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HORTICULTURAL SERVICES

Australian Government
Grape and Wine Research and Development Corporation

REVIEW OF VINE HEALTH PARAMETERS, IMPLEMENTATION PRIORITIES AND CAPABILITIES FOR VINE IMPROVEMENT GROUPS AND ACCREDITED NURSERIES

FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **NVH 03/01**

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Introduction

This report was commissioned[1] to provide a comprehensive framework including standardised terminology and labelling, for the development of guidelines for the production, handling and provision of grapevine planting material that is true to type and of known health status. The guidelines will be relevant to nuclear collections and to the commercial production of planting material, as well as to wine grapes, table grapes, grapes for drying and related ornamentals. The framework described in the report has been developed following widespread consultation and a review and comparison of existing planting material schemes for perennial crops in Australia and overseas.

The provision of grapevine planting material of superior health status has been an industry issue for many decades and was the stimulus for the development of Vine Improvement Groups (VIGs) and their predecessors, the vine selection societies. Improved diagnostic techniques, substantially increased wine grape and table grape plantings since 1990, market pressures for new varieties and clones, organisational changes and developments in the vine improvement movement, and the apparently increasing threat of exotic organism entries (i.e. grapevine leaf rust, recent re-occurrence of citrus canker) have increased the necessity for, and relevance of, this review.

The aim of a certification scheme and the supporting accreditation scheme is to reduce the threat of spread of these pathogens and pests, to provide traceability, and to ensure provision of high quality planting material that is true to type. Vineyards established with such material, if properly maintained, should remain sustainable and productive for many years.

Framework

A revised conceptual framework for the propagation and provision of planting material has been developed and comprises a process divided into three phases:

- Foundation Phase (identify, procure, test and prepare varieties and clones of industry interest; enter and maintain material in nuclear collections, propagate, plant and maintain mother vines);
- Multiplication Phase (establish source blocks from material from mother vines to provide propagules for nursery propagation and distribution); and
- Propagation and Distribution Phase (produce young vines, own-rooted or grafted to distribute to commercial vineyards).

The process recommends three pathways via which three classes of planting material would be produced, maintained, evaluated and labelled. A flow chart using conventional flow chart symbols further characterises the process, and enables identification of critical control points as decision points. The flow chart is shown in full in Annex 4 and is shown in sequential sections in the report.

The recommended labels for planting material from the suggested pathways are:

[1] By the Vine Collections and Propagation Technical Reference Group (VCPTRG) of the National Vine Health Steering Committee (NVHSC)

- Certified elite;
- Certified best available; and
- Non-certified.

Certified elite material is planting material that meets the highest health and quality criteria and such material would ideally provide the foundation of the Australian viticulture industry. It comprises graftlings (if rootstock also certified elite) or own rootlings that:

- can be traced to an elite collection and is thus certified true-to-type;
- has undergone testing and procedures as described, and thus has no detectable prescribed or non-prescribed pests/pathogens;
- is a product of a certified elite source block; and
- have been propagated and distributed by an AVIA or VINA accredited nursery.

Certified best available material is planting material that meets most of these criteria but has tested positive for one non-prescribed grapevine pathogen. It comprises graftlings (if rootstocks of elite status) or own rootlings that:

- can be traced to the best available collection and is thus certified true-to-type;
- has undergone testing and procedures as described, and is thus labelled for the detected pathogen provided no additional pests/pathogens have been detected;
- is a product of a certified best available source block; and
- have been propagated and distributed by an AVIA or VINA accredited nursery.

Non-certified material includes graftlings or own rootlings from sources that may originally have been of elite, best-available or non-certified status. Deviation from production, maintenance and handling protocols renders material non-certifiable regardless of the original source. Material propagated or distributed by a non-accredited nursery, regardless of its source, can only be sold as non-certified. The health status of non-certified material is not defined. There are no limitations on the production of non-certified material.

Effects of Pests and Pathogens on Vine Health

Many pathogens and pests impact significantly on the health of grapevines, affecting the yield and quality of grapes and the quality of grapevine propagation material. Many of these pathogens can be transmitted through propagation material. Many economically-important pests and pathogens are not currently present in Australia, and as such are ‘quarantineable’. Before release in Australia, all imported planting material must be determined by the Australian Quarantine and Inspection Service (AQIS) to have non-detectable levels of these pathogens/pests. Many other pests and diseases are endemic. Imported material may be released without due knowledge of the status of these pests/pathogens in it. The report recommends however that eligibility of entry into an elite nuclear collection is based on known health status of the planting material, and documented freedom (of detection) of both exotic and endemic pests/pathogens. It is recommended that the entry into a best available nuclear collection is also based on the known health status. In this case however it is possible for an identified non-prescribed pest or pathogen, deemed not to be detrimental to the scion or rootstock, individually or in combination, to be present.

The effects of viruses, phytoplasmas, viroids, bacteria, fungi, and insects and other pests are described in the report.

Comparisons of Collections and Schemes

The high health status schemes reviewed and compared in the report are listed below:

- Australian Pome Fruit Improvement Program (APFIP)
- AusCitrus
- Canadian Plant Protection Export Certification Program (PPECP) for grapevine nursery stock
- Etablissement National Technique pour l'Amélioration de la Viticulture (ENTAV)
- European and Mediterranean Plant Protection Organization (EPPO) guidelines for pathogen-tested material of grapevine varieties and rootstocks
- Foundation Plant Services (FPS), UC Davis, California
- International Council for the Study of Viruses and Virus Diseases of the Grapevine (ICVG) – Safe Movement of Grapevine Germplasm
- International Council for the Study of Viruses and Virus Diseases of the Grapevine (ICVG) – Recommendations from the 14th meeting (2003)
- CIHEAM – Options Mediterraneennes – Proceedings of the Mediterranean Network on Certification of Citrus, Stone fruit (Series B)
- South African Plant Certification Scheme for Winegrapes (SAPCSW)

The practices, knowledge and opinions of AVIA and SAVII were also considered.

In particular, pathogen elimination methods including heat treatment and shoot tip or meristem culture, and hot water treatment, pathogen testing methods including ELISA and/or PCR, biological indexing, visual inspections for ampelography and for disease, and organisational structures and authorities, and planting protocols were reviewed and compared.

Recommended Procedures

The provision of certified pathogen-tested, true-to-type material requires active pathogen testing at every level of both the 'elite' and 'best available' streams. The schedule of testing recommended in this report gives consideration to the grapevine pathogens endemic in Australia, their known economic impact and their mode of transmission. For a number of considered and enunciated reasons, this report recommends all material entering an elite nuclear collection be heat treated using established procedures.

The report also explains the need for consistency in the time of sampling and tissue sampled, diagnostic test techniques. Recommended diagnostic tests and procedures must be effective, meet best practice criteria, be conducted by trained and competent staff, and use integrated data that are professionally collected and assessed, and able to be accurately and easily interrogated. Many of the tests used require experience and a high level of technical understanding and expertise. Ideally, all pathogen testing would be performed in NATA-accredited laboratories. It is recommended that labelling of clones, is such that the original name of the clone and imposed treatments, are identified.

Implementation Issues

Consultations indicated there is relatively widespread industry acceptance of the desirability of certified, superior health status planting material. The foundation for increasing its availability is the establishment and operation of certified elite or certified best available nuclear collections.

At present, two vine improvement bodies (AVIA and SAVII) are developing higher health status plantings at Dareton and Kapunda respectively. There are existing plantings nuclear or foundation plantings at Manjimup and in some private nurseries, and there are genetic resource collections at Nuriootpa and Merbein. The health status and trueness to type of these collections, the genetic resource collections, and other collections at private nurseries and Manjimup, WA have not been assessed against the suggested recommendations of this report.

Development and sustainability of collections to the elite status would require:

- Endorsement by NVHSC (or agreed authority) of the test and treatment requirements;
- Development of agreed protocols;
- Development of NATA-accredited diagnostic facilities with a competent industry-oriented research capability to provide testing services;
- Development of an accreditation or auditing system independent of the collections and with industry credibility to provide certification that material in elite and best available collections (and mother plant collections) has been subjected to, and satisfied, the required protocols;
- Assurance through accreditation that the management and operation of source blocks and propagation nurseries meet standards required to maintain the certification level of multiplied material; and
- Stimulation of demand for certified elite or certified best available planting material through development and implementation of a communication program.

Development of this system requires the application of high level technical and industry development expertise. Terms of reference for such a person or group must be developed. Industry funding will be required and the ‘project’ should be accountable to a group such as NVHSC or a technical sub committee such as VCPTRG.

Recommendations

1 An Australian Grapevine Foundation Planting Scheme (AGFPS) is required to ensure planting material of the required health status and provenance is available to meet the needs of the winegrape, dried vine fruit and table grape as well as the vine nursery industries.

2 The AGFPS should ensure that one or more certified elite collections and/or if required by industry, certified best available collections, both with accompanying mother plantings are established and operated according to protocols to be developed by VCPTRG based on this report and using the terminology recommended in this report.

3 Eligibility for inclusion in elite or best available collections requires clones and varieties meet known health status requirements for both quarantineable and endemic pathogens. Consequently, it is recommended that AQIS provide endemic pathogen testing services (for a fee if necessary) concurrently with the implementation of testing for quarantineable pathogens.

4 Implementation of these recommendations should proceed in the following steps:

- Development of the required protocols for entry to and maintenance of collections that must be followed in order to qualify as elite or best available;
- Concurrent development of one or more collections managed by industry bodies or commercial entities;
- Development and implementation of an accreditation procedure for elite and best available collections and their mother vines;
- Implementation of an industry research and evaluation project to assess the risks and benefits of using planting material of various levels of health status and clonal or varietal provenance;
- Development and implementation of a communication and education campaign to improve industry understanding of the risks, benefits and costs of using certified true to type planting material of high health status; and
- Proposition and negotiation of accountability for funding, management, coordination and implementation of the above.

5 An NVHSC committee representing a range of industry interests should develop a communication campaign to support the establishment of the AGFPS and to foster the inclusion and commitment of the dried vine fruit and table grape industries as well as the wine grape and vine nursery industries.

6 Success of the proposed AGFPS depends on the establishment and effective operation of a "driver", preferably responsible to the NVHSC through a relevant TRG and funded by industry and Government through both the GWRDC and HAL. The model enabling and supporting the "driver" should draw on features of the AusCitrus Scheme and APFIP. The support for the coordinator should be commensurate with the national responsibility of the position.

1 INTRODUCTION

1.1 Purpose of Report

The Vine Collections and Propagation Technical Reference Group (VCPTRG) of the National Vine Health Steering Committee (NVHSC) has commissioned this report to enable incorporation of the best of national and international superior health status planting material programs and technology into Australian guidelines for the production and maintenance of vine material with known health status and provenance. This report will be the comprehensive resource from which guidelines for the production, handling and provision of planting material that is true to type and of known disease status, will be developed. The report provides the background reviews enabling development of the guidelines relevant to nuclear collections and to the commercial production of planting material. The guidelines will be based on scientifically justified best practice.

The report also recommends standardised terminology and labelling. It identifies and describes the need for and timing of an awareness and industry communication campaign focussing on implementation of the protocols.

1.2 Scope of Report

1.2.1 Types of grapevine material

This report refers to grape vine planting material (*Vitis sp*) to produce wine grapes (*V. vinifera*), table grapes, grapes for drying, and ornamental vines. Planting material includes varieties, clones (genetic selections within a variety) and rootstocks. Vines can be planted either on their own roots or grafted onto rootstocks (usually not *V. vinifera* – for example, var. shiraz grafted onto Schwarzmann[2] rootstock).

It has been agreed over many years of industry discussion, and during the consultation phase of this project, that the wine industry, the table grape industry, the dried vine fruit industry and the nursery industry would benefit from adoption of and inclusion in a high health planting material scheme. Planting material is propagated and supplied to each of these industries through various suppliers including independent nurseries, propagation facilities and nurseries operated by grape producers (including wine companies), and through regional and state vine improvement groups (VIGs). Collections of source material are maintained by all these groups plus CSIRO and some state agriculture departments.

1.2.2 Quality parameters

There is a range of quality parameters that are relevant to the provision of planting material including health status and physical characteristics such as shoot diameter and number of nodes. The quality parameters of concern in this report are those relevant to the provision of planting material that is:

- true to type, including clonal identity; and
- of known disease status.

A further relevant quality characteristic is that of comparative merit eg improved winemaking characteristics, improved yield, or improved ability to cope with environmental stress or management systems etc. While evaluation of varieties and clones for such quality characteristics is critically important and should be considered an integral part of vine improvement, it is not the major focus of this report.

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[2] Schwarzmann is one of several *V. riparia x V. rupestris* crosses.

1.2.3 Assessment of protocol requirements and methods

Requirements for and methods of assessing, cleaning up, pathogen eradication and maintaining disease status and trueness to type are also described.

1.2.4 Terminology

A range of terminology has been assessed and specific usage recommended in the report to form the basis for the development of protocols with clear and widespread application for all stages of the system.

Pests and pathogens, diagnostic methods, planting criteria and release to industry criteria have been assessed under the following categories – 'tolerated' or 'not tolerated'; Ideal, Practical, Negotiable and Non-negotiable. These categories should be used by the VCPTRG when deciding which pathogens will be prescribed and non-prescribed.

1.3 Methodology

This report describes a recommended framework for the provision of superior health status planting material for the relevant industries and focuses on "vine improvement". This proposed framework has been developed after a widespread consultation process and comparison with schemes for other perennial horticulture sectors in Australia and also some overseas grapevine and citrus, and stone fruit schemes. A list of those consulted including growers, nursery operators, researchers and industry people is at Annex 8.

2 THEMES FROM CONSULTATIONS

2.1 Background

The provision of grapevine planting material of high health status has been an industry issue for many decades and was the stimulus for the development of VIGs and their predecessors, the vine selection societies. Improved diagnostic techniques, substantially increased plantings since 1990, market pressures for new varieties and clones, and organisational changes and developments in the vine improvement movement have increased the necessity for this review. Themes and issues that emerged from the consultation process during this review are summarised below. They included the widespread industry support for the provision of planting material of higher health status and improved quality.

2.2 Issues

2.2.1 Risk management for planting material

Demand, supply, health status, clonal identity and range of planting material affect the assessment and management of risk associated with planting material. The availability and quality of information about the planting material and improving the capability of growers and providers of planting material to manage risk are widely recognised as important industry considerations.

2.2.2 Accreditation

The necessity for and value of accreditation of multiplication and propagation facilities was an important issue, as was identification of the most suitable organisations to be responsible for accreditation. A major factor relevant to accreditation was the need to protect against the threat of litigation should planting material not fulfil expectations. There was general agreement that independent audits including inspections should be mandatory.

Similar arguments were suggested to support the necessity for accreditation of providers of diagnostic services (including biological indexing) regardless of whether the service is outsourced or provided by the organisation owning and managing the nuclear collection.

2.2.3 Clonal Identity and Names

Consultations revealed a relatively widespread agreement on the importance of the maintenance of accepted clonal names and of adherence to the national accession numbering system. The use of suffixes such as HT (indicating the clone had undergone heat therapy) was preferred to signify treatment of the foundation plants, rather than the introduction of a new clonal name. Traceability and relationship to the original clone should be maintained in clonal names.

2.2.4 Right to Import

There was widespread agreement that public, industry and private organisations or individuals had the right to import new or improved varieties and clones provided the required quarantine provisions were observed. The ability and right of private organisations or individuals to benefit from their entrepreneurship was accepted. Foundation plantings must be able to cater for the needs and protection of private importers as well as public or industry body importers.

2.2.5 National Industry approach

The need for a co-ordinated national approach to industry development was identified in many consultations. This extended to the expressed support for a national scheme focussed on the provision of high health status, improved planting material.

2.3 Industry Support

2.3.1 Improved Quality of Planting Material

There was widespread agreement that, planting material with low health status or unsatisfactory provenance has caused, on many occasions, substantial loss across the grapevine industries. This has occurred in new plantings, re-planting sites, top working situations in wine grapes, dried vine fruit and table grapes in many regions, and with imported and domestic planting material. It was not surprising therefore that most of those consulted expressed support for the development of a better system for identifying the health status of planting material and for the provision of improved quality and high health status planting material. It was notable that many also commented on the purchasers' rights to make their own commercial decisions. This was viewed as an expression of support for growers to have available to them the full range of planting material from non-certified stock to certified, high health status material. This may represent existing concern about the issue of price compared with perceived risk.

Of those consulted, many associated with the wine grape industry suggested that a winery-mandated use of certified planting material, (i.e. as a condition in a supply contract) would be the most effective way of ensuring national advancement in the demand for and planting of better quality wine grape material. Satisfying such a condition would result in benefits to nurseries, wineries and the viticultural industries.

A major point of widespread agreement was the necessity for truth in labelling[3].

2.3.2 Organisational Structure for Provision of High Health Status Planting Material

While there was general agreement on the need for one or more nuclear plantings and for a nationally-accepted set of protocols and even standard operating procedures, there were substantial differences of opinion regarding funding options and organisational accountability, for such schemes. Such views have been demonstrated by the independent developments fostered by SAVII and AVIA, and the uncertain future and status of the state and CSIRO collections. WA, Tasmania, Queensland and selected private nurseries who import material have also established "nuclear" plantings of different scales and status, and meeting differing ranges of objectives.

2.3.3 Mandatory Certification

The re-occurrence of citrus canker in Queensland has stimulated concern within viticulture. The nature and source of threats and the risk management options for such industries are similar. Mandatory certification of propagated material is an alternative being discussed by the citrus industry. The issue is relevant to all perennial horticulture industries that import clones and varieties (and have additional threats from related species imported as 'ornamentals') such as citrus, stone fruit, apples and pears and viticulture. There may be an opportunity in the near future, for such industries in the interest of biosecurity and industry development, to co-operatively advance discussions with government and to educate industry on the relative benefits and costs of mandatory certification.

[3] See s52, Trade Practices Act 1974 (Cth)

3 INDUSTRY FRAMEWORK

3.1 Drivers for change

Several trends identified during consultations and literature searches are likely to stimulate changes in the system for provision of planting material. These include:

- Insufficient planting material to meet demand, leading to compromised quality;
- Insufficient planting material of a defined health status
- Increased willingness to litigate;
- Increased requirements for VIGs to meet commercial criteria to compete;
- Capability of some VIGs is constrained by reliance on volunteers and the cooperative structure of VIGs;
- Increased influence of competition policy on industry structure and operations;
- State quarantine demands;
- Technological advances such as development of improved diagnostic and propagation techniques;
- Increased industry awareness of plant biosecurity as a national issue;
- Identification of pathogens associated with emerging diseases and previously undescribed pathogens associated with known diseases;
- Opportunities and threats arising from trade becoming increasingly liberalised.

3.2 Structure

3.2.1 Current

The current industry framework for the provision of planting material of a range of health levels is described in Annex 1.

3.2.2 Development of preferred structure

Reviewing the provision of grapevine planting material has stimulated development of a revised and preferred conceptual framework describing the process or system. The proposed conceptual framework comprises a process divided into three phases:

- Foundation Phase;
- Multiplication Phase; and
- Propagation and Distribution Phase.

As well, we propose that the process comprises three pathways producing three classes of planting material to be labelled:

- Certified elite;
- Certified best available; and
- Non-certified.

The framework and classes are described below.

3.3 Phases in the Provision of Planting Material

The system of providing high health quality grapevine planting material using recommended terms is shown in Table 1 below.

Table 1: Description of system providing grapevine planting material of specified health quality

Phase	Generation	Stage	Function
Foundation	Pre	1. Identify and procure varieties and clones of interest to industry; test and prepare	Project demand for specified clones/varieties of known health status; Identify and procure from collections: - overseas (through AQIS, tested, cleaned); - Australian breeding program/collection (tested, cleaned)
	F0	2. Nuclear collections: - Elite; - Best Available	Genetic library of high health quality vines; Source of propagules for mother vines; Evaluation of varieties and clones
	F1	3. Mother vines	Multiplication to provide propagules for source blocks
Multiplication	F2	4. Source blocks	Multiplication to provide propagules for nursery propagation and distribution
Propagation and Distribution		5. Nursery propagation	Produce young vines (own rooted or grafted) to distribute to commercial vineyards
	F3	6. Commercial vineyard	New plantings, replacements (replanting, top grafting)

Each stage is discussed and issues relevant to individual stages are identified and discussed in detail in Annex 2.

3.3.1 Issues

Issues regarding the framework as a whole or more than one stage include:

- Acceptance that the stages as identified and described above represent the situation;
- Development of agreed terminology within viticulture and across other horticultural industries;
- Development of industry agreement to legitimise scope, content and auditing of accreditation schemes, testing protocols, and treatment protocols for pathogen eradication.

Issues that are relevant to stages 1 to 3 are:

- Selection criteria and comparative merit evaluation for new varieties and clones;
- Feasibility and relative industry benefit of a centralised (industry monopoly) approach compared with fragmented approach i.e. commercial freedom;
- Factors affecting forward planning for acquisition of new varieties and clones.

Entry of vine material into each stage, and maintenance of the quality of vine material within each stage of the process requires adherence to protocols based on the best scientific knowledge and specified procedural standards. The scientific bases for many of these protocols have been

described as part of this project. The procedural standards have been developed as accreditation schemes by AVIA and VINA. AVIA's guidelines were developed with industry input to industry-owned projects supported by GWRDC in the 1990s. The VINA accreditation procedures were prepared by independent nursery industry members, from their collective experience. Each scheme requires periodic review and revision. Consistent terminology and definitions are required to underpin these developments and our recommendations are shown below.

3.4 Recommended Terminology and Definitions

Specification of processes to provide improved planting material requires general agreement on the terms to be used and their specific meaning. Consideration of a range of terms and recommended usages follow.

3.4.1 Recommended terminology

The following terminology is used in the report and is recommended for general adoption by the viticultural industries. It is also put forward for potential promotion to other perennial horticultural high health schemes. The terminology used in the report has been selected after consideration of the terminology used internationally and locally, although in several cases it is new, so as to avoid confusion with similar terms used in other countries.

Pathogen - *Any species, strain or biotype, or pathogenic agent, injurious to grapevine, grape or grape product, or capable of vectoring a grapevine pathogen.*

Vector – *An organism that transmits a particular disease or parasite from one animal or plant to another.*

Quarantineable pathogen – *A pathogen determined by AQIS to be of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled" (FAO, IPPC)*

Pathogen-infected - *plant material infected with a grapevine pathogen*.

Pathogen-tested - Scheme, industry: *planting material available to industry that has been indexed and found not to carry detectable levels of economically important, endemic (prescribed and non-prescribed) pathogens in the year of testing. [It is assumed to be free of detectable levels of quarantineable pathogens]*

Pathogen-tested – AQIS: *planting material not yet released, that has been indexed and found not to carry detectable levels of quarantineable pathogens*. (NOTE -status of endemic pathogens unknown)

Clean – (colloquial) **–** as for *pathogen-tested.*

Prescribed pathogen – Scheme: *a pathogen deemed unacceptable ('non-negotiable' not tolerated) in grapevines within elite and/or best available collections.*

Non-prescribed pathogen - Scheme: 1) *a pathogen deemed acceptable ('negotiable', tolerated) in grapevines in non-certified collections or* 2) *with industry approval, in best available collections.*

Released from quarantine – *planting material that has passed through a series of indexing and other tests under the direction of AQIS, and is determined to be free of detectable levels of quarantineable pathogens.*

Indexed - *screened biologically (herbaceous and/or woody indicators), serologically or molecularly for the presence of nominated pathogens and symptoms produced by them.*

Improved varieties - *varieties that have been heat treated and pathogen tested or simply pathogen tested and assessed for horticultural merit.*

Heat treatment – *incubation of plants at high temperatures (36° to 40°C) for 4-12 weeks combined with the excision and culture of very small stem tips (shoot tip or meristem culture) to eradicate viruses from infected varieties or clones.*

High health status grapevine certification scheme – *a scheme utilising phytopathological and horticultural competencies aiming for improved grapevine quality, vine health definition, varietal conformity and clonal identification.*

3.4.2 Classes of Planting Material

Until now there has been no structured approach to producing elite grapevine planting material by the Australian VIGs. Existing genetic resource collections have been maintained by State departments and CSIRO. While justification of their continued maintenance is frequently questioned at the treasury level, it is likely that states will continue to support such collections, and they are viewed generally as useful resources. Material from these collections would be eligible for entry into best available or elite schemes, after testing and cleaning-up processes.

The process of production of grapevine planting material of specified health status as described in this report provides for three classes of planting material:

- Certified elite;
- Certified best available; and
- Non-certified.

These three classes of planting material are defined below.

Certified elite material is planting material that meets the highest health and quality criteria and will provide the foundation of the Australian viticulture industry. It comprises graftlings (if rootstock also certified elite) or own rootlings that:

- can be traced to an elite collection and is thus "certified true to type";
- have undergone testing and procedures as described in the protocols and is thus has no prescribed or non-prescribed pests/pathogens detected;
- are a product of a certified elite source block; and
- have been propagated and distributed by an AVIA or VINA accredited nursery.

The pathogens for which tests have been performed must be clearly identified on associated labels or documentation. Ampelography by visual examination and DNA testing should be conducted after heat treatment to provide an accurate description of the variety/clone. Prior to entering the elite collection, this material, depending on its maintenance after heat treatment, may not need hot water treatment for fungi, bacteria and phytoplasmas, as heat treatment (and maintenance systems thereafter) should eradicate these pathogens.

Certified best available material is planting material that meets most of the highest criteria but has tested positive for one non-prescribed grapevine pathogen. It comprises graftlings (if rootstocks of elite status) or own rootlings that:

- can be traced to the best available collection and is thus "certified true to type";
- have undergone testing and procedures as described in the protocols and is thus labelled for the detected pathogen provided no additional pests/pathogens have been detected;
- are a product of a certified best available source block; and
- have been propagated and distributed by an AVIA or VINA accredited nursery.

Non-certified material includes graftlings or own rootlings from sources that may originally have been of elite, best-available or non-certified status. Deviation from production, maintenance and handling protocols renders material non-certifiable regardless of the original source. Material propagated or distributed by a non-accredited nursery, regardless of its source, can only be sold as non-certified. AVIA and VINA accredited nurseries are also able to distribute non-certified material. The health status of non-certified material is not defined. There are no limitations on the production of non-certified material. This material may be sold without undergoing ampelography, pathogen testing or specified treatment other than that required by state regulations. This material is likely to be in demand when supply of certified elite or certified best available material does not meet demand.

3.4.3 Rationale

The idea of having both elite and best available collections was supported by recommendations made by the ICVG during their14th meeting in Locorotondo, Italy 2003, which suggested two sanitary classes and by inference, a non-certified class. The ICVG recommendations state that: "Class 1 should only included grape nursery stock that tests negative for the most damaging diseases/pathogens". The ICVG recommends that: "Class 2 would be a specific pathogen tested certification system for stock that remains within regulatory regions and is only distributed with disclosure of virus status". In this report, replacement terminology for Class 1 and 2, is recommended.

3.4.4 Test Regimes

Heat treatment of material destined for elite collections will reduce the risk of introducing pathogens, especially viruses, which may escape detection using ELISA, PCR, biological indexing or other testing. Although heat therapy does not provide a 100% guarantee that viruses and other pathogens will be eradicated it is considered to be the most reliable method for the production and selection of "high health" varieties and clones.

Heat treated plants have been incubated at 36°C-40°C for 4-12 weeks. For vines the current practice is 38°C for 6-8 weeks. These high temperatures reduce the replication of virus and other pathogens in the plant tissue, allowing many shoot tips to grow free of the pathogens. The shoot tips, containing one or two leaf primordia, or apical meristems are excised and grown in tissue culture to obtain rooted plants. These plants must be established[4] in an area removed from other elite collection plants until they have been tested by PCR and/or ELISA and woody indexing. Preliminary screening by PCR and/or ELISA would reduce the need to screen larger numbers of plants by woody indexing. Once heat treated, virus tested plants have been selected, determination of their horticultural merit is required.

Without endemic pathogen testing in quarantine, it is likely that material from non-accredited sources and released by AQIS, would not be eligible for either an elite or best available collection, since its health status would be unknown. The time delays for entry to either

[4] Prescription of the type of substrate (soil, soil less media, or potting mix) and whether or not the vines should be confined to pots is being considered.

collection stream would be negligible if full testing or heat treatment were performed during the quarantine period. This would benefit the industry as a whole and would utilise to a greater degree the available expertise and resources within AQIS.

3.4.5 Use of the term "Vine Improvement"

The process as a whole has been commonly designated "Vine Improvement", but consultations have led to the suggestion that the term "vine improvement" be restricted or perhaps even discarded. If retained, "vine improvement" may more correctly be thought of as encompassing the functions of the Foundation phase which are:

- identification and procuring of superior clones and varieties;
- disease testing, evaluation and elimination
- evaluation for viticultural and rootstock merit, and for example, oenological, drying or other horticultural merit; and
- establishment and maintenance of the elite and best available collection including the propagation and growth of mother vines to produce planting material for elite and best available source blocks.

The other two functions that would now fall outside the term "vine improvement" are:

- management of source blocks to produce cuttings; and
- commercial propagation and distribution of certified and non certified planting material.

It is noted that in California the term 'improvement' has a restricted meaning and these functions would generally be included within 'clonal protection'.

3.5 Critical Control Points

Production of planting material of each class can be described as a separate stream within the overall process. The critical control points for each stream within the above process, the control measures required and the recommended critical limits have been identified and specified for certified elite material and certified best available material and are detailed in Annex 3. The following definitions have been used:

- **Critical control point** – A step at which control can be applied and is essential to prevent or eliminate a hazard or reduce it to an acceptable level;
- **Control measure** – any action or activity that can be used to prevent or eliminate a hazard or reduce it to an acceptable level; and
- **Critical limit** – a criterion which differentiates acceptability from unacceptability.

Effective operation of this process requires the development of protocols relevant to each critical control point. Much of the scientific and practical basis for the development of these protocols is provided in this report. However, writing the protocols is beyond the scope of this report and has previously been identified as the responsibility of VCPTRG.

3.6 Flow Chart

A flow chart using conventional flow chart symbols has been produced to further characterise the process and to enable identification of critical control points as decision points. The flow chart is shown in full in Annex 4 and is shown in sequential sections in Figure 1, Figure 2, and Figure 3 below. The Foundation Phase has been divided in two for purposes of clarity. A flow

chart for part 1 of the Foundation Phase which includes identification and procuring of clones and varieties in demand is shown in Figure 1 below.

Figure 1 : Flowchart, Foundation Phase – Part 1

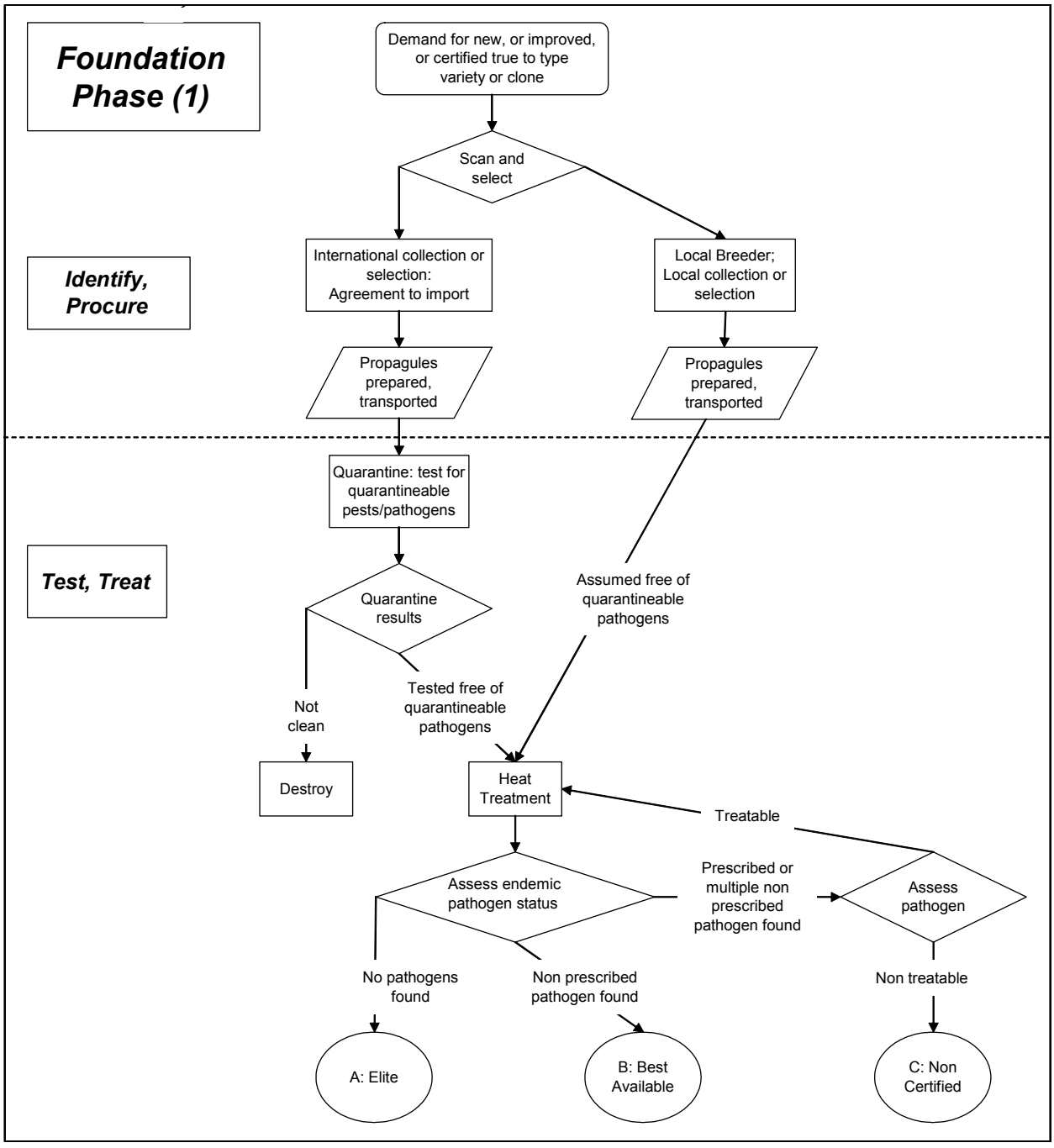
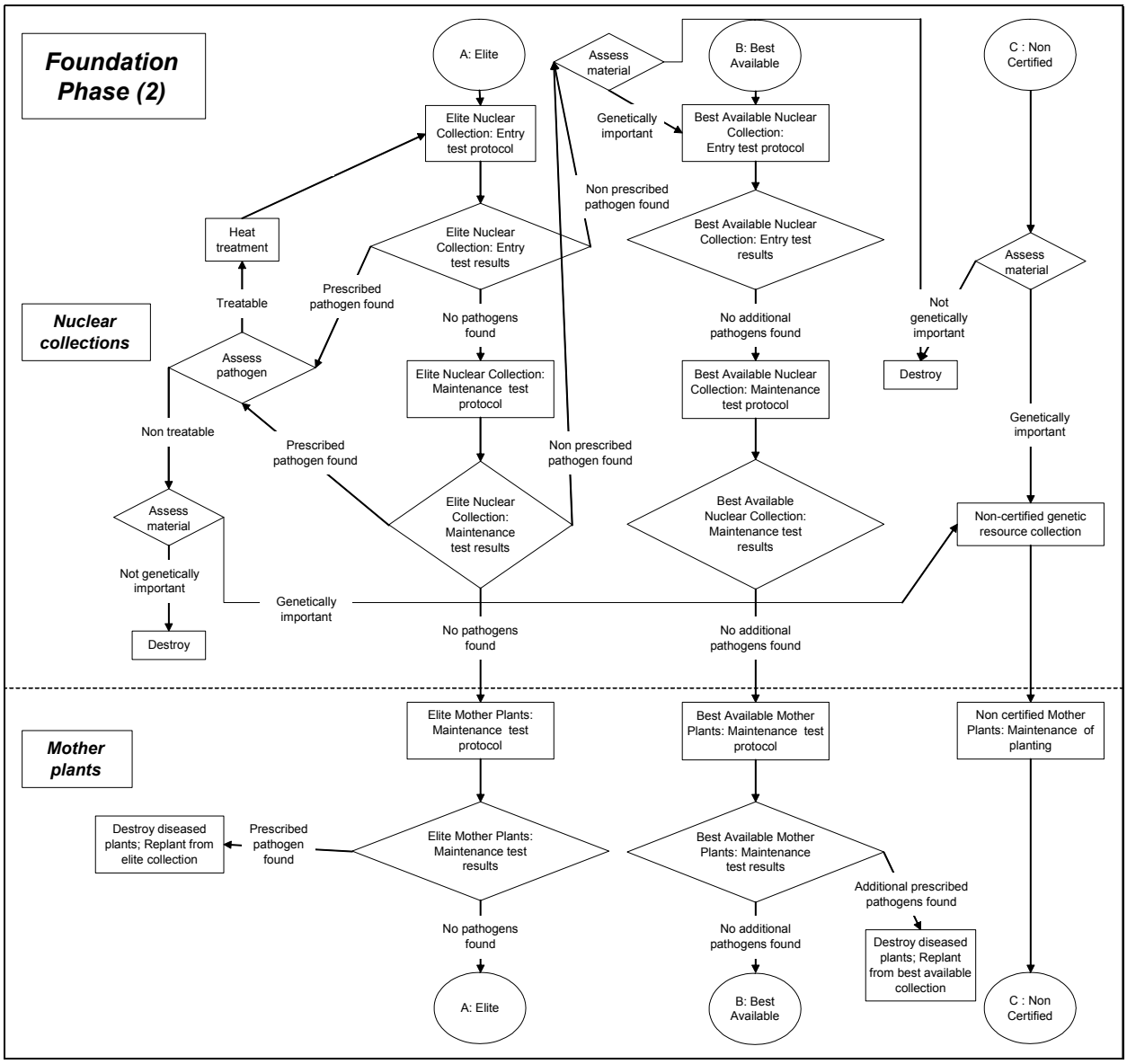


Figure 1 starts with a perceived demand for a variety or clone which is identified, by the potential importers or local decision-makers, to be of industry interest. Importers include private individuals or companies, government or industry bodies. The variety or clone is either procured from overseas or within Australia, and is then tested and prepared for entry into elite (stream A) or best available (stream B) nuclear collections. Stream C depicts material with prescribed pathogen(s) or multiple non prescribed pathogens present. Depending on the genetic merit of the material, it may enter a genetic resource collection or it may be destroyed.

Streams A, B and C continue in the flow chart for Foundation Phase part 2, which is shown in Figure 2 below. For streams A and B, it shows the entry test process and results, and the maintenance test process and results for the nuclear collections (elite, best available), and non-

certified genetic resource collections, and the progression into mother plants for each stream which are also tested. Mother plants provide planting material for source blocks.

Figure 2 : Flowchart, Foundation Phase – Part 2



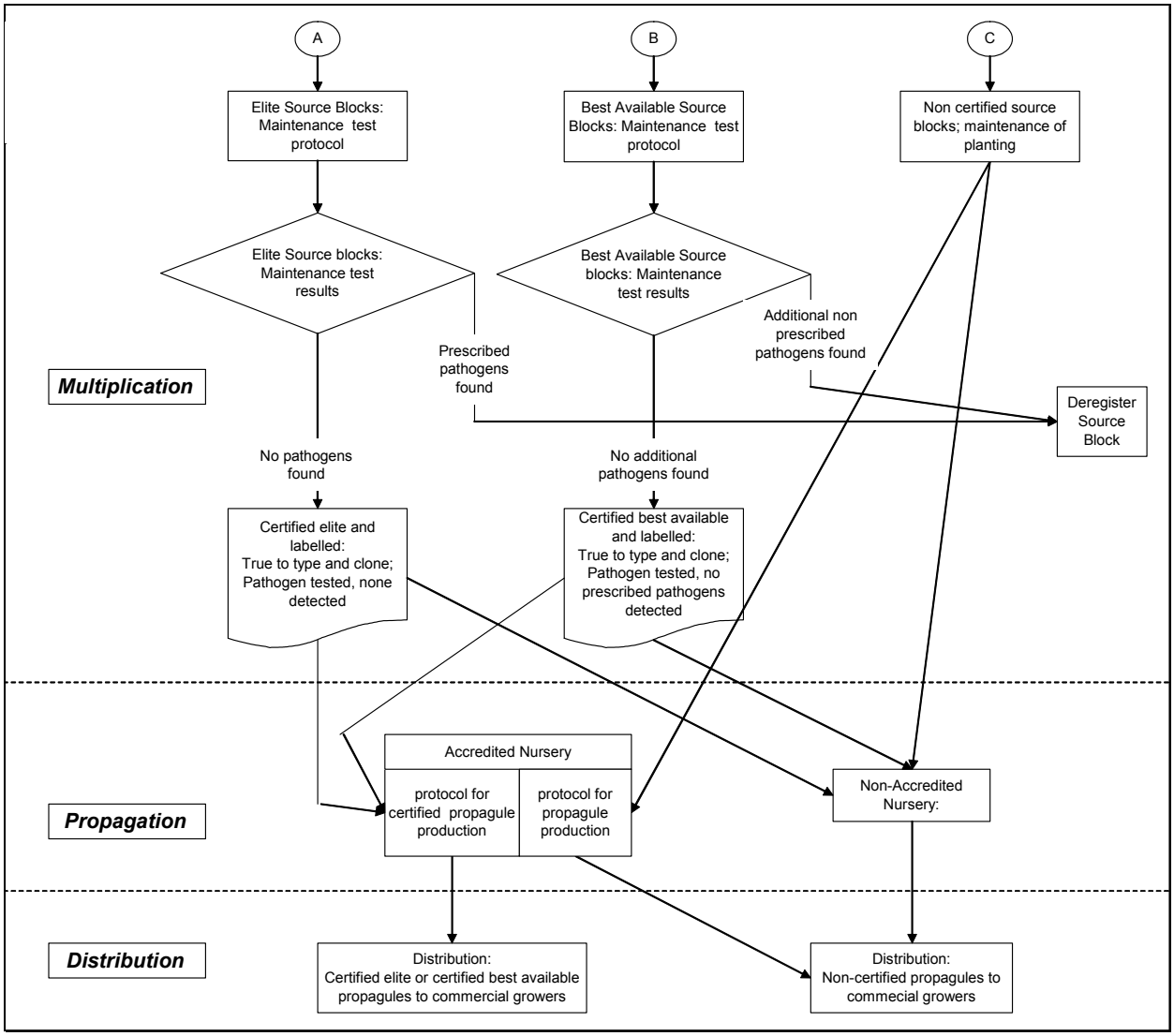
The flow chart finishes with circles indicating the same streams of the elite, best available and non-certified pathways and which are connectors to the flow chart for the next phase, Multiplication and the following phase, Propagation and Distribution, shown as Figure 3 below.

All plants within the foundation collection and the mother plantings are visually monitored during the growing season for the presence of pests and disease. Diseased grapevines will be tagged and tested for the presence of previously unrecorded pathogens. Pests that are potential vectors of serious pathogens will be eradicated and the infested vines monitored and tested for the pathogens.

Figure 3 incorporates the remaining two phases of the process – multiplication using source blocks, and the nursery based operations of propagation and distribution. Elite source blocks are recommended not to be established within commercial plantings. The 3 streams conclude with the provision of planting material of 3 different classes labelled to reflect their differing health status. Only accredited nurseries are able to provide certified elite or certified best available

material. However, both accredited and non accredited nurseries can propagate and distribute non-certified planting material.

Figure 3 : Flowchart - Multiplication Phase; Propagation and Distribution Phase



The establishment of planting material on commercial vineyards is the final stage of the process but is not within the scope of this report.

4 EFFECTS OF PESTS/PATHOGENS ON VINE HEALTH

4.1 Introduction

Many pathogens and pests have a significant impact on the health of grapevines, affecting the yield and quality of grapes and the quality of grapevine propagation material. Many of these pathogens can be transmitted through propagation material.

The aim of a certification scheme and the supporting accreditation scheme is to reduce the threat of spread of these pathogens and pests, to provide traceability, and to ensure provision of high quality planting material that is true to type. Vineyards established with such material, if properly maintained, should remain sustainable and productive for many years.

Current research indicates that 11 viruses, 5 viroids, 3 phytoplasmas, 6 bacteria and 98 fungi which infect grapevines, have been reported in Australia. The most important of these pests/pathogens are listed in Table 2 below.

Table 2 : Important pests/pathogens of Grapevine Planting Material in Australia

Pathogen/pest	Name
Virus	Grapevine virus A vitivirus (GVA); Grapevine virus B vitivirus (GVB); Grapevine fan leaf nepovirus (GFLV); Grapevine fleck maculavirus (GFkV)*; Grapevine leafroll-associated ampelovirus 1 (GLRaV-1); Grapevine leafroll-associated closterovirus 2(GLRaV-2); Grapevine leafroll-associated ampelovirus 3(GLRaV-3); Grapevine leafroll-associated ampelovirus 4(GLRaV-4); Grapevine leafroll-associated ampelovirus 5(GLRaV-5); Grapevine leafroll-associated ampelovirus 9(GLRaV-9); Grapevine rootstock stem lesion closterovirus (GRSLaV = strain of GLRaV-2; Grapevine red globe maculavirus (GRGV) Grapevine rupestris stem pitting associated foveavirus (GRSPaV = Grapevine stem pitting associated closterovirus?)*
Viroid	Australian grapevine viroid (AGVd) Citrus exocortis viroid (CEVd) Grapevine yellow speckle viroid-1 (GYSVd-1) Grapevine yellow speckle viroid-2 (GYSVd-2) Hop stunt viroid (ASVd)
Phytoplasma	Australian grapevine yellows (AGY) Tomato big bud (TBB) Buckland Valley grapevine yellows (BVGY)
Bacteria	Crown gall - *Agrobacterium vitis*

Pathogen/pest	Name
Fungi	*Botryosphaeria dothidea* *Botryosphaeria obtusa* *Botryosphaeria rhodina* *Botryosphaeria ribis* *Botryosphaeria stevensii* *Uncinula necator* *Eutypa lata* *Plasmopara viticola* *Phaeacremonium aleophilia* *Phaeomoniella chlamydospora* *Phomopsis viticola*
Pests	Citrophilous mealybug - *Pseudococcus calceolariae* (vectors of GLRaV-3 in New Zealand); Citrus mealybug - *Planococcus citri* (vectors of GVA, GLRaV-3 in other countries) longtailed mealybug - *Pseudococcus longispinus* (vectors of GLRaV-3, 5, GVA in other countries) Plum scale - *Parthenolecanium corni* (vectors of GLRaV-1, 3 in other countries) Obscure mealybug - *Pseudococcus viburni* (= *P. affinus*) (vectors of GVA, GVB GLRaV-3 in other countries) Grape phylloxera - *Daktulosphaira vitifoliae* *Brevipalpus* spp – including bunch mite Blister mite and bud mite - *Colomerus vitis* Grape leaf rust mite - *Calepitrimerus vitis* Dagger nematode - *Xiphinema index* (vector of GFLV) Dagger nematode - *Xiphinema vuittenezi*, (vector of nepoviruses) Root-knot nematodes - *Meloidogyne* spp Root-lesion nematodes - *Pratylenchus* spp Citrus nematode - *Tylenchulus semipenetrans*

* Specific tests for two strains of each of these viruses is available

Some viruses have multiple strains. The pathogenicity and host range of each is likely to be different. Serological and molecular tests may detect all known strains but not differentiate them. For other viruses all strains may not be detected by a single test. It is our opinion that all strains of viruses be tested for, where possible.

The effects of various pathogens on grapevines are summarised below and discussed in detail with references in Annex 5. The successful outcomes of planting material of a known high health status are compared with those for planting material known to be infected. A more definitive study would allow quantitative estimation of the benefits and costs of specific levels of planting material health, in specified situations.

4.2 Effects of Virus

4.2.1 Quarantineable and Endemic Viruses

Many important grapevine viruses are yet to be detected in Australia and have therefore been specified as “quarantineable”. Many others are endemic and are therefore considered non-quarantineable and outside the usual screening and testing protocols of AQIS.

Imported planting material that has been determined by AQIS to be free of detectable levels of quarantineable pathogens and pests is eligible for release to industry. It is therefore assumed that all grapevine planting material that has legally entered Australia is free (of detection) of the quarantineable pathogens known at the time of entry. It is however known that exotic diseases do on rare occasions, enter the country, as was recently seen with grapevine leaf rust. It is also likely that over time the list of quarantineable pathogens for any particular plant species will change.

At present, AQIS is not required to test for endemic pathogens in planting material entering the country. As a consequence, material being released from quarantine is not usually of a defined health status. As such, planting material released by AQIS would not be eligible for entry into the proposed best available or elite collections.

Importers in the grape industry should however be aware that AQIS does offer the service of testing for endemic pests and pathogens. It is at the discretion and cost of the importer. The available testing for pathogens endemic to Australia, by current serological and molecular methods, may not however result in detection of those pathogens in low titre or of a particular strain. Heat treatment provides a means to remove most pathogens, regardless of their detectable titre.

Heat treatment is proposed as a means of providing further confidence in the health status of planting material entering the elite or best available collections. If material is heat treated while going through post entry quarantine, the time taken for introducing pathogen-tested varieties into the collections would be greatly reduced.

A list of viruses and their quarantine status in Australia is tabulated in Annex 6.

4.2.2 Endemic Viruses

Table 2 above lists the viruses that are known to occur in Australia. Various reports associate viruses with changes in yield, changes in vine and fruit quality, graft incompatibility and graft take rates, and vine decline and death. In addition, viruses can cause deformation that makes clonal identification difficult. Some viruses may not cause disease in isolation. However, combinations of viruses infecting a grapevine can significantly affect performance.

4.2.3 Incidence of effects

Specific examples of the effects of virus are presented in Annex 5. These examples indicate that the effect of virus may be dependent upon the grape cultivar, the combination of viruses and/or strains of virus present. Some viruses may not induce disease in a scion until they are grafted onto susceptible rootstocks, or rootstocks containing other viruses. The examples also indicate that the effect of virus strains may be important, i.e. different strains may be associated with different severity of disease. Grapevine management practices and stress can also affect the vine's susceptibility to the viral impact.

4.2.4 Transmission

All grapevine viruses can be transmitted through planting material. Some viruses, such as the majority of the leafroll associated viruses, are also transmitted via insect vectors. Mealybugs are the vectors of several viruses in other countries.

4.2.5 Effects on fruit yield, fruit quality and vine health and morphology

There are many examples, especially from other countries, that show a substantial reduction in yield associated with single and mixed viral infections. GFLV is considered one of the most serious pathogens of grapevines in many countries, causing 20 to 90% yield reduction in some cultivars and under certain environmental conditions. Excessive yield and vegetative vigour has

been raised as a potential detrimental effect of viral elimination. However, grapevine management (irrigation, pruning, nutrient regimes etc) should be used to control any such effects.

Viruses are associated with changes in the quality of fruit, depending on the cultivar and the virus present. Several studies have shown that leafroll associated viruses, most notably GLRaV1 and GLRaV3, alone or in combination with other viruses, are associated with reduced sugars and increased titratable acidity.

Several viruses are associated with vine decline including leafroll viruses and vitiviruses.

Graft incompatibilities associated with viruses can lead to decline and death of the grafted scion or rootstock. The opportunities for successful top-working are greatly reduced by the presence of virus in rootstock and/or scion.

Viruses can also induce morphological changes leading to incorrect clonal/varietal identification. One study has demonstrated that despite genotypic influence, only clones free of virus (especially GFLV), can be correctly identified by leaf morphology.

4.2.6 Future

There is general awareness that advances in technology have resulted in the detection of viral particles that are yet to be defined as the causal agents of disease. Until field pathology and research substantiate the impact of the undefined virus particles, they will remain classified as non-negotiable, in the interest of long-term industry biosecurity.

4.3 Effects of Phytoplasmas

Australian grapevine yellows (AGY) disease and phytoplasmas are found in most viticultural regions of Australia. Although Koch's postulates have not been fulfilled, phytoplasmas have an accepted and strong association with AGY symptoms and are considered to be the cause of this disease. Chardonnay and Riesling appear to be more susceptible to AGY disease, than other varieties. However, AGY symptoms have been observed and phytoplasmas have been detected in other varieties, both white and red. Significant reductions in yields have been reported from AGY affected vineyards.

Many phytoplasmas are spread to plants by insect vectors, most of which belong to the superfamilies Cicadelloidea (leafhoppers) and Fulgoroidea (planthoppers). No insect vectors have been identified for AGY or BVGY phytoplasmas, although AGY phytoplasma has been detected in the common brown leafhopper, *Orosius argentatus* (Evans) using PCR techniques. Recent studies have shown that TBB phytoplasma can be acquired from grapevine by *O. argentatus* and subsequently transmitted to Faba bean but the ability of the leafhopper to transmit TBB phytoplasma back to grapevines has not been confirmed. The transmission of phytoplasmas through grapevine cuttings has not been demonstrated. However, Flavesecnce dorée phytoplasma has been shown to spread through infected cuttings and rootstocks and a hot water treatment of cuttings is recommended to control spread of this phytoplasma.

Restricted growth (RG or RSG) disorder also commonly affects Chardonnay grapevines in the Riverland and Sunraysia districts. The cause of RG is not understood but phytoplasmas are considered as one possible cause. RG symptoms include retarded growth resulting in shortened shoots and smaller leaves. RG affected grapevines have an overall appearance of being stunted and lacking in vigour, early in the season particularly.

Similarly, phytoplasmas have been proposed as one of a number of causes of late season leaf curl (LSLC) disease in Chardonnay. Interestingly, Chardonnay grafted onto the rootstock 3309C in the US display similar symptoms to LSLC observed in Australian Chardonnay. Graft union

incompatibility was also observed. The results indicated an association with a graft transmissible agent and there may have been an association with GRSLaV.

4.4 Effects of Viroids

Five viroids have been detected in Australian grapevines and are listed in Table 2. All viroids can be transmitted via mechanical means, including pruning and grafting equipment and through planting material. It is generally accepted that viroids pose little threat to productivity and the quality of grapevines. However, each of the grapevine yellow speckle viroids (GYSVd 1 and 2) cause yellow speckle symptoms. A reduction in photosynthesis is possible when the disease is severe, resulting in reduced growth and productivity. When GFLV and GYSVd infect grapevines together, vein banding disease is often observed. Australian grapevine viroid, citrus exocortis viroid (CEVd), and hop stunt viroid (HSVd) are not known to cause symptoms in grapevine.

The viroids that infect grapevines can have a significant impact on other crops, affecting performance of the host plant and, in some cases, the quality of the associated end product. For example, HSVd causes stunting in hops and cone weight may be reduced by 50%. Variants of HSVd also infect citrus (cachexia disease), plum and peach (dapple fruit disease), and almond and apricot (latent infections). While most commercial species and cultivars of citrus are tolerant to CEVd, if they are grafted onto sensitive rootstocks, the viroid can reduce tree size and lower yields in infected, declining citrus. This has implications for industry as grapevines are often grown in regions where citrus and hops are also grown. Some grape growers and nurseries grow and manage both grapes and citrus.

4.5 Effects of Bacteria

Agrobacterium vitis (*A. tumefaciens* biovar 3) can have a significant effect on grapevines in Australia. *A. vitis* can be transmitted via planting material. Hot water treatment can significantly reduce the titre of the bacterium thereby improving the success of grafting in nurseries. However, the only sure way to eliminate the bacterium from grapevines is to use *in vitro* shoot tip culture as the bacterium does not systemically invade green shoots. *A. vitis* causes galls on trunks at or above the graft union and necrosis of the roots. Severe infections lead to a reduction in vine growth and yield compared to mild infections. It causes significant losses to nurseries. In the vineyard, severe infections can lead to decline and death of grapevines.

4.6 Effects of Fungi

Only a small number of the fungi that infect grapevines are considered to be pathogenically effective and economically important. These fungi are associated with diseases such as Esca, Eutypa dieback, Petri disease and vine decline and are listed in Table 2.

Eutypa lata is not transmitted via planting material. However, *E. lata* affects the productivity and sustainability of vineyards. Infection of *E. lata* can result in stunted shoots and does cause Eutypa dieback, thus reducing the number of cuttings per vine. Diseased grapevines have a shortened life span and will need to be replaced sooner than unaffected grapevines. Consequently this disease should be controlled in an elite collection of high health grapevines, in mother vines and source plantings.

Botryosphaeria dothidea, B. obtusa, *B. rhodina*, *B. ribis* and *B. stevensii* have been found in association with canker and decline of grapevines, although their role in the disease is not fully understood. Isolation and pathogenicity tests suggested that *B. obtusa* may have a role in the decline of grapevines, for example cv Semillon, in the Hunter Valley of Australia. If *Botryosphaeria* sp do cause decline of grapevines then, like *Eutypa lata*, they could also have an effect on the productivity and sustainability of vineyards.

Although Koch's postulates for cause of disease have not been fulfilled, *Phaemoniella chlamydospora* (=*Phaeoacremonium chlamyidosporum*) is thought to be the cause of Esca disease and Petri disease. Esca can result in apoplexy of affected grapevines. The lifespan of Esca affected grapevines is reduced.

Other fungi are often found in association with Esca disease and young vine decline, including *Phaeoacremonium aleophilum, Formitiporia punctata*, *Stereum hirsutum* and *Eutypa lata*. The role of *P. aleophilum* is uncertain, although it is detected less frequently in affected grapevines compared to *P. chlamydospora* in Australia. *Formitiporia punctata* is often associated with white heart rot in Esca affected grapevines but other fungi have been found in association with white heart rot of Esca affected grapevines in Australia. *Stereum hirsutum* was also found in association with white heart rot in other countries, but to a lesser extent than *F. punctata*, and is also no longer considered a potential cause of Esca. Additionally white heart rot is not always associated with Esca disease. In the case of young Esca (ie Esca in vines less than 10 years old) in Australia, *P. chlamydospora* was the only fungus consistently isolated from affected grapevines.

Esca disease of older grapevines in Australia is rare (Edwards *et al* 2001a). *P. chlamydospora* can sporulate in the vineyard in cracks in the wood of infected vines. Although the means of dissemination of the conidia to other vines is not understood, the fungus is thought to invade wounds of mature vines.

Petri disease, also known as black goo decline, is a serious disease of young vines worldwide and can cause establishment problems in new vineyards. The associated fungus *P. chlamydospora* is transmitted through planting material. Affected planting material grows poorly and has difficulty establishing. Graft union failures, shoot dieback, decline and death of young grapevines can also be associated with Petri disease. Recent results have shown that hot water treatment can reduce the amount of *P. chlamydospora* infection in planting material.

Phomopsis viticola causes lesions on canes and leaf spots. Affected canes can be weakened and/or girdled and poor berry set has been observed, resulting in yield loss. The fungus is spread through planting material but can be controlled by use of hot water treatment.

Uncinula necator (powdery mildew) can over-winter as mycelium in buds or as cleistothecia in bark. Transmission of the powdery mildew through planting material has not been reported. However, severe infections in vineyards can lead to reduced growth and winter hardiness of vines. Consequently, in any certification scheme, this powdery mildew should be controlled.

Plasmopara viticola (downy mildew) usually survives as oospores in the soil and old infected leaf material but can overwinter as mycelium in buds and persistent leaves in mild grape growing regions. Severe defoliation can decrease the hardiness of buds through winter. Consequently this fungus should also be controlled in any certification scheme. Transmission of downy mildew through planting material has not been reported.

Root rotting fungi such as *Rhizoctonia solani*, *Phytophthora* spp, *Armillaria* spp and *Pythium ultimum* can infect grapevines and cause establishment problems. Infected vines may show a lack of vigour. Consequently these pathogens should be considered when establishing collections and plantings for the provision of high health material. Hot water treatment at 54°C for 5 minutes or 50°C for 30 minutes may be effective against root rotting fungi eg, *Phytophthora cinnamomi* that can be transmitted on rootlings.

In summary, any endemic pathogen that reduces the chance of graft success or vineyard establishment and productive life, or affects yield or quality of fruit, should be eliminated from elite and best available collections of planting material.

4.7 Effects of Insect and Other Pests

The long tailed mealybug, *Pseudococcus longispinus* and the citrophilous mealybug *P. calceolariae* can infest Australian grapevines. Mealybugs excrete sticky honeydew in which sooty mould and other fungi can grow and affect bunches and leaves. Heavy infestations of mealybug and, subsequently, sooty mould and fungi, can result in crop loss. Both the long tailed mealybug and the citrophilous mealybug have been shown to transmit grapevine viruses in other countries. In addition, Table 2 lists some other mealybugs and a soft scale species that occur in Australia, although not reported on grapevines. These insects can infest grapevines and transmit grapevine viruses in other countries. Mealybugs and scale can overwinter on their host plants and thus have the potential to be transmitted on grapevine cuttings.

Several mite species, including *Brevipalpus* spp, *Colomerus vitis* and *Calepitrimerus vitis* can also be harmful to grapevines, causing a yield and growth reduction in young vines. Mites can overwinter in buds and under rough bark. Consequently they may also be transmitted through propagation material.

The grape Phylloxera (*Daktulosphaira vitifoliae* Fitch) is found in small areas in central Victoria (Nagambie, Upton, Mooroopna) and northeast Victoria (North East, King Valley), in southeast New South Wales (Corowa) and in Camden and Cumberland near Sydney. The movement of grapevine material from these regions is restricted. Consequently, collections or source blocks of high health material cannot be located in these regions and should be located in Phylloxera Exclusion Zones (PEZs). The provision of high health phylloxera resistant rootstocks can ameliorate the effects of phylloxera in the affected regions.

Various nematodes can infest soils where grapevines are grown. Dagger nematodes can cause root damage resulting in loss of vigour and yield. The dagger nematode *Xiphinema index*, vector of GFLV, has a restricted distribution in Australia and is considered quarantineable. Recently, another dagger nematode, *X. vuittenezi*, was discovered in a young Shiraz planting where vines displayed symptoms of unthrifty growth and decline. It was unclear if this nematode was the cause of the observed symptoms. However, *X. vuittenezi* may transmit nepoviruses, including grapevine chrome mosaic virus and cherry leafroll virus. Grapevine chrome mosaic virus has not been reported in Australia.

Root-knot nematodes, *Meloidogyne* spp., can have an economic impact on grapevines, reducing vigour and yield when they are in high numbers. The combination *of M. incognita* and the fungus *Rhizoctonia solani* was associated with stunting of grapevines in a field nursery. Root-lesion nematodes, *Pratylenchus* spp and the citrus nematode, *Tylenchulus semipenetrans* can also cause a reduction of vigour and yield in grapevines. Accredited nurseries should therefore consider fumigation of areas prior to the planting of elite material.

Hot water treatment at 54°C for 5 minutes or 50°C for 30 minutes is effective against nematodes and phylloxera. This treatment may also prevent the transmission of mealybugs, scale, and mites on propagation material.

4.8 Examples of commercial loss due to pests/pathogens

Industry consultations have provided some first-hand examples of substantial loss due to failure to use high health quality planting material. A more comprehensive study of the benefits and costs of poor quality planting material is warranted.

4.8.1 Importance of Hot Water Treatment

Vineyard established in three stages: the planting material for two stages had been subjected to HWT, while planting material for the third stage was not. In the third stage planting:

- Attainment of full yield was delayed by 2 years;

- Full yield was 30% less; and
- Mitigation required extra mulch and irrigation in an attempt to improve vine condition.

4.8.2 Presence of leaf roll virus affected top working results

Several vineyards that were top worked with a scion infected with leaf roll have not taken satisfactorily and required regrafting within three years thus incurring double the expected cost. As well, at least two years total production was lost.

4.8.3 Presence of leaf roll affected productivity

A vineyard was established with planting material of which 10% was infected with leaf roll virus. Total yield of the vineyard is estimated to be 10% less, and increased costs are incurred in monitoring for any viral spread. Clearly, the productive life of this vineyard has been reduced and the priority for full redevelopment is increased compared with non infected vineyards.

4.8.4 Petri disease

A large vineyard was established with planting material that subsequently was found to be infected with Petri disease. This resulted in retarded establishment and attainment of full yield. Mitigation also required a number of management and vine nutrition changes which affected the robustness of the vines and their ability to produce in more adverse conditions.

4.8.5 Graft success rates in nursery

A nursery that focussed on high health planting material for a specific period experienced improvement in graft success rates from between 65% and 70% to more than 90%, thus decreasing labour costs per unit sold and attracting increased profit per unit sold.

4.9 Conclusion

Development of an effective process for the provision of improved planting material requires development of protocols to deal with pests and pathogens based on the best available scientific and practical knowledge.

5 COMPARISON OF COLLECTIONS AND SCHEMES

5.1 Introduction

The grapevine certification scheme proposed in this report has been developed after critical evaluation of the key components, protocols and ideals of other certification schemes nationally and internationally. We have proposed that the Australian grapevine scheme accommodate both 'elite' and 'best available' material, with some of the recommended standards for the elite grapevine material surpassing those accepted in other schemes. The rationale supporting the strategic and technical recommendations is outlined in the report.

This section summarises the features of selected Australian and International collections, and describes best practices for tests and treatments as a basis for the development of detailed protocols for a proposed Australian scheme. It also suggests some best practices for plantings at the nuclear stage, the mother plant stage and the source block stage.

5.2 List of Schemes

The high health status schemes reviewed are listed below:

- Australian Pome Fruit Improvement Program (APFIP)
- AusCitrus
- Canadian Plant Protection Export Certification Program (PPECP) for grapevine nursery stock
- Etablissement National Technique pour l'Amélioration de la Viticulture (ENTAV)
- European and Mediterranean Plant Protection Organization (EPPO) guidelines for pathogen-tested material of grapevine varieties and rootstocks
- Foundation Plant Services (FPS), UC Davis, California
- International Council for the Study of Viruses and Virus Diseases of the Grapevine (ICVG) – Safe Movement of Grapevine Germplasm
- International Council for the Study of Viruses and Virus Diseases of the Grapevine (ICVG) – Recommendations from the 14th meeting (2003)
- CIHEAM – Options Mediterraneennes – Proceedings of the Mediterranean Network on Certification of Citrus, Stone fruit (Series B)
- South African Plant Certification Scheme for Winegrapes (SAPCSW)

AVIA and SAVII were also consulted. Their practices, knowledge and opinions have also been given consideration in our assessments.

Some of the specific standards for testing and treatment by the various schemes are compared below. Further details are tabulated in the report.

5.3 Pathogen Elimination

5.3.1 Heat treatment and shoot tip or meristem culture

5.3.1.1 Proposed scheme

Heat treatment and meristem culturing is required for all varieties entering the elite collection.

5.3.1.2 Other schemes

In 1998 APFIP introduced a range of industry standard varieties and rootstocks into heat treatment for the purpose of their re-establishment in a known health state, and free from the endemic viruses of economic significance. This process, testing and evaluation are ongoing, with more varieties undergoing heat treatment.

AusCitrus, EPPO, and FPS use heat treatment as required.

SAPCSW has no prescribed process for heat treatment. However, most of the nucleus plants were subjected to heat therapy and meristem culture. From 2007 all nucleus plants will have to be tested if their status is unknown and, where the results are positive, undergo a process of virus elimination.

5.3.2 Hot water treatment

5.3.2.1 Proposed scheme

Hot water treatment is required for all certified elite and certified best available material used to establish mother plantings. State regulations may dictate other treatments.

5.3.2.2 Other schemes

EPPO requires hot water treatment for the elimination/treatment of phytoplasmas.

SAPCSW does prescribe the use of hot water treatment but it is used on all imported plant material and the plant material prior to the establishment of foundation plantings or mother vine plantings.

5.4 Pathogen Testing Methods

5.4.1 ELISA and/or PCR techniques

5.4.1.1 Proposed scheme

Certified elite material requires no detectable levels of prescribed viruses (except GRSPaV), phytoplasmas, *Agrobacterium* sp. and some specified fungi.

Certified best available material allows detection of one non-prescribed pathogen that has been specifically approved by industry.

5.4.1.2 Other schemes

ENTAV uses ELISA and/or PCR to complement biological indexing.

EPPO requires ELISA and/or PCR testing for all viruses, phytoplasmas, *Agrobacterium* sp. and some fungi.

FPS requires ELISA and/or PCR testing for all viruses (except GRSPaV) and phytoplasmas. FPS suggests some random testing of nursery material by these methods

ICVG requires ELISA and/or PCR testing for all viruses (except GRSPaV and GFkV) and phytoplasmas.

PPEPC requires ELISA and/or PCR testing for all viruses and phytoplasmas.

SAPCSW requires ELISA testing for Grapevine fanleaf virus, GLRaV types 1, 2 and 3 and GVA prior to inclusion for registration of candidate clones. PCR is optional. From 2004, a distinction was made between low risk and high risk units. Plants from high risk units must be tested annually using the ELISA tests for Fanleaf and Leafroll. Plants from low risk units must be tested at least every third year using the ELISA tests for Fanleaf and Leafroll. Fanleaf samples

are taken on a 5% basis, i.e. one out of 20 vines, while the Leafroll samples are taken from each vine in a unit. These tests are voluntary on mother vine plantings.

5.4.2 Biological indexing

5.4.2.1 Proposed scheme

Prior to entry into a certified elite or best available collection, material must be field indexed onto St George, Cabernet Franc, Kober 5BB and LN 33.

5.4.2.2 Other Schemes

ENTAV requires field and green indexing onto Rupestris du lot, Cabernet Sauvignon, Cabernet Franc, Pinot Noir, Merlot, Kober 5BB and LN 33. Indexing should be done at the prescribed distance from any elite or best available collection and from any material destined for either of the collections that is undergoing testing. Woody indexing for elite heat treated material could be done by the owners of the collections, the organisation performing treatment or in post entry quarantine, if the material is a new import.

EPPO requires biological indexing because diseases are not identified by any other means (woody differential hosts). The green grafting method is encouraged. Indicators used are St George, Cabernet Franc, Pinot noir and other red-berried cultivars, Kober 5BB, LN 33, Gloire de Montpellier and 110 R. Herbaceous indexing is used for nepovirus.

FPS requires field indexing onto St George, Cabernet Franc, Kober 5BB and LN 33. Herbaceous indexing is used for nepoviruses.

SAPCSW requires biological (hardwood) indexing to be used for certified clones. Inclusion requires that the following diseases must not be detected - Leafroll, Fleck, Corky bank, Stem pitting/grooving, Shiraz disease, Vein necrosis, Vein mosaic.

AVIA requires field indexing onto St George, Cabernet Franc, Kober 5BB and LN 33.

SAVII requires field indexing onto St George, Cabernet Franc, Cabernet Sauvignon, Merlot, Kober 5BB and LN 33.

5.4.3 Visual inspections: ampelography and disease

5.4.3.1 Proposed scheme

Certified elite and best available material must be inspected twice per annum as a minimum.

5.4.3.2 Other Schemes

FPS requires their nuclear collection to be inspected twice per year, and the mother vines and source plantings to be inspected once per year. FPS use international ampelographers from ENTAV.

EPPO requires inspection for ampelography and disease at every stage within a certification scheme but has not nominated an inspection frequency.

SAPCSW requires all nucleus and foundation units to be inspected annually during the early active growing stage, the early leaf fall stage and the dormant stage.

5.5 Organizational Structures and Authorities

5.5.1 Government and industry involvement

5.5.1.1 Proposed Scheme:

Government and industry involvement and commitment is required. There is a strong case for AQIS to provide additional services (perhaps on a fee for service basis), and for the Commonwealth to commit matching funds for grower levies through HAL and GWRDC**.**

The time required to complete the suggested testing prior to material entering nuclear collections could be substantially reduced by AQIS undertaking tests for endemic pathogens at the same time as it tests for quarantineable (exotic) pathogens. AQIS is well placed to provide this additional service and industry should take advantage of the service and the considerable expertise and resources, within AQIS. Similarly, the PEQs at SARDI and Merbein (and elsewhere) could give consideration to the commercial provision of propagules suitable for nuclear collections.

This report should be viewed as the resource from which further guidelines will be developed. It is considered unlikely, in Australia, that the guidelines or scheme will be the mantra of a single entity. Currently there are two grapevine 'higher health' schemes being established in Australia. In the past, collections have been further fragmented, with each state having its own repository of grapevine material. In most countries, and for other horticultural crops in Australia, there is only one scheme for the production of certified material.

In the proposed scheme the role of regional VIGs is encouraged, particularly as they reconsider their vine improvement roles. The Australian VIG system forms a valuable dimension within a certification scheme, not apparent in any other country.

5.5.1.2 Other Schemes

Government involvement is an important component of most of the other schemes including ENTAV, PEPPC and FPS.

European schemes are required to operate under EPPO guidelines. These include the Italian schemes at University of Bari, ICVG, Mediterranean Agronomic Institute, and the French scheme, Institut National de la Recherche Agronomique (INRA).

The Canadian scheme operates under the Canadian Food Inspection Agency (CFIA). The American scheme (FPS) is supported by the US Department of Agriculture (USDA), the California Department of Food and Agriculture (CDFA) and the University of California, Davis.

The USA and Canada comply with the North American Plant Protection Organization (NAPPO) guidelines for regional risk management regarding entry, establishment and spread of regulated pathogens.

SAPCSW is a private industry body. However, the Department of Agriculture and the Agricultural Research Council each have a co-opted member on the executive board. Also the board includes representatives from Plant Improvement Organisations, the Wine Growers' Association Nursery, Association Winetech and an expert Viticulturist/Virologist.

5.5.2 Management and organisational expertise

5.5.2.1 Proposed scheme

Adoption and success of the proposed scheme requires confidence at all levels in its organisational and technical competence, and clear lines of accountability. The report suggests that the appointment of a technically-skilled "driver", accountable to the appropriate body,

probably NVHSC or a sub committee of NVHSC, is central to the scheme's success. The structure of AusCitrus with an over-arching Board and a highly skilled, national technical manager for citrus improvement has a number of positive features that require consideration in the design of the proposed grapevine scheme.

Government departments in Australia can no longer maintain the same level of input to grape collections as in the past. Other certification schemes in Australia (apart from AusCitrus) do not have the dedicated input of a plant pathologist, but apple and pear growers are supporting these services at APFIP. The reliance on expertise within Universities, CSIRO and state government departments remains high, but at the same time the institutional commitment to the maintenance of 'discipline expertise' is declining. This industry-relevant loss of knowledge presents a threat to the development and advancement of certification schemes in Australia.

Currently in Australia, two laboratories provide diagnostic services. Waite Diagnostics within the University of Adelaide uses PCR technology for assessment of virus, phytoplasmas and *Agrobacterium sp* presence. Crop Hygiene and Crop Health Services at DPI Knoxfield uses ELISA-based tests for several viruses, PCR for phytoplasmas, and direct culturing for bacterial and fungal pathogens. In the proposed scheme, we recommend that any laboratory performing diagnostics tests should be NATA accredited and that more than one test method be used.

5.5.2.2 Other Schemes

FPS and PEPPC are managed and driven by highly qualified virologists/plant pathologists including Drs. D.A. Golino, A. Rowhani and R.C. Johnson. Diagnostic testing for PEPPC must be done by Centre for Plant Health (CPH) in Sidney, British Columbia (B.C.), or a laboratory approved by CFIA's Plant Protection Division. The University of California, the USDA, or the CDFA carry out the indexing, tests and inspections for the FPS grape program.

European schemes regularly involve and consult with Professor G. Martelli, University of Bari, (and other highly qualified virologists), and together with the available training support, are considered very important to the certification schemes.

SAPCSW includes an expert Viticulturist/Virologist. as an executive board member. This body also has a technical committee comprising members of each Plant Improvement Association, Agricultural Research Council, Department of Agriculture, Winetech, winegrowers, nurserymen and viticultural consultants. This committee advises the executive Board on technical matters and on the application of the requirements of the Scheme.

The commitment of highly qualified pathologists in Europe and North America (and the resources available to them), is considered critical to the implementation and interpretation of pathogen testing by all methods. These experts contribute to the identification and understanding of new and emerging diseases, aetiology and epidemiology of existing diseases, developments in pathogen testing technology and development in treatment for disease.

5.6 Planting Protocols

Protocols for plantings have been provided by some organisations and are shown in Table 3 below. Best practice protocols will be formulated by VCPTRG.

Table 3 : Planting Protocols

Stage/Stream	Type	Containment/location	Equipment	Soil type	Clone	
					# Vines	Separation
Nuclear Collections:						
Elite	Best practice					
Best Available	Best practice					
	AVIA	At least 100 metres from other vines		Virgin	4	1.8 metres
	SAVII	At least 100 metres, contained (security fencing)	Dedicated	Fallow - previously wheat?	5	4 metres
	Other					
Mother Plants - Maintenance						
Elite	Best practice					
Best Available	Best practice					
	VIGs (AVIA members)	At least 100 metres from other vines		Virgin		3 metres
	SAVII	At least 100 metres		Fallow - previously wheat?		
	Other					
Source Blocks:						
Elite	Best practice					
Best Available	Best practice					
	VIGs (AVIA members)					
	SAVII					
	Other					

5.7 Best Practice Tests and Treatments

5.7.1 Symbols

The range of tests and treatments used and symbols used for each are shown in Table 4 below.

Table 4 : Symbols for Tests and Treatments

Test	Symbol
Ampelography/ DNA Testing	A
Biological Indexing [1]	BI
Heat treated	HT
Hot water treated	HWT
Pathogen tested - PCR and/or ELISA	PT
Visual inspection (pathogens)	VI

1: LN 33, Kober 5BB, Cabernet Franc, St George

5.7.2 Best Practices

The symbols shown in Table 4 above are used to specify the test/treatments recommended as best practice for each of stages and functions discussed. These are given in Table 5 below.

Table 5 : Best Practice Test/Treatment Frequencies

Stage/Stream	Type	A	BI	HT	HWT	PT	VI
Nuclear Collections - Entry:							
Elite	Best practice	Yes	Yes	Yes	Yes	Yes	
Best Available	Best practice	Yes	Yes	No	Yes	Yes	
	EPPO	Yes	Yes	Yes if required		Yes* - must test negative for all viruses (except GRSPaV), Agrobacterium, Phomopsis Eutypa, phytoplasmas and mites.	
	FPS	Yes	Yes	Yes if required - use shoot tip culture		Yes - must test negative for all viruses except GRSPaV	
	AVIA	Yes	Yes	Yes - some varieties in last 10 years	Yes	Yes	
	SAVII	No	?	No		Yes	
	Other	No	No	Yes - some material		Yes	
Nuclear Collections - Maintenance:							
Elite	Best practice	Yes	3 years			**Annual:** GLRaV1, 3, 5, 9, GVA, GVB; **Tri-annual:** fungi, bacteria, other viruses, viroids, phytoplasmas	2 pa
Best Available	Best practice	Yes	No			**Annual:** GLRaV1, 3, 9 and GVA; **Tri-annual:** fungi, bacteria, other viruses, viroids, phytoplasmas	
	EPPO	Yes			Yes	As required or as improved tests become available	Yes
	FPS	Yes	No			Yes - one third each year for Leafroll associated virus; half per year for nepovirus	2 per annum
	AVIA	Yes	No	If Required	Yes	**Annual:** One third ELISA tested. PCR when material required	3 pa
	SAVII	Yes				**Annual:** all viruses	2 pa
	Other	Yes	Unknown				
Mother Plants - Maintenance							
Elite	Best practice	Yes	No		Yes	**Tri-annual:** GLRaV1,3, 5, 9, GVA, GVB, viroids and phytoplasmas	2 pa
Best Available	Best practice	Yes	No			**Tri-annual:** GLRaV1,3, 5, 9, GVA, GVB, viroids and phytoplasmas	2 pa
	EPPO	Yes	No		Yes	As required	Yes
	FPS	Yes	No			As required	1 per annum
	VIGs (AVIA members)	Yes	No	No	Yes	ELISA of 5% of vines per annum	2 pa
	SAVII	Yes	No				2 pa
	Other	Yes	No				

6 RECOMMENDED PROCEDURES

6.1 Diagnostic Tests and Treatments

6.1.1 Assumptions

The recommendations for diagnostic tests and treatments are based on the assumption that:

- the procedures employed meet best practice criteria;
- the staff conducting the tests and treatments are trained and competent;
- the tests and treatments are effective; and
- all data (sampling, submission codes, results) are integrated, professionally assessed and can be accurately and easily interrogated.

Meeting these assumptions is important and their impact on the overall effectiveness of a superior health status scheme, should not be under-estimated.

This report recommends that heat treatment be performed on all material entering an elite nuclear collection. Procedures for heat treatment must be based on good science. Ideally, heat treatment prior to entry into nuclear collections would be conducted by government (eg AQIS). Heat treatment for virus eradication is described in section 4.8.1 .

6.1.2 Pathogen Testing

The continued provision of certified pathogen tested, true-to-type material requires active pathogen testing at every level of both the elite and best available streams.

Pathogen testing is expensive. Much of the expense associated with molecular, serological and biological indexing is attributable to labour costs. Consumables for molecular techniques, including RNA extraction kits and PCR kits, are also expensive. The space, time and expertise required for biological indexing onto woody indicators also make this method of health status screening, expensive. Pathogen testing every vine at each point within a certification scheme, annually and by each method, although considered ‘best practice’, is unrealistic.

The schedule of testing recommended in this report gives consideration to the grapevine pathogens endemic in Australia, their known economic impact and their mode of transmission. This recommended testing regime is dynamic. New test technology and testing for new (or newly recognised) diseases and pests could be incorporated into the recommendation at the appropriate level within the scheme, when necessary.

A vine collection that includes the positive and negative controls for such testing, is also required. Such a collection should be maintained by the industry body managing elite and best available schemes. These positive controls should be held in a screen-house at the recommended distance from the elite or best available collections and accessible to both industry and government diagnosticians.

6.1.3 Sampling

Currently, no protocols exist for appropriate or optimal sampling frequencies and pooling of samples for molecular and serological tests. Pooling remains a useful means of reducing costs of testing at certain levels within the scheme, but individual tests must follow any positive, pooled sample results. Time of sampling and tissue sampled must be consistent and protocols relevant to each of the pathogen groups (i.e. viruses, phytoplasmas) will be required.

Biological indexing also requires consistent evaluation periods and protocols. Since environmental factors, including light and temperature, and virus titre influence the development of symptoms, it is important to determine the optimal time for each region, for the indexing of both red and white grape vines, for each of the prescribed pathogens.

6.1.4 Laboratory standards

Protocols for testing of grapevine pathogens are not available from the various laboratories providing these services. The high health planting material scheme therefore is reliant upon, but has no control over, the operating standards within each of the laboratories.

Ideally, all pathogen testing should be done in a NATA accredited laboratory. NATA uses an international standard, ISO/IEC 17025, to assess the ability of a laboratory to perform "specific tests, calibrations, measurements and inspections". Accreditation is based on:

- Technical competency of staff;
- Validity and appropriateness of the methods;
- Traceability of measurements and calibrations to national standards;
- Appropriate application of measurement uncertainty;
- Suitability, calibration and maintenance of test equipment;
- The testing environment;
- Sampling, handling and transportation of test items;
- Quality assurance of test, inspection and calibration data.

6.1.5 Labelling of Clones

Protocols directing the labelling of clones should reflect the need for clonal identity by name to be maintained in the interests of traceability, and for imposed treatments to be identified (i.e. heat treatment).

6.2 Recommended levels of phytosanitary requirements

The proposal resulting in this review project included an objective to define the ideal, practical, negotiable and non-negotiable phytosanitation elements that impact on the health status and acceptability of grape material. It was stated that this would include assessment of viruses and other pests/diseases, diagnostic technologies, isolation distances, practices influencing re-infection etc. These recommendations are addressed in Table 6 – 11 below.

Table 6 : Elite Collection – requirement levels for testing, tolerance of detections and labelling

Pest/pathogen type	Requirement level		
	Tolerated, not tested	Tolerated, tested and labelled	Tested, not tolerated
Quarantineable			All
Viruses		GRSPaV	GLRaV – all GVA GVB GFkV GFLV GRGV GRSLaV
Viroids	All		
Phytoplasma			AGY TBB BVGY
Bacteria			Crown gall
Fungi	*Botrytis sp.* Powdery mildew Downy mildew		*P. chlamydospora* (Petri disease, Esca) *Botryosphaeria sp.* *Eutypa sp.*
Known vectors of tolerated and not tolerated diseases; other pests			Mealybug (vector) Scale (vector) Dagger nematodes Phylloxera *Orosius sp.*

All the viruses (with the exception of GRSPaV) should be non-negotiable in an elite collection as this collection is supposed to be of the highest health status. Some viruses are acceptable in the best available collection but these must be labelled.

Table 7 : Best Available collection – requirement levels for testing, tolerance of detections and labelling

Pest/pathogen type	Requirement level		
	Tolerated, not tested	Tolerated*, tested and labelled	Tested, not tolerated
Quarantineable			All
Viruses		GRSPaV+ GLRaV 2, 4 GVB GFkV GRGV GRSLaV	GLRaV – 1, 3, 5, 9 GVA GFLV
Viroids	All		
Phytoplasma		AGY TBB BVGY	
Bacteria		Crown gall	
Fungi	*Botrytis sp.* Powdery mildew Downy mildew	*Botryosphaeria sp.* *Eutypa sp.*	***P. chlamydospora*** (Petri disease, Esca)
Known vectors of tolerated and not tolerated diseases; other pests			Mealybug (vector) Scale (vector) Dagger nematodes Phylloxera *Orosius sp.*

*****Industry approval required. Recommended that no more than one pathogen from each pathogen group in column two be tolerated in any one clone, in the collection.

+If GRSPaV is detected, industry may approve some clones with an additional, known and detected virus, remain eligible for this collection.

Table 8 : Requirement levels for Phytosanitary treatments at all phases

Treatment	Requirement level		
	Ideal	Negotiable	Not negotiable
Hot water treatment – 50°C for 30 minutes*****		Nursery	Quarantine Elite nuclear collection – entry Mother vine establishment material Source block establishment material
Hot water treatment – 54°C for 5 minutes			In all phases – for own-rooted and grafted rootlings
Heat treatment (and tip-cultured) – 38°C – 6-8 weeks	Quarantine¤	Elite collection – entry	

* Dormant wood, pre-propagation according to protocols. HWT is not suitable for green material.

¤ Approximate timeframe for imported cuttings to be struck, heat-treated, tip-cultured, pot established and PCR/ELISA tested – 2 years.

Table 9 : Planting Characteristics – Requirement levels

Criteria	Ideal	Practical	Negotiable	Non-negotiable
Established on virgin soil (no history of horticultural production)			Source blocks	Elite nuclear collection Elite mother plantings Best available nuclear Best available mother planting
Isolation from other vineyards - 30 metres minimum				Nuclear collections Mother plantings Source blocks
Spacing between varieties, clones - 2 metres				Elite nuclear collection Elite mother plantings
Vines/clone	Elite: 5-10 Mother vines			Elite: 3 minimum Mother vines

Table 10 : Release to growers – Requirement levels for material with Elite or Best Available history

Type	Ideal	Practical	Negotiable	Non-negotiable
Certified elite	Originating from heat treated vines, planting material		Hot water treated	• Originating from pathogen tested elite material of known origin and true to type in which prescribed (not tolerated) pathogens have not been detected • Handled by accredited nursery according to protocols
Certified best available			Hot water treated	• Originating from pathogen tested best available material of known origin and true to type, and labelled to reflect detected non-prescribed pathogen; and free of detection of prescribed pathogens • Handled by accredited nursery according to protocols

Table 11 : Pathogen Testing Methods – Requirement levels

Phase/material type	Testing	Ideal	Practical	Negotiable	Non-negotiable
Quarantine		**Woody indexing:** St George; Cabernet Franc; Kober 5BB			**Biological:** woody (LN33) and herbaceous **PCR:** Virus, bacteria, phytoplasma **ELISA:** virus **Culturing:** fungal pathogens
Elite and best available collections	Prior to addition (after heat treatment)		**ELISA:** GLRaV6, 7;	**Woody indexing** (best available only)	**ELISA (if PCR is not used) and/or PCR (if ELISA is not used)** GLRaV1, 3, 5; GVA; GFkV **PCR only:** GLRaV 2,4, 9; GVB; GFLV; GRSLaV; GRSPaV; Phytoplasma Crown gall Viroids Other pathogens if available **Woody indexing:** (elite only) **Culturing:** Fungal pathogens

Phase/material type	Testing	Ideal	Practical	Negotiable	Non-negotiable
Elite collections	Maintenance	**Woody indexing:** every year **Visual inspection:** monthly	**PCR:** Viroids	**Culturing:** Fungal pathogens **PCR and/or ELISA:** Elite collection – all viruses, every year	**Woody indexing** : once every three years **PCR and/or ELISA(every year):** GLRaV1, 3, 5; GVA; GVB **PCR and/or ELISA (every three years)** Other viruses **Visual inspection:** twice/year **Soil testing (every year):** nematodes **Regular monitoring:** other pests and pathogens
Best available collections	Maintenance	**Visual inspection**: monthly	**PCR:** Viroids	**Culturing:** Fungal pathogens **PCR and/or ELISA**: Other viruses	**Visual inspection:** twice/year **PCR and/or ELISA (every three years):** GLRaV1, 3, 5; GVA; GVB **Soil testing (every year):** nematodes **Regular monitoring:** other pests and pathogens
Elite and best available mother blocks	Maintenance	Active testing for all pathogens	**PCR:** Viroids	**Fungal culturing/ PCR:** Petri disease **PCR:** GRSPaV; GLRaV 2,4, 9; GFkV; GFLV; GRSLaV **ELISA:** GLRaV6, 7	**PCR and/or ELISA (all vines every three years):** GVA; GVB;GLRaV1, 3, 5; Phytoplasma Crown gall **Visual inspection:** twice/year Symptomatic vines must be tested for the most likely pathogens

Phase/material type	Testing	Ideal	Practical	Negotiable	Non-negotiable
Elite source blocks	Maintenance	Active testing for all pathogens	**PCR:** Viroids	**Fungal culturing/ PCR:** Petri disease; Crown gall	**PCR and/or ELISA (1% of all vines in a block):** GVA; GVB; GLRaV1, 3, 5; Phytoplasma **Visual inspection:** twice/year Symptomatic vines must be tested for the most likely pathogens **Soil testing (every year):** nematodes
Best available source blocks	Maintenance		**PCR:** Viroids	**Fungal culturing/ PCR:** Petri disease; Crown gall	**PCR and/or ELISA (1% of all vines in a block)** for the following pathogens if not already detected in the original variety or clone: GVA; GVB; GLRaV1, 3, 5; Phytoplasma; **Visual inspection:** twice/year Symptomatic vines must be tested for the most likely pathogens **Soil testing (every year):** nematodes
Nursery	**PCR and/or ELISA, fungal culturing**	Random testing of propagated material just prior to sale: GLRaV1, 3; GVA; GVB phytoplasma, Crown gall, Petri disease			

7 GRAPEVINE HEALTH PRACTICES REQUIRING FURTHER INDUSTRY DEVELOPMENT

7.1 Development of reliable tests for endemic pathogens

Industry members and scientists have been justifiably critical of grapevine material indexing based on a single diagnostic method. It has been demonstrated that certain viruses and phytoplasmas are harder to detect in particular tissue, at certain times of the year. The reproducibility of test results has, in some cases, been very low.

However, reliance on test results is increasing throughout the industry. Only with extensive confidence in test methodologies and capabilities and the scientific support for them, can the proposed high health status scheme expect to develop and be adopted.

7.1.1 Test methods

An overview of diagnostic techniques is given in Annex 9.

Some industry and research community members believe the only reliable method of detection is biological (woody) indexing. This method is time-consuming and demands considerable expertise for the identification of symptoms. Biological indexing may not identify the specific pathogen present. In some schemes, reliance is on serological tests (ELISA) or molecular tests (PCR). ELISA and PCR can identify specific pathogens, but are prone to registering false negatives and false positives. In such cases, reliance on one test technique could result in the unnecessary removal of a variety from a certification scheme, or retention of one that may not , in fact, meet the health criteria. Within a certification scheme, false negatives are of the greatest concern. Pathogens may go undetected and infected planting material may be disseminated.

Validation of diagnostic testing is required and would be assisted by:

- The development and validation of sampling protocols (timing, frequency, plant material) for viruses and viroids;
- Comparative analyses of biological indexing, ELISA (virus only) and PCR for Australia's economically important grapevine viruses;
- Training for symptom identification on biological indicators;
- Greater understanding of the effect of viral strain variation on test results and symptom development on own-rooted and grafted vines, and woody indicators; and
- Validation of new test technology (array technology?).

It has also been identified that the high health status planting material scheme for viticulture would be enhanced if:

- Centres of expertise for particular testing skills and technology (as per citrus industry service) were identified and laboratories were accredited; and
- AQIS services and facilities were used more extensively to include heat treatment of planting material before release.

7.2 Development of Post Entry Diagnostic Protocols

Time in quarantine is a limiting factor in grapevine importation. A review completed in 2001 (Sivapalan *et al*), and yet to be released by Biosecurity Australia, identified the possibility of reducing time in quarantine to 16 months for material imported at the appropriate time for immediate woody indexing. Risk management of this would be assisted by the development of

improved diagnostic protocols such as PCR or ELISA, for rapid detection and identification of quarantineable (and endemic) pathogens. The existing AQIS pathway and the proposed Sivapalan plan are outlined in Appendix 1.

7.3 Effects of pathogens on grapevines

In Australia, very little research has been done on the effects of some pathogens, especially viruses, on grapevines. The specific effects of viruses and their strains, alone and in combination, in our environmental conditions, under normal Australian viticultural practices, may differ from those reported overseas. In the absence of specific local knowledge, it is reasonable to recommend heat treatment of planting material destined for elite collections, prior to release from quarantine. This treatment is widely used and is likely to have a positive impact on pathogen elimination, vine growth and yield.

The oenological effects of heat treatment are yet to be determined for many clones. It is however likely that some of the clones received from international sources have been subjected to prior heat treatment. It will be important to ascertain what prior treatment imported clones have received and to evaluate the additional benefit of further treatments in Australia, to the Australian industry as a whole. For those clones that have not been heat treated and have come from non-approved sources, heat treatment before release is considered beneficial.

Analysis of viral strains and their molecular characterisation will assist in the interpretation of diagnostic results and field symptoms. For example, the strain of GVB identified in Australia does not appear to be associated with corky bark disease, as it is in other countries. It is notable however that there is no Australian research being carried out currently to clarify this situation.

7.4 Transmission of pathogens

To control the spread of pathogens we must understand their mode of transmission. Although mechanical transmission of viruses is the means by which grapevine viruses are spread extensively, there have been very few Australian studies on potential insect vectors of our grapevine viruses and phytoplasmas. Surveys and transmission studies for virus vectors, in particular for GLRaV1, GLRaV 3, GLRaV 5, GLRaV 9, GVA and the Australian isolate of GVB are needed. Further studies on transmission of phytoplasmas through planting material are also warranted.

The mode of transmission of Australian phytoplasmas is not understood. Insect vectors are suspected but no insect vector has been confirmed for any phytoplasmas. However studies have shown that the TBB phytoplasma can be acquired from grapevine and transmitted to faba bean (Beanland, 2001). Aerial transmission of BVGY phytoplasma is suspected (Constable *et al* 2003b). Some studies on the transmission of phytoplasmas through planting material have been done. One study used planting material from phytoplasma-infected grapevines and this material was observed and tested after one or two years. Single leaf samples were tested for phytoplasmas and very few rootlings tested positive. It is possible that phytoplasmas were present in other parts of the rootlings that were not tested or may have been below detectable levels.

Similarly, there is little information available on the mode of transmission of some fungi, in particular *Phaemoniella chlamydospora.* The potential role of mites, mealybugs, insects and nematodes on fungal spread has largely not been studied in Australia.

7.5 Hot water treatment

Research on the effects of hot water treatment (HWT) is being conducted, but the results of these studies are yet to be finalised. It is possible that further research is required, especially with regard to regional differences, dormancy and wood density and their interaction with HWT.

8 IMPLEMENTATION ISSUES

8.1 Current Situation

The existing industry situation is summarised in Annex 1. At present, two vine improvement bodies (AVIA and SAVII) are in the process of developing nuclear plantings at Dareton and Kapunda respectively. As well, there are existing plantings at Manjimup, WA and in some private nurseries, and there are genetic resource collections at Nuriootpa and Merbein.

While the planting material for these collections has undergone a range of diagnostic tests and treatments, their health status and trueness to type has not necessarily been assessed against the recommendations of this report. Full disclosure and evaluation of collections has not been made. However it is almost certain that the existing and developing collections would not meet the suggested requirements for 'elite' status. This is despite some individual clones within the collections possibly being eligible for a certified elite or certified best available label.

8.2 Characteristics of Nuclear Collections

Certified elite or certified best available nuclear collections are assumed to contain a small number (say 3) of vines of each clone or variety with accompanying but separate plantings of mother vines propagated from the elite (or best available) nuclear vines. Mother plantings would be confined to clones and varieties for which increased future demand is likely, to provide planting material for source blocks geographically separate from the nuclear collection facility.

The operators of nuclear collections obtain income from sales of certified planting material produced from mother plantings and from levies collected from sales of commercial planting material produced from source block material.

Operation of certified elite or certified best available nuclear collections requires high level expertise and may result in the formation of mutually beneficial alliances with relevant government departments, academic and research institutions, and/or technologically-advanced winemakers or fruit exporters.

The operators of source blocks such as VIGs and commercial nurseries would purchase certified planting material from the nuclear collections. Continued certified status of planting material produced from source blocks would require continued best practices and accreditation of the management of the source block. The operators of source blocks may propagate their own planting material or provide propagating material to commercial nursery operators who in turn would follow agreed best practices and maintain accreditation for their business, to ensure they too would be providing certified material of known health status.

The proposed system of provision of certified elite or certified best available planting material would provide industry benefits by enabling increased use of higher health planting material and also by providing effective traceback systems to the original material.

8.3 Steps to Achieve Certification Status

Consultations indicated there is relatively widespread industry acceptance of the desirability of certified, high health status planting material. The foundation for increasing its availability is the establishment and operation of certified elite or certified best available nuclear collections. Development of elite or best available collections and/or progression of one or more of the newer collections to this status, would require the following:

- Endorsement by NVHSC of the test and treatment requirements;
- Development of test and treatment protocols for the agreed requirements;

- Development of NATA accredited diagnostic facilities with a competent industry oriented research capability to provide and support testing services;
- Development of an accreditation or auditing system independent of the collections and with industry credibility to provide certification that material in elite and best available collections (and mother plant collections) has been subjected to, and met, the documented requirements;
- Assurance through accreditation that the management and operation of source blocks and propagation nurseries meet standards required to maintain the health status (and thus certification level) of multiplied material;
- Stimulation of demand for certified elite or certified best available planting material through development and implementation of a communication program including identification and estimation of risks and consequences of use of lower status planting material, and an extension campaign to promote awareness of the risks and consequences and its incorporation into decisions regarding planting material.

8.4 Need for Driver

Development of this system requires the application of high level technical and industry development expertise. Terms of reference for such a person or group, must be developed. Industry funding will be required and the 'project' should be accountable to a group such as NVHSC or a technical sub-committee such as VCPTRG.

8.5 Relationships with other industry bodies

The industry development dimension of this project may attract funding from HAL and GWRDC. As well, it is clearly relevant to Plant Health Australia and to the Phylloxera and Grape Industry Board of South Australia. Mechanisms by which, and justification for, all to contribute, should be explored.

8.6 Communication Strategy

The essential components of the communication strategy relate to the following:

- A need to gain industry and R&D acceptance of the specified test and treatment requirements and the resulting protocols including the required improvements in diagnostic facilities and performance;
- A need to identify, estimate (in monetary, vineyard longevity and production terms) and explain the potential impact of the use of poor quality planting material (including rootstocks);
- A need to stimulate demand for high quality planting material through an extension campaign.

9 SUMMARY AND RECOMMENDATIONS

9.1 Drivers for Change

Against a background of global competitiveness it can be expected that:

- Full traceability of end product to planting material source and status will be demanded in the near future for planting material used in Australian plantings and to meet export demand;
- Reduction in the effectiveness of import barriers, is likely to result in increased pressures to allow importation of bulk quantities of cheap planting material of a defined health status; and
- The Australian industry's sustainability will be underpinned by an on-going, adequate and reliable supply of high health status planting material, including rootstocks.

9.2 Current status

The Australian grapevine industry includes winegrapes, table grapes, dried vine fruit and vine nurseries and has:

- Most of the necessary components and expertise to produce and deliver planting material of a higher certified health status than has been provided to-date;
- The necessary infrastructure to produce and provide better planting material but it is fragmented and lacks a coordinating force;
- Unlike other major producing countries, not had a reliable and accountable elite planting material source;
- The immediate need and opportunity to develop and adopt a co-ordinated national scheme (involving industry and government) aimed at the efficient production of high health status grapevine planting material.

9.3 Recommendations

This report is the culmination of review of best practices for such schemes, vine health parameters and implementation strategies and has developed the following recommendations:

9.3.1 Recommendation 1

An Australian Grapevine Foundation Planting Scheme (AGFPS) is required to ensure planting material of the required health status and provenance is available to meet the needs of the winegrape, dried vine fruit and table grape as well as the vine nursery industries.

9.3.2 Recommendation 2

The AGFPS should ensure that one or more certified elite collections and/or if required by industry, certified best available nuclear collections, both with accompanying mother plantings are established and operated according to protocols to be developed by VCPTRG based on this report and using the terminology recommended in this report.

9.3.3 Recommendation 3

Eligibility for inclusion in elite or best available collections requires clones and varieties to meet known health status requirements for both quarantineable and endemic pathogens. Consequently,

it is recommended that AQIS provide endemic pathogen testing services (for a fee if necessary) concurrently with the implementation of testing for quarantineable pathogens.

9.3.4 Recommendation 4

Implementation of these recommendations should proceed in the following steps:

- Develop the required protocols for entry to and maintenance of collections that must be followed in order to qualify as elite or best available;
- Concurrent development of one or more collections managed by industry bodies or commercial entities;
- Development of and implementation of an accreditation procedure for elite and best available collections and their mother vines;
- Implementation of an industry research and evaluation project to assess the risks and benefits of using planting material of various levels of health status and clonal or varietal provenance;
- Development and implementation of a communication and education campaign to improve industry understanding of the risks, benefits and costs of using certified true-to-type planting material of defined, high health status; and
- Propose and negotiate accountability for funding, management, coordination and implementation of the above.

9.3.5 Recommendation 5

An NVHSC committee representing a range of industry interests should develop a campaign to support the establishment of the AGFPS and to foster the inclusion and commitment of the dried vine fruit and table grape industries as well as the wine grape and vine nursery industries.

9.3.6 Recommendation 6

Success of the proposed AGFPS depends on the establishment and effective operation of a "driver", preferably responsible to the NVHSC through a relevant TRG and funded by industry and Government through both the GWRDC and HAL. The model enabling and supporting the "driver" should draw on features of the AusCitrus Scheme and APFIP. The support for the coordinator should be commensurate with the national responsibility of the position.

Annex 1

Summary of Existing Industry System

GRAPEVINE PLANTING MATERIAL - SUMMARY OF EXISTING INDUSTRY SYSTEM

Summary of the existing industry system of importation, selection, elimination, isolation, maintenance, monitoring and testing, propagation, and auditing of planting material – Pathology

1 Importation

1.1 Introduction

The Australian and Quarantine Inspection Service (AQIS) assess all imported plant material prior to arrival in Australia. Grapevines are imported into Australia as tissue cultured plantlets, canes or, rarely, as green softwood cuttings. Imported grapevine material is generally handled in AQIS facilities at Knoxfield, Victoria, Eastern Creek in NSW and in Western Australia. The majority of grapevine material imported into Australia, enters as dormant canes. AQIS consider grapevines to be 'high risk' material because viticulture is Australia's principal horticultural industry and grapevines are host to a number of serious pathogens and pests, which have not yet been detected in Australia. Some of these are economically-important pathogens and pests of other crops.

Table 1 : Grapevine cultivar imports at Post Entry Quarantine (PEQ) Knoxfield since 1995/96

1995/96	16
1997	16
1998	24
1999-2000	104
2000-2001	71
2001-2002	37
2002-2003	34
2003-2004	61
2004-2005	30 to-date; potentially 30 more by end of 2005

(Source: Mark Whattam, AQIS plant pathologist, pers. comm.)

These grapevines were imported from 15 countries: USA, Italy, South Africa, Iran, New Zealand, Germany, Canada, Chile, Argentina, France, Slovenia, Israel, Japan, Austria and Portugal.

Approximately 95% of these importations were organised by private companies/individuals. Government bodies (eg. CSIRO) account for approximately 5% of importations. Nearly 95% of all imported grapevines are from non-accredited sources and their health status is not known. Current AQIS regulations require grapevine material to spend a minimum of two years in a post entry quarantine (PEQ) facility to enable testing for a variety of pathogens using herbaceous indicators, woody indicators and visual screening. The Sivapalan plan (Sivapalan *et al*, 2001) proposes this time be reduced.

Importation of plant material to Australia is open to anyone. Some vine improvement groups import grapevine material. Private nurseries, eg Yalumba and Chalmers nurseries, also import

grapevine material. Grapevine material being imported into Australia is tested for quarantineable pathogens. It is not screened for pathogens already present in Australia, and not under specific (internal quarantine) control. As such, imported grapevine material released from AQIS, may carry such pathogens. Importers may request tests for non-quarantineable pathogens using commercial services, while plants are in quarantine facilities. The fees for this testing must be paid by the importer. Testing for and treatment of non-quarantineable pathogens is at the importer's discretion.

1.2 Pathogens

Quarantineable and non-quarantineable grapevine pathogens for Australia are described in a draft review of post entry quarantine protocols for importation of grapevines (Sivapalan *et al*, 2001). Since this review, several viruses previously unreported in grapevine, have been found and some previously reported viruses have been further characterised. Table 1 is an updated list of the quarantineable and non-quarantineable pathogens of grapevines, in Australia.

1.3 Screening

Figure 1 outlines the current process of PEQ screening of grapevine material for quarantineable pathogens. Dormant canes are imported from the northern hemisphere in January to May and from the southern hemisphere between July and September. The material is inspected for pathogens and insects. Any abnormal symptoms are investigated further. To eliminate insect pests, canes are fumigated with methyl bromide and air dried. Canes are hot water treated for 20 minutes at 52ºC and then dipped in cold water. Up to six canes with 2-4 nodes are propagated, all remaining canes are destroyed once material has been established. The propagated plants are maintained for two months in a 25ºC AQIS-approved glasshouse while new growth is produced. In winter, plants are placed in a 4ºC cool room for 6-7 weeks. A single plant of each cultivar is potted within 12 months and placed in an AQIS glasshouse, at 25ºC to produce further growth. The remaining plants are maintained as backup. In the second year, the plants are placed into open quarantine facilities for bacterial and fungal screening.

Grapevines are inspected weekly during post entry quarantine for symptoms of bacterial, fungal, phytoplasma and viral diseases. If symptoms are observed additional testing is done. Viruses are tested by grafting eight buds onto two virus tested LN33 indicators (woody indexing) and by mechanical inoculation onto herbaceous indicators (herbaceous indexing), including *Chenopodium quinoa* and cucumber. Woody indexing takes up to 15 months for symptom expression and is required to detect viruses for which, herbaceous indexing and other detection methods are not definitive. Herbaceous indexing takes up to eight weeks and is used primarily for detection of nepoviruses. Electron microscopy, ELISA or PCR tests may also be used. Daughter plants are propagated from the mature mother plant. The mother plant and one daughter plant are released to the importer at the completion of the PEQ period.

AQIS has proposed that the PEQ period be reduced to 16 months with modification to the above procedure. This is outlined in Figure 2. Under this plan, material imported in January or February would be indexed for viruses soon after arrival. In the following year, the second round of visual observations would be done and, provided no quarantineable pathogens are detected, plants could then be released.

2 AVIA and Vine improvement groups

2.1 Accreditation

Vine improvement groups were established to provide higher quality planting material to grape growers. AVIA was formed to coordinate the VIGs, and it developed the National Vine

Accreditation Scheme. The relevant standards and detailed protocols are published in a procedure manual, "Part One: The National Vine Accreditation Scheme for Vine Improvement Groups".

AVIA also developed protocols for nurseries that want to propagate material to AVIA standards. These standards were published in "Part Two: The National Vine Accreditation Scheme for Nurseries". AVIA may certify planting material, from registered source blocks, for future distribution via VIGs and AVIA-accredited nurseries. Most VIGs are affiliated with AVIA. The VIGs undergo an accreditation process and must meet the required standards before being approved for the distribution of "AVIA certified" material. The VIGs may also supply non-certified material. There has been industry confusion about the terms used to-date, with "AVIA-accredited", being mis-interpreted as reflecting planting material health status, rather than business practices, by some growers.

2.2 AVIA Nuclear Collection

AVIA have indicated their intention to establish a nuclear collection at NSWAg, Dareton. The collection will be established on virgin soil. Each clone or variety planted will have been virus tested by PCR (Waite Diagnostics), ELISA (Knoxfield) and woody indexing and some have undergone heat therapy for virus eradication in the last 10 years. Prior to the development of the collection, AVIA negotiated a 15-year, (with a 10-year option) Deed of Agreement for the maintenance of the AVIA collection at the Dareton Research Station. The planting will be maintained by the farm staff at the research station on a fee-for-service basis. Distances between existing grape and citrus plantings have been considered and the collection will be grown at least 100 metres from other vines.

Two hundred and ten different varieties or clones will be planted at the nuclear collection at Dareton. The list of varieties and clones is available from AVIA upon request. Four plants of each will be grown. All rootstocks undergo hot water treatment at 50ºC for 30 minutes prior to establishment, to reduce the risk of propagation of fungal and bacterial pathogens and phytoplasma. *V. vinifera* clones/varieties are not always hot water treated. AVIA aim to test each grapevine in the nuclear collection once every three years for various viruses after the collection is established. Of the viruses, only RSPaV will be tolerated in this collection.

Genetic resource collections are also utilised by some VIGs. These collections include those at CSIRO Merbein; Dareton, NSW; Griffith NSW; Nuriootpa, SA; Loxton, SA; Manjimup, WA; Alice Springs, NT; and Stanthorpe, QLD. AVIA guidelines for the "*National Vine Accreditation Scheme for Vine Improvement Groups*" state that genetic resource collections must be established outside of Vine Disease Districts, on virgin soil or soil that has been fallowed (without horticultural crops) for at least six years. The population of soil pathogens must be low and the soil must have been recently fumigated. The genetic resource collection must also be well separated from other vines, but the exact distance is not specified.

Grapevine material selected (from breeding, imports, clonal selection) for inclusion into a genetic resource collection are assumed to be free of quarantineable pathogens, and pathogen-tested (and reported to be free of detection) for GLRaV1, 2 and 3, GVA, GVB, GFLV. Biological indexing, ELISA, PCR and/or visual symptoms are used for the detection of viruses. *Agrobacterium* sp must also be tested for or treated. No other pathogen testing, for fungi or phytoplasmas is specified. Planting material must be hot water treated, but the time and temperature are not specified. RSPaV type 1 and 2, GVD, GLRaV4 and GFkV types A and B are tolerated within some material distributed by the certification scheme, however only under specific conditions when other 'clean' material is not available.

Genetic resource collections must be maintained contamination free and free of potential virus vectors. A spray program is also implemented to control other pests and pathogens, such as mites and powdery mildew. Six months after establishment, genetic resource collections are inspected.

In subsequent years, they are inspected in November and January for AGY symptoms and in February to April for virus symptoms. In the first growing/fruiting year an ampelographer also inspects the vines for trueness to type. Vines that are off-type or have disease symptoms, or test positive for GLRaV1, 2 and 3, GVA, GVB, GFLV, or quarantineables (i.e.TomRSV, ArMV), are not used for cuttings and are removed from the genetic resource collection.

Cuttings from suitable vines are harvested from the genetic resource collections, hot water treated and used to establish pre-multiplication rows (mother vines), the length and diameter of cuttings are specified in the procedure manual. Establishment of pre-multiplication rows may currently be done on a grape grower's property. Mother vines are used to supply healthy, true-to-type, material for source blocks. The mother vines are inspected in the same manner as the genetic resource collection and samples may be taken for testing of pathogens, particularly virus. Vines that show symptoms of leafroll virus infection, AGY, restricted growth, crown gall, phytoplasma, or are off-type, are tagged. The procedure manual does not specify the fate of such vines. Presumably these vines are not used for cuttings, and are removed.

Cuttings from the mother vines are harvested and used to establish source blocks on growers' properties. Source blocks are inspected for disease and tested for pathogens in the same manner as the mother vines. Vine improvement committees reject source areas if they exceed certain levels of problems present.

Cuttings from source blocks are distributed to nurseries for propagation and sale to growers. Depending on its length and diameter, scion material is sold as ungraded, bench graft grade, field/chip grade or thin grade. Similarly rootstock cuttings are sold as bench graft grade, field graft grade, low grade or thin grade. Depending on their origin, cuttings are identified as A, B or C class. Cuttings from source blocks, which were established directly from mother vines, are graded as Class A, regardless of the source block's location of the surrounding vine health. Class B cuttings come from second-generation plantings, regardless of their location or the surrounding vine health. VIGs may also distribute class C cuttings but they come from vineyards that were not established with material originating from the genetic resource collection. In the nurseries, if Class A or B material is grafted onto a rootstock of a lower class, then the graftling is sold as the lower class.

2.3 VIGs

MIAVIS and VAMVVIA follow the AVIA protocols for vine improvement groups. In the case of VAMVVIA, all rootstocks undergo hot water treatment at 50ºC for 30 minutes prior to establishment to reduce the risk of transmission of fungal and bacterial pathogens and phytoplasma in cuttings. *V. vinifera* cuttings are not always hot water treated. Each year, VAMVVIA randomly test 3 vines from each source block for GLRaV1, GLRaV3, GFkV and GVA in addition to visual inspections.

VAMVVIA are considering establishment of self managed mother plantings so that they have better control over the maintenance of their vines. VAMVVIA use a combination of green propagation and dormant cuttings for the production of planting material. The use of hot water treatment is being re-considered because recent studies indicate detrimental effects to some varieties.

In 2000, WAVIA established a foundation planting of varieties and clones for Western Australia, at the Manjimup Horticultural Research Institute (MHRI). The collection includes table grape, wine grape, dried vine and rootstock material. Prior to this, foundation plantings were located at the Wokalup Research Station and in the Swan Valley. All material included in the foundation planting is tested for virus and only those with an acceptable status, according to AVIA protocols, are distributed. WAVIA would prefer to import material concurrently with AVIA, to improve timely access to material in WA. The WAVIA collection is divided into two parts: one contains material acceptable for distribution, and the other includes vine material with viruses

not tolerated under AVIA guidelines. Like VAMVVIA, WAVIA test 3 vines in 300 (1%) in source blocks by ELISA. WAVIA have not been AVIA accredited because they cannot meet the nursery accreditation standards. They are currently working towards VINA accreditation.

2.4 SAVII

SAVII continues to collect levies but is no longer affiliated with AVIA due to industry politics. SAVII is establishing a higher health status collection independent of AVIA. This collection is located at Kapunda in the Barossa Valley, South Australia. SAVII considers that the standards adopted in this collection's preparation are higher than those previously adopted in collection establishment in Australia. Entries to the planting were selected after a survey of clients. Grapevines included in the collection have been tested at least three times by PCR for the presence of viruses and phytoplasmas. At present five vines each of 160 varieties/clones have been planted. The five vines are derived from one original vine and are virus tested separately every year. Each variety is well spaced from others so that the risk of mixing varieties is reduced. Only grapevines free of detectable levels of all viruses except RSPaV are included in the collection. In some cases however, GFkV may be tolerated. Rootstocks must test negative for all viruses. SAVII has reported that they intend to conduct their own ELISA and PCR testing and biological indexing, in the future, as it may be more economical than the current arrangements.

This collection is within a high security compound to prevent introduction pests and diseases via people and equipment. Mother vines will be produced in the same area, but outside the compound. These vines will be tested for listed endemic viruses every year by ELISA.

Like the other VIGs, SAVII only test a small number of grapevines from source blocks for the presence of GLRaV type1, 2, 3, 4, 7, 9, GVA, GVB, GVD and GFkV due to the cost and time. SAVII will knowingly supply virus infected material but only with the signed consent of the purchaser. SAVII perform hot water treatment on all rootstock cuttings unless otherwise requested. The treatment is elective for *V. vinifera* cuttings.

3 Independent importers and producers

3.1 Nurseries

Independent nurseries and growers in Australia import grapevine material. Both Chalmers and Yalumba nurseries are examples of independent commercial entities that are importing grapevine material and producing their own material for sale to growers. Both nurseries buy material from the VIGs but, due to supply not meeting demand (especially for rootstocks), they have also decided to produce their own material. Chalmers Nurseries virus test vines used for propagation and only use those that are identified as "clean" (no virus detected except for RSPaV). Chalmers Nurseries grow all their mother vines from a single vine to ensure trueness to type and disease status. Both Chalmers and Yalumba use heat therapy to eradicate pathogens, including virus.

SunWorld International is a US based company that breeds and grows their own fruit varieties. The company grants long-term licenses to growers and marketers worldwide. The Australian representative is ANFIC (Australian Nurserymen's Fruit Improvement Company), who licence Australian growers to trial and grow the products. The licenses are granted in exchange for royalties associated with the use of the Company's plant patents and trademarks. SunWorld table grapes are imported through Western Australia quarantine and SunWorld maintain a backup of 17 varieties in quarantine in case they need to be provided to other countries. The SunWorld table grape varieties are reportedly tested for quarantineable and endemic viruses on arrival. Currently, no further testing is conducted. SunWorld varieties originate either from a breeding program that uses embryogenesis for development of new varieties from seedless varieties or through traditional methods involving hand transfer of pollen. Whilst these methods reduce the

risk of transmission of many pathogens it is possible that some varieties may become re-infected due to field spread. In the USA, many of the SunWorld varieties are maintained by the Foundation Planting Service (FPS). However, SunWorld is reconsidering this arrangement.

It is notable that 95% of grapevine imports over the last 10 years have been via private companies and, prior to 2001, 90-95% of material came from non-accredited sources. It is therefore important that the proposed grape scheme is accessible and advantageous both to the industry as a whole and also to importers taking this initiative.

Several of the commercial nurseries consulted with in this project stressed that the new scheme should not include barriers to their participation.

3.2 VINA

Nurseries have the option to be accredited by the Vine Industry Nursery Association (VINA) which is the peak industry body for vine nurseries. VINA protocols include guidelines additional to those originally included in AVIA's nursery accreditation procedures, i.e. documentation and labelling for improved tracking of material and documentation of moisture status of cuttings. VINAS is the VINA-endorsed nursery accreditation scheme which has accredited 53 nurseries since 2003. AVIA appears to agree that VINA is the most suitable body to accredit vine nurseries.

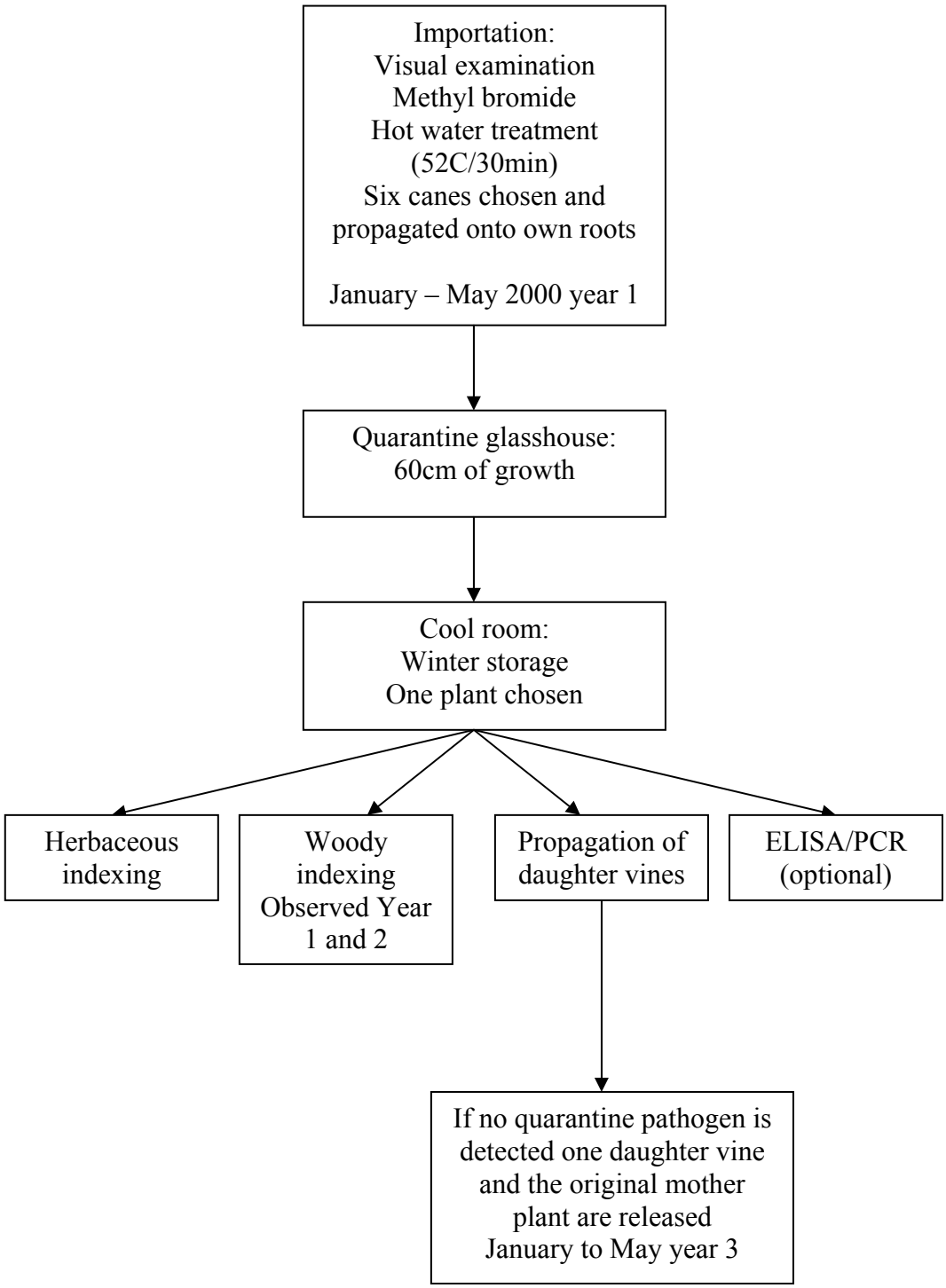


Figure1 Current AQIS protocols for the treatment of imported grapevine material Source: Sivapalan *et al*, 2001

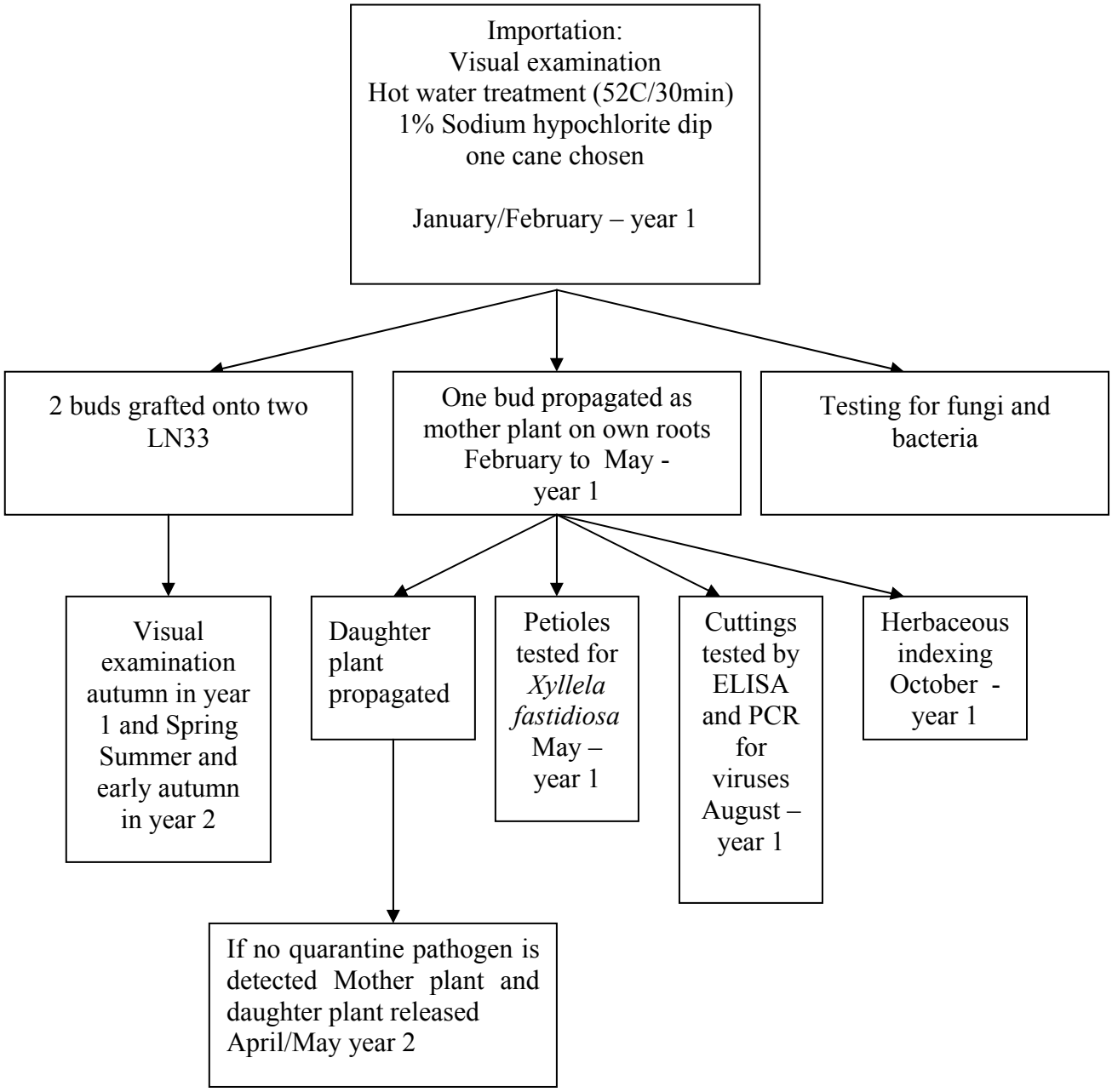


Figure 2 Proposed AQIS protocols for the treatment of imported grapevine material to reduce time in PEQ. Source: Sivapalan *et al*, 2001

Annex 2

Framework and Issues

FRAMEWORK STAGES

1 Stage 1 – Identify and procure new varieties and clones

1.1 Description

Access to new varieties and clones of both scions and rootstocks from overseas and within Australia is a fundamental requirement for industry sustainability and growth. Identification of prospects requires an understanding of the characteristics of likely future demand (or even the capability to influence it) and access to data describing the relevant characteristics of available varieties and clones including location and availability. Access also requires the ability to fulfil any transaction requirements i.e. both a willing buyer and a willing seller. Transaction requirements may relate to intellectual property issues, price, reputation of parties, licensing, vine health status, strategic considerations, etc.

International sources of high health status material include nuclear elite collections such as FPS at UC Davis, ENTAV in France, Geisenheim in Germany, and similar collections in Spain, Italy, South Africa etc.

Sources of grape material within Australia do not always provide material of a fully defined health status. The future management and funding of sources like genetic resource collections these collections is under consideration. Similarly, the standardization and imposition of testing and treatment protocols for genetic resource collection material, prior to its provision as planting material for mother vines or source blocks, are under consideration.

All imported planting material is required to pass through quarantine to ensure that it is free of quarantineable diseases. However, passage through quarantine does not provide any guarantee on the health status as it relates to endemic pathogens. Some quarantineable pathogens may also escape detection. The management of AQIS quarantine and PEQ facilities, and the protocols to be followed, are under review[1].

1.2 Issues and suggested actions/responses

- Development of health status, sampling, testing and treatment protocols;

  Discussion with local and overseas grapevine pathologists and viticulturists

- Assessment of priorities to be allocated to viruses and other pathogens;

  Discussion with local and overseas grapevine pathologists and viticulturists

- Quarantine process – scope, time taken, transparency eg public knowledge of material (varieties, clones, current stage) going through quarantine; process for material from certified/accredited and non-certified/non-accredited overseas sources;

  Adoption of the Sivapalan plan

  Development of improved diagnostic procedures for quarantineable pathogens

- Quarantine testing – widen scope to simultaneously test for endemic pathogens, i.e. non quarantineable organisms;

[1] This is in response to Radcliffe et al "Review of Plant Research Biosecurity Protocols", July 2003.

Education program to develop understanding of the effects of endemic and quarantineable pathogens

- Differences between quarantine facilities in quality and range of services provided;

  Education of quarantine pathologists in diagnostic tests and the effects of various pathogens; standardised services

- Balance between constraints to importation and incentive to circumvent those constraints;

  Adoption of the Sivapalan PEQ plan

2 Stage 2 – Elite Source Material Collections

2.1 Description

Elite collections comprise a small number of vines of each selected variety or clone that are true to type and tested free of high priority (prescribed and non-prescribed) diseases. Elite collections must operate under strict protocols that determine and verify their required high health status and their trueness to type.

Elite collections provide the source material for the growth of mother vines and thus are the foundation of any new multiplication program. Vines for elite collections may be sourced internationally and thus must be shown to be free of quarantine specified diseases before release by AQIS, or they may be sourced from within Australia, and would include tested and "cleaned up" material from existing genetic resource collections.

The terms of reference (TOR) for VCPTRG include advising NVHSC on the "scientific and technical aspects of producing and maintaining grapevine source collections of the highest health status".

Currently, SAVII is establishing a higher health collection at Kapunda (planting started in autumn 2003) and AVIA is in the initial stages of establishing a collection at Dareton. Their status as 'elite', 'best available' or other has not been determined and will be dependent upon the criteria and protocols adopted by the VCPTRG. WAVIA and the WA Department of Agriculture (AgWA) also have a "foundation collection" at the AgWA horticulture research station at Manjimup.

The nature, extent and location of vine collections within commercial nurseries is not well documented. To-date protocols for their establishment have not been provided and the history of the vines in the collections, are not widely known. Also, at this time, it is unclear if any would meet the criteria of this report for an 'elite' collection, although it is likely some material within the collections could be of the highest health status.

If the recommendations in the report are implemented, the status of collections as well as the accreditation status of nurseries should be in the public domain.

2.2 Issues and suggested actions/responses

- Evaluation of feasibility of restrictions on the establishment, location, ownership and management of elite source material collections;

- Scope of elite collection protocols and accreditation schemes including enforceability, credibility, auditability, industry ownership;

  Development of protocols by the VCPTRG/NVHSC and appointment of an expert (preferably a viticultural pathologist) to advise the committees and the owners of the schemes and assist with testing

- Development of testing protocols and accreditation schemes relevant to elite collections;

  Discussion with local and overseas pathologists as well as adoption of similar or better protocols currently in use by overseas grapevine certification schemes and local and overseas certification schemes for other crops

- Accountability and auditing of accreditation and certification schemes;

  NATA accreditation for laboratories

  VINA (and/or AVIA) for nursery accreditation

  External accreditation for the scheme with reporting to NVHSC; Unless certification become mandatory this may present difficulties

- Consequences of audit failure;
- Risk management of collections (industry issue as well as confined to specific collection) – geographical location, numbers of collections, diversity and specialisation (ie profile of rootstocks, varieties and clones for each collection),

  Epidemiological research to understand the risk and rate of spread of the various pathogens in each region where collections are to be maintained

- Possible use of quarantine post entry quarantine facilities as locations for elite collections;

  This is unlikely due to space restrictions. However the use of appropriate testing (improved PEQ protocols, testing for endemic pathogens and heat treatment) would assist in the provision of high health material.

- Lack of a national database of varieties and clones.

  Registration of varieties, perhaps maintained by GWRDC/VCPTRG and included in the protocols for a certification scheme or other publication. Privacy laws must be considered

3 Stage 3 – Mother vines

3.1 Description

Mother vines provide the planting material for the establishment of source blocks. For vine selection societies, the mother vines were those individual vines on commercial blocks selected for superior characteristics. However, now the establishment and growth of mother vines is likely to be performed by the management of the elite collection and in the same location. Mother vines must be grown under strict protocols that enable an audit trail to ensure maintenance of health status and trueness to type. The number of mother vines produced in a specified time is constrained by the productivity of the vines in the elite collection and the propagation processes used. Thus, the use of rapid propagation techniques may reduce the time taken for new or improved varieties or clones to become established in commercial vineyards.

3.2 Issues and suggested actions/responses

- Development and assessment of methods to reduce the time taken for elite collections to produce planting material for source blocks;

  Achieved by managers/owners of elite and best available collections, in association with accredited nurseries and an impartial research agency.

- Efficacy of rapid propagation techniques – scientific assessment of consequences;

Achieved by managers/owners of the elite and best available collections, in association with accredited nurseries and an impartial research agency.

- Accreditation, certification and paper trails;

  Developed by the managers/owners of the collections in association with the VCPTRG/NVHSC and VINA

- Testing and treatment protocols

  Test protocols developed in association with the testing agencies

4 Stage 4 – Source Blocks

4.1 Description

Source blocks are those that supply the canes to be cut, grafted if necessary and grown by nurseries (see stage 5). Source blocks at present are classified as certified (A class), uncertified (B class) or unclassified. A class blocks are F2 material and certified as complying with the protocols specified in the current accreditation scheme. B class material is usually based on planting material from A class blocks and with less rigorous compliance to protocols. C class blocks are used to provide material when demand is greater that the supply for A and B class blocks. Source blocks are usually owned by nurseries or by VIG groups and may be planted in commercial vineyards. As this material is planted out commercially, health status and trueness to type should be assessed and clearly documented. Diagnosis of disease may lead to the decision to clean up material through hot water treatment (HWT) in the nursery stage if appropriate, the removal of affected vines or change of classification of the source block.

The classification level of planting material available to purchasers is affected by the level of demand for planting material, the availability of planting material of various classes, and the premium prospective purchasers place on the classification level of planting material.

4.2 Issues and suggested actions/responses

- Management of source blocks to ensure maintenance of health status and trueness to type (eg isolated blocks cf those in commercial vineyards);

  Managed by the VIGs – VIGs need to ensure that the managers of these blocks are trained to identify disease and off-types.

- Accreditation and registration of source blocks;

  Achieved by the managers/owners of the elite and best available collections, to reflect status of the original vines, treatments and monitoring.

- Propagation and collection practices;

  Developed and accredited by VINA and/or AVIA

- Testing and treatment protocols;
- Equity of distribution of canes.

  Better forecasting of demand is required

5 Stage 5 – Nursery

5.1 Description

Canes from source blocks are cut, grafted if necessary, grown and stored as dormant rootlings. They are subjected to HWT or other phytosanitary treatments if necessary before or after storage and distributed to commercial vineyards for planting. Operations in this stage are conducted by commercial nurseries and VIGs.

Disease status of planting material depends partly on the propagation procedures employed. Thus, accreditation of the nursery (eg VINA, AVIA, other schemes such as ISO 9000) is important.

5.2 Issues and suggested actions/responses

- Potential for conflict of interest in commercial nursery membership of VIGs

  VIGs in each region need representation nursery, grower, winemaker, government and elite/best available collections and a pathologist for advice

- Distribution of dormant rootlings – equity, level of satisfaction of demand; quality
- Efficacy of HWT methods and timing;

  These methods are being developed through research and an education program is required to assist with adoption.

- Accreditation, certification;
- Certification and labelling to reflect health status of the source material

  A consistent certification and labelling protocol needs to be developed by the VCPTRG in consultation with the owners of elite and best available collections/certification schemes.

- Propagation and storage practices;
- Product description standards and adherence

  Consistent product description standards need to be developed by the VCPTRG in consultation with the owners of elite and best available collections/certification schemes.

6 Stage 6 – Commercial Vineyard

6.1 Description

Planting material is provided for new developments and replacement through replanting or top grafting. If for replacement, specific management protocols should be implemented to ensure minimum carry over of diseases etc from the previous planting.

6.2 Issues and suggested actions/responses

- Grower knowledge and understanding of relative priorities of diseases eg virus, trunk diseases;
- Demand for certified planting material from an accredited nursery;

  Demand by wineries, supermarkets, importers/exporters etc for quality assessments back to planting material, may increase demand for certified planting material; education of growers will result in increased demand

- Source and credibility of descriptions of planting material;
- Trueness to type, health status;
- Strike rate and growth
- Adequacy of preparation for new planting;
- Protocols for replanting and top working are required to minimise carryover of problems from previous planting and to ensure success of replanting or top working.
- Post planting husbandry

  Many of the above issues require an education campaign to assist growers in understanding of the effects of disease, pests and pathogens and viticultural practice

Annex 3

Critical Control Points and Control Measures

LIST OF CRITICAL CONTROL POINTS WITHIN THE VINE IMPROVEMENT FRAMEWORK, ON WHICH THE ASSESSMENT AND DETERMINATION OF VINE HEALTH ARE DEPENDENT

Definitions according to HACCP.

- **Critical control point** – A step at which control can be applied and is essential to prevent or eliminate a (food safety) hazard or reduce it to an acceptable level
- **Control measure** – any action or activity that can be used to prevent or eliminate a (food safely) hazard or reduce it to an acceptable level.
- **Critical limit** – a criterion, which specifies the acceptability and unacceptability

Table 1 : Control points, control levels and critical limits for each level of elite material within a grapevine certification scheme and additional control measures for the provision of high health planting material.

LEVEL	CONTROL POINT	CONTROL MEASURE	CRITICAL LIMIT	ADDITIONAL CONTROL MEASURE
Imported material	Post entry quarantine, prior to release	Testing for all quarantineable pathogens (biological, serological and molecular) - entry denied for any material containing such pathogens Hot water treatment, 50ºC/30min	Nil quarantineable pests and pathogens	Additional testing and treatment for pests and pathogens already present in Australia, which are considered undesirable in a certification scheme – if possible reintroduce some pathogens to the quarantine list. Obtain material from international high health schemes - but testing must not be relaxed Ampelography/ DNA testing for trueness to type
Local breeding program	Prior to addition	Breeding from high health material	No detections of prescribed or non-prescribed pathogens, phylloxera or known vectors (NB - as material is from seed it is unlikely that any pathogen, other than viroids, will be present during the initial growth phase)	Breeding and assessment done away from possible sources of infection by pests and pathogens If using rootstocks, ensure these are from a high health source

LEVEL	CONTROL POINT	CONTROL MEASURE	CRITICAL LIMIT	ADDITIONAL CONTROL MEASURE
	Maintenance	Visual inspection for disease Pathogen testing Maintenance away from possible sources of infection	No detections of prescribed or non-prescribed pathogens, phylloxera, known vectors, economic pests *(except viroids, RSPaV, trunk disease associated fungi*)	Maintained in pots in a screen house or in an isolated field site. Pathogen infected material is removed Pests treated accordingly
Nuclear (Elite) stock	Prior to addition	Pathogen testing (biological, serological and molecular) Heat therapy and meristem culture if any disease detected Ampelography/DNA testing for trueness to type Hot water treatment*, 50ºC/30min	No detections of prescribed or non-prescribed pathogens, phylloxera or known vectors *(except viroids, RSPaV*) in the planting material	Prior to the establishment of a collection the site should be appropriately prepared - soil tested, and treated if necessary, for potential pests or pathogens that could affect establishment, appropriate infrastructure in place Site selection should be where grapevines have not been previously planted preferably virgin soil. Established away from possible sources of infection
	Maintenance	Regular pathogen testing (biological once every three years, serological and/or molecular yearly) - GLRaV1, 3, 5, 9, GVA, GVB, Petri disease, crown gall, phytoplasma, nematodes Visual inspection for disease Regular spray application for pests and fungi Appropriate labelling of cuttings taken from the plantings and used to establish mother plantings, for disease status and trueness to type (ie certified or non certified) ie QA system in place	No detections of prescribed or non-prescribed pathogens, phylloxera or known vectors, economic pests *(except viroids, RSPaV, trunk disease associated fungi,* crown gall?	Maintained in pots in a screen house and/or tissue culture plantlets –in addition to field planting If a pathogen infected plants is detected, removal of infected plant, testing of nearest neighbours (whole planting?) and all other plants of the same clone/variety to determine of incidence, replacement vine produced from uninfected clone, new import or through heat therapy Disinfection of pruning tools
Mother plantings	Establishment	Established with material only from Elite (nuclear) collection Hot water treatment, 50ºC/30min of cuttings used for establishment; (or 54ºC/5 min if rooted cuttings)	No detections of prescribed or non-prescribed pathogens, known vectors, or phylloxera *(except viroids, RSPaV*) in the planting material	Prior to the establishment of a collection the site should be appropriately prepared - soil tested, and treated if necessary, for potential pests or pathogens that could affect establishment, appropriate infrastructure in place Site selection should be where grapevines and citrus have not been previously planted preferably virgin soil. Established away from possible sources of infection

LEVEL	CONTROL POINT	CONTROL MEASURE	CRITICAL LIMIT	ADDITIONAL CONTROL MEASURE
	Maintenance	Visual inspection – disease and trueness to type Pathogen testing – routine, every vine should be tested every third year for pathogens that are most likely to spread aerially or mechanically eg GLRaV1, 3, 5, 9, GVA, GVB, Petri disease, phytoplasmas Appropriate labelling of cuttings taken from the plantings and used to establish increase blocks, for disease status and trueness to type (ie certified or non certified) ie QA system in place	No detections of prescribed or non-prescribed pathogens, phylloxera or known vectors; economic pests *(except viroids, RSPaV, trunk disease associated fungi)*	Any diseased grapevine removed, neighbouring grapevines actively tested or removed Disinfection of pruning tools
Increase blocks	Establishment	Established with material only from Elite (nuclear) collections or mother plantings Hot water treatment, 50ºC/30min of cuttings used for establishment (or 54ºC/5 min if rooted cuttings)	No detections of prescribed or non-prescribed pathogens, phylloxera or known vectors *(except viroids, RSPaV)* in the planting material	Prior to the establishment of a collection the site should be appropriately prepared - soil tested, and treated if necessary, for potential pests or pathogens that could affect establishment, appropriate infrastructure in place Established away from possible sources of infection
	Maintenance	Visual inspection – disease and trueness to type Pathogen testing – random sampling throughout the vineyard every year*for GLRaV1, 3, 5, 9, GVA, GVB and phytoplasmas. Active testing of any vine displaying disease Appropriate labelling of cuttings taken from the blocks, for disease status and trueness to type (ie certified or non certified)	No detections of prescribed or non-prescribed pathogens, phylloxera, known vectors, economic pests *(except viroids, RSPaV, trunk disease associated fungi)*	Maintained as a vineyard but away from other potential sources of infection Any diseased grapevine removed, surrounding grapevines actively tested Diseased grapevine must not be used for cuttings

LEVEL	CONTROL POINT	CONTROL MEASURE	CRITICAL LIMIT	ADDITIONAL CONTROL MEASURE
Nurseries	Acquisition	Material gained from certified high health mother planting	No detections of prescribed or non-prescribed pathogens, phylloxera, known vectors *(except viroids, RSPaV ,trunk disease associated fungi)*	Not to be handled concurrently with any other material Kept separate from other material
	Treatment	Hot water treatment, 50ºC/30min of cuttings Appropriate labelling for disease status and trueness to type (ie certified or non certified) Maintain certified material away from non-certified	Appropriate disinfestation of nursery equipment to prevent possible contamination by fungi and bacteria and viroids that could affect the establishment of material in vineyards	Appropriate spray application to prevent insect pests and vectors.

Table 2 : Control points, control levels and critical limits for each level of best available material, that may be infected by pathogens, within a grapevine certification scheme and additional control measures for the provision of this planting material.

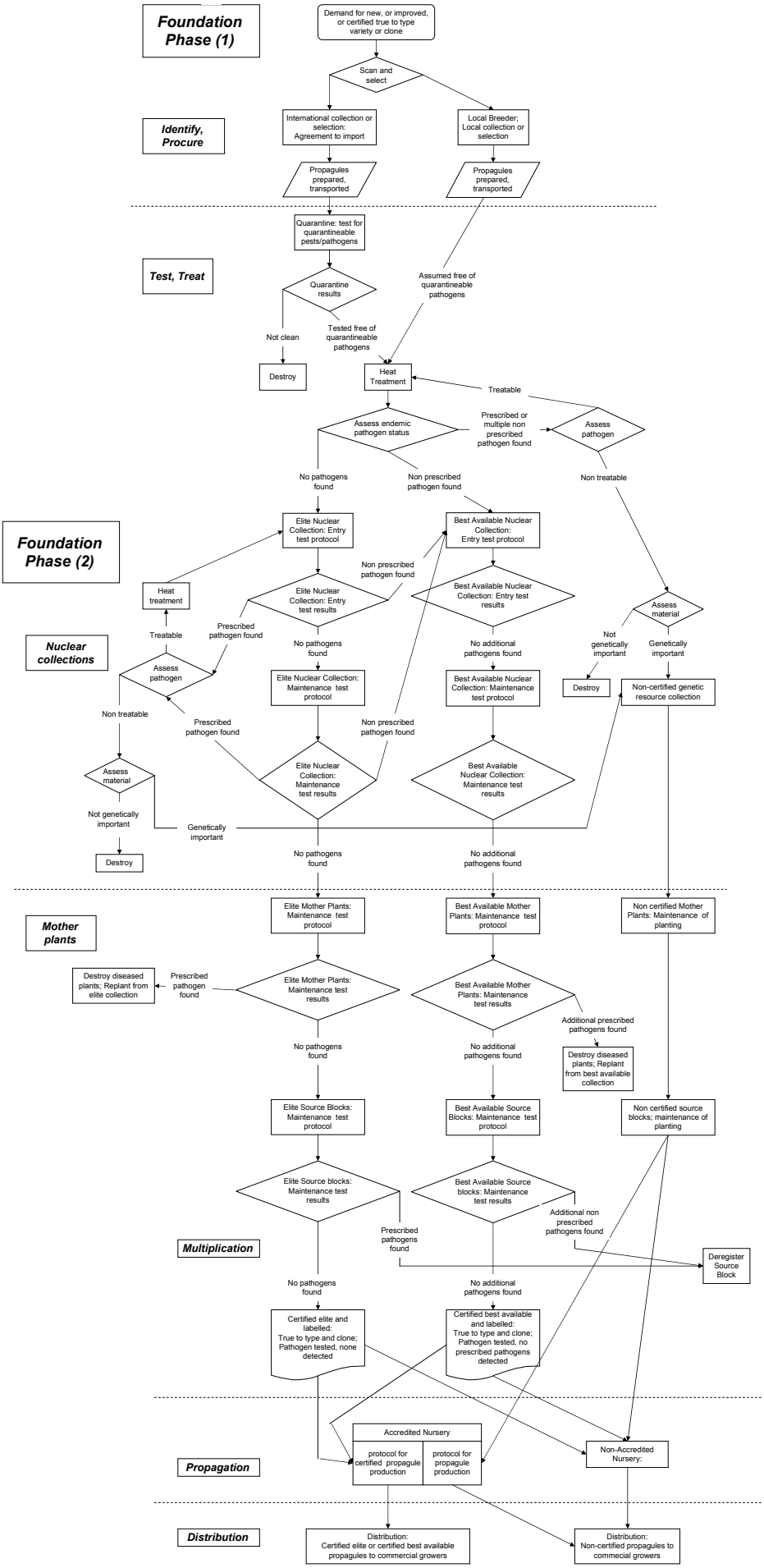
LEVEL	CONTROL POINT	CONTROL MEASURE	CRITICAL LIMIT	ADDITIONAL CONTROL MEASURE
Imported material	Post entry quarantine, prior to release	Testing for all quarantineable pathogens (biological, serological and molecular) - entry denied for any material containing such pathogens Hot water treatment, 50ºC/30min	Nil quarantineable pests and pathogens	Additional testing for pests and pathogens already present in Australia Obtain material from international high health schemes - testing must not be relaxed Ampelography/ DNA testing for trueness to type
Best available collection	Prior to addition	Pathogen testing (biological, serological and molecular) Ampelography/DNA testing for trueness to type Hot water treatment, 50ºC/30min	No detection of 'not-tolerated/prescribed' pathogens; isolated detections of 'tolerated' pathogens. Pathogens infecting each clone or rootstock must be identified and labelled	Prior to the establishment of a collection the site should be appropriately prepared - soil tested, and treated if necessary, for potential pests or pathogens that could affect establishment, appropriate infrastructure in place Site selection should be where grapevines have not been previously planted preferably virgin soil. Established away from possible sources of infection and away from an elite collection
	Maintenance	Visual inspection Regular pathogen testing (biological once every three years, serological and/or molecular yearly) GLRaV1, 3, 5, 9, GVA, GVB, Petri disease, crown gall, phytoplasma, nematodes Regular spray application for pests and fungi Appropriate labelling of cuttings taken from the plantings and used to establish mother plantings, for disease status and trueness to type (ie certified or non certified) ie QA system in place	No additional pests/pathogens	Maintained in the field away from other vineyards etc If a previously unreported pathogen is detected in any plant this must be labelled and testing of nearest neighbours and all other plants of the same clone/variety must be done to determine of incidence. A decision will need to be made as to whether an additional pathogen will be tolerated or id the material should be removed Disinfection of pruning tools

LEVEL	CONTROL POINT	CONTROL MEASURE	CRITICAL LIMIT	ADDITIONAL CONTROL MEASURE
Germplasm Mother plantings	Establishment	Established with material only from germplasm collection Hot water treatment, 50ºC/30min of cuttings used for establishment (or 54ºC/5 min if rooted cuttings)	For grades of mother plantings, it will depend on knowledge of health status at time of inclusion. Material infected by the same tolerated pathogen/s could be planted near each other, while those that differ could be separated	Prior to the establishment of a collection the site should be appropriately prepared - soil tested, and treated if necessary, for potential pests or pathogens that could affect establishment, appropriate infrastructure in place Site selection should be where grapevines and citrus have not been previously planted preferably virgin soil. Established away from possible sources of infection and away from an elite collection
	Maintenance	Visual inspection – disease and trueness to type Pathogen testing – routine, every vine should be tested every third year for pathogens that are most likely to spread aerially or mechanically eg GLRaV1, 3, 5, 9, GVA, GVB, Petri disease, phytoplasmas Appropriate labelling of cuttings taken from the plantings and used to establish increase blocks, for disease status and trueness to type (ie certified or non certified) ie QA system in place	No additional pests/pathogens	Maintained in the field away from other vineyards etc If a previously unreported pathogen is detected in any plant this must be labelled and testing of nearest neighbours and all other plants of the same clone/variety must be done to determine of incidence. A decision will need to be made as to whether an additional pathogen will be tolerated or id the material should be removed Disinfection of pruning tools
Increase blocks	Establishment	Established with material only from germplasm collections or germplasm mother plantings Hot water treatment, 50ºC/30min of cuttings used for establishment	None	Prior to the establishment of a collection the site should be appropriately prepared - soil tested, and treated if necessary, for potential pests or pathogens that could affect establishment, appropriate infrastructure in place Established away from possible sources of infection

LEVEL	CONTROL POINT	CONTROL MEASURE	CRITICAL LIMIT	ADDITIONAL CONTROL MEASURE
	Maintenance	Visual inspection – disease and trueness to type Pathogen testing – random sampling throughout the vineyard every year* GLRaV1, 3, 5, 9, GVA, GVB and phytoplasmas and active testing of any vine displaying disease other than that which is already known Appropriate labelling of cuttings taken from the blocks, for disease status and trueness to type (ie certified or non certified)	No additional pests/pathogens	Maintained as a vineyard but away from other potential sources of infection Any diseased grapevine removed, surrounding grapevines actively tested Diseased grapevine must not be used for cuttings
Nurseries	Acquisition	Material gained from germplasm mother planting	No additional pests/pathogens	Not to be handled concurrently with elite material
	Treatment	Hot water treatment, 50ºC/30min of cuttings Appropriate labelling for disease status and trueness to type (ie certified or non certified) Maintain certified material away from non-certified	Appropriate disinfestation of nursery equipment to prevent possible contamination by fungi and bacteria and viroids that could affect the establishment of material in vineyards	Appropriate spray application to prevent insect pests and vectors.

Annex 4

Flow Chart


Foundation Phase (1)
Demand for new, or improved, or certified true to type variety or clone
Scan and select
Identify, Procure
International collection or selection: Agreement to import
Local Breeder; Local collection or selection
Propagules prepared, transported
Propagules prepared, transported
Quarantine: test for quarantineable pests/pathogens
Test, Treat
Quarantine results
Assumed free of quarantineable pathogens
Not clean
Tested free of quarantineable pathogens
Destroy
Heat Treatment
Treatable
Assess endemic pathogen status
Prescribed or multiple non prescribed pathogen found
Assess pathogen
No pathogens found
Non prescribed pathogen found
Non treatable
Foundation Phase (2)
Elite Nuclear Collection: Entry test protocol
Best Available Nuclear Collection: Entry test protocol
Non prescribed pathogen found
Heat treatment
Elite Nuclear Collection: Entry test results
Best Available Nuclear Collection: Entry test results
Assess material
Nuclear collections
Treatable
Prescribed pathogen found
No pathogens found
No additional pathogens found
Not genetically important
Genetically important
Assess pathogen
Elite Nuclear Collection: Maintenance test protocol
Best Available Nuclear Collection: Maintenance test protocol
Destroy
Non-certified genetic resource collection
Non treatable
Prescribed pathogen found
Non prescribed pathogen found
Assess material
Elite Nuclear Collection: Maintenance test results
Best Available Nuclear Collection: Maintenance test results
Not genetically important
Genetically important
Destroy
No pathogens found
No additional pathogens found
Mother plants
Elite Mother Plants: Maintenance test protocol
Best Available Mother Plants: Maintenance test protocol
Non certified Mother Plants: Maintenance of planting
Destroy diseased plants; Replant from elite collection
Prescribed pathogen found
Elite Mother Plants: Maintenance test results
Best Available Mother Plants: Maintenance test results
Additional prescribed pathogens found
Destroy diseased plants; Replant from best available collection
No pathogens found
No additional pathogens found
Elite Source Blocks: Maintenance test protocol
Best Available Source Blocks: Maintenance test protocol
Non certified source blocks; maintenance of planting
Elite Source blocks: Maintenance test results
Best Available Source blocks: Maintenance test results
Additional non prescribed pathogens found
Multiplication
Prescribed pathogens found
Deregister Source Block
No pathogens found
No additional pathogens found
Certified elite and labelled: True to type and clone; Pathogen tested, none detected
Certified best available and labelled: True to type and clone; Pathogen tested, no prescribed pathogens detected
Accredited Nursery
protocol for certified propagule production
protocol for propagule production
Non-Accredited Nursery:
Propagation
Distribution
Distribution: Certified elite or certified best available propagules to commercial growers
Distribution: Non-certified propagules to commecial growers

Annex 5

Effects of Pathogens on Grape Vines

EFFECTS OF PESTS/PATHOGENS ON VINES

7 Introduction

Many pathogens and pests have a significant impact on the health of grapevines, affecting the yield and quality of grapes and the quality of grapevine propagation material. Some of these pathogens can be transmitted through propagation material. A certification scheme is a mechanism for multiple users, that should increase both knowledge of the identity and history, and assurance of the health status, of the planting material prepared under its guidelines. As such, certification schemes aim to reduce the threat of spread of pathogens and pests through planting material. Vineyards established with certified material, if properly maintained, should remain sustainable and productive for many years.

Current research indicates that 11 viruses, five viroids, and three phytoplasmas, six bacteria and 98 fungi, which infect grapevines, have been reported in Australia. The significant pathogens are listed in Table 1 below.

Table 1 : High Health Grapevine Planting Material – Important pests/pathogens

Pathogen/pest	Name
Virus	Grapevine virus A vitivirus (GVA); Grapevine virus B vitivirus (GVB); Grapevine fan leaf nepovirus (GFLV); Grapevine fleck maculavirus (GFkV); Grapevine leafroll-associated ampelovirus 1 (GLRaV1); Grapevine leafroll-associated closterovirus 2 (GLRaV2); Grapevine leafroll-associated ampelovirus 3 (GLRaV3); Grapevine leafroll-associated ampelovirus 4 (GLRaV4); Grapevine leafroll-associated ampelovirus 5 (GLRaV5); Grapevine leafroll-associated ampelovirus 9 (GLRaV9); Grapevine red globe maculavirus; Grapevine rootstock stem lesion closterovirus (= strain of GLRaV2) Grapevine rupestris stem pitting associated foveavirus (GRSPaV); (= Grapevine stem pitting associated closterovirus?)
Viroid	Australian grapevine viroid (AGVd) Citrus exocortis viroid (CEVd) Grapevine yellow speckle viroid-1 (GYSVd-1) Grapevine yellow speckle viroid-2 (GYSVd-2) Hop stunt viroids (HSVd)
Phytoplasma	Australian grapevine yellows (AGY, *Candidatus* Phytoplasma australiense) Tomato big bud (TBB) Buckland Valley grapevine yellows (BVGY)
Bacteria	*Agrobacterium vitis*

Pathogen/pest	Name
Fungi	*Botryosphaeria dothidea* *Botryosphaeria obtusa* *Botryosphaeria rhodina* *Botryosphaeria ribis* *Botryosphaeria stevensii* *Uncinula necator* *Eutypa lata* *Plasmopara viticola* *Phaeacremonium aleophilia* *Phaeomoniella chlamydospora* *Phomopsis viticola*
Pests	Citrophilous mealybug - *Pseudococcus calceolariae* (vectors of GLRaV-3 in New Zealand); Citrus mealybug - *Planococcus citri* (vectors of GVA, GLRaV-3 in other countries) longtailed mealybug - *Pseudococcus longispinus* (vectors of GLRaV-3, 5, GVA in other countries) Plum scale - *Parthenolecanium corni* (vectors of GLRaV-1, 3 in other countries) Obscure mealybug - *Pseudococcus viburni* (= *P. affinus*) (vectors of GVA, GVB GLRaV-3 in other countries) Grape phylloxera - *Daktulosphaira vitifoliae* *Brevipalpus* spp – including bunch mite Blister mite and bud mite - *Colomerus vitis* Grape leaf rust mite - *Calepitrimerus vitis* Dagger nematode - *Xiphinema index* (vector of GFLV) Dagger nematode - *Xiphinema vuittenezi*, (vector of nepoviruses) Root-knot nematodes - *Meloidogyne* spp Root-lesion nematodes - *Pratylenchus* spp Citrus nematode - *Tylenchulus semipenetrans*

To further understand the benefits of planting material of a known high health status, the effects of various pathogens on grapevines are discussed in this review.

8 Effects of Virus

Table 1 lists the viruses that are known to occur in Australia. Various reports associate viruses with changes in yield, changes in quality, graft incompatibility, and vine decline and death. In addition, viruses can cause deformation that makes clonal identification difficult. Some viruses may not cause disease, especially when they infect grapevines in the absence of other viruses. However, combinations of viruses infecting a grapevine can significantly affect performance.

Specific examples of the effects of virus are given below. These examples indicate that the effect of virus may be cultivar specific and related to the combination of viruses and/or strains of virus present. Some viruses may not induce disease in a scion until they are present on susceptible rootstocks, or rootstocks containing other viruses. The examples also indicate that the effect of virus strains may be important - different strains may be associated with different severity of disease. Grapevine management practices can also interact with viruses to have a significant impact on the performance of grapevines.

All grapevine viruses can be transmitted through planting material. Some viruses, such as the majority of the leafroll associated viruses, are also transmitted via insect vectors, including mealybugs. Mealybugs can be introduced into and spread within vineyards on infested planting material, equipment, workers, birds, other animals, ants, wind and by crawling (Barrass *et al,* 1994, Furness, 1976, Pietersen, 2004).

8.1 Reduced yield

There are many examples, especially from other countries, demonstrating a substantial reduction in yield associated with single and mixed viral infections. Both GLRaV1 and GLRaV3 have been associated with fruit yield loss in various cultivars (Abrashevea, 1977; Tomazic *et al,* 2000, Guidoni *et al,* 2000; Mannini and Credi, 2000; Simon *et al,* 2003; Kovacs *et al,* 2000). Depending on the cultivar, fruit yield loss has been attributed to reduced numbers of clusters/vine (GLRaV1, Tomazic *et al,* 2000) or reduced berry weight (GLRaV3, Kovacs *et al*, 2001; GLRaV1, Mannini and Credi, 2000).

Australian studies have shown that leafroll virus in combination with grapevine yellow speckle viroid was associated with reduced annual growth and fruit yield in the cv. Cabernet Franc (Woodham *et al,* 1983; Clingeleffer and Krake, 1992). However, the mean berry weight and inflorescence numbers were not affected (Woodham *et al* 1983). When compared, yield reduction was greater in minimally-pruned virus/viroid infected Cabernet Franc, than in spur-pruned virus/viroid infected grapevines of the same variety. This suggests that, grapevine management - in this case pruning practices - may ameliorate the effect of virus and virus-like pathogens (Clingeleffer and Krake, 1992). Another Australian study showed that heat-treated sultana performed better than leafroll-affected sultana, especially under minimal pruning (Clingeleffer and Krake, 2002).

Grapevine leafroll-associated viruses, alone and in combination with other viruses or virus like diseases, can affect plant growth (Abrasheva, 1980; Credi and Bambini, 1996; Berres and Stellmach, 1990; Mannini and Credi, 2000; Kim *et al,* 2003). A 79-89% reduction in plant growth and cane pruning weight was observed when 420 A, Kober 5BB and Teleki 5A rootstock hybrids were inoculated with a combination of GLRaV3 and GFLV (Credi and Babini, 1996). When 420 A, Kober 5BB and Teleki 5A rootstock hybrids were inoculated with Kober stem grooving disease (KSG), rupestris stem pitting disease (RSP), vein necrosis (VN) disease and GLRaV3 there was a 42-57% reduction in cane pruning weight (Credi and Babini, 1996). The rootstock hybrids 420 A (66%) and Kober 5BB (48%) had a 66% and 48% reduction in plant growth, respectively, when they were inoculated with KSG, RSP, GLRaV-1 and vein mosaic disease (VM) (Credi and Babini, 1996). Grapevine fleck virus (GFkV) with VN and VM also caused a 51% decrease in 420 A and a 37% decrease in Kober 5BB (Credi and Babini, 1996). Other studies have shown that leafroll-associated viruses have a significant effect on the ability of grapevines to uptake nutrients and that infected grapevines had reduced root mass (Berres and Stellmach, 1990).

GFLV is considered one of the most economically-serious pathogens of grapevines in many countries, causing between 20-90% yield reduction in some cultivars and under certain environmental conditions (Walter and Martelli, 1996). When grapevine cultivars were inoculated with GFLV in combination with other viruses, reduced vigour, including reduction in leaf area and pruning weight, and yield were significantly reduced compared to uninoculated virus-free controls. A recent study showed that, compared to uninfected grapevines of the same variety, GLRaV1 and GFLV infected cv. Banyalbufar Malmsey had reduced photosynthetic activity, which was related to decreased carboxylation and mesophyll conductance (Sampol *et al,* 2003). Ultimately the grapevines had shorter shoots, smaller and less leaves, which were likely to lead to reduced yield. Other research has shown a definite decrease in yield of several cultivars infected by GFLV alone or in combination with other viruses. These cultivars include cv Bolgar on rupestris du lot (GFLV, Abrasheva, 1976), Chardonnay and Ezerfürtű, (GFLV, yellow mosaic

and phytoplasma, Simon *et al* 2003) and Thompson seedless (GFLV, Auger *et al* 1992). In Australia, GFLV is present in some areas of Australia but there is no record of spread. The nematode vector of GFLV, *Xiphinema index*, is not found in most grape growing regions of Australia. Spread of GFLV via planting material or *X.index* has been restricted in Australia, primarily it is thought, as an unexpected benefit of the restrictions placed on soil, equipment and grape material movement from phylloxera-infested zones, where both the virus and nematode vector have been found.

Increased yield and vegetative vigour of grapevines are considered a potential problem when viruses are eradicated. However, changes in grapevine management can control these effects. For example, bunch thinning improved quality of grapes from heat treated vines of cv Gignolino when compared to virus-infected vines. Improved soluble solids and anthocyanin content were observed when heat treated cv Nebbiolo Michet vines were spaced further apart, which was probably due to more light entering the canopy (Mannini *et al,* 2003).

8.2 Quality

Viruses are associated with changes in the quality of fruit, depending on the cultivar and the virus present. Several studies have shown that leafroll-associated viruses, most notably GLRaV1 and GLRaV3, alone or in combination with other viruses, are associated with reduced sugars and increased titratable acidity (Borgo and Angelini, 2002; Guidoni *et al* 2000; Kim *et al,* 2003; Kovacs *et al,* 2000; Mannini, 2001; Malossini *et al,* 2003; Woodham *et al,* 1983). GLRaV3 has been associated with the reduced terpenoids, causing diminished aroma, and reduced anthocyanin content, resulting in poor colour (Mannini, 2001; Mannini and Credi, 2001). When GLRaV3 was eliminated some wines were ranked better in sensory evaluation compared to wine made from infected grapevines (Mannini and Credi, 2001). In some cultivars ripening of fruit may be retarded due to virus infection (Abrasheva, 1980), but in other cultivars virus can cause early ripening (Tomazic *et al,* 2001). Preliminary studies done in Australia have also shown that GRLaV3 might be associated with lower colour rankings of wine made from infected Pinot Noir compared to Pinot Noir that was not infected with GRLaV3 (Farquhar, 2004).

In contrast, no differences were observed between titratable acidity or pH of fruit from heat-treated sultana vines compared to non heat-treated GLRaV-infected sultana vines (Clingeleffer and Krake, 2002). Also cv. Gewurtztraminer clone 913 had an increased yield but lower sugar content after elimination of a leafroll-associated virus (Balthazard, 1993). Consequently, the interaction of variety is an important factor when determining the effects of virus on grape quality.

8.3 Vine decline

Several viruses are associated with vine decline including leafroll viruses and vitiviruses, particularly GLRaV2 and GVB (Golino *et al*, 2003). Other studies have shown an association between grapevine rootstock stem lesion associated virus (GRSLaV = strain of GLRaV2), alone and in combination with other strains of GLRaV2, and grapevine decline (Golino, 2003; Uyemoto *et al,* 2000; Uyemoto *et al,* 2001; Gomez-Talquenca *et al,* 2003). In some cases the disease appeared to be rootstock dependent; that is, the scion was generally symptomless but decline was observed when it was grafted onto susceptible rootstocks. Recent research has also suggested a possible association with strains of RSPaV and Syrah (Shiraz) decline in California and France (Stamp, 2004; Lima *et al,* 2003). Grapevine angular mosaic virus (GAMV) is also associated with decline, severe stunting or death in the variety Baresana x Baresana (Girgis *et al*, 2003). GAMV has not been detected in Australia.

8.4 Graft incompatibility

Graft incompatibilities lead to decline and death of the grafted scion. GLRaV2 has been associated with incompatibility between the rootstock Kober 5BB and several cultivars (Grief *et al,* 1995). In addition, a newly identified Closterovirus, similar, but not identical, to GLRaV2, has been associated with graft incompatibility in merlot in New Zealand (Bonfiglioli *et al,* 2003). It is possible that grapevine decline, as discussed in the previous section, is associated with graft incompatibility also.

8.5 Clonal identification

Viruses, for example GFLV, can induce morphological changes leading to incorrect clonal/varietal identification (Pearson and Goheen, 1994; Santos *et al,* 2003). One study has shown that while genotype is important, only clones free of virus, especially GFLV, can be correctly identified by leaf morphology (Mannini *et al,* 2000).

9 Effects of Phytoplasmas

Australian grapevine yellows (AGY) disease and phytoplasmas are found in most viticultural regions of Australia (Bonfiglioli *et al*, 1996; Magarey & Wachtel, 1986b). Although Koch's postulates have not been fulfilled, phytoplasmas have a strong association with AGY symptoms and are considered to be the cause of this disease. Of the three phytoplasmas associated with AGY symptoms, the AGY phytoplasma is the most frequently detected (Constable *et al,* 2003a, Gibb *et al,* 1999). Tomato big bud (TBB) phytoplasma infects grapevines from several regions but is detected less frequently (Constable *et al,* 2003a; Gibb *et al,* 1999). The Buckland Valley grapevine yellows (BVGY) phytoplasma was reported only from the Buckland Valley of Victoria (Constable *et al,* 2002).

Chardonnay and Riesling appear to be affected by AGY disease more often than other varieties (Magarey & Wachtel, 1986a). However, AGY symptoms have been observed and phytoplasmas have been detected in other white and red varieties (Bonfiglioli *et al,* 1996). Symptomless phytoplasma infections can occur and it is possible that the expression of disease is related to titre and/or location of phytoplasmas (Constable *et al,* 2003a). Significant reductions in yields have been reported from AGY affected vineyards (Constable *et al,* 2000; Magarey and Wachtel, 1986b).

Many phytoplasmas are spread to plants by insect vectors, most of which belong to the superfamilies Cicadelloidea (leafhoppers) and Fulgoroidea (planthoppers) (Lee *et al,* 1998a). No insect vectors have been identified for AGY or BVGY phytoplasmas, although AGY phytoplasma has been detected in the common brown leafhopper, *Orosius argentatus* (Evans) using PCR techniques (Beanland *et al,* 1999). Recent studies have shown that TBB phytoplasma can be acquired from grapevine by *O. argentatus* and subsequently transmitted to Faba bean (Beanland, 2001) but the ability of the leafhopper to transmit TBB phytoplasma back to grapevines has not been confirmed. The transmission of phytoplasmas through grapevine cuttings has not been demonstrated (Magarey, 1986). However, Flavesecnce dorée phytoplasma has been shown to spread through infected cuttings and rootstocks. A hot water treatment of cuttings is recommended to control spread of this phytoplasma (Caudwell *et al,* 1994; Caudwell *et al,* 1997; Pavan *et al,* 1997).

Restricted growth (RG) disease also commonly affects Chardonnay grapevines in Sunraysia (Constable *et al,* 2004; Bonfiglioli *et al,* 1995; Padovan *et al,* 1995). The cause of RG is not understood but phytoplasmas are considered one possible cause. RG symptoms include retarded growth resulting in shortened shoots and smaller leaves. Compared to unaffected grapevines, RG-affected grapevines have an overall appearance of stunting or lack of vigour. Some

grapevines with RG can also display uneven or no bud development resulting in canes and cordons that are bare in places or entirely bare with little or no bunch development. No significant association between phytoplasmas and shoots of grapevines affected by RG alone has been shown (Bonfiglioli *et al,* 1995; Gibb *et al,* 1999; Padovan *et al,* 1995). However, in another study, phytoplasmas were more frequently detected in grapevines that had displayed both AGY and RG compared to grapevines displaying AGY alone (Constable *et al,* 2003a). Analysis of survey data indicates that some grapevines can exhibit a combination of AGY and RG, however RG can occur independently of AGY. Statistical analyses using Log-linear models also indicated that RG were not always associated with AGY.

Similarly, phytoplasmas have been implicated as a cause of late season leaf curl (LSLC) disease in Chardonnay. In one study phytoplasmas were detected in 48/59 shoot samples affected by LSLC (late AGY; Bonfiglioli *et al,* 1995). In another study the AGY phytoplasma was detected in four of 126 shoot samples from LSLC-affected grapevines and TBB phytoplasma was detected in 8 of the 126 samples (Gibb *et al,* 1999). An additional study showed that phytoplasmas were detected more frequently in grapevines affected by LSLC and AGY compared to grapevines affected by AGY alone (Constable *et al,* 2003a).

It is possible that phytoplasmas are not the cause of RG or LSLC and their association is coincidental. Interestingly, Chardonnay grafted onto the rootstock 3309C in the US display similar symptoms to LSLC observed in Australian Chardonnay (Uyemoto and Rowhani, 2003). Graft union incompatibility was also observed. The results indicated an association with a graft transmissible agent and there may have been an association with GRSLaV.

10 Effects of viroids

Five viroids can infect Australian grapevines (Little and Rezaian, 2003) and are listed in Table 2. All viroids can be transmitted via mechanical means, including pruning and grafting equipment and through planting material. It is generally accepted that viroids pose little threat to productivity and the quality of grapevines. However, both grapevine yellow speckle viroids (GYSVd) 1 and 2 cause yellow speckle symptoms, and it is possible, when the disease is severe there might be a reduction in photosynthesis, and therefore growth and productivity (Little and Rezaian, 2003). When GFLV and GYSVd infect grapevines together, vein banding disease is often observed (Krake and Woodham, 1983; Szychowski *et al,* 1995). Australian grapevine viroid, citrus exocortis viroid (CEVd), and hop stunt viroid (HSVd) are not known to cause symptoms in grapevine (Little and Rezaian, 2003).

The viroids that infect grapevines can have a significant impact on other crops, affecting performance of the host plant and, in some cases, the quality of the associated end product. For example, HSVd causes stunting in hops and cone weight can be reduced by 50% (Randles, 2003). Variants of HSVd also infect citrus (cachexia disease, Duran Vila and Semancik, 2003), plum and peach (dapple fruit disease; Sano, 2003), and almond and apricot (latent infections; Pallàs *et al,* 2003). While most commercial species and cultivars of citrus are tolerant to CEVd, if they are grafted onto sensitive rootstocks, the viroid can reduce tree size and lower yields in infected, declining citrus. This has implications for industry as grapevines are often grown in regions where citrus (and hops, in some areas) is also.

11 Effects of Bacteria

Agrobacterium vitis (*A. tumefaciens* biovar 3) can have a significant effect on grapevines in Australia. *A. vitis* can be transmitted via planting material (Gillings and Ophel-Keller, 1995; Burr and Otten, 1999). Hot water treatment significantly reduces the titre of the bacterium thereby improving the success of grafting in nurseries (Ophel *et al,* 1990). However, the only sure way to

eliminate the bacterium from grapevines is to use *in vitro* shoot tip culture as the bacterium does not systemically invade green shoots. *A. vitis* causes galls on trunks at or above the graft union and necrosis of the roots. Severe infections lead to a reduction in vine growth and yield, and significant losses in nurseries (Krake *et al,* 1999; Burr and Otten, 1999). In the vineyard, severe infections can lead to decline and death of grapevines.

12 Effects of Fungi

Only a small number of the fungi that can infect grapevines are considered to be strong pathogens. The significant fungi are associated with diseases such as Esca, Eutypa dieback, Petri disease and vine decline. They are listed in Table 2.

Eutypa lata is not transmitted via planting material. However, *E. lata* affects the productivity and sustainability of vineyards (Lardner *et al,* 2002). Infection by *E. lata* results in stunted shoots and dieback, thus reducing the number of available cuttings per vine. Diseased grapevines have a shortened life span and will need to be replaced sooner than unaffected grapevines. Consequently this disease should be controlled in an elite collection of high health grapevines, in mother vines and source plantings.

Botryosphaeria dothidea, B. obtusa, *B. rhodina*, *B. ribis* and *B. stevensii* have been found in association with canker and decline of grapevines, although their role in the disease is not fully understood (Castillo-Pando *et al*, 2001; Shoemaker, 1964; Pearson and Goheen, 1994; Paradela, 1995). Isolation and pathogenicity tests suggest that *B. obtusa* may have a role in the decline of grapevines, cv Semillon, in the Hunter Valley of Australia (Castillo-Pando *et al,* 2001). If *Botryosphaeria* spp. cause decline of grapevines then, like *Eutypa lata*, they could also have an effect on the productivity and sustainability of vineyards.

Although Koch's postulates for cause of disease have not been fulfilled, *Phaemoniella chlamydospora* (=*Phaeoacremonium chlamydosporum*) is thought to be the cause of Esca disease and Petri disease (Edwards and Pascoe, 2002a). Esca can result in apoplexy of affected grapevines. The lifespan of Esca affected grapevines is reduced.

Other fungi are also often found in association with Esca disease and young vine decline, including *Phaeoacremonium aleophilum, Formitiporia punctata*, *Stereum hirsutum* and *Eutypa lata*. The role of *P. aleophilum* is uncertain. It is detected less frequently in affected grapevines than *P. chlamydospora* in Australia (Edwards and Pascoe, 2002a). *Formitiporia punctata* is often reported to be associated with white heart rot in Esca-affected grapevines but, in Australia other fungi have been found in association. *Stereum hirsutum* has been found in association with white heart rot in other countries, but to a lesser extent than *F. punctata*. It is no longer considered a potential cause of Esca (Mugnai *et al,* 1999). It is notable that white heart rot is not always associated with Esca disease (Edwards and Pascoe, 2002a). In the case of "young Esca" (i.e. Esca in vines less than 10-years-old) in Australia, *P. chlamydospora* was the only fungus consistently isolated from affected grapevines (Edwards *et al,* 2001a).

Esca disease of older grapevines in Australia is rare (Edwards *et al,* 2001a). *P. chlamydospora* can sporulate in the vineyard in cracks in the wood of infected vines. Although the means of dissemination of the conidia to other vines is not understood, the fungus is thought to invade wounds of mature vines (Edwards *et al,* 2001b).

Petri disease, also known as black goo decline, is a serious disease of young vines worldwide and can cause establishment problems in new vineyards. The associated fungus *P. chlamydospora* is transmitted through planting material (Edwards and Pascoe, 2002b). Affected planting material grows poorly and has difficulty establishing. Graft union failures, shoot dieback, decline and death of young grapevines can also be associated with Petri disease. Recent

results have shown that hot water treatment can reduce the level of *P.chlamydospora* infection in planting material (Edwards and Pascoe, 2002b).

Phomopsis viticola causes lesions on canes and leaf spots. Affected canes can be weakened and/or girdled and poor berry set has been observed, resulting in yield loss (Nicholas *et al,* 1998) The fungus is spread through planting material but can be controlled by hot water treatment (Rawnsley *et al,* 2002).

Uncinula necator (powdery mildew) can overwinter as mycelium in buds (Pearson and Gärtel, 1985) or as cleistothecia in bark (Nicholas *et al,* 1998). Transmission of the powdery mildew through planting material has not been reported. However, severe infections in vineyards can lead to reduced growth and winter hardiness of vines (Pearson and Goheen, 1994). Consequently, in any certification scheme, this powdery mildew should be controlled.

Plasmopara viticola (downy mildew) usually survives as oospores in the soil and old infected leaf material (Nicholas *et al,* 1998) but can overwinter as mycelium in buds and persistent leaves in mild grape growing regions (Pearson and Goheen, 1994). Severe defoliation can decrease the hardiness of buds through winter (Pearson and Goheen, 1994). Consequently this fungus should also be controlled in any certification scheme. Transmission of downy mildew through planting material has not been reported.

Root pathogens such as *Rhizoctonia solani*, *Phytophthora* spp, *Armillaria* spp and *Pythium ultimum* can infect grapevines and cause establishment problems. Infected vines may show a lack of vigour. Consequently these pathogens should be considered when establishing planting for the provision of high health material. Hot water treatment at 54°C for 5 minutes or 50°C for 30 minutes may be effective against these and related root-rotting fungi, eg. *Phytophthora cinnamomi* (Von Broembsen and Marais, 1978).

13 Effects of Insect Pests

The long tailed mealybug, *Pseudococcus longispinus* and the citrophilous mealybug *P. calceolariae* can infest Australian grapevines (Furness, 1977; Nicholas *et al,* 1998). Mealybugs excrete sticky honeydew in which sooty mould and other fungi can grow. Bunches and leaves may be affected. Heavy infestations of mealybug and, subsequently, sooty mould and fungi, can result in crop loss (Nicholas *et al,* 1998). Both the long tailed mealybug and the citrophilous mealybug have been shown to transmit grapevine viruses in other countries. Table 2 lists some other mealybugs and a soft scale species that occur in Australia, although not reported on grapevines here. These insects can infest grapevines and transmit grapevine viruses in other countries (Sforza *et al,* 2003; Golino *et al,* 2002; Acheche *et al,* 1999; Cabaleiro and Segura, 1997; Notte *et al,* 1997; Engelbrecht and Kasdorf, 1987; Petersen and Charles, 1997). Mealybugs and scale can overwinter on their host plants and thus have the potential to be transmitted on grapevine cuttings.

Several mite species, including *Brevipalpus* spp, *Colomerus vitis* and *Calepitrimerus vitis* can also be harmful to grapevines, causing reduced yield and growth in young vines (Nicholas et al, 1998). Mites can overwinter in buds and under rough bark, consequently they may also be transmitted through propagation material.

The grape Phylloxera (*Daktulosphaira vitifoliae* Fitch) is found in small areas in central Victoria (Nagambie, Upton, Mooropna) and northeast Victoria (North East, King Valley), in southeast New South Wales (Corowa) and in Camden and Cumberland near Sydney. The movement of grapevine material from these regions is restricted. Plantings of high health material cannot be located in these regions. High health, phylloxera-resistant rootstocks will ameliorate the effects of phylloxera in these regions.

Various nematodes can infest soils where grapevines are grown. Dagger nematodes can cause root damage resulting loss of vigour and yield (Nicholas *et al,* 1998). The dagger nematode *Xiphinema index*, vector of GFLV, has a restricted distribution in Australia and is considered quarantineable. Recently, another dagger nematode, *X. vuittenezi*, was discovered in a young Shiraz planting where vines displayed symptoms of unthrifty growth and decline (Walker, 2004). It is unclear if this nematode was the cause of the observed symptoms. *X. vuittenezi* may transmit nepoviruses, including grapevine chrome mosaic virus and cherry leafroll virus (Bandte and Buttner, 2000; Wang *et al,* 2003). Grapevine chrome mosaic virus has not been reported in Australia.

Root-knot nematodes (*Meloidogyne* spp.) can have an economic impact on grapevines, reducing vigour and yield when they are in high numbers (Nicholas *et al,* 1998). The combination *of M. incognita* and the fungus *Rhizoctonia solani* was associated with stunting of grapevines in a field nursery (Walker, 1997). Root-lesion nematodes, *Pratylenchus* spp., and the citrus nematode, *Tylenchulus semipenetrans,* can also cause a reduction of vigour and yield in grapevines (Nicholas *et al,* 1998).

Hot water treatment (54°C for 5 minutes or 50°C for 30 minutes) is effective against nematodes and phylloxera. This treatment may also prevent the transmission of mealybugs, scale, and mites on propagation material.

Annex 6

Relevant Plant Viruses in Australia

A LIST OF QUARANTINEABLE AND NON-QUARANTINEABLE VIRUSES IN AUSTRALIA

Quarantineable viruses			
Virus Name	**Disease**	**Reported in Australia**	**Quarantine status**
Arabis mosaic nepovirus	degeneration	Yes - other crops limited distribution	Quarantineable
Artichoke Italian latent nepovirus	degeneration	No	Quarantineable
Blueberry leaf mottle nepovirus	Decline	No	Quarantineable
Bratislava mosaic		No	? not on list
Cherry leafroll nepovirus		No	Quarantineable
Grapevine ajinashika disease luteovirus	ajinashika disease	No	Quarantineable
Grapevine Algerian latent tombusvirus		No	Quarantineable
Grapevine Anatolian ringspot nepovirus		No	? not on list
Grapevine angular mosaic ilarvirus		No	? not on list
Grapevine asteroid mosaic marafivirus		No	Quarantineable
Grapevine virus B vitivirus	Corky bark	Some strains present	Strains associated with corky bark Quarantineable
Grapevine berry inner necrosis virus		No	Quarantineable
Grapevine Bulgarian latent nepovirus	degeneration	No	Quarantineable
Grapevine virus C vitivirus		No	Quarantineable
Grapevine chrome mosaic nepovirus		No	Quarantineable
Grapevine corky bark-associated closterovirus		?	?
Grapevine virus D vitivirus		No	Quarantineable
Grapevine deformation nepovirus		No	? not on list
Grapevine fanleaf nepovirus	degeneration	Yes - limited distribution	Quarantineable
Grapevine labile rod shaped virus		N	Quarantineable
Grapevine leafroll-associated (?) closterovirus 6	Leafroll	unknown	Quarantineable
Grapevine leafroll-associated (?) closterovirus 7	Leafroll	unknown	Quarantineable
Grapevine leafroll-associated (?) ampelovirus 8	Leafroll	unknown	Quarantineable
Grapevine line pattern ilarvirus	Line pattern	No	Quarantineable
Grapevine rupestris vein feathering marafivirus	vein feathering	?No	? not on list
Grapevine stunt virus	Stunt	No	Quarantineable

Grapevine Tunisian ringspot nepovirus		No	Quarantineable
Joannes seyve nepovirus (strain of tomato black ring)	degeneration	No	Quarantineable
Peach rosette mosaic nepovirus	Decline	No	Quarantineable
Petunia asteroid mosaic tombusvirus		No	Quarantineable
Raspberry bushy dwarf Idaovirus		No	? Not on list
Raspberry ringspot nepovirus	degeneration	No	Quarantineable
Strawberry latent ringspot nepovirus	degeneration	Yes - other crops limited distribution	Quarantineable
Tomato black ring nepovirus	degeneration	No	Quarantineable
Tomato ringspot nepovirus	Decline	Yes - other crops limited distribution	Quarantineable

Non Quarantineable viruses

Virus Name	Disease	Reported in Australia	Quarantine status
Alfalfa mosaic alfamovirus	yellow mottle disease	Yes - other crops	Non-Quarantineable
Broad bean wilt fabavirus		Yes - other crops	Non-Quarantineable
Carnation mottle carmovirus	Roditis leaf discolouration	Yes - other crops	Non-Quarantineable
Cucumber mosaic virus		Yes	Non-Quarantineable
Grapevine virus A vitivirus	Rugose wood	Yes	Non-Quarantineable
Grapevine fleck maculavirus		Yes	Non-Quarantineable
Grapevine leafroll-associated ampelovirus 1	Leafroll	Yes	Non-Quarantineable
Grapevine leafroll-associated closterovirus 2	Leafroll	Yes	Non-Quarantineable
Grapevine leafroll-associated ampelovirus 3	Leafroll	Yes	Non-Quarantineable
Grapevine leafroll-associated ampelovirus 4	Leafroll	Yes	Non-Quarantineable
Grapevine leafroll-associated ampelrovirus 5	Leafroll	Yes	Non-Quarantineable
Grapevine leafroll-associated ampelovirus 9	Leafroll	Yes- not formally reported	Not on list?
Grapevine red globe maculavirus		Yes	Non-Quarantineable
Grapevine rootstock stem lesion closterovirus (= strain of GLRaV2)	rootstock stem lesion	Yes	Non-Quarantineable
Grapevine rupestris stem pitting associated foveavirus = Grapevine stem pitting associated closterovirus	rupestris stem pitting	Yes	Non-Quarantineable

Potato virus X potexvirus		Yes – other crops	Non-Quarantineable
Sowbane mosaic sobemovirus		Yes – other crops	Non-Quarantineable
Tobacco mosaic tobamovirus		Yes – other crops	Non-Quarantineable
Tobacco necrosis necrovirus		Yes – other crops	Non-Quarantineable
Tobacco ringspot nepovirus	Decline	Yes - other crops limited distribution	Non-Quarantineable
Tomato mosaic tobamovirus		Yes – other crops	Non-Quarantineable
Tomato spotted wilt tospovirus		Yes – other crops	Non-Quarantineable

Annex 7

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Annex 8

Consultations

							Consultation			
Rec #	Cons #	First name	Surname	Job Title	Organisation	form	date	location	by whom	Notes
1	1	Helen	Waite	Lecturer	Uni Melbourne	in person	23-Mar-04	DPI Knoxfield	fc	Dookie college
2	2	Brendan	Rodoni	Plant Virologist	DPI	in person	01-Apr-04	DPI Knoxfield	fc	
3	3	Mark	Whattam	Senior Plant Pathologist	AQIS	in person	01-Apr-04	PEQ Knoxfield	fc	
4	4	Peter	Smith	Managing Director	Sunraysia Nurseries	in person	05-Apr-04	at Nursery	cmd, fc	
5	4	John	Messina	Nursery Manager	Sunraysia Nurseries	in person	05-Apr-04	at Nursery	cmd, fc	
6	5	Greg	Buchanan		DPIV	in person	05-Apr-04	DPIV Irymple	cmd, fc	VCPTRG member
7	6	Bruce	Chalmers	Director	Chalmers Nurseries	in person	05-Apr-04	DPIV Irymple	cmd, fc	
8	7	Paul	Wright	President	VINA	in person	05-Apr-04	DPIV Irymple	cmd, fc	VINA
9	7	Andy	Gordon		KC Nurseries	in person	05-Apr-04	DPIV Irymple	cmd, fc	VINA
10	8	Mike	Pullen	Exec Officer	VAMVVIA	in person	05-Apr-04	DPIV Irymple	cmd, fc	AVIA/VAMVVIA
11	8	Julian	Connellan			in person	05-Apr-04	DPIV Irymple	cmd, fc	AVIA/VAMVVIA
12	8	George	Kerridge	Exec Officer	AVIA	in person	05-Apr-04	DPIV Irymple	cmd, fc	AVIA/VAMVVIA
13	8	Paul	Croxton	Managing Director	Boulevarde Nurseries	in person	05-Apr-04	DPIV Irymple	cmd, fc	AVIA/VAMVVIA
14	8	Kym	Ludvigsen	Viticultural Consultant		in person	05-Apr-04	DPIV Irymple	cmd, fc	AVIA/VAMVVIA
15	9	Leonie	Pitt		Boulevarde Nurseries	in person	05-Apr-04	Boulevarde	cmd, fc	
16	10	Graeme	Sanderson		NSW Ag, Dareton	in person	05-Apr-04	Dareton	cmd, fc	AVIA elite collection
17	11	Rob	Walker		CSIRO Merbein	in person	05-Apr-04	CSIRO Merbein	cmd, fc	Chair VCPTRG
18	11	Peter	Clingleffer		CSIRO Merbein	in person	05-Apr-04	CSIRO Merbein	cmd, fc	
19	12	Robin	Nettelbeck	Group Viticulturist	Yalumba Wines	in person	06-Apr-04	Yalumba	cmd, fc	ex Chair, SAVII
20	12	Anna	Hurn	Nursery Manager	Yalumba Wines	in person	06-Apr-04	Yalumba	cmd, fc	
21	13	Mike	McCarthy		SARDI	in person	06-Apr-04	Nuriootpa	cmd, fc	Genetic Resource Collection
22	14	Wayne	Farquhar	Executive Officer	SAVII	in person	06-Apr-04	Nuriootpa	cmd, fc	Elite collection, Kapunda
23	14	Philip	Deverill	Chairman	SAVII	in person	06-Apr-04	Nuriootpa	cmd, fc	also Orlando Wyndham
24	15	Ross	Heinze	Chair	PGIBSA	in person	06-Apr-04	Nuriootpa	cmd, fc	also Vine improvement
25	16	Richard	Hamilton		Southcorp	in person	07-Apr-04	Magill	cmd, fc	VCPTRG member
26	16	Peter	Hayes		Southcorp	in person	07-Apr-04	Magill	cmd, fc	
27	17	Paul	Petrie		Southcorp	in person	07-Apr-04	Magill	cmd, fc	
28	18	Eileen	Scott	Lecturer	Uni Adelaide, Waite	in person	07-Apr-04	Waite	cmd, fc	
29	19	Nigel	Scott		ex CSIRO Horticulture	in person	07-Apr-04	Waite	cmd, fc	
30	20	John	Randles	Director	Waite Diagnostics	in person	07-Apr-04	Waite	cmd, fc	Professor, Virology
31	20	Nuredin	Habili	Manager	Waite Diagnostics	in person	07-Apr-04	Waite	cmd, fc	
32	21	David	Hunt Sharman	Chief executive	ATGA	in person	15-Apr-04	DPI Knoxfield	fc	
33	21	Brian	Woodford		ATGA	Phone	15-Apr-04		fc	grower
34	21	Nick	Muraca		ATGA	Phone	15-Apr-04		fc	grower
35	22	Bob	Wickson	regional Liscencing Ma	Sun World	phone	19-Apr-04		fc	
36	23	DeAnn	Glenn	Program Manager	GWRDC	in person	20-Apr-04	GWRDC	cmd	
37	24	Ian	Pascoe	Mycologist	DPIV	in person	23-Apr-04	DPI Knoxfield	fc	
38	24	Jackie	Edwards	Mycologist	DPIV	in person	23-Apr-04	DPI Knoxfield	fc	
39	25	Possingham	John	Chair	SAFF Winegrapes Div	phone	03-May-04		cmd	
40	26	Fortune	Jim	Executive Director	GWRDC	in person	03-May-04	GWRDC	cmd	
41	27	Bruno	Brombal	Chair	WGMB MIA	in person	03-May-04	WGMB Griffith	fc	also a grower and nurseryman
42	27	Leo	DePaoli			in person	03-May-04	WGMB Griffith	fc	grower and nurseryman
43	28	Adrian	Ceccato			in person	03-May-04	WGMB Griffith	fc	grower and nurseryman
44	29	Stuart	McGrathKerr	Secretariat	NSW wine idustr asso	in person	03-May-04	WGMB Griffith	fc	
45	30	Emma	Jameison	Industry Development	WGMB MIA	in person	03-May-04	WGMB Griffith	fc	
46	30	Jeremy	Cass		MIAVIS	in person	03-May-04	WGMB Griffith	fc	
47	30	Harry	Creecy		NSW Ag. Griffith	in person	03-May-04	WGMB Griffith	fc	
48	30	Lou	Cremasco	Chairman	MIAVIS	in person	03-May-04	WGMB Griffith	fc	also a grower
49	31	Pat	Barkley		Auscitrus	in person	04-May-04	HAL Sydney	fc	
50	32	Wayne	Farquhar	EO	SAVII	phone	05-May-04		cmd	discuss vi dev
51	33	Richard	Cirami	ret Vine Imp Officer	SA Dag	phone	05-May-04		cmd	discuss vi dev
52	34	Prue	Henschke	Viticulturist	Henschke Wines	phone	06-Sep-04		cmd	
53	35	Sandra	Savocchia	Lecturer	CSU	e-mail	14-May-04		fc	
54	36	Phil	Nicholas	Plant pathologist	PIRSA	in person	18-May-04	SRHS Fullarton	cmd,fc, pam	
55	37	Graeme	Wellman			in person	18-May-04	SRHS Fullarton	cmd,fc, pam	
56	38	Ross	Skinner		HAL	phone	21-May-04	Mildura	fc	

Annex 9

Diagnostic Techniques - Overview

DIAGNOSTIC TECHNIQUES - OVERVIEW

1 Introduction

The three major diagnostic techniques used to detect and identify pathogens in plant material are biological indexing, ELISA and PCR. Each is discussed below.

2 Biological Indexing

This method of indexing takes advantage of a sensitive plant response to the presence of pathogens. Indicator plants are inoculated with material of another source, which may or may not contain one or more pathogens, and are observed for characteristic symptom development. Two biological indexing methods are used for the detection of grapevine viruses: herbaceous indexing and woody indexing.

2.1 Herbaceous Indicators

The use of herbaceous indicators within grapevine pathology today is limited. It is useful in the detection of nepoviruses (eg. GFLV) of grapevine. Herbaceous indicator plant species that are sensitive to nepoviruses include *Chenopodium* spp., in particular *C. quinoa* and *C. amaranticolor*. Grapevine material to be tested is ground in an appropriate buffer, then rubbed onto the leaves of the indicator plants that have been dusted with carborundum powder, or some other abrasive powder. Nepovirus symptoms can develop within seven days of inoculation, however plants are traditionally observed for up to six weeks post inoculation. Herbaceous indicators are not reliable for the detection of other important grapevine viruses.

2.2 Woody Indicators

Woody indicators are used to detect many important virus and phytoplasma pathogens of grapevine. Traditionally buds or bark pieces from a candidate are grafted onto sensitive varieties and symptom development is observed for at least two growing seasons. Different viruses and phytoplasmas may induce similar symptoms on indicators thus making differentiation difficult. It is particularly difficult to distinguish between viral strains with this method. For example, the various leafroll viruses are associated with reddening and rolling of leaves of the indicators Pinot Noir and Cabernet franc. GVA is associated with stem pitting on Rupestris St George. Some indicators develop symptoms that cannot be readily attributed to a known causal agent, or combination of causal agents. Indicator LN33 is useful in the detection of both stem grooving and corky bark diseases.

Woody indicators themselves must be free of all pathogens, especially viruses and phytoplasmas, as these can affect symptom expression in the woody host. Woody indicators must be maintained in an isolated area to ensure that natural infection events do not lead to false positive results. Symptom expression may be influenced by environmental factors such as temperature, light and nutrition. Virus detection in woody indicators is usually evaluated from late summer through to leaf fall. Woody indexing requires experienced personnel to read and interpret the symptoms.

Indicators used for virus detection include LN33, Kober 5BB, Cabernet franc, Pinot Noir, Rupestris St George. Other sensitive varieties may also be used.

LN33, Riesling and Chardonnay are varieties useful in the detection of phytoplasmas. While grafting a candidate onto these indicator varieties can be done for phytoplasma detection, phytoplasmas are more successfully detected when the indicator is grafted onto the candidate

plant. Symptom expression is likely to be observed during summer months December-February and plants should be observed for at least two growing seasons.

3 ELISA

Enzyme-linked immunosorbent assay (ELISA) is a sensitive, serological technique often used to detect viruses in plants and animals. The most common method used for plant virus detection is the double antibody sandwich (DAS)-ELISA. Polystyrene plates containing small wells are coated with antibodies, which have been raised to purified plant viruses. Once the antibodies are bound to the plates and the plates rinsed of excess unbound antibodies, plant material ground in a suitable buffer, is applied to the plates. If particular viruses are present they will bind to the antibodies coating the plate. The plant sap is rinsed from the plate. Antibodies, which are bound to an enzyme, are added. The enzyme bound antibodies bind to viruses that have been captured on the plate. The antibody bound enzyme reacts with a substrate, added after washing the plate of excess antibody, causing a colour change that indicates the presence of virus.

Typically these antibodies react to the presence of the protein coat that encapsidates the virus. The RNA sequence of the viral genome, encoding the coat protein, can be variable amongst virus strains and isolates but the protein arising from this variable gene often remains conserved within the virus species. Consequently these tests, especially the commercially available tests, tend to detect groups of viruses within a species and specific strains are not distinguished. It is possible, although infrequent, that the antibodies will cross react with other virus species that have a similar coat protein, giving false positives. Some genome differences within a virus species can cause significant changes to the coat protein of some isolates, and the non-recognition by the antibody can therefore result in false negative results. Techniques are available that allow for the production of strain specific antibodies, if required. Sera that have not been prepared well might also contain antibodies to plant proteins, in addition to those raised against the virus, and this can cause background colouration, masking weak positive results.

ELISA is a more cost effective test than PCR, especially as the samples require less processing. The consumable items required for ELISA, such as antibodies, buffers, pipette tips, bags and plates are less expensive than the consumable items required for PCR. ELISA results can be gained within 24 hours. However, ELISA is not always as sensitive as PCR.

Commercial ELISA kits are available for the detection of GLRaV1, 3, 5, GFkV and GVA.

4 PCR

Polymerase chain reaction (PCR) is a rapid and sensitive molecular technique that is used to detect a variety of pathogens that can infect grapevine including viruses, phytoplasmas, bacteria, fungi and viroids. During PCR a thermostable enzyme (usually *Taq* DNA polymerase) is used to generate multiple copies of a specific nucleotide (DNA or RNA) sequence. PCR requires that total DNA (for phytoplasmas, bacteria and fungi) or RNA (for viruses and viroids) is extracted from grapevine material. PCR can be carried out immediately on DNA but in the case of RNA a reverse transcription step is required to make copy DNA from the RNA before the PCR can proceed. After the PCR reaction is complete the products are electrophoretically separated on an agarose gel and stained with a dye that fluoresces under UV illumination. A positive result is gained when a product of the correct size is observed.

PCR requires knowledge of at least part of the genome of the organism to be detected so that primers can be designed. Primers are short nucleotide sequences specific to the DNA template of interest, which identify the point from which the DNA template is to be copied. Often primers are targeted to conserved regions of the genome of an organism so that multiple strains can be

detected. However if genetic variability occurs amongst strains of an organism at the primer-binding site, the primer may not bind and false negatives can be obtained. Highly specific primers can also be designed to identify a specific isolate within a species.

PCR is also prone to false positives due to sequence similarity of the primer sequences with other genetic material, such as that of the host plant or other organisms. Often this "mis-priming" with other genetic material generates a product that is different in size to that expected. However, products very similar to the expected size can be generated and false positives sometimes occur. When products of a different size are generated it is important to determine the origin of the product as mutation or recombination events can lead to detection of smaller or larger genomic fragments of the test organism. Ignoring these products can lead to false negative results. Unexpected positive results, be they bands of the correct size or of a different size, should be confirmed by another test, such as re-extracting from the same grapevine and repeating the PCR test or re-testing by ELISA. Sequencing will confirm the origin of the positive result.

Theoretically PCR can detect one molecule of a template in a reaction and because of this sensitivity false positives can be gained due to contamination of equipment and/or biochemicals during nucleic acid extraction or PCR reaction set up. Conversely, false negative results can also be obtained due to inhibition of the reverse transcriptase or DNA polymerase activity by compounds co-extracted with the nucleic acids.

Nested PCR is used to detect phytoplasmas because of their low concentration in grapevine tissue and inhibitors present in the grapevine tissue. Nested PCR is done by using one pair of primers to amplify DNA in an initial PCR reaction. In the second or "nested" PCR step, a small amount of the first reaction is added to a second PCR reaction containing primers that are internal, along the DNA fragment of interest, to the first primer pair. For phytoplasmas, most PCR tests are based on detecting the 16S ribosomal RNA (rRNA) gene. The PCR is a universal test that should detect all known phytoplasmas, and can detect previously uncharacterised phytoplasmas. However, the use of the test should be approached with caution as the 16S rRNA gene is highly conserved amongst many organisms and false positives can be obtained, even with nested PCR. Consequently, it is recommended that the identity of the phytoplasma be confirmed by restriction fragment length polymorphism (RFLP) analysis. RFLP uses enzymes that recognise and cut at specific, short, sequences along the DNA fragment to generate a series of smaller DNA fragments. Each phytoplasma, especially those infecting Australian grapevines, produce distinct RFLP patterns allowing them to be distinguished from one another. If an unknown RFLP pattern is observed, sequencing should be done to confirm the identity of the organism that was detected.

There is a very high risk of producing false positives when nested PCR is used due to carry-over contamination of amplified products generated in the first round. Contamination can occur on equipment, hands and as an aerosol in the laboratory. Great care must be taken when using this technique. Unexpected positive results should be checked by repeating the nested PCR on the extracted DNA or by re-extracting DNA from the original sample and repeating the PCR test.

PCR is rapid, results can be obtained within 4-5 hours depending on the method of extraction and the PCR. However, consumable items such as enzymes and nucleic acid purification products are expensive.

5 Sampling for PCR and ELISA

Crucial to both PCR and ELISA is sampling of the grapevine tissue for pathogen detection. Viruses and phytoplasmas of grapevines are often unevenly distributed in vines, and the concentration within the vines may be low. Seasonal fluctuation of pathogen concentration is also known. Consequently it is important to understand when and from where the vine should be

sampled to increase the chance of detection. Currently, late autumn and early winter is considered the best time for virus detection using PCR and ELISA. Testing for phytoplasmas is best done in January from symptomatic shoots. At other times of the year, sampling from the trunk, cordons and shoots, can improve the probability of detection of phytoplasmas. Some plant material contains inhibitors that prevent the detection of pathogens and it is important not to sample this material.

6 Controls

Biological indexing, ELISA and PCR all require the use of positive controls so that the positive results from test samples can be compared under the same conditions. The use of positive controls also ensures that both the PCR and ELISA tests are working as expected. Both ELISA and PCR also require the use of a negative control so that "background" results due to reaction with the host material can be compared. For example a non-specific band observed in both the "healthy" controls and the test samples during PCR could be considered a non-significant band. ELISA antibodies might react with some plant material and give a slight colour reaction in healthy controls, which can be subtracted from the test sample results. Similarly both tests require a buffer control in which no plant material is added. A positive result obtained in the buffer control indicates that contamination has occurred at some point during the setting up of the test and the test will need to be redone.