	+	-	-	-	-		
Pinot Noir	115	-	-	-	-			+	-	-	-	+	-	-		
Pinot Noir	MV6	-	-	-	-			+	-	-	-	+	-	-		
Riesling	114	-	-	-	-			+	-	+	-	-	-	-		
Riesling	138	-	-	-	-			+	-	+	-	-	-	-		
Riesling	140	-	-	-	-			-	-	+	-	-	-	-		
Sangiovese	Mudgee	+	-	-	-			+	-	-	-	-	-	-		Biological indexing positive for leafroll
Sauvignon Blanc	132	+	-	-	-	-	+	-	-	+	-	-	-	-	-	Biological indexing positive for leafroll and KSG
Shiraz	1125	-	-	-	-	-	-	+	-	-	-	+	-	-	-	
Tempranillo	D8V12	-	-	-	-	-	-	+	-	-	-	-	-	-	-	Biological indexing positive for leafroll (tested positive for GLRaV-5 in other RT-PCR tests - Habili, pers. com.)
Traminer	T6	-	+	+	-			+	-	-	-	+	-	-		
Verdelho	E6V15	-	-	-	-			+	-	+	-	-	-	-		
Viognier	1968	+	+	-	-			+	-	-	-	-	-	-		Heat treated clone HTK weak indexing symptoms of KSG and also GVA positive with RT-PCR (Constable, pers. com.)
Table grapes																
Moss Sultana		-	-	-	-	-	+	+	-	+	+	+	-	-	-	
Red Globe	Olmo	-	-	-	-	-	-	-	-	-	-	-	-	-	+	See also Habili and Symons (2001)
Drying grapes																
Sultana	H4	-	-	-	+			-	-	-	-	-	-	-		
Sultana	H5	-	-	-	+			-	-	-	-	-	-	-		
Sultana	M12	-	-	-	+			+	-	-	-	-	-	-		
Zante Currant	F2V6	-	-	-	-			+	-	+	-	-	-	-		

		RT-PCR														Comments
Variety	**Clone**	**GLRaV-1**	**GLRaV-2**	**GLRaV-3**	**GLRaV-4**	**GLRaV-5**	**GLRaV-9**	**RSPaV-1**	**RSPaV-2**	**GVA**	**GVB**	**GFkV-A**	**GFkV-B**	**GFLV**	**GRSLaV**	
Rootstocks																
101-14	100-3HT	-	-	-	-	-	-	+	-	-	-	+	-	-	-	
101-14	107-1HT	-	-	-	-			-	-	-	-	+	-	-		
140 Ruggeri	Q45-3A	-	-	-	-	-	-	-	-	-	-	+	-	-	-	See also Habili and Bogacz (2006)
3309 Couderc	Rutherglen	-	-	-	-			+	-	-	-	+	-	-		
420 A	Irymple	-	-	-	-			+	-	-	-	+	-	-		
5C Teleki	10-48-49 Gm	-	-	-	-			-	-	-	-	-	+	-		Fleck positive by ELISA
Ramsey	A11V12	-	-	-	-			-	-	-	-	-	+	-		See also Habili and Bogacz (2006)

Virus and symptom abbreviations used

Abbreviation	Virus	Abbreviation	Virus or symptom
GLRaV-1	Grapevine leafroll-associated virus 1	GVA	Grapevine virus A
GLRaV-2	Grapevine leafroll-associated virus 2	GVB	Grapevine virus B
GLRaV-3	Grapevine leafroll-associated virus 3	GFkV-A	Grapevine fleck virus (strain A)
GLRaV-4	Grapevine leafroll-associated virus 4	GFkV-B	Grapevine fleck virus (strain B)
GLRaV-5	Grapevine leafroll-associated virus 5	GFLV	Grapevine fanleaf virus
GLRaV-9	Grapevine leafroll-associated virus 9	GRSLaV	Grapevine rootstock stem lesion-associated virus
RSPaV-1	Rupestris stem pitting associated virus (strain 1)	KSG	Kober stem grooving symptoms
RSPaV-2	Rupestris stem pitting associated virus (strain 2)		

12. Appendix 4: References

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13. Appendix 5: Staff

Tony Bass provided technical support throughout the project.
The biological indexing program for this project was begun in year 2000 and both Graeme Fletcher and Les Krake were initially closely involved with this.
Nuredin Habili from Waite Diagnostics supervised the RT-PCR testing.
Brendon Radoni from Crop Health Services supervised the ELISA testing.
Julian Connellan supervised the establishment of the Australian National Nuclear Grapevine Collection and George Kerridge provided ampelography checks on this.

14. Appendix 6: Budget reconciliation