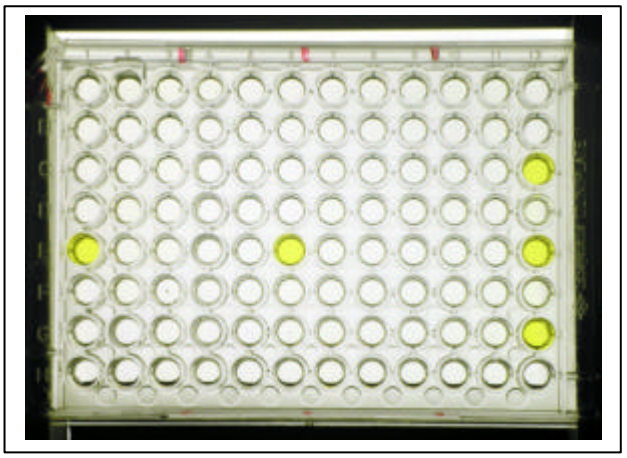
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

Australian Government
Grape and Wine Research and Development Corporation

Identification of virus free clones of the winegrape varieties and rootstocks required in Australia to enable the establishment of elite nuclear source collections for vine improvement schemes



INTERIM FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **SAR 00-6**

Principal Investigator: **Philip R. Nicholas**

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

Date: 6/5/05

Executive summary

It is crucial that disease free planting material is used when establishing a vineyard. 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.

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.

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 laboratory RT-PCR and ELISA methods. The remaining clones have then been further screened using biological indexing. This involves 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 a national nuclear collection of 200 of the clones selected in the project at the NSW DPI Research Station at Dareton. In the GWRDC submission SAR 03/05 to continue this project, funding was also requested for the indexing of an additional 60 clones. Funding is still required for this additional indexing to complete the collection at Dareton. The clones selected in the project will be made available under a licence agreement to any vine improvement group in Australia wishing to distribute them.

As part of this project a catalogue is being finalised which contains more detailed information on the nuclear clones. This catalogue will be set up on the SARDI website in 2005 and it will be of particular value in publicising the clones. The feasibility and cost of integrating an updated national register of clones with the catalogue on the website is being investigated.

Contents

1. BACKGROUND 1

1.1 GRAPEVINE COLLECTIONS 1
1.2 GRAPEVINE VIRUSES 1
1.3 VIRUS ELIMINATION 2

2. OBJECTIVES 3

3. METHODS 3

3.1 SELECTION AND SCREENING OF CANDIDATE CLONES 3
3.2 VIRUS DETECTION BY RT-PCR AND ELISA 4
3.3 DISEASE DETECTION BY BIOLOGICAL INDEXING 4

4. RESULTS AND DISCUSSION 5

4.1 RT-PCR AND ELISA TESTING 5
4.2 BIOLOGICAL INDEXING 5
4.3 NUCLEAR COLLECTION 6
4.4 CATALOGUE 6
4.5 FUTURE WORK 6

5. REFERENCES 10

1. Background

1.1 Grapevine collections

Many vineyards in Australia have vines with virus infections, which affect both yield and quality and can cause stock/scion compatibility problems. The recognition that leafroll virus was spreading through grapevine collections, which are 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 P. Nicholas to visit 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. There are also other collections held in Victoria, New South Wales, Western Australia, Tasmania and Queensland (deLaine and Nicholas, 2000). A genetic resource collection is considered an unsatisfactory site to hold virus tested clones, as virus can be spread by insect vectors 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.

1.2 Grapevine viruses

Grapevine viruses and sanitary selection were reviewed with reference to Australia by Nicholas (2004). The review included the following information on leafroll and rugose wood viruses:

Leafroll viruses are considered the most detrimental with reported yield losses varying from minor to more than 50%. Leafroll viruses can also affect grape quality by causing delayed maturity, and reduced fruit colour of red 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 and in clone 3A of Emperor.

Rugose wood is a complex disease caused by viruses. It is characterised by symptoms on the woody cylinder 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—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. 1991), 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).

1.3 Virus elimination

There are procedures which can be used for eliminating virus diseases from grapevines. In the past, 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 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 originally went through thermotherapy and some of the clones have undergone *in vitro* procedures and this is reflected in the clonal names (MTC for meristem tip culture and FSAC for fragmented shoot apex culture).

2. Objectives

The project objectives are:

- 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.

3. Methods

3.1 Selection and screening of candidate clones

Over 350 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 has involved rapid screening of the clones for viruses using both RT-PCR and ELISA laboratory methods and the remaining ones have then been biologically indexed. Test results from all three methods are complementary and their combined use provides a more reliable indication of virus status.

Table 3-1 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	RSPaV		Rupestris St George
Kober stem grooving	GVA		5BB Kober
Grapevine corky-bark	GVB		LN33
LN33 stem grooving			LN33
Fleck			
Grapevine fleck virus	GFkV-A, GFkV-B		Rupestris St George
Fanleaf			
Grapevine fanleaf virus	GFLV		Rupestris St George

3.2 Virus detection by RT-PCR and ELISA

ELISA testing procedures have 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. RT-PCR testing is a more recent development and methods have been reviewed by Minafra et al. (1997). RT-PCR involves amplification and detection of a small part of the genome of the virus, making the test far more sensitive than ELISA.

RT-PCR and ELISA procedures used in the project involved taking scrapings of the cortex of dormant (woody) cuttings (Habili and Randles 2002) and the tests conducted are given in Table 3-1. RT-PCR testing was done by Waite Diagnostics in Adelaide and ELISA testing by Crop Health Services at Knoxfield. These tests resulted in the elimination of many clones with virus infection and the selection of specific vines, which appeared to be free of virus. These selected vines were then biologically indexed.

3.3 Disease detection by biological indexing

Biological indexing with woody indicators was used prior to the availability of laboratory methods. Indexing 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, indexing detects the disease rather than the associated virus and is still considered to be essential in sanitary selection. The early indexing work in Australia was reported by Shanmuganathan and Fletcher (1980) and Cirami et al. (1988).

Indexing of the clones has been conducted at SARDI Loxton Centre. Indexing procedures have been reviewed by Martelli (1993) and Garau et al. (1997). A range of grafting methods can be used and we used both chip budding and bench grafting. Material from the selected vines was grafted to the four indicators in Table 3-1 and the vines planted at a spacing of 1.5 x 2.5 m and trellis and drip irrigation installed. For each grafted clone/indicator combination, 3 replicate vines and an indicator control vine were planted. The planting area for the project covers 1.7 hectare. Test vines have been observed over 3 growing seasons for leaf symptoms of leafroll, fleck and fanleaf viruses and cane symptoms of corky bark virus. When these observations are complete, the vines are dug (in May/June each year), the bark removed and inspected for trunk symptoms of the rugose wood viruses Rupestris stem pitting, Kober stem grooving and LN33 stem grooving to complete the indexing.

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

4. Results and discussion

4.1 RT-PCR and ELISA testing

RT-PCR testing of grapevine collections for viruses was first conducted in Australia by SARDI at Nuriootpa in 1998 and was soon adopted by other states. The use of this pre-existing RT-PCR data in addition to that obtained in the project 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 long been infected with viruses, RT-PCR was also useful where vines of a particular clone were infected with leafroll virus in one collection but not in another, allowing the selection of a disease free vine. ELISA tests provided an additional check for GLRaV-1 and GLRaV-3 infection.

4.2 Biological indexing

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. 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. The grafting procedures used and the time of planting of grafted vines depends on a range of factors such as availability and diameter of candidate and indicator wood, the number of clones to be grafted and environmental conditions at the field planting. In our situation, we found:

- Chip budding – was best done by propagating indicator vines in winter and chip bud inoculating them with the candidate in summer and planting and training up the indicator in spring the following growing season.
- Bench grafting – could be done by grafting the candidate onto the indicator with a bud being left on the indicator from which a shoot is trained up (although the Cabernet Franc indicator may be better benched grafted on top of the candidate). Grafting is done in winter and the vine is preferably planted in late spring.

Where vines were planted in spring, we observed them in the season of planting if vines were sufficiently advanced and in the following two growing seasons before digging them and removing the bark to check for rugose wood symptoms. Some observation we made were:

- Leafroll viruses – some clones were found to give leafroll symptoms on the indicator Cabernet Franc, where leafroll was not detected by RT-PCR. The degree of symptom expression for leafroll viruses 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 that GLRaV-4 and GLRaV-9 displayed weaker symptoms on Cabernet Franc than the others).
- Rugose wood viruses – we found symptoms on Rupestris St George the indicator for Rupestris stem pitting on more than 70% of indexed vines, but so far have not seen symptoms on indicators for Kober stem grooving, corky bark or LN33 stem grooving (clones with Kober stem grooving appear to have been eliminated by using RT-PCR).

Indexing was completed on 100 clones in June 2004, an additional 130 clones will be completed in June 2005 and the remaining 50 clones in June 2006. A detailed analysis and tabulation of data from all virus testing methods will be undertaken for the final report when all the indexing data are available.

4.3 Nuclear collection

The Australian Vine Improvement Association has recognised the unique quality of this material and established a national nuclear collection of 200 of the clones in 2004 at the NSW DPI Research Station at Dareton. Clones planted are ones which to date have tested negative to ELISA and RT-PCR tests (other than Rupestris stem pitting) and to biological indexing conducted so far. Planting was done with the provisor that any clones subsequently found by indexing to contain viruses will be removed. The clones planted in the nuclear collection are given in Table 4-1. Most of these clones of the varieties Cabernet Sauvignon, Chardonnay, Merlot, Riesling, Semillon and Shiraz have also been planted in fully replicated clonal trials at Monash by the Riverland Vine Improvement Committee.

In future, all vines in the nuclear collection will be periodically laboratory tested and checked visually to ensure that they remain free of viruses. Also, any vine from which cuttings are taken will be RT-PCR tested again, before cuttings are made available to industry for propagation. The vines will be checked by ampelographer George Kerridge for trueness to type.

SARDI has prepared draft licence conditions for the maintenance 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 any vine improvement group in Australia wishing to distribute them provided there is agreement to these conditions.

4.4 Catalogue

Information is available on clones distributed in many overseas schemes, including those in California (Bettiga et al. 2003), France (Boidron et al. 1995) and Italy (Calo 2000). Australia has the National Register of Grapevine Varieties and Clones (deLaine and Nicholas, 2000), which is a list of 1500 clones held in genetic resource collections, but there is no publication with more comprehensive information on clones available. As part of this project a catalogue is being finalised which contains more detailed information on the clones selected in the project for planting in nuclear collections. A web development officer has been employed to put the catalogue in a searchable format on the SARDI web site in 2005. The catalogue will be of particular value in publicising the clones. The feasibility of updating and integrating the National Register with the catalogue on this website is being investigated.

4.5 Future work

In the GWRDC submission SAR 03/05 to complete the indexing in this project, funding was also requested for the indexing of an additional 60 clones. Funding is still required for this additional indexing to complete the national nuclear collection at Dareton.

Table 4-1 Clones selected in the project which have been planted at the national nuclear collection at Dareton (further indexing results are required before release)

Variety	Clone
Wine grapes	
Albana	FSAC
Arneis	CVT CN 15
Arneis	CVT CN 19
Barbera	AT 84
Barbera	CVT AT 424
Barbera	F6V4
Brown Frontignac	WA
Cabernet Franc	1334 Bord
Cabernet Franc	C24-1
Cabernet Franc	C7V15
Cabernet Franc	C7V15 MTC
Cabernet Sauvignon	125 MTC
Cabernet Sauvignon	126
Cabernet Sauvignon	CW44
Cabernet Sauvignon	FPS 12
Cabernet Sauvignon	G9V3 FSAC
Cabernet Sauvignon	G9V3 MTC
Cabernet Sauvignon	LC10
Cabernet Sauvignon	LC14
Cabernet Sauvignon	LC84
Cabernet Sauvignon	LC9
Cabernet Sauvignon	LCR2V11
Cabernet Sauvignon	Q390-05
Cabernet Sauvignon	R3V19E
Cabernet Sauvignon	R3V19E MTC
Canada Muscat	1970/CX
Chambourcin	Q106-35B
Chardonnay	76
Chardonnay	95
Chardonnay	G9V7
Chardonnay	G9V7 MTC
Chardonnay	I10V1
Chardonnay	I10V3
Chardonnay	I10V5 MTC
Chardonnay	Q233-03
Chardonnay	Q390-09
Chardonnay	Q661-04
Chenin Blanc	C4V16
Chenin Blanc	C4V16 MTC

Variety	Clone
Colombard	F13V7
Colombard	F13V7 MTC
Colombard	F13V8
Colombard	G3V1
Dolcetto	CN 69
Dolcetto	CVT AL 275
Dolcetto	SGW 1034 1-1-183
Durif	H7V13
Fiano	FSAC
Gamay	284
Gamay	Beauj. H200A
Gamay	BGW19 MTC
Gamay	RVC12 MTC
Graciano	WA6V6 MTC
Grenache	1-248 MTC
Grenache	38
Lagrein	H9V7
Lagrein	H9V9
Malbec	1056 HT162
Malbec	1056 MTC
Malbec	C6V11
Malbec	E2V2
Malbec	Kalimna 1
Mataro	R2V13
Mataro	R2V7
Merlot	D3V7
Merlot	Q45-14
Merlot	RVC13
Merlot	FPS 08
Meunier	H10V5
Montepulciano	FSAC
Muscadelle	32HT
Nebbiolo	CVT CN 230
Nebbiolo	K6V1
Orange Muscat	C13V1
Petit Verdot	G7V1
Pinot Blanc	54 ENTAV
Pinot Gris	D1V7
Pinot Noir	18 Gm
Pinot Noir	Cortaillod H120B

Variety	Clone
Pinot Noir	D2V5 MTC
Pinot Noir	D2V6
Pinot Noir	D5V12A
Pinot Noir	G8V7
Pinot Noir	H7V15
Pinot Noir	Mariafeld
Pinot Noir	MV6 MTC
Riesling	156
Riesling	237 Gm
Riesling	356 Trier
Riesling	68 Trier
Riesling	810 Colmar H160A
Riesling	812 Colmar
Riesling	813 Colmar
Riesling	D2V2 (198 Gm)
Riesling	D2V3
Riesling	E37 Trier
Riesling	G9V15
Riesling	I10V14
Riesling	I10V15
Riesling	K34 Trier H200B
Roussanne	Vassal
Rubired	C5V14
Ruby Cabernet	E5V4
Ruby Cabernet	E5V4 MTC
Sangiovese	H6V9
Saperavi	I11V10
Sauvignon Blanc	5385 Bord HT231A
Sauvignon Blanc	F7V7
Sauvignon Blanc	H5V10
Sauvignon Blanc	H5V10 MTC
Sauvignon Blanc	I4V9
Semillon	14
Semillon	143
Semillon	3049
Semillon	32
Semillon	82
Semillon	D10V12
Semillon	D10V12 MTC
Semillon	DA16162
Semillon	F4V1
Semillon	I11V14
Semillon	TO 9081
Shiraz	373
Shiraz	1125

Variety	Clone
Shiraz	1127
Shiraz	1654
Shiraz	2626
Shiraz	12
Shiraz	30
Shiraz	712
Shiraz	ESA3021
Shiraz	PT15
Shiraz	PT23 MTC
Shiraz	R6V28W
Shiraz	SARDI 3
Shiraz	SARDI 4
Shiraz	SARDI 6
Shiraz	SARDI 7
Shiraz	SARDI 8
Shiraz	SARDI 9
Shiraz	SARDI 10
Tannat	H9V3
Tinta Molle	F2V14
Traminer	457 Colmar
Traminer	C3V15
Traminer	H8V9
Verdelho	Kosovich
Verdelho	WA 4
Vermentino	H62.1
Viognier	642
Viognier	HTK
White Frontignac	73-7
White Frontignac	F3V14
Zinfandel	C11V7
Zinfandel	F11V6
Table grapes	
Calmeria	H64-1
Crimson Seedless	USDA
Flame Seedless	K5V8
Muscat Hamburg	
Drying grapes	
Carina	MM4-38
Muscat Gordo Blanco	138
Muscat Gordo Blanco	173
Muscat Gordo Blanco	LC3
Sultana	H5 2-2-255
Zante Currant	F2V6 MTC

Variety	Clone
Rootstocks	
101-14	123-3HT
101-14	2-4-84
101-14	2-5-84
1045 Paulsen	C8V4
110 Richter	Requena
110 Richter	Requena MTC
1103 Paulsen	15VC
1103 Paulsen	200HT
1103 Paulsen	200HT MTC
140 Ruggeri	18 ENTAV
225 Ruggeri	D4V10
3306 Couderc	RVC
5BB Kober	13-44-3 Gm
5BB Kober	13-45-5 Gm
5BB Kober	A10V19
5BB Kober	A10V19 MTC
5BB Kober (5A)	A3V13
5BB Kober (5A)	A3V13 MTC

Variety	Clone
5C Teleki	10-48-49 Gm
5C Teleki	6-4-22 Gm
5C Teleki	A6V18
5C Teleki	A6V18 MTC
779 Paulsen	C8V7
99 Richter	2-10-285
99 Richter	2-9-285
99 Richter	2-9-285 MTC
Dog Ridge	A6V8 MTC
Freedom	D11V1
Freedom	D11V1 MTC
J17-48	D12V11
K51-32	D13V14
Ramsey	A11V2 MTC
Schwarzmann	WA
Schwarzmann	WA5 MTC
SO4	94 TC1
Teleki C	8-285

5. References

Barlass, M., Skene, K.G.M., Woodham, R.C. and Krake, L.R. (1982) Regeneration of virus-free grapevines using *in vitro* apical culture . Annals of Applied Biology **101**, 291-295.

Bettiga, L. J., Christensen, L. P., Dokoozlian, N. K., Golino, D. A., McGourty, G., Smith, R. J., Verdegaal, P. S., Walker, A., Wolpert, J. A., and Weber, E. (2003). 'Wine Grape Varieties in California' (University of California Agriculture and Natural Resources: Oakland).

Boidron, R., Boursiquot, J. M., Doazan, J. P., Leclair, P., Leguay, M., and Walter, B. (1995). 'Catalogue of Selected Wine Grape Varieties and Clones Cultivated in France' (ENTAV: Le Grau Du Roi, France).

Bonavia, M., Digiaro, M., Boscia, D., Boari, A., Bottalico, G., Savino, V. and Martelli, G.P. (1996) Studies on 'corky rugose wood' of grapevine and on the diagnosis of grapevine virus B. Vitis **35**, 53-58.

Boscia, D., Digiaro, M., Fresno, J., Greif, C., Grenan, S., Kassemeyer, H. H., Prota, V. A. and De Sequeira, O. A. (1997) ELISA for the detection and identification of grapevine viruses. In: 'Sanitary Selection of the Grapevine'. Ed. B. Walter (INRA Editions: Colmar, France).

Calo, A. (2000). 'Catalogo dei cloni del nucleo di premoltiplicazione delle Venezie' (Istituto Sperimentale per la Viticoltura, Susegana, Italy).

Chevalier, S., Greif, C., Clauzel, J.M., Walter, B. and Fritsch, C. (1995) Use of an immunocapture-polymerase chain reaction for the detection of grapevine virus A in Kober stem grooving-infected grapevines. Journal of Phytopathology **143**, 369-373.

Cirami, R.M., vanVelsen, R.J. and Niejalke, J. (1988) Grapevine virus indexing in the South Australian Vine Improvement Scheme, 1974-1987. Australian Journal of Experimental Agriculture **28**, 645-649.

deLaine, A. M. and Nicholas, P. R (2000). 'National Register of Grapevine Varieties and Clones' (Australian Vine Improvement Association: Mildura, Victoria).

Garau, R., Padilla, V., Rumbos, I., Walter, B. and Savino, V. (1997) Indexing for the identification of virus and virus-like diseases of the grapevine. In: 'Sanitary Selection of the Grapevine'. Ed. B. Walter (INRA Editions: Colmar)

Garau, R., Pronta, V.A., Piredda, R., Boscia, D. and Prota, U. (1994) On the possible relationship between Kober stem grooving and grapevine virus A. Vitis **33**, 161-163.

Garau, R., Prota, V. A. and Prota, U. (1991) Distribution of Kober stem grooving and Rupestris stem pitting of grapevine in symptomless cv. Torbato scions. Proceedings 10th meeting ICVG, Volos, Greece (Ores Publishing). pp.175-181.

Golino, D.A., Sim, S., Grzegorczyk, W. and Rowhani, A. (1998) Optimising tissue culture protocols used for virus elimination in grapevines. Phytopathology **88**, 532.

Goszczynski, D. E. and Jooste, A. E. C. (2003) Shiraz disease (SD) is transmitted by mealybug *Planococcus ficus* and associated with Grapevine virus A. Extended Abstracts 14th meeting ICVG, Locorotondo, Italy (University of Bari) pp.219.

Greif, C., Garau, R., Boscia, D., Prota, V.A., Fiori, M., Bass, P., Walter, B. and Prota, U. (1995) The relationship of grapvine leafroll-associated closterovirus 2 with a graft incompatibility condition of grapevines. Phytopathologia Mediterranea **34**, 167-173.

Greif, C. and Walter, B. (1997) The European reference collection of grapevine viruses. In: 'Sanitary Selection of the Grapevine'. Ed. B. Walter, (INRA Editions: Colmar).

Habili, N., Ewart, J., Fazeli, C., Scott, N., Krake, L. and Rezaian, M. (1996) Virus types associated with grapevine leafroll disease in Australia. Australian Grapegrower and Winemaker **390a**, 25-28.

Habili, N. and Randles, J.W. (2002) Developing a standardised sampling protocol for consistent detection of grapevine viruses by the PCR assay. Australian and New Zealand Grapegrower and Winemaker **464**, 88-91.

Habili, N. and Randles, J.W. (2004) Descriptors for Grapevine virus A associated syndrome in Shiraz, Merlot and Ruby Cabernet in Australia and its similarity to Shiraz Disease in South Africa. Australian and New Zealand Grapegrower and Winemaker **488**, 71-74.

Habili, N. and Rowhani, A. (2002) First detection of a new virus, Grapevine leafroll-associated virus type 9, in a popular clone of Cabernet Sauvignon in Australia. Australian Grapegrower and Winemaker **461a**, 102-103.

Habili, N. and Symons, R. H. (2000) Grapevine viruses detected by Waite Diagnostics in Australia. Extended Abstracts 13th meeting ICVG, Adelaide (University of Adelaide and CSIRO). pp. 124.

Martelli, G. P. (1993). 'Graft-Transmissible Diseases of Grapevines' (FAO: Rome).

Minafra, A., Greif, C. and Romero, J. (1997) Molecular tools for the detection of grapevine viruses. In: 'Sanitary Selection of the Grapevine'. Ed. B. Walter (INRA Editions: Colmar).

Nicholas, P. R. (2004) Grapevine planting material. In: 'Viticulture Volume 1- Resources'. Eds. P. R. Dry and B. G. Coombe (Winetitles: Adelaide).

Nyland, G. and Goheen, A.C. (1969) Heat therapy of virus diseases of perennial plants. Ann. Rev. Phytopathol. **7**, 331-354.

Reynolds, A.G., Lanterman, W.S. and Wardle, D.A. (1997) Yield and berry composition of five *Vitis* cultivars as affected by Rupestris stem pitting virus. American Journal of Enology and Viticulture **48**, 449-458.

Savino, V., Boscia, D., D'onghia, A. M. and Martelli, G. P. (1990) Effect of heat therapy and meristem tip culture on the elimination of grapevine leafroll - associated closterovirus type III. Proc. 10th Meeting ICVG , Volos, Greece (Ores Publishing :Volos). pp.433-436.

Shanmuganathan, N. and Fletcher, G. (1980) Indexing grapvine clones in the fruit variety foundation of Australia for virus and virus-like diseases. Australian Journal of Experimental Agriculture and Animal Husbandry **20**, 115-118.

Shi, B.J., Habili, N., Gafny, R. and Symons, R.H. (2004) Extensive variation of sequence within isolates of grapevine virus B. Virus Genes **29:2**, 279-285.

Whattam, M. (2001) Grapevine corky bark disease: a serious quarantine threat of grapevines for Australia. Australasian Plant Pathology **30**, 379-380.