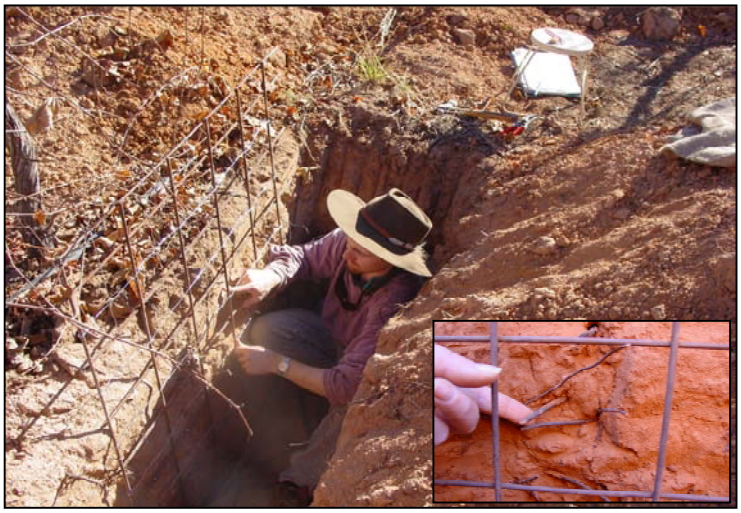
Australian Government
Grape and Wine Research and Development Corporation

A systematic and smarter approach to breeding and developing grapevine rootstocks adapted to Australian conditions.



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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A systematic and smarter approach to breeding and developing grapevine rootstocks adapted to Australian conditions.

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Cover Picture

Assessment of root distribution using trenching techniques.

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1. Abstract:

Problems associated with adoption of high vigour rootstocks by the wine industry include negative impacts on berry composition associated with high potassium uptake, high grape juice pH, poor organic acid composition and reduced colour of wine. This project aimed to develop new rootstocks for winegrape production to minimise these problems. A further aim was to develop a smarter, focussed approach to the breeding and selection of rootstocks based on knowledge of the inheritance of key characteristics. Key outputs from the project included phenotyping of ungrafted populations of rootstock hybrids for rooting ability, grafting ability, nematode tolerance, mineral element (particularly K^+) discrimination, transpiration efficiency, drought tolerance and root architecture; identification of 20 rootstock genotypes for further evaluation as grafted vines; identification of 4 new, low-medium vigour rootstock genotypes for commercial release with PBR protection; and preliminary analyses of genotype x environment interactions using existing data sets.

2. Executive summary:

Problems associated with high vigour rootstocks, introduced for nematode tolerance (eg. Ramsey) and currently used widely across the wine industry include negative impacts on berry composition and wine quality. These effects are associated with high potassium uptake and related detrimental effects on pH and organic acid composition in juice and spectral properties of red wines. In addition, high levels of tartaric acid addition are required for pH adjustment during winemaking. This project aimed to develop new rootstocks for winegrape production to minimise these problems. A further aim was to develop a smarter, focussed approach to the breeding and selection of rootstocks based on knowledge of the inheritance of key characteristics.

A major output of the project was the identification and release of four new low-medium vigour rootstocks for commercial use with Plant Breeders Rights protection. Compared to standard rootstocks they have low to medium vigour, have reduced levels of juice potassium, require lower levels of tartaric acid for pH adjustment in winemaking, have enhanced wine spectral properties and have mild to high phylloxera and nematode tolerance. These new low-medium vigour rootstocks have potential to be components of an integrated approach for winegrape management using high density plantings with closer row spacings to increase yields per hectare, vineyard water use efficiency and sustainability. Because of the reduced shoot growth, the low-medium vigour of these rootstocks may also facilitate adoption of low input pruning systems (eg. mechanical hedging) and minimise canopy management inputs (eg. for vertical shoot positioning and summer trimming), and hence reduce the cost of winegrape production in cool regions.

Based on the characteristics assessed in phenotyping/screening studies of over 350 ungrafted hybrids, there was significant diversity between and within families indicating very significant potential to breed and select new rootstocks combining beneficial traits across the rootstock gene-pool. Across all genotypes a large proportion of the hybrids propagated successfully as there were no major problems with rooting (mean success rate of 74%) or grafting (mean success rate of 65%). Assessment of hybrid capacity for mineral element discrimination was determined by measurement of 12 elements in petioles. Potassium concentration was identified as being of high importance because of established links to wine quality. Across the hybrid population there was a four-fold range in petiole K concentrations, with 10% of hybrids having K concentrations considered to be deficient, while a further 3% had excessive concentrations. For each element, narrow sense heritability was determined across the rootstock hybrid population. For this purpose, end of season samples gave better estimates of heritability compared to spring samples, although there were also significant differences between seasons. The most reliable results over two seasons indicate high heritabilites for B, K, P, and S. In particular, the significant heritabilities measured for K (ie. 0.82 and 0.43 respectively for seasons 2000 and 2001) and over both seasons (0.63) indicate potential to develop rootstocks with low potassium uptake by judicious selection of parents.

Rootstock hybrids were screened against three biotypes of root knot nematode in large sand beds established under glasshouse conditions. Of the controls included in the study, Ramsey rootstock was not infested by any biotype, while Sultana was infested by all three biotypes. While all species combinations showed evidence of nematode susceptibility, about 30% of the hybrids were not infested by any biotype, while less than 10% were infested by all three biotypes.

New techniques applied to assess rootstock hybrid performance included the use of carbon isotope discrimination as a measure of transpiration efficiency and therefore potential water use efficiency, the measurement of leaf temperature under well-watered and drought stress conditions to determine drought tolerance and detailed measurement of root architecture. The carbon isotope discrimination studies indicated a 50% variation in transpiration efficiency across the ungrafted rootstock hybrids. The technique offers considerable potential to rapidly screen rootstocks with respect to transpiration efficiency and by implication water use efficiency, although verification studies using grafted plants in the field is required.

Measurement of leaf temperature was undertaken under well-watered and drought conditions across the hybrid populations. The technique, which indicates the level of canopy cooling through transpiration, shows considerable promise for assessing the capacity of the root system of rootstock hybrids to maintain an

adequate water supply and hence transpiration. Compared to ambient temperature, under well-watered conditions very large differences in leaf temperature were recorded across the population (from 2 ^{0}C to 11 ^{0}C below ambient air temperature). A similar range in leaf temperature (from 2 ^{0}C below ambient to 11 ^{0}C above ambient) was also recorded under drought stress conditions. Under drought conditions it would be expected that hybrids with the lowest temperature are potentially the most drought tolerant and able to maintain canopy function under severe water deficits. Comparing mean values for rootstock families there was a strong linear relationship between leaf temperature under well-watered (WWT) and drought stress conditions (DST). The equation which describes this relationship is DST (^{0}C) = 7.83 + 0.61 WWT (^{0}C). This relationship indicates that rootstock hybrids that have significantly lower leaf temperatures than ambient under well-watered conditions are also most likely to be the most tolerant of drought. Verification of these responses with grafted plants in the field is required.

Soil trenching techniques were used to assess root numbers (ie. the number of roots intercepted in a 1.0 m^2 vertical plane located 0.3m from the vine row) and distribution of roots within the soil profile of individual hybrids. There was a 7.5 fold difference in root numbers across the hybrid population with significant ranges within and between the families tested. The data will be used to elucidate relationships between root number, root distribution, vine vigour, mineral element status, transpiration efficiency and drought tolerance.

Screening of the hybrid population identified 20 selections for evaluation as grafted plants using a number of key criteria (ie. vigour, rooting and grafting ability, nematode tolerance and K status). A replicated trial has been established with these selections grafted with Chardonnay, Cabernet Sauvignon and Shiraz to assess vine performance, fruit composition and wine quality. Because the ungrafted vines are still available for comparative studies, the site will also be useful to assess relationships between grafted and ungrafted plants for vigour, mineral element status, transpiration efficiency and drought tolerance and root architecture.

As part of the evaluation of the new low-medium vigour rootstocks, root architecture studies were undertaken. Three very different patterns of root distribution were recorded across five standard rootstocks and four new rootstocks. Firstly 6 rootstocks (1103 Paulsen, Ramsey, 140 Ruggeri and 3 new selections) had similar patterns, with the most roots in the top 10 cm, reducing progressively with each layer, with fewest roots in the 70-80 cm layer. Of these six rootstocks Ramsey had the most uniform distribution while 1103 Paulsen had the highest number of roots in the surface layer. Secondly, with Dog Ridge, root numbers increased with depth reaching a maximum in the 30-40 cm layer then reducing progressively with lowest numbers in the 70-80cm layer. Thirdly, root distribution of Freedom and one new selection was uniform at all positions within the profile. The relevance of the significant differences in root distribution to water management, water use efficiency, drought tolerance, mineral element uptake from the profile and vine vigour require further study.

The analysis of genotype x environment interactions (GxE) showed significant effects for most variables measured across four sites in major viticulture regions, ie. Sunraysia, the Barossa Valley and Padthaway. The results suggest it is unlikely that a universal rootstock can be developed suited to all regions with varying climates, soils and management practices. They also indicate that new rootstocks being released from the breeding program need to be tested at a range of sites to ensure their benefits are maximised across the industry.

In summary, key outputs from the project included:-

- Phenotyping of ungrafted populations/families of rootstock hybrids for rooting ability, grafting ability, nematode tolerance, mineral element discrimination, transpiration efficiency, drought tolerance and root architecture.
- Identification of rootstock genotypes meeting key selection criteria for further evaluation as grafted vines.
- Establishment of a replicated trial with 20 rootstock hybrids identified above, together with a number of standard rootstocks grafted with the three main wine grape varieties (Chardonnay, Cabernet Sauvignon and Shiraz).
- Identification of new, low-medium vigour rootstock genotypes for commercial release with PBR protection.
- Preliminary analyses of Genotype x Environment interactions using existing data sets.

3. Background

Rootstocks were introduced for wine grape production in the late 19th century to combat the devastating effects caused by phylloxera in Europe. They have now been used for approximately 100 years in NE Victoria having been introduced to combat phylloxera. Research by CSIRO during 1960-72 established that Ramsey rootstock (*Vitis champini*) was tolerant of Australian root-knot nematode which depressed yields in sandy soils, particularly in replant situations (summarised by Sauer, 1977). As a consequence, the use of Ramsey rootstock has dominated vineyard redevelopment for drying, wine and table varieties in the warm irrigated regions. Research and commercial experiences have, however, demonstrated a negative impact of Ramsey and various other nematode tolerant rootstocks on wine quality. With Ramsey, the negative impact on wine quality is associated with high potassium uptake and related detrimental effects on pH and organic acid composition in the juice, and subsequently the wine. In red wine, high potassium uptake also has a detrimental effect on spectral parameters (Ruhl, 1989; Clingeleffer, 1997). Furthermore, other studies (Walker *et al.,* 1998) have confirmed that the negative quality aspects associated with high vigour rootstocks apply across a wide range of red and white scion varieties. From a breeding and rootstock selection perspective, recent studies involving rootstock selections covering a wide range of genotypes demonstrated that rootstocks of different genetic background may be used to manipulate vine growth, crop development and juice composition (Clingeleffer, 1997). These studies confirmed that screening of ungrafted rootstock hybrids for potassium uptake provides a sound basis to select for rootstock effects on berry composition, and hence wine quality. Furthermore, variation in vine growth and yield characteristics, ie. vigour and berry weight, across rootstock selections was closely correlated with juice potassium concentration and pH.

Worldwide, there is concern that evolution of new biotypes of soil-borne pests is occurring for which current rootstocks have only limited tolerance/resistance. Examples include:-

- the breakdown of tolerance of AXR1 rootstock to new phylloxera biotypes in California,
- the poor phylloxera performance of the common European rootstocks, Kober 5BB and SO4 with the introduction of non-cultivation practices in Germany (Ruhl, pers.comm.),
- poor tolerance of the most frequently planted rootstock in South Africa (R99), to nematodes (Loubser, pers. comm.),
- the lack of tolerance of Ramsey to some root knot nematodes in California (Cain *et al*.1984).

In Australia there is concern that the widespread adoption of a vigorous rootstock such as Ramsey, may not be in the best interest if its tolerance to soil-borne pests, eg. nematodes, is reduced through the evolution of more aggressive pest biotypes (Walker, 1992). Recent studies have shown that Ramsey is not resistant to all local populations of root knot nematodes (Walker, 1997).

When undertaking conventional and molecular breeding programs, understanding the inheritance of essential and desired traits is vital so that appropriate strategies can be adopted. For important rootstock traits there are very few published studies in this regard. Boubals (1996) demonstrated that resistance to root attack by phylloxera was under polygenic control while Bouquet (1981) found that *V. rotundifolia* carries dominant genes for resistance. Lider (1954) showed that resistance to root knot nematode by species such as *V. vinifera* and *V. labrusca* involved a single recessive gene for susceptibility while other species such as *V. champini* and *V. candicans* carried the dominant form for resistance. Firoozabaday and Olmo 1982 identified a dominant gene that suppressed nematode resistance in *V. rupestris*. Meredith *et al.*(1982) attributed *Xiphinema index* resistance to one dominant or 2 (one dominant and one recessive) genes. There appear to be no studies of the inheritance of nutrient discrimination characteristics except for salt tolerance. Newman and Antcliff (1984) identified a dominant gene for chloride exclusion in *V. berlandieri* while Sykes (1985) demonstrated polygenic control in crosses between *V. champini* and *V. vinifera.*

Advances in technology and efficiency have contributed to international competitiveness and the strong, export driven growth in the Australian wine industry in recent years. The winegrape industry, in common with the rest of the horticulture sector places emphasis on "environmental and economic sustainability" as being critical to success. Selection and management of vines grafted to rootstocks will become important tools for winegrape management in the pursuit of sustainable and efficient production, a pre-requisite to meeting the 2025 vision (Clingeleffer, 1996). Rootstocks will assist the industry to meet customer expectations with respect to price points, wine quality and style; optimise productivity; minimise inputs of labour, chemicals, energy and physical

infrastructure; efficiently utilise water and nutrients and thereby minimise negative environmental impacts. The increasing use of rootstocks in modern wine grape vineyards will include selections for:

- tolerance/resistance to soil borne pests such as nematodes and phylloxera.
- soil type specificity, eg. for acidic and heavy soils.
- high vigour and productivity.
- efficient water and nutrient uptake. Grafted vines have a more efficacious root distribution and may explore a larger proportion of the soil profile resulting in improved water and nutrient uptake. Genetic control of ion and water uptake at the root/soil interface and transport by different selections may also play a significant role.
- reduced salt uptake and the resulting minimisation of sodium and chloride levels in wine. A recent project by CSIRO with support of the GWRDC has developed a basis for improving salt tolerance.
- vigour control to reduce management inputs and/or enhance quality, eg. to improve fruit exposure.
- enhancement of juice, and subsequent wine quality. Recent research results show potential to use rootstocks to manipulate juice ion and organic acid composition, pH, and berry colour, flavour and size (Clingeleffer, 1997).

Rootstocks have been adopted widely for wine grape production in Australia, eg. 42% of all Chardonnay and more than 20% of Semillon, Cabernet Sauvignon and Shiraz production is from vines on rootstocks (Australia Wine and Grape Industry 1996). Significant benefits have been demonstrated from the adoption of rootstocks for replant situations in nematode infested soils in the warm irrigated regions. Wine grape production would in many cases be uneconomical if redeveloped as own-rooted plantings. The negative impact on wine quality associated with the use of rootstocks must be removed through the identification and introduction of new selections along with changes in vineyard management practices to maintain and improve economic viability and contribute further to Australian export successes.

Rootstocks will continue to have an important role to play in ensuring the long term competitive position of Australia's key viticultural industries. They can potentially lead to significant improvement in scion variety yield and grape quality. All rootstocks for commercial use in Australia, however, have been imported from overseas. Until now, the lack of a systematic and coordinated approach to screening, and subsequently evaluation, has meant that unique Australian bred rootstocks have been unavailable. This project aimed to utilise CSIRO's extensive and unique collection of rootstock germplasm developed from hybridisation programs. It applied objective screening tests for the range of essential and desirable traits. Essential traits identified for inclusion in the project were:- Phylloxera tolerance, nematode tolerance, good propagation characteristics (straight wood, rooting ability, scion compatibility), appropriate vigour, salinity tolerance, efficient water use (including drought tolerance), and appropriate mineral element discrimination (in particular moderate potassium uptake for low grape juice pH). Hybrids that fulfil these criteria, and with application of suitable vine management systems, would reasonably be expected to perform within desired yield expectations.

4. Project Aims and Performance targets:

The main objectives of the project when it commenced in 1999 were:-

- To collect and analyse data of essential rootstock characteristics and determine their modes of inheritance.
- Where necessary, refine screening tests to enable reliable and accurate data acquisition.
- To identify candidate hybrids to be (a) offered to industry for second phase evaluation, and (b) used as parents for next generation crosses.
- To be in a position to develop a follow-up project to investigate linkages between molecular markers and phenotypes within populations/families segregating for key characteristics.

The project was extended for 2 years in 2003 to:-

- Collect data for lodgement of a PBR application for new, low-medium vigour selections.
- Multiply new, low-medium vigour rootstock selections
- Propagate low-medium vigour selections to establish replicated field trials
- Establish new hybrid families in the field
- Identify new candidate selections for stage 2 evaluation as grafted vines
- To analyses existing data sets to provide information on Genotype x Environment effects on grafted vine performance.

5. Method:

CSIRO has available unique seedling progenies from crosses aimed at developing new rootstock types for the Australian grape industries. In the main, these are multi-species hybrids developed to combine the traits of phylloxera tolerant rootstocks used in Europe for high quality wine production and the nematode tolerant rootstocks widely adopted in Australia. Other material includes the range of species material in the germplasm collection and current rootstock trials which would provide reference data.. The latter includes a diverse range of selections (in total 55) and standard rootstocks (5) grafted with Shiraz. Most of the 55 genotypes included in the Shiraz rootstock trial were selected as ungrafted plants for low potassium uptake in previous studies.

Phenotypic data of key traits/characteristics will be collected from progeny within each population over the 3 year period. The number of progeny sampled will vary depending on the particular trait and the minimum sample size required for statistical validity. The data will be analysed to determine the inheritance of key characteristics. The characteristics will include those identified as critical for wine production augmented by the needs of the dried grape industry. The traits to be assessed include:

The characteristics assessed included those identified with industry in a review of CSIRO dried grape breeding augmented by the needs of the wine industry for quality wine production, improved productivity and improved water use efficiency. The traits assessed included:

- Phylloxera tolerance screening in cooperation with DPI-Victoria (Rutherglen). Bioassays based on the methods of Granett *et al*. 1987 using excised root pieces were used for rapid evaluation of the most advanced lines. Sultana (*Vitis vinifera*) was used as a control and a range of standard rootstocks included for reference. For logistical and cost reasons Phylloxera screens are only applied to material at later stages of evaluation.
- Nematode tolerance screening under glasshouse conditions in $2m^3$ sand beds inoculated with 3 *Meloidogyne* spp., biotypes. Single replicate cuttings of 40 rooted hybrids per bed were grown for three months. Nematode egg masses were detected by staining 10 g subsamples of roots with 0.25 g/L eosin-y for 30 min and examining under a magnifying lens (x10). Note, as part of the project a new approach using dual culture of grapevine rootstock and tomatoes was trialled, although the results are not reported here.
- Straight wood production from field observation during cutting collection in winter.
- Rooting ability was assessed using dormant 4-node cuttings from field-grown hybrids. Following storage at 4°C for 6 weeks, basal buds were removed and 20 cuttings of each hybrid planted in 20 L crates containing 15 kg washed sterilised sand. Crates were placed on sand beds heated to 25°C for 6-8 weeks and hand-watered daily, before hardening-off in a shadehouse. Root production was assessed after 14-16 months.
- Scion compatibility, assessed utilising routine grafting techniques, eg. chip budding and a ring grafting technique adapted from citrus (Bevington *et al*. 1978). Five replicates of 18-month-old plants of each hybrid that rooted successfully were used for studies with Chardonnay as the scion. Grafting success was evaluated visually 12-18 weeks after chip-budding. The ring grafting technique was trialled, but the results are not reported here.
- Appropriate vigour from measurement of biomass production (ie. pruning weights) during dormancy in winter.
- Water use efficiency, using carbon isotope discrimination techniques to determine transpiration efficiency and on a limited scale, sap flow techniques to measure vine water use. Recent studies have demonstrated a significant correlation between carbon isotope discrimination and transpiration efficiency measured as g of dry matter per kg of H_20 transpired (M. Gibberd et al. 2001).
- Drought tolerance using chlorophyll fluorescence and leaf temperature to indicate water stress and related effects on transpiration and photosynthesis (During, 1998, Iacono and Sommer, 1999). These measurements were undertaken with vines in full canopy, pre- and post irrigation.
- Observation of rooting depth and root distribution in respect to drought tolerance and water extraction from the soil profile. Soil pits were dug alongside hybrid vines from three hybrid families during dormancy in May, 2003. The depth, number, size and distribution of roots intercepted in the

vertical plane positioned 0.3 m into the inter-row space were recorded to a depth of 1.0 m and 1.0 m along the row from the trunk using a 10 cm grid pattern (see figure below).

- Mineral element discrimination using petioles and leaf blades collected at different times of the year and in different seasons and analysed by ICP, Nitrogen analyser or chloridometer.

To meet the main objectives of the project the key components of the research conducted over a five year period were:-

1. Screening of seedling progeny against key selection criteria (first stage evaluation)
(See above)

2. Establishment of a trial site with the best selections identified in the screening process (second phase evaluation).
As a result of first stage screening during the early years of the current project, 20 promising selections were identified for evaluation as grafted vines. These have been grafted with the three main wine varieties (ie. Chardonnay, Cabernet Sauvignon and Shiraz) and established in a replicated trial established in the field in spring 2005.

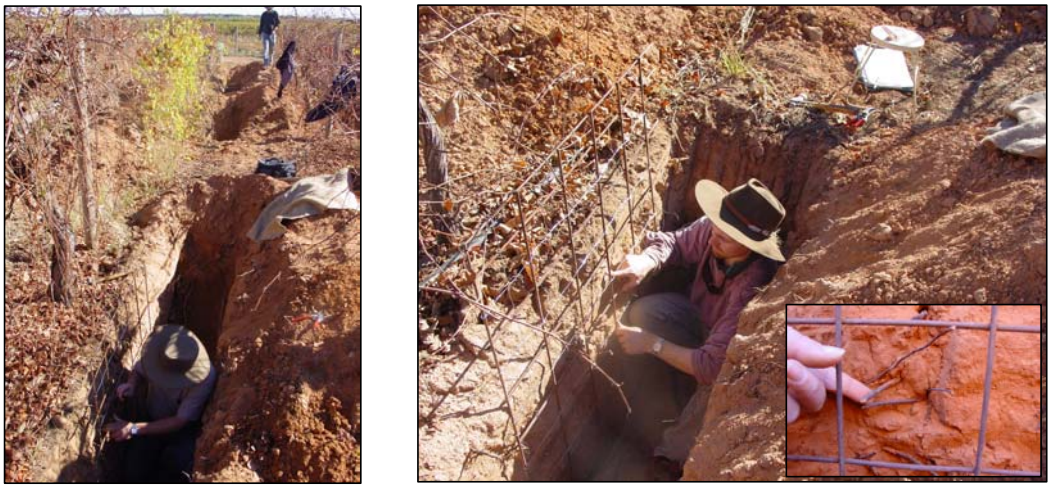
3. Identify hybrids for release for limited release to industry
Thirteen selections (identified in previous studies supported by GWRDC, CSH94/3) were assessed to identify new rootstock types for release to industry for larger scale evaluation. The detailed assessment included measurements of vine performance (yield, berry weights, juice composition, dormant pruning weights etc.), and where appropriate, application of key criteria listed above including phylloxera and nematode tolerance, water use efficiency, mineral element discrimination and root distribution. In addition, small-scale wine lots were produced in the CSIRO facility with fruit harvested at optimum maturity (ie. about 24-25 ^{0}Brix). The amount of tartaric acid required for pH adjustment of the must and during winemaking was recorded. Wines were assessed with regard to basic compositional, spectral and sensory properties. For the latter, industry winemakers were used to form a tasting panel. The standard rootstocks (Ramsey, 140 Ruggeri and 1103 Paulsen), which were also planted in the Shiraz trial, were used for comparative purposes.

4. Establishment of a replicated trial with the best new selections for PBR purposes.
Four new low-medium vigour rootstock selections were identified for commercial release with PBR protection based on winegrape data (see 3 above). A replicated shadehouse trial was established with these selections and comparator varieties to assess distinctness, uniformity and stability (DUS).

5. Smarter focussed breeding and selection of rootstocks based on knowledge of the inheritance of key rootstock characteristics.
Over the course of the project more than 1000 new inter- and intra-specific hybrids were produced, grown as seedlings, planted in the field and trained onto the trellis for ongoing evaluation in new studies.

6. Preliminary Genotype x Environment (G x E) interaction analyses.
One of the aims of setting up the regionally based trials with the new, low-medium vigour selections involving commercial operators will be to assess the extent that genotype x environment (x management) interactions affect grafted vine performance and the oenological qualities of harvested grapes. To ensure the experimental designs of these trials are as scientifically rigorous as possible, it will be advantageous to have a preliminary idea of the likely magnitude of GxE effects on grafted vine performance. Existing data sets from other rootstock trials may be of assistance in gaining some insight with regard to this and a component of the extension to this project involved collation and analysis of some of these data.

Figure 1. Views of soil pits and techniques used to record root numbers and distribution.



6. Results/Discussion:

Results 1. Screening of rootstock hybrids

Screening studies against key selection criteria, identified in the materials and methods, were undertaken across a diverse range of rootstock progeny. A significant component of this work was presented at the Australasian Plant Breeding Conference in 2002 (Wheal et al. 2002, Grapevine rootstocks for Australian conditions. Plant breeding for the 11th Millennium. J A McComb (Ed.) Proceedings of the 12th Australasian Plant Breeding Conference, Perth, Western Australia. 15-20th September 2002. pp161-166). The paper outlined the specific methodology used and provided analysis of issues associated with inheritance of key selection criteria. An adaptation of this paper is inserted below. Where appropriate, further information on the screening of rootstock progeny is presented in latter sections.

1.1 Summary paper: Grapevine rootstocks for Australian conditions, Wheal et al. 2002.

Abstract

Grapevine rootstocks (*Vitis* species) are used primarily to increase vine tolerance to root pests, but also influence plant nutrition, water use, vine vigour and crop yield. Rootstocks used in Australia were originally selected under Northern Hemisphere criteria and current usage is based on local experience. No rootstocks exist that were bred specifically for Australian conditions.

CSIRO's rootstock breeding program is based on crosses between established rootstock cultivars and individuals from species collections to identify hybrids with improved characteristics for Australian viticulture. Most established rootstocks are interspecific hybrids between selections of *V. riparia, V. rupestris* and *V. berlandieri.* The CSIRO breeding program seeks to exploit further the potential of interspecies crosses by crossing established rootstock cultivars with pure *V. champini* rootstocks and species selections of *V. berlandieri* and *V. cinerea*. Some hybrids at Merbein have four species as grandparents, which reflects the range of genetic and phenotypic diversity in rootstock populations.

Results are presented from screening procedures performed on a collection of 306 rootstock hybrids to assess propagation and graft compatibility, nutrition of grafted and own-rooted vines, and tolerance to root-knot nematodes. Hybrid response was not predictable based on parental species, e.g. hybrids containing *V. cinerea* and *V. berlandieri* increased rooting and grafting rates. Hybrids with 0.5 *V. rupestris* or 0.5 *V. berlandieri* had decreased K concentrations which may be beneficial for wine production.

Introduction

Grapevines (*Vitis vinifera*) are grown under many conditions both worldwide and in Australia. Adverse biotic and abiotic soil conditions often decrease production and the use of other *Vitis* species as tolerant rootstocks is necessary to maintain production (Galet 2000). Grapevine rootstocks were used originally to increase vine tolerance to the root pest phylloxera (*Daktulosphaira vitifoliae*), a virulent North American aphid. Because *V. vinifera* is susceptible to phylloxera, tolerant North American *Vitis* species have formed the basis for rootstock breeding programs (Reisch and Pratt 1996). In addition to phylloxera tolerance, rootstocks have other consequences for crop quality as they can alter plant nutrition, water use, vigour and increase yield of grafted scions compared to own-rooted vines (Clingeleffer 1996).

There are no rootstocks bred specifically for Australian conditions. Rootstocks used in Australia were bred and selected in Europe and North America under different growing conditions and were recommended from local field trials and grower experience. Sales of rootstocks in Australia in 2000 exceeded 5.3 million (DeLaine 2001) and 40% of Chardonnay, 20% of Semillon, Cabernet Sauvignon and Shiraz plantings are grafted to rootstocks (ABS 1997). Replanting of vineyards generally requires rootstocks, which means the potential market for a new Australian rootstock is high.

For a rootstock to be of practical use, it must be able to tolerate root pests (phylloxera, nematodes; *Meloidogyne* spp., *Xiphinema index*) and other adverse soil conditions (*e.g.* high lime/pH, low nutrients,

salinity, drought). It must also have favourable viticultural characteristics including a high rate of propagation as rooted cuttings, graft compatibility with scions, appropriate growth and yield traits. These criteria form the basis of selection in CSIRO's breeding program.

The aim of CSIRO's rootstock breeding program is to develop new hybrids with advantageous characteristics for Australian viticulture by crossing established rootstock cultivars and selected individuals from species collections. The purpose of this paper is to enumerate the responses of complex hybrids composed of four North American species (*V. champini*, *V. rupestris*, *V. riparia* and *V. berlandieri*) and intraspecific hybrids of three species (*V. champini*, *V. berlandieri* and *V. cinerea*) for some essential rootstock selection criteria.

Materials and Methods

Populations

CSIRO's rootstock populations were produced by pollinating female or emasculated flowers of established rootstock cultivars with pollen of other rootstock cultivars. Hybrid vines were planted as family groups at 1.5 m intra- and 2.4 m inter-row spacing with individuals randomised within families. Vines were trained to single wire trellises and spur pruned annually in winter. Hybrids are described in terms of the proportions of all species present in hybrids (see Galet 2000). The CSIRO hybrids can have up to four separate species as grandparents, i.e. four species present in 0.25 proportion each.

A total of 208 inter-specific hybrids from 12 crosses of established rootstocks, and 99 intra-specific hybrids of *V. champini*, *V. berlandieri* and *V. cinerea* (*i.e.* 1.0 proportions of each) from six crosses, planted in the CSIRO Merbein vineyards (near Mildura, Victoria) were available for screening (Table 1). Hybrids with a 0.5 proportion of a given species were derived from two parents each having a 0.5 proportion of that species, except for the 0.5 *V. champini* hybrids which had a single 1.0 *V. champini* parent.

Table 1. Numbers of hybrids classified by proportion of five North American *Vitis* species in 305 complex multispecies hybrid genotypes at Merbein.

Species			**Species**		
proportion	*V. champini*	*V. rupestris*	*V. riparia*	*V. berlandieri*	*V. cinerea*
0.25	97	141	141	115	-
0.50	70	8	27	25	10
1.00	2	-	-	56	41

Screening procedures

Rooting ability was assessed using 4-node cuttings of canes from field-grown hybrids in June and July 1999 and 2000. Following storage at 4°C for 6 weeks, basal buds were removed and cuttings planted in 20 L crates containing 15 kg washed sterilised sand. Crates were placed on sand beds heated to 25°C for 6-8 weeks and hand-watered daily, before hardening off in a shadehouse. One replicate of 20 cuttings of each hybrid was planted in 1999 and one replicate of 25 in 2000. Root production was assessed after 14-16 months.

Graft compatibility was tested by chip-budding Sunmuscat scions (December 2000) and Chardonnay scions (December 2001). Five replicate 18-month-old plants of each hybrid that rooted successfully were used for each scion. Grafting success was evaluated visually 12-18 weeks after chip-budding.

Mineral nutrient concentrations were assessed in the petioles of youngest mature blades of field-grown hybrid vines sampled in March 2000 (end-of season), November 2000 (flowering) and again in April 2001. Leaf samples were washed to remove surface contaminants, and rinsed in deionised water before drying at 80°C. Ground sub-samples of petioles were acid digested for 6 hrs at 125°C. Potassium and sodium concentrations were measured by ICP spectrometer. Chloride concentrations of March sampled petioles were measured using silver ion titration. Samples were dissolved in a nitric/acetic acid mix for 3 hr before analysis.

Nematode tolerance was examined by planting vines into $2m^3$ sand beds previously inoculated separately with three biotypes of *Meloidogyne* spp. Single replicates of forty hybrids per bed were grown for three months. Excess sand was shaken from roots before weighing. Nematode egg masses were detected by staining 10 g subsamples of roots with 0.25 g/L eosin-y for 30 min and examining under a 10X magnifying lens.

The experimental units in this study were hybrids, composed of different species in various proportions. Because both species and proportions vary within the hybrids, statistical tests cannot be validly applied across species as each hybrid would occur once for each species present in its pedigree. T-tests or one-factor ANOVAs were therefore applied only to the proportions within species. Means ± SD are presented. Number of samples is shown in Table 1.

Results

Rooting success

Average rooting success ranged from 82% in 0.25 *V. champini* hybrids to 66% in 1.0 *V. cinerea* vines (Table 2). The proportion of species had no effect on rooting within *V. rupestri*s and *V. riparia* hybrids. Rooting of hybrid *V. berlandieri* vines, but not hybrid *V. cinerea* vines, was significantly increased compared to pure vines of each.

Table 2. Average percentage success of propagation of 4-node cuttings over two sampling years. Plants classified by species and proportion in pedigree. Identical superscripts in the same row indicate non-significant differences at P = 0.05. A "-" indicates no plants existed for testing.

	Proportions in hybrids		
Species	0.25	0.50	1.00
V. champini	82a	60b	78a
V. rupestris	72a	80a	-
V. riparia	75a	76a	-
V. berlandieri	79a	75a	67b
V. cinerea (1 year)	-	78a	66a

Graft success

Scion variety had no effect on overall graft success rates (Table 3). However, Chardonnay was less variable than Sunmuscat (used for comparative purposes through the companion HAL funded rootstock project). The proportion of each species present in hybrids influenced graft success of Sunmuscat scions, with greater success on hybrids with 0.5 species proportion than 0.25, but had no influence on graft success of Chardonnay.

Table 3. Percentage success of grafting of two scions to hybrid rootstocks classified by species and proportion of species in pedigree. Identical letters within rows indicate non-significant differences at P = 0.05. A "-" indicates no plants in a particular pedigree existed for testing.

Scion	Sunmuscat			Chardonnay		
Species	0.25	0.5	1.0	0.25	0.5	1.0
V. champini	52a	62ab	90b	71a	57b	60b
V. rupestris	61a	90b	-	65a	66a	-
V. riparia	63a	76a	-	67a	80a	-
V. berlandieri	54a	80b	-	73a	78a	-
V. cinerea	-	-	-	-	53a	56a
All hybrids	65 ± 32a			66 ± 28a		

Potassium concentration

Potassium concentrations in petioles of all hybrids from March/April 2000 and 2001 were not significantly different, hence data were pooled (Table 4). Potassium concentrations in November were higher than in March for all hybrids. Potassium concentration was not significantly influenced by the proportion of species present in hybrids except for decreased K concentrations in 0.5 *V. rupestris* in November and 0.5 *V. berlandieri* in March/April, compared to 0.25 proportions of each species.

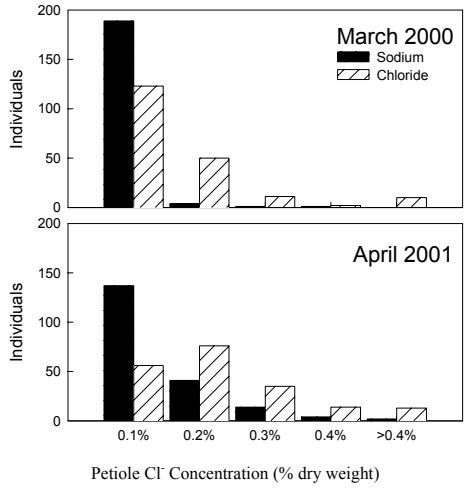
Table 4. Average petiole K concentrations (% dry weight) of rootstock hybrids at two growth stages, as classified by species and proportion of species in pedigree. P indicates level of probability that K in same species at same harvest date is significantly influenced by proportion in pedigree (t-test). NS = Not significant.

Species	November (Flowering)			March / April (Harvest)		
	0.25	0.5	P	0.25	0.5	P
V. champini	3.09 ± 0.83	3.17 ± 1.08	NS	2.63 ± 0.47	2.54 ± 0.58	NS
V. rupestris	3.19 ± 0.90	2.36 ± 0.56	<0.05	2.53 ± 0.49	2.44 ± 0.37	NS
V. riparia	3.15 ± 0.88	3.15 ± 1.15	NS	2.57 ± 0.42	2.67 ± 0.58	NS
V. berlandieri	3.04 ± 0.88	2.77 ± 1.01	NS	2.58 ± 0.49	2.27 ± 0.51	<0.01
All hybrids	3.06 ± 0.95			2.53 ± 0.65		<0.01

Salt concentrations

Most hybrids had Na and Cl concentrations in petioles of less than 0.3% dry weight when measured in March 2000 and April 2001. Species composition had little effect on uptake of either element in either year (Fig. 1). Chloride ion concentrations were lower ($P < 0.05$) in 2000 (0.15 ± 0.30 %) than 2001 (0.19 ± 0.18 %). Sodium concentrations were not correlated with Cl levels in either year ($r^2 = 0.07$, $P < 0.01$; $r^2 = 0.16$, $P < 0.01$, for 2000 and 2001 respectively). Ten hybrids that accumulated > 0.4 % Cl in either year also accumulated > 0.15% in the other year; these individuals came from three families with equal proportions of four species (*i.e.* 0.25 of each) and grew in three non-adjacent rows.

Figure 1. Frequency distribution of petiole Na and Cl concentrations in 199 multispecies complex hybrids sampled in March 2000 and April 2001.



Nematode tolerance

Nematode egg masses were observed on 53 of 80 hybrids tested. Maximum infestation was 101 egg masses per plant (average = 14.6 ± 18.6). Four hybrids were infested by all three biotypes of nematode (average = 41.7 ± 47.3). All species combinations showed evidence of nematode susceptibility.

Discussion

Non-*vinifera* species of *Vitis* have been the basis of grapevine rootstocks since the introduction of phylloxera to Europe in the 1850's (Galet 2000). Tolerance to phylloxera is still the primary criterion for selection of new rootstocks (Galet 1998), however, confirming phylloxera tolerance in the field takes several years. Removing potential candidate hybrids based on other selection criteria will decrease the effort required (*i.e.* to reduce number of candidates) in testing phylloxera tolerance.

Successful propagation (high rooting and grafting percentage) is a basic selection criterion for grapevine rootstocks as genotypes with poor propagation will be more expensive to produce under commercial conditions. Unless providing some essential benefit these will be replaced by other, more reliable cultivars. Various sources suggest *V. cinerea* and *V. berlandieri* are too difficult to propagate and require outcrossing to the more reliable *V. rupestris* and *V. riparia* to be of any use (Galet 1998; Pongrácz 1983). However, individual 1.0 *V. berlandieri* and 1.0 *V. cinerea* genotypes had rooting rates exceeding 75%, as did hybrids containing *V. rupestris* and *V. riparia*. Similarly, references to poor graft success of *V. berlandieri* and *V. cinerea* (Galet 1998; Pongrácz 1983) do not appear justified, as some genotypes had 100% graft success with the two scion varieties tested. *Vitis rupestris* and *V. riparia* parentage did not significantly influence propagation rates in hybrids, but increased grafting rates slightly.

Interactions between scion variety and proportion of rootstock species on grafting success were considerable. In most cases, hybrids with a species proportion of 0.5 derived from two parents had higher average graft success than single-parent 0.5 *V. champini* hybrids. These results suggest that generalisations about either rooting or grafting success in rootstock species and hybrids are not justified and each specific rootstock and stock/scion combinations needs investigation.

Hybrids of *V. champini* and *V. rupestris* experienced nematode infection even though *V. rupestris* is thought to carry a dominant resistance gene, and *V. champini* is homozygous or heterozygous for a dominant resistance gene (Firoozabady and Olmo 1982). Testing nematode tolerance in sand beds indicates true susceptible hybrids but does not guarantee that some plants will not "escape" infestation even with thorough mixing of inoculated soil and high inoculum rates (Firoozabady and Olmo 1982). Double-checking potential tolerant cultivars can overcome such escapes. Even if a variety is proclaimed nematode tolerant, susceptibility to other untested biotypes or species of nematodes is still possible. The presence of 0.5 *V. cinerea* in the phylloxera tolerant variety Börner (Becker 1988) suggests testing of *V. cinerea* hybrids may yield other phylloxera and/or nematode tolerant genotypes.

Low K uptake is an advantageous characteristic as high K (> 3.0 %) in berries is correlated with poor juice and wine characteristics (Clingeleffer *et al.* 1999). Rootstock genotypes selected for low K uptake when ungrafted improved quality parameters of juice and wine made from Shiraz grafted to those genotypes (Clingeleffer 1997). Hybrids containing 0.5 *V. berlandieri* or 0.5 *V. rupestris* may therefore be worth further investigation as rootstocks for winegrape production because of their low K concentrations.

Hybrids were not deliberately stressed with a salt load, therefore concentrations of Na and Cl were low and uptake or exclusion mechanisms may not have been fully expressed. Sykes (1987a) showed evidence for a single dominant gene for Cl exclusion in hybrid *V. berlandieri* vines. Open-pollination of *V. berlandieri* hybrids heterozygous for the dominant Cl exclusion gene will produce vines homozygous for the recessive allele and accumulate Cl. A small number of vines with 0.25 *V. berlandieri* were observed to have relatively high uptake of Cl (Fig. 1). Location in the field may also be a contributing factor as the Cl accumulators in each cross were adjacent to each other in their respective rows. As field salinity stresses may not be uniform, additional hybrids may be identified in glasshouse trials where all hybrids are screened under standard conditions (see also Sykes 1987b).

The capacity for different *Vitis* species to produce viable offspring from cross-pollinations means potential rootstocks can be derived from interspecific crosses of individuals with advantageous characteristics. The results presented here are averages of a wide range of responses to a subset of selection criteria for grapevine rootstocks. Individual hybrids can show responses that exceed the average response for the species and proportion. Further studies will identify individual hybrids that may be suitable for use as rootstocks.

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1. 2. Screening of rootstock progeny for mineral status

The mineral element status of ungrafted rootstocks was determined as described above to provide background information for screening purposes and for use in inheritance studies. Mineral element concentrations of hybrid *Vitis* rootstocks from a number of crosses sampled at different times, both between and within years, were determined to assess the most appropriate time of year to sample. The results were also correlated to vine vigour (pruning weight).

Concentrations are presented as either µg g^{-1} (dry mass) or as percentage of dry mass (% dm). Means ± standard deviation are shown with number of samples in parentheses. Data were also plotted on probability plots and log transformed as required to provide normally distributed datasets for analysis of variance (Systat v7, SPSS Inc, 1997).

Nutrient concentrations at different times of sampling

Average petiolar nutrient concentrations for all hybrids sampled at flowering (November) and at end-of-season (April 2000 or March 2001) are shown in Table 1.2.1. Petiole nutrient concentrations at flowering were generally within typical limits for Sultana vines in the Sunraysia district (Reuter and Robinson, 1997), although average Fe concentration was slightly lower, while Mn and K were slightly higher and Cu much higher. Petioles sampled at end-of-year were also within typical concentration limits for Sultana, except for B and Mn which were slightly higher than adequate. The concentration of many elements was influenced by time of sampling: Al, B, Fe, Mg all increased from flowering to end-of-season, while K, P, S and Zn decreased. Differences in concentration of nutrients occurred between end-of-season sampling times, but remained within the acceptable limits for *V. vinifera* vines.

Average concentration of each element (as pooled samples from end-of-season) differed significantly between families ($P < 0.05$, Table 1.2.2), but were within typical ranges determined for Sultana (Reuter and Robinson 1997). Average K^+ concentration exceeded 3.0 % dm in only one family (K51-32 x SO4), although 38 individuals in 11 families exceeded this value. No individual reached 4.5 % dm K which is regarded as detrimental to growth. Average Fe concentrations from all families were above adequate limits, especially in 2001 (data not shown). Average B concentrations exceeded adequate in many families. The highest Zn concentration (63 ± 15 µg g^{-1}) was in the *vinifera x berlandieri* family Koshu Sanjaku x M56-111. Average P concentrations were lower than adequate (0.2 % P dm) in two families (K51-32 x SO4 and Koshu Sanjaku x M56-111), and only two families did not have individuals with inadequate P. Families with other nutrients that were lower than adequate included K51-40 x 140 Ruggeri (Mg) and Ramsey x Schwarzmann (Ca).

Table 1.2.1. Means ± standard deviation for all elements across all hybrids (N = 185) at different times of sampling expressed on a dry matter basis (dm). Identical letters in columns indicate differences at the $P < 0.05$ level. A mean value for the end of season measurements (March 2000 and April 2001) is also included.

	Al	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	S	Zn
	µg g^{-1}	µg g^{-1}	µg g^{-1}	µg g^{-1}	µg g^{-1}	% dm	% dm	µg g^{-1}	µg g^{-1}	% dm	% dm	µg g^{-1}
March 2000	54 ± 26b	116 ± 41a	1.20 ± 0.32b	9.3 ± 3.2b	32.4 ± 13.9b	2.58 ± 0.54a	0.48 ± 0.26b	64.4 ± 36.3b	415 ± 1258b	0.29 ± 0.11a	0.09 ± 0.02a	32.2 ± 13.3b
Nov. 2000	22 ± 13c	69 ± 20b	1.52 ± 0.40a	62.0 ± 33c	25.3 ± 9.0c	3.08 ± 0.99b	0.42 ± 0.18b	84.7 ± 52.5a	239 ± 394b	0.73 ± 0.26b	0.13 ± 0.04b	52.4 ± 23.8c
April 2001	109 ± 48a	118 ± 34a	1.56 ± 0.49a	6.0 ± 2.3a	74.7 ± 30.0a	2.50 ± 0.66a	0.57 ± 0.31a	88.5 ± 65.3a	1036 ± 1278a	0.28 ± 0.12a	0.09 ± 0.02a	36.3 ± 13.9a
Mean (end-of-season)	82 ± 29	116 ± 35	1.38 ± 0.33	7.6 ± 2.27	53.7 ± 18.1	2.57 ± 0.52	0.52 ± 0.25	76.4 ± 43.2	716 ± 11570	0.28 ± 0.11	0.09 ± 0.02	34.2 ± 11.3

Table 1.2.2. Mean (March 2000 and April 2001) petiole nutrient concentrations (dry matter basis, dm), classed by family and ranked by size of family.

Cross	No.	Al	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	S	Zn
		μg g^{-1}	μg g^{-1}	% dm	μg g^{-1}	μg g^{-1}	% dm	% dm	μg g^{-1}	μg g^{-1}	% dm	% dm	μg g^{-1}
Ramsey x Schwarzmann	49	71 ± 19	136 ± 24	1.13 ± 0.21	8.2 ± 2.3	44 ± 10	2.60 ± 0.53	0.59 ± 0.18	53 ± 20	705 ± 877	0.39 ± 0.09	0.08 ± 0.01	34 ± 9
Kober 5BB x K49-56	35	82 ± 24	111 ± 20	1.34 ± 0.24	8.4 ± 2.0	54 ± 15	2.56 ± 0.35	0.52 ± 0.15	60 ± 23	450 ± 319	0.29 ± 0.07	0.08 ± 0.01	30 ± 7
K51-32 x SO4	21	94 ± 15	98 ± 17	1.59 ± 0.33	6.3 ± 1.2	64 ± 10	3.06 ± 0.42	0.24 ± 0.08	110 ± 61	688 ± 414	0.18 ± 0.03	0.07 ± 0.01	25 ± 6
Dog Ridge x Schwarzmann	16	65 ± 20	168 ± 31	1.27 ± 0.26	5.9 ± 1.7	43 ± 10	2.23 ± 0.64	0.81 ± 0.39	85 ± 51	2746 ± 3251	0.35 ± 0.09	0.09 ± 0.02	33 ± 13
Kober 5BB x J17-69	15	88 ± 38	100 ± 13	1.65 ± 0.27	7.4 ± 1.4	61 ± 24	2.21 ± 0.52	0.62 ± 0.22	129 ± 56	640 ± 267	0.26 ± 0.04	0.11 ± 0.01	42 ± 11
K51-40 x 1103 Paulsen	11	70 ± 17	146 ± 20	1.33 ± 0.22	4.4 ± 1.5	46 ± 10	2.62 ± 0.43	0.66 ± 0.16	75 ± 25	709 ± 362	0.30 ± 0.06	0.08 ± 0.02	32 ± 4
K51-40 x 140 Ruggeri	11	111 ± 15	86 ± 10	1.41 ± 0.21	6.9 ± 1.2	74 ± 12	2.61 ± 0.30	0.20 ± 0.07	60 ± 18	659 ± 579	0.20 ± 0.02	0.09 ± 0.01	30 ± 6
Ramsey x 1103 Paulsen	10	78 ± 17	128 ± 48	1.46 ± 0.35	8.3 ± 3.6	50 ± 10	2.67 ± 0.53	0.61 ± 0.30	54 ± 17	487 ± 326	0.39 ± 0.19	0.08 ± 0.02	35 ± 13
Kober 5BB x M27-12	8	56 ± 12	75 ± 16	1.40 ± 0.43	9.5 ± 3.4	40 ± 7	2.46 ± 0.37	0.49 ± 0.23	102 ± 48	221 ± 220	0.23 ± 0.05	0.10 ± 0.01	30 ± 8
Kober 5BB x 1103 Paulsen	6	105 ± 35	92 ± 18	1.54 ± 0.41	6.1 ± 1.3	69 ± 20	2.72 ± 0.22	0.47 ± 0.13	89 ± 33	362 ± 147	0.22 ± 0.05	0.10 ± 0.01	34 ± 4
Koshu Sanjaku x M56-111	6	87 ± 10	77 ± 9	1.53 ± 0.25	8.4 ± 1.0	60 ± 9	2.08 ± 0.39	0.44 ± 0.13	99 ± 36	343 ± 172	0.15 ± 0.02	0.10 ± 0.02	63 ± 15
K51-32 x M54-12	5	69 ± 16	78 ± 5	1.46 ± 0.21	6.3 ± 1.0	44 ± 7	2.69 ± 0.31	0.25 ± 0.03	65 ± 13	268 ± 228	0.23 ± 0.03	0.08 ± 0.00	29 ± 4
Ramsey x 140 Ruggeri	5	98 ± 26	74 ± 9	1.30 ± 0.42	10.1 ± 1.9	63 ± 16	2.16 ± 0.37	0.33 ± 0.14	60 ± 15	406 ± 323	0.20 ± 0.03	0.08 ± 0.01	36 ± 9
V. rupestris x M59-06 (open)	4	49 ± 22	95 ± 21	1.59 ± 0.28	8.4 ± 2.3	31 ± 9	2.39 ± 0.41	0.39 ± 0.05	71 ± 48	135 ± 102	0.22 ± 0.03	0.10 ± 0.02	44 ± 10
Kober 5BB	2	100 ± 4	159 ± 19	1.52 ± 0.37	8.1 ± 1.6	62 ± 1	2.95 ± 0.45	0.45 ± 0.15	67 ± 16	252 ± 170	0.27 ± 0.02	0.13 ± 0.01	37 ± 9
M54-12	2	162 ± 6	66 ± 5	1.38 ± 0.34	12.7 ± 3.4	95 ± 3	3.13 ± 0.29	0.23 ± 0.06	47 ± 8	63 ± 1	0.20 ± 0.02	0.11 ± 0.02	37 ± 12
Significance (P value)		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	Ns	<0.001	<0.001	<0.001

Correlations between mineral element concentrations at different times of sampling

Correlations of petiole concentration of each element were made within-year (November 2000: April 2001) and between-year (March 2000: April 2001) for all hybrids (n = 185, Table 1.2.3). Significant positive within-year correlations were observed for B, Ca, Fe, Mg, Mn, P and S. Most elements were significantly correlated between-year, the highest being for B, Na, P and K.

Table 1.2.3. Pearson r correlations of March 2000 to April 2001 (End-of-season) and Nov: End-of-season nutrient concentrations across 220 rootstock genotypes. "ns" denotes non-significant correlation, **, *** P <0.01, < 0.001 respectively.

Element	Within year (Nov. 2000:April 2001)	Between year (March 2000:April 2001)
Al	0.094 ns	0.110 ns
B	0.664 ***	0.769***
Ca	0.298***	0.232**
Cu	0.260 ns	0.414***
Fe	0.152 ***	0.133 ns
K	0.245 ns	0.526***
Mg	0.515 ***	0.503***
Mn	0.431***	0.337***
Na	0.168 ns	0.691***
P	0.378***	0.682***
S	0.310***	0.431***
Zn	0.182 ns	0.412***

Correlations between mineral elements

The correlation matrix for concentrations of all elements between years (ie end-of-season sampling, Table 1.2.4) show the highest correlations were between Al & Fe, Ca & Mn and Ca & S. Negative correlations were observed only between K & Ca, and K & Mg. The elements not significantly correlated between years (Al and Fe, Table 3), were highly correlated with each other between end-of-season (Table 4). Magnesium and Ca were positively correlated with five elements each. Boron was positively correlated with Mg and P.

Several significant correlations between elements at end-of-year occurred in different families. Most highly positively correlated were Fe:Al and Na:Cl, followed by Ca:Mg and Ca:Mn. The most common significant negative correlations were between K:Ca, K:Mg and K:Mn.

Table1.2.4. Matrix of significant ($P \leq 0.05$) element-to-element correlations (Pearson r) observed in both years (2000 and 2001) in 180 hybrid genotypes. Dashes indicate non-significant r values.

		Al	B	Ca	Cl	Cu	Fe	K	Mg	Mn	Na	P	S	Zn
Al		1												
B		-	1											
Ca		-	-	1										
Cl		-	-	-	1									
Cu		-	-	-	-	1								
Fe		0.98	-	-	-	-	1							
K		-	-	-0.23	-	-	-	1						
Mg		-	0.42	0.33	-	-	-	-0.48	1					
Mn		-	-	0.50	-	-	-	-	-	1				
Na		-	-	-	.70	-	-	-	-	-	1			
P		-	0.41	-	-	-	-	-	0.39	-	-	1		
S		-	-	0.52	-	-	-	-	-	0.25	-	-	1	
Zn		-	-	0.36	-	-	-	-	0.28	-	-	-	0.39	1

Heritability determinations

Heritability of element concentration was calculated using ASREML (Table 1.2.5). Nutrient concentrations of individuals were compared to nutrient concentrations of parents sampled from an adjacent vineyard. Heritability varied depending on the time of sampling and year, although some nutrients showed similar high h^2 values in end-of-year samples.

Flowering-time samples (November 2000) showed low heritability (poor correlation with parental concentrations) of all elements except Mg. Harvest-time samples were somewhat better estimators of heritability, but results differed dramatically between years: Al, Fe, Mn and Na had very high h^2 values at each harvest-time sampling, but had low or zero h^2 when averaged across years. B, K and S had high h^2 in March 2000 and when averaged, but not at other times. P showed high h^2 when averaged but not at any other time. Standard errors of h^2 estimates were quite large due to the small sample sizes but decreased when both harvest-time samples were used.

Table 1.2.5. Heritability ± standard error of element concentrations in petioles sampled at three different times (March 2000, November 2000 and April 2001). The combined values determined from end-of season data (March 2000 and April 2001) are also included.

Heritability ± se	March 2000	November 2000	April 2001	End-of-season (2000 and 2001)
Al	**0.7532 ± 0.3812**	0	**1.0000 ± 0.0000**	0
B	**0.7005 ± 0.3572**	0.0077 ± 0.1075	0.1818 ± 0.2063	**0.4964 ± 0.0710**
Ca	0.2710 ± 0.2658	0	0.3180 ± 0.3245	0
Cu	**0.9989 ± 0.0117**	0.2736 ± 0.2571	0.4289 ± 0.3257	0.0475 ± 0.0600
Fe	**0.7995 ± 0.3739**	0	**0.9999 ± 0.0000**	0
K	**0.8146 ± 0.3764**	0	0.4291 ± 0.2881	**0.6288 ± 0.0529**
Mg	**0.9982 ± 0.0172**	**0.8956 ± 0.3071**	0	0.3685 ± 0.0850
Mn	**0.8910 ± 0.3387**	0	**0.7932 ± 0.3689**	0.3523 ± 0.0819
Na	**1.0000 ± 0.0000**	0	**1.0000 ± 0.0015**	0.0639 ± 0.0529
P	0.3614 ± 0.3034	0	0.2284 ± 0.2654	**0.5056 ± 0.0695**
S	**0.9468 ± 0.3343**	0.0691 ± 0.1933	0.1553 ± 0.1929	**0.5142 ± 0.0688**
Zn	0.5653 ± 0.3362	0.1216 ± 0.1643	0.0321 ± 0.1414	0.1670 ± 0.0886

1. 3 Screening of rootstock progeny for water use efficiency and drought tolerance

1.3.1 Carbon isotope discrimination

Carbon isotope discrimination (Δ) has been suggested as a reliable indicator of transpiration efficiency in grapes (W = carbon assimilation for dry matter production/ water transpired, Gibberd et al. 2001). In those studies conducted with ungrafted rootstocks and grafted plants grown under well-watered conditions an increase in Δ of $2x10^3$ was linked to a reduction in transpiration efficiency of 19%. In general in these studies the values of Δ across the ungrafted hybrids grown under field conditions were higher than those previously reported (Figure 1.3.1). The large differences in Δ across the ungrafted rootstock hybrids, ranging from $25x10^3$ to $30x10^3$, indicate a variation of 50% in transpiration efficiency (W) across the hybrid population. Large differences in Δ were also observed between and within family groups, as shown by the standard errors as a proportion of the overall range (Table 1.3.1). These results indicate potential to identify hybrids with high transpiration efficiency within most family groups. The results also suggest that it should be possible to identify rootstock parents to improve transpiration efficiency. For example, the Kober 5BB x 1103 Paulsen cross had the lowest family mean (Table 1.3.1).

Figure 1.3.1. Carbon isotope discrimination (Δ) of laminae dry matter from across the range of field grown hybrids, sampled April 2001.

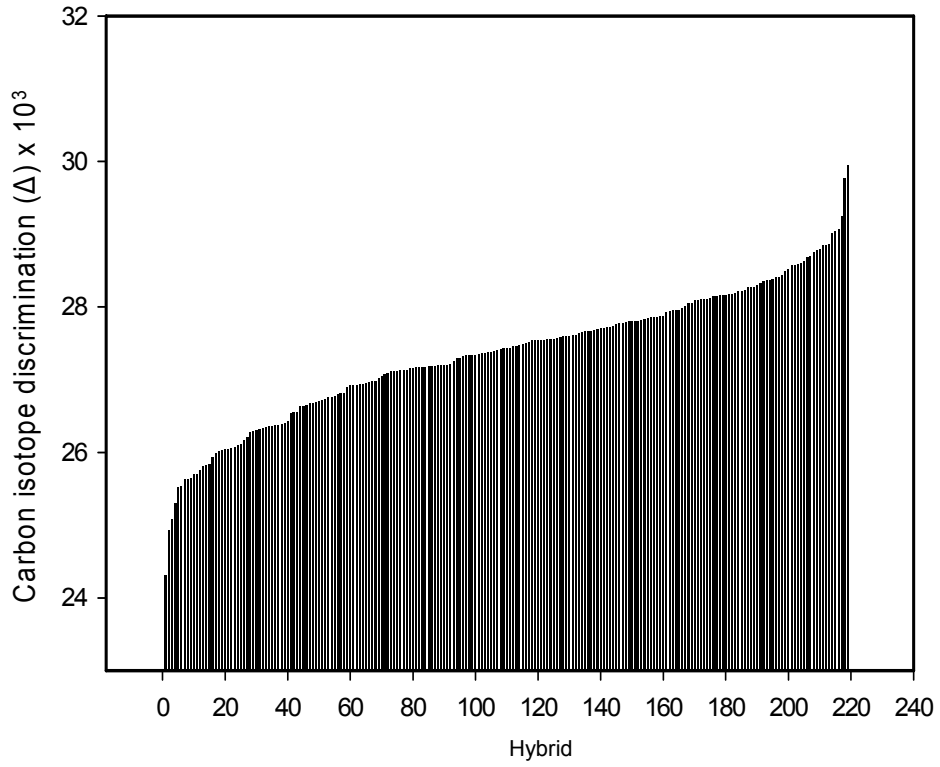


Table 1.3.1. Mean carbon isotope discrimination (Δ) of laminae dry matter for each family group across the range of field grown hybrids, sampled April 2001.

Cross	Carbon isotope discrimination
Ramsey x Schwarzmann	27.40 ± 0.99
Kober 5BB x K49-56	27.5 ± 0.94
K51-32 x SO4	27.92 ± 0.63
Dog Ridge x Schwarzmann	27.66 ± 0.89
Kober 5BB x J17-69	27.08 ± 0.61
K51-40 x 1103 Paulsen	27.77 ± 0.69
K51-40 x 140 Ruggeri	26.91 ± 0.52
Ramsey x 1103 Paulsen	27.21 ± 0.92
Kober 5BB x M27-12	27.22 ± 0.94
Kober 5BB x 1103 Paulsen	26.60 ± 0.65
Koshu Sanjaku x M56-111	26.66 ± 1.16
K51-32 x M54-12	27.55 ± 0.97
Ramsey x 140 Ruggeri	26.88 ± 0.48
V. rupestris x M59-6 (open)	27.66 ± 0.72

1.3.2 Canopy leaf temperature

Canopy leaf temperature is related to leaf transpiration and hence vine water status and the ability of the roots to supply adequate amounts of water to the canopy. Canopy leaf temperature was measured for rootstock hybrids during February and March 2003. Measurements were made pre- and post irrigations and after a 3 week period with no irrigation when the vines were under severe stress. In total measurements were made on 15 days during the February-March period. Leaf temperatures were recorded in the middle of the afternoon with an infrared temperature sensor and compared to ambient conditions. The results for the vines under well-watered conditions (05/02/03, 38.6 ^{0}C at 3 pm) and after an extended period of drought (12/03/03, 35.8 ^{0}C at 3 pm) are presented in Figure 1.3.2. Under well-watered conditions all rootstock hybrids had leaf temperatures below ambient, indicative of substantial cooling from transpiration (Figure 1.3.2A). The wide range in the response of the different hybrids, ranging from 2 ^{0}C to 11 ^{0}C below ambient, indicates that even under well-watered conditions, there were large differences between genotypes in transpiration. There were also differences between hybrid family groups, with crosses involving Kober 5BB having the highest reduction in leaf temperature (ie. up to 9 ^{0}C below ambient) while Ramsey x 1103 Paulsen cross had the lowest reduction in leaf temperature (4.8 ^{0}C below ambient) (Table 1.3.2).

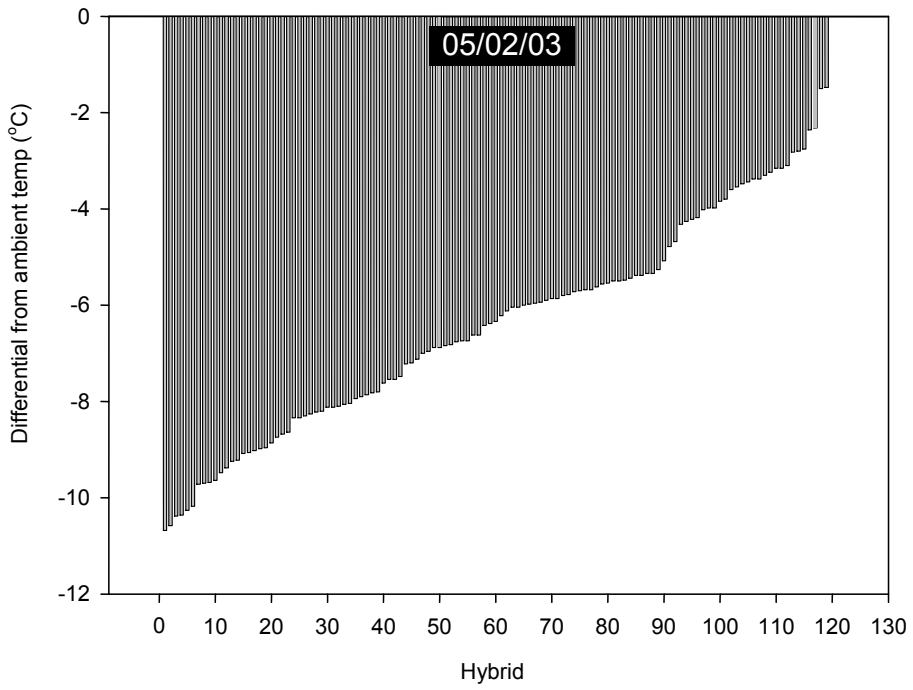
Under drought conditions, leaf temperatures of most hybrids were above ambient (ie. up to 11 ^{0}C above ambient), indicating significant reductions in transpiration. There were however 8 hybrids that had leaf temperatures up to 2 ^{0}C below ambient (Figure 1.3.2B). There were differences between the family groups with the Ramsey x Schwarzmann cross having the highest leaf temperatures and the Kober 5BB x J17-69 cross the lowest leaf temperature compared to ambient (Table 1.3.2).

Table 1.3.2. Mean difference between leaf temperature and ambient temperature of each family group across the range of rootstock hybrids measured under well-watered conditions (05/02/03) and drought conditions (12/03/03). 'Minus' indicates mean leaf temperatures were below ambient and 'plus' indicates mean leaf temperatures were above ambient.

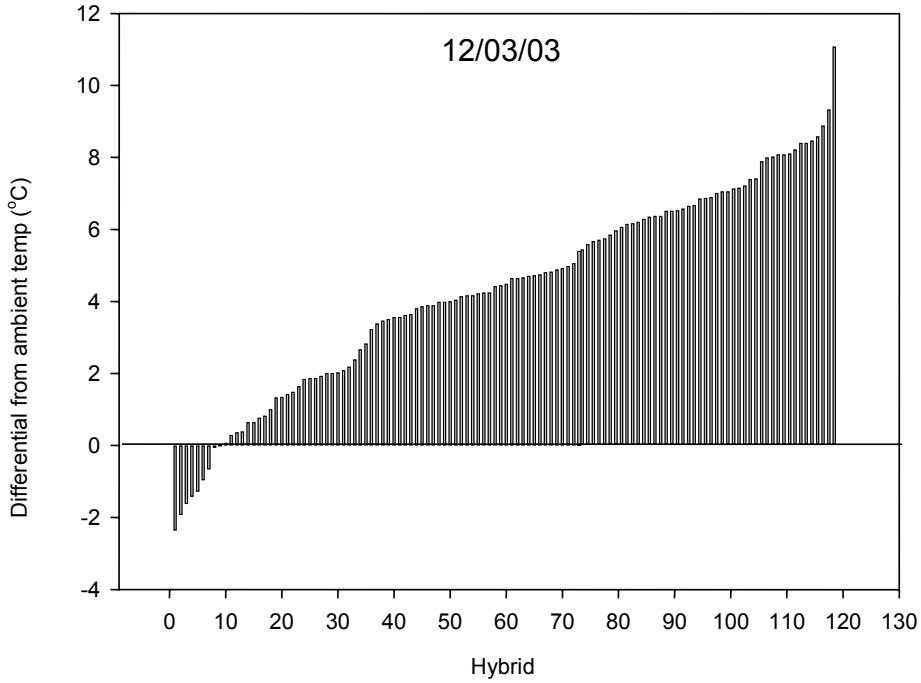
Cross	05/02/03	12/03/03
Ramsey x Schwarzmann	- 5.4	+ 5.0
Kober 5BB x K49-56	- 8.9	+ 3.5
Dog Ridge x Schwarzmann	- 5.4	+ 4.3
Kober 5BB x J17-69	- 9.0	+ 1.3
K51-40 x 1103Paulsen	- 5.7	+ 4.1
Ramsey x 1103Paulsen	- 4.8	+ 4.9

Figure 1.3.2. Mid-afternoon differential leaf temperature (ambient =0) across the range of hybrids under well-watered conditions (A, 05/02/03) and after prolonged drought (B, 12/03/03). 'Minus' indicates mean leaf temperatures were below ambient.

A.



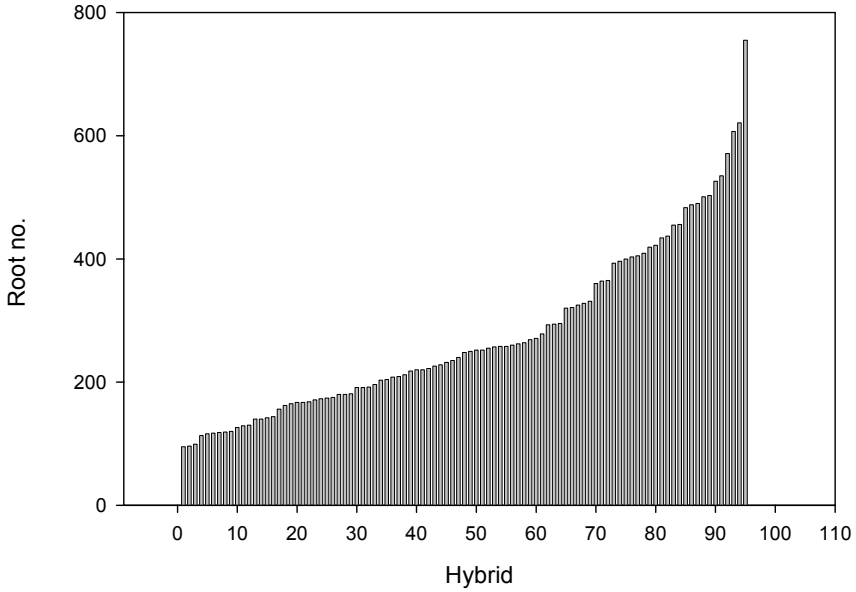
B.



1. 4 Root architecture of rootstock progeny

Soil pits were dug alongside hybrid vines from three hybrid families during dormancy in May, 2003 as described in the materials and methods. The depth, distribution and size of roots intercepted in the vertical plane positioned 0.3 m into the inter-row space were recorded to a depth of 1.0 m and 1.0 m along the row from the trunk using a 10 cm grid pattern. A summary of the results is provided by the total number of roots across the three families. There was an 8-fold range in the number of roots detected for individual hybrids (ie. from less than 100 to 750 roots per m^2, Figure 1.4.1). All families had similar distributions except that none of the individual hybrids in the cross (Kober 5BB x K49-56) had more than 400 roots per m^2, whereas individuals in the cross (Dogridge x Schwarzmann) had up to 600 roots per m^2 and in the cross (Ramsey and Schwarzmann), 750 roots per m^2.

Figure 1.4.1. Root number per m^2 across three families in the rootstock hybrid population recorded from interceptions recorded in the vertical plane, 0.3m from the trunk in the inter-row space.



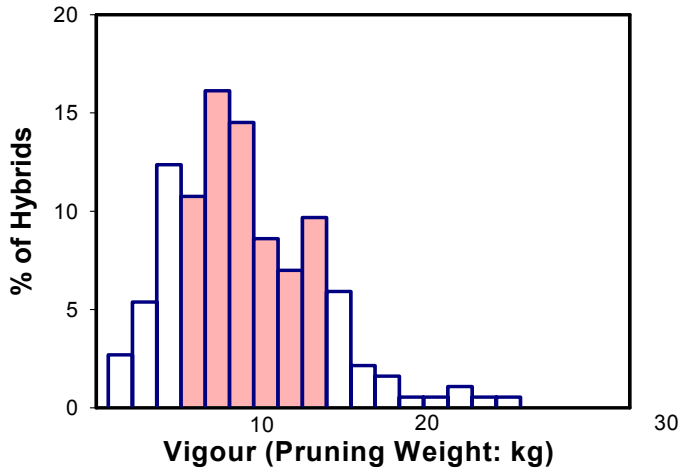
Results 2. Selection of hybrids for further evaluation

Screening data for key selection criteria reported in Section 1 have been used to identify hybrids for further evaluation as grafted plants. The key criteria used were hybrid vigour, rooting ability, grafting success, nematode tolerance and petiole potassium status. As a result 20 selections were identified for establishment as grafted plants with three key wine grape varieties (ie. Chardonnay, Cabernet Sauvignon and Shiraz). These have been established in a replicated field trial, which also includes three standard rootstocks (Ramsey, 1103 Paulsen and 140 Ruggeri) for ongoing evaluation. The trial will produce a significant crop in season 2008.

2.1 Selecting for optimum vine vigour

Dormant pruning weight of hybrid vines, recorded as a measure of vine vigour over two seasons, ranged from very low (ie.< 1 kg per vine) to very high (ie. > 20 kg per vine)(Figure 2.1). In the selection process, hybrids with low and excessively high vigour were excluded, with acceptable vigour taken in the range between 5 and 14 kg per vine.

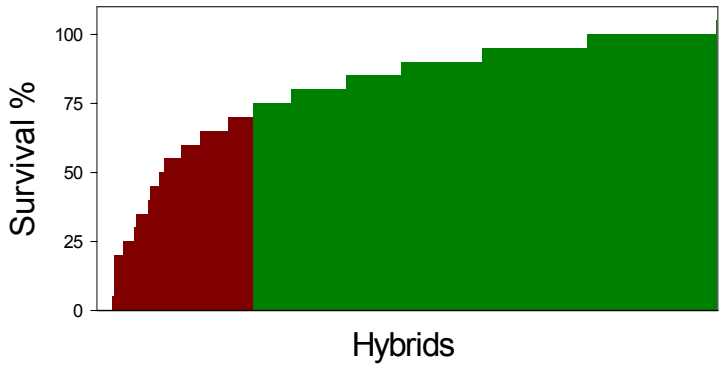
Figure 2.1. Distribution showing percentage of hybrids falling into vine vigour classes based on dormant pruning weights (kg per vine) collected from spur pruned vines in 2 seasons, 2000 and 2001. The selection range used to identify hybrids for further evaluation is highlighted.



2.2 Selection for rooting and grafting ability

The rooting ability of dormant cuttings taken from all hybrids was assessed (Figure 2.2). Only a small proportion of the hybrids had poor rooting ability with low survival. A cut-off selection value of 75% survival was used in the selection process. Surviving, rooted vines of each hybrid were grafted by chip-budding with Sunmuscat and Chardonnay. About 25% of the rooted hybrids had poor grafting results with less than a 40% success rate while 55% of all rooted hybrids had a high success rates which exceeded 75%; these included 31% with 100% success. A grafting success rate of 75% was deemed acceptable in the selection process.

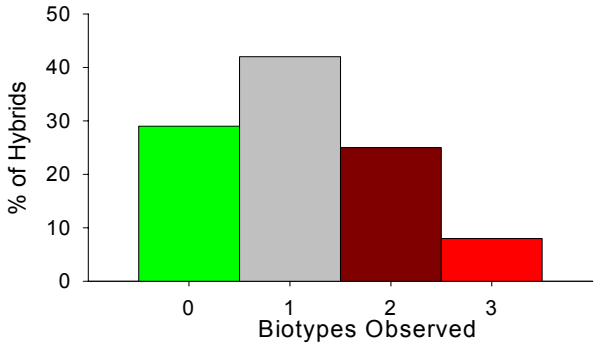
Figure 2.2. Percentage survival of dormant winter cuttings when assessed in propagation studies (n=5).



2.3 Selection for nematode tolerance

The nematode tolerance of all hybrid selections was assessed against three biotypes of *Meloidgne* spp. (the root knot nematode) in large screening beds established under glasshouse conditions. The three biotypes were a biotype of *Meloidogyne incognita* and two biotypes of *M. javanica*, the most common nematode biotype found in Australian vineyards. It should be noted that the nematode resistant rootstock Ramsey (*V. champini*) was not infected by any biotype, while Sultana (*V. vinifera*) was infected by all three biotypes in the screening process when used as controls. Thirty percent of all hybrids were not infested by any biotype while more than 40% were infested by 1 biotype, 25 % by 2 biotypes and less than 10% by 3 biotypes (Figure 2.3). In the screening process, hybrids with zero infestation were retained.

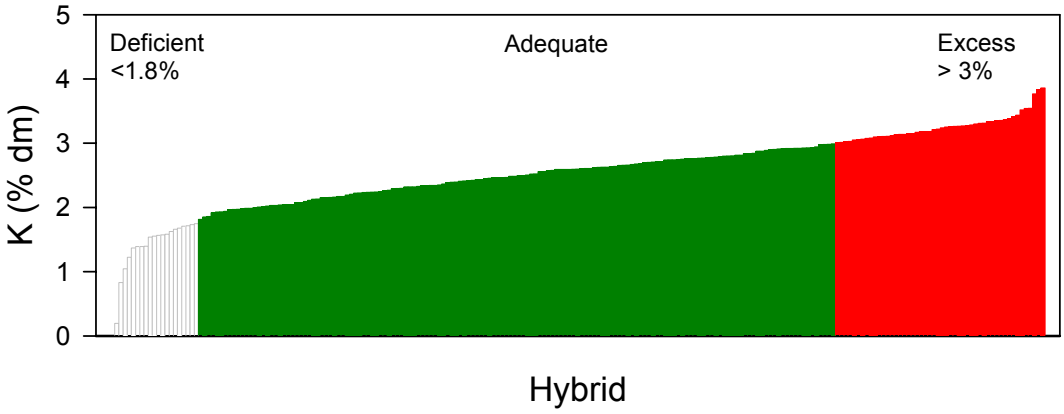
Figure 2.3. Percentage of hybrids infested by 0, 1, 2 or 3 nematode biotypes.



2.4 Selection based on potassium status

Correlations between K^+ concentrations in ungrafted rootstock petioles and grafted scion fruit pH have been reported previously (r^2 = 0.58, 0.68; Rühl 2000). Hence a link exists between K concentration of an ungrafted rootstock petiole and berry K of a scion grafted to the same rootstock genotype. Early selection for low petiole K, even with a low correlation to final juice pH, should provide a rootstock that decreases K by an appreciable amount compared to existing rootstock types. The potassium status of hybrids was determined from petiole samples collected either in spring or at the end of the season. Across the hybrid populations there was a wide range in potassium levels, from a small proportion (about 10%) with deficient levels (ie. <1.8%) to a significant proportion (about 20%) with excessive levels (Figure 2.4).

Figure 2.4. Petiole potassium levels across the hybrid populations, sampled in November 2000.



Results 3. Evaluation of grafted rootstock hybrids and identification of selections for commercial release

3.1 Release of new hybrid rootstocks

Four new rootstocks for commercial release have been identified after intensive evaluation of 55 rootstock hybrids and 5 standard rootstocks grafted with Shiraz which were planted in 1989. The characteristics of these rootstocks included low to medium vigour, reduced levels of juice potassium, enhanced spectral properties in wine, and moderate to high phylloxera and nematode tolerance. A provisional Plant Breeders Rights (PBR) application has been submitted for each of these new, low to medium vigour, rootstock selections. Distinctness, uniformity and stability required for PBR purposes was established in a comparator trial with the four new rootstock selections and 11 rootstock varieties/genotypes. The trial includes 15 replicates of each rootstock genotype. The rootstock comparators were identified for inclusion in the trial after field observations of morphological growth and flower characteristics were used to eliminate most of the standard rootstocks held in the Merbein collection, ie. about 80 varieties and unnamed genotypes. The standard rootstocks included in the trial include Börner, Freedom, Kober 125A, Teleki 5C, Cosmos 10A and Dogridge. Licensing arrangement for the supply and sale of grafted material with PBR protection have been finalised with nurseries in Sunraysia and Western Australia. It is expected that the first commercial sales of the new rootstocks, grafted with Shiraz, will occur in spring 2007.

A summary of the features of the new rootstocks for winemaking purposes (seasons 2000-2002) is provided in the attached poster prepared for the 12th Wine Industry Technical Conference (Figure 3.1). Small-scale wine lots were produced from the most promising selections each season from the Shiraz rootstock trial.

Figure 3.1. Poster showing key characteristics of the new rootstocks identified for commercial release.

Development of medium to low vigour rootstocks for quality wine production

CSIRO PLANT INDUSTRY

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Background

High scion vigour often imparted by rootstocks has been linked to low quality fruit and, from this, poor quality wine. The negative quality attributes include excessively large berries with high juice pH, high potassium and malate levels and in red wines, low concentrations of anthocyanins and phenolics. These problems may be overcome by breeding and developing new rootstocks that impart less vigour to a scion.

CSIRO's Rootstock Breeding Program

A number of potential new rootstocks that produce less vigorous grafted vines have been selected from CSIRO's breeding program. When grafted to Shiraz, yields were adequate and fruit quality was enhanced with juice having less potassium and lower pH. This fruit quality was reflected in wines. These new rootstock selections are also *phylloxera* and nematode tolerant.

Results

When grafted with Shiraz the new selections:

- Had lower pH and K^+ levels in berries
- Required less tartrate addition for pH adjustment in winemaking and lower wine pH (Table 1)
- Had enhanced wine spectral and sensory properties (Table 2)

Table 1. Must and Wine Analysis

Rootstock	Sugar	Must pH	Must Acid	Added Tartrate	Wine pH	Wine Acid
	Brix°		(g/L)	(g/L)		(g/L)
1103 Paulsen	26.0	4.27	4.45	5.33	3.59	8.75
Ramsey	25.2	4.24	4.24	4.73	3.61	8.28
Dogridge	26.7	4.26	4.66	6.08	3.67	8.92
A	24.8	3.81	5.07	2.23	3.52	7.76
B	24.8	3.84	4.84	2.58	3.46	7.82
C	26.1	4.17	3.91	4.00	3.45	8.09
D	24.5	3.93	4.18	2.83	3.49	7.78
Lsd (p=0.05)	1.6	0.30	0.77	3.06	0.05	0.71

Table 2. Wine Spectral and Sensory Properties

Rootstock	Colour Density	Hue	% Ionised Anthocyanins	Ionised Anthocyanins	Total Anthocyanins	Total Phenolics	Sensory Score
	(A.U.)			(mg/L)	(mg/L)	(A.U.)	2003
1103 Paulen	6.75	0.61	7.32	47.9	655	63.2	13.1
Ramsey	6.06	0.59	7.83	46.0	591	53.4	13.1
Dogridge	6.66	0.63	6.75	48.1	713	63.3	.
A	8.28	0.55	10.78	68.6	636	71.4	14.6
B	7.22	0.53	9.76	61.4	634	65.5	14.4
C	8.44	0.57	9.50	65.1	684	68.6	14.9
D	7.95	0.54	9.35	66.0	716	73.5	14.2
Lsd (p=0.05)	0.66	0.02	0.98	6.8	ns	4.6	.

Future

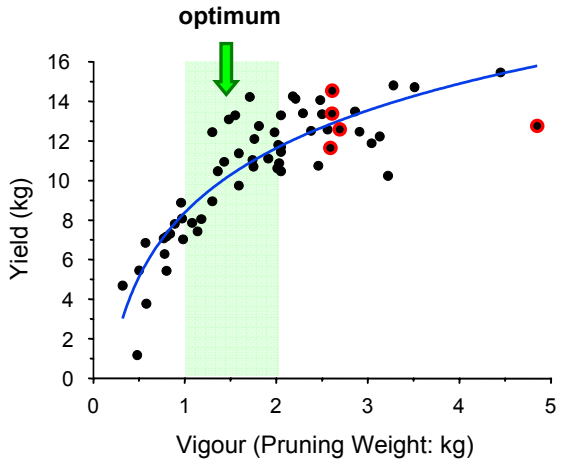
These results are encouraging and indicate that improved rootstocks are possible through breeding. These selections, for which Plant Breeders Rights are being sought, will be available for commercial release and wider industry evaluation in the very near future.

GWRDC

3.2 Identification of selections based on vigour and yield

Previous research conducted over 4 seasons (1995-98) across 55 hybrids and 5 standard rootstocks grafted with Shiraz (Clingeleffer 2000, GWRDC Final report CSH94/3) had shown that yield response to increasing pruning weight was accurately described by a logarithmic response. A maximum yield limit was achieved for high vigour selections with pruning weights greater than 1.5 kg (Figure 3.2.1). Such results showed that rootstock selection can be used to manipulate the yield:vigour relationship and that high vigour rootstocks are likely to produce high yields in an inefficient manner. Furthermore, high vigour was strongly linked to high K in berries. In the current studies an optimum vigour range between 1.0 kg and 2.0 kg per vine was used to identify 24 hybrids for detailed study (Figure 3.2.1) leading to release of the 4 new low-medium vigour rootstocks.

Figure 3.2.1. Relationship between mean yield and mean pruning weight across 60 rootstock genotypes grafted with Shiraz, over 4 seasons and 12 replicates.



Yield, pruning weight and the ratio of yield to pruning weight measured as part of recent studies of the 4 new low-medium vigour rootstocks identified for commercial release compared to the standards, 1103 Paulsen and Ramsey (Table 3.2.1). Ramsey had the highest yield followed by selection A and 1103 Paulsen while selections B, C, and D had lowest yields. Pruning weights of the new selections were 2-3 fold lower. Consequently the yield to pruning weight ratio, ie. a measure of the efficiency of vine performance, was higher for all the new rootstocks compared to the standards, particularly 1103 Paulsen.

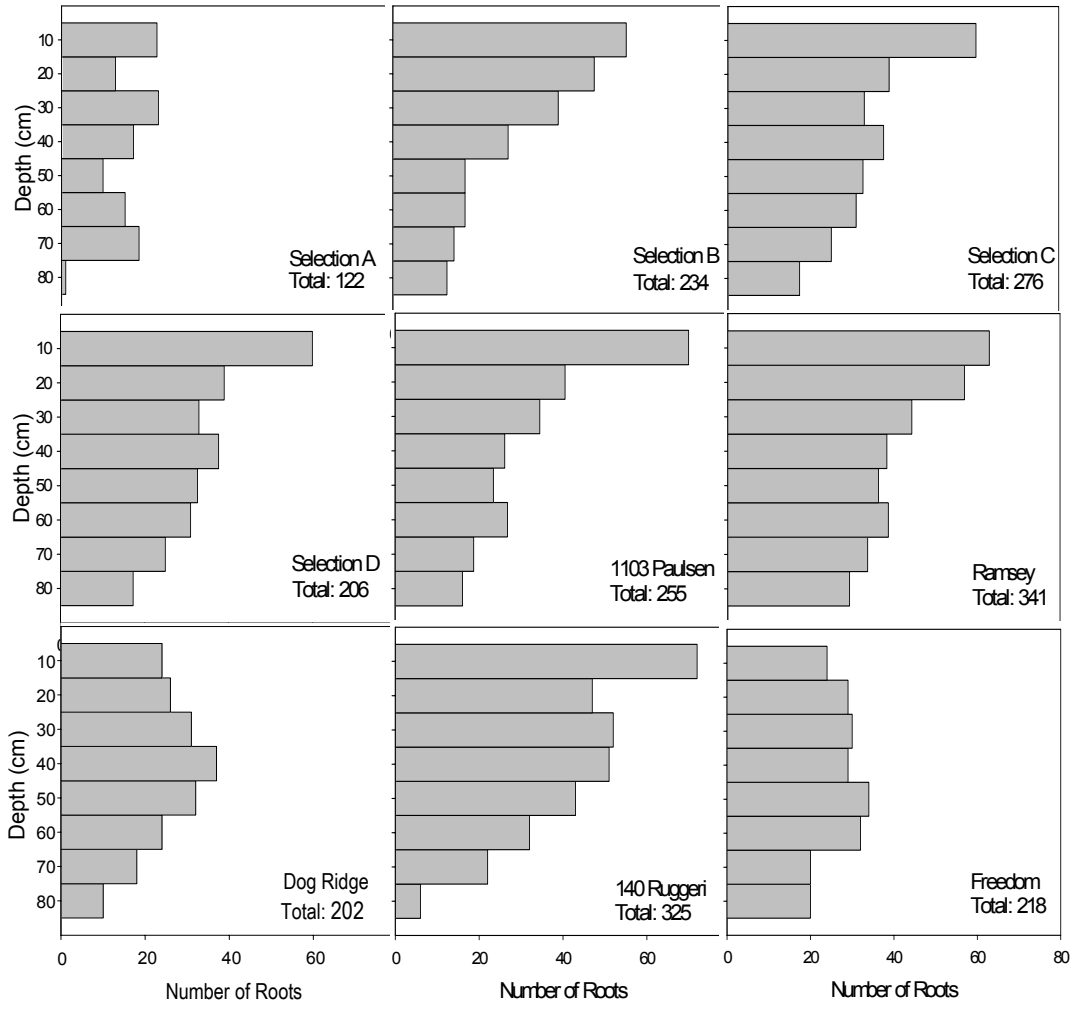
Table 3.2.1. Mean yield, pruning weight and the yield : pruning weight ratio of the four new hybrids and the standard rootstocks, 1103 Paulsen and Ramsey grafted with Shiraz (2001-2003). Means with different letters are significantly different (p=0.05).

Rootstock	**Yield** (kg/vine)	**Pruning Wt.** (kg/vine)	**Ratio Yield : pruning wt.**
1103 Paulsen	11.8b	4.2c	2.8
Ramsey	16.7c	4.3c	3.9
Selection A	13.3b	2.0b	6.7
Selection B	8.8a	1.5ab	5.9
Selection C	9.9a	2.0b	5.0
Selection D	9.9a	1.2a	8.3

3.3. Root number and distribution of the new low-medium vigour rootstocks.

Root number and distribution was assessed as previously described in the materials and methods for Shiraz vines grafted to the new rootstock selections and standard rootstocks. The results, comparing root distribution at various depths within the soil profile, describe large differences between all the rootstocks (Figure 3.3.1). Of the standard rootstocks, total root numbers recorded in the 1 m^2 vertical face were lowest for Dog Ridge (202) and Freedom (218), followed by Paulsen (255) with 140 Ruggeri (325) and Ramsey (341) having the highest values. Of the new selections, selection A (122) had the fewest roots followed by selection D (206), selection B (234) and selection C (276). Three very different patterns of root distribution were recorded across the 9 rootstocks. Six rootstocks (1103 Paulsen, Ramsey, 140 Ruggeri and selections B, C, D) had similar patterns with the most roots in the top 10 cm, reducing progressively with each layer, with fewest roots in the 70-80 cm layer. Of these six rootstocks Ramsey had the most uniform distribution while 1103 Paulsen had the highest number of roots in the surface layer. With Dog Ridge, root numbers increased with depth reaching a maximum in the 30-40 cm layer, then reducing progressively, with lowest numbers in the 70-80cm layer. Root distribution of Freedom and selection A was more uniform at all layers, although selection A had fewer roots in all layers with very few roots in the 70-80 cm layer.

Figure 3.3.1. Total number of roots at various depths of the 4 low-medium vigour selections (A-D) and 5 standard rootstocks when grafted with Shiraz. Root numbers were recorded in the vertical plane along a 1.0 m transect parallel to the row, 0.3 m from the vine trunk within 10 cm layers within the soil profile (N=3). Each bar represents the total number of roots intercepted in the 0.1 m^2 zone.



3.4. Water use efficiency of the new low-medium vigour rootstocks

As identified above, the low-medium vigour rootstocks had improved crop to pruning weight ratio compared to the standard rootstocks. It was hypothesised that development of the smaller canopies associated with lower vigour of the new rootstocks could lead to improved water use efficiency, due to reduced transpiration while maintaining acceptable crop loads. This hypothesis assessed by Walker et al. (2005) in a comparative study between 1103 Paulsen and rootstock selection A where transpiration was measured using sap flow techniques. Values for harvest index (ie. total bunch weight / total bunch weight + vegetative biomass) and crop water use index (yield/cumulative water transpired) (Table 3.3.1) were also determined. Compared to 1103 Paulsen in season 2004, selection A used 40% less water, produced 30% more crop, had a 30% higher harvest index and a 2.4-fold improvement in crop water use index.

Table 3.4.1. Vine water use, yield, harvest index and crop water use index of Shiraz vines grafted on 1103 Paulsen and rootstock selection A. (Adapted from Walker, R.R., Gibberd, M.R. and Stevens, R.M. 2005. Improving vineyard water use efficiency. In: Blair, R.J., Williams, P.J. and Pretorius, I.S.,editors. Proceedings 12th Australian Wine Industry Technical Conference. pp152-8.)

Shiraz/rootstock	Vine water use (kL/75 days)	Yield (kg/vine)	Harvest index (Bunch wt./bunch wt. +shoot biomass)	Crop water use index (yield/cumulative water use)
1103 Paulsen	1.39	14.9	0.53	10.7
Selection A	0.85	19.0	0.73	25.2

3.5. Assessment of phylloxera tolerance

The phylloxera tolerance of 12 rootstock selections showing promise with regard to wine properties when grafted with Shiraz (3.1 above) and 6 standard rootstocks were assessed by DPI Victoria, Rutherglen. A root piece biossay technique (Granett 1987) was used for this purpose. Up to three phylloxera strains were included in the studies (ie. the Rutherglen strain G30, the King Valley Strain G4 and the Nagambie strain G1). Roots from *Vitis vinifera* (cv. Sultana) were used as a control. Measurements of population growth, egg numbers, crawler numbers and adult numbers were recorded over a four week period. The value for total number of phylloxera surviving was used to determine overall tolerance (Table 3.5.1).

Table 3.5.1. Phylloxera tolerance of 12 rootstock selections (the four new low-medium vigour selections (A-D) and 8 other promising rootstock types including Selection E for which PBR data has also been collected) and 6 standard rootstocks.

	Rutherglen Strain (G30)	**King Valley Strain (G4)**	**Nagambie Strain (G1)**	**Overall tolerance**
Standards				
1103 Paulsen	High	High		High
140Ruggeri	High/Moderate	High/Moderate		High/Moderate
Dog Ridge	Low	Low/Moderate		Low/Moderate
Freedom	Low	Moderate		Low/Moderate
Ramsey		High		High
Schwarzmann	Low	High		High/Low
New selections				
Selection A	High	High	High	High
Selection B	High	High		High
Selection C	High	High	High	High
Selection D	Moderate	Moderate		Moderate
Selection E	High	High	High	High
Other selections				
Selection 1	Moderate			Moderate
Selection 2	Moderate	High	High	High/Moderate
Selection 3	Moderate	Low		Low/Moderate
Selection 4	High	High		High
Selection 5	Moderate/High	High/Moderate		High/Moderate
Selection 6	Moderate	Moderate		Moderate
Selection 7	Moderate	Moderate		Moderate

The phylloxera tolerance of the six standard rootstocks was assessed using the Rutherglen (G30) and King Valley (G4) strains, with the exception of Ramsey which was only tested against the King Valley (G4) strain (Table 3.5.1). Of the standard rootstocks, only 1103 Paulsen had high phylloxera tolerance against both strains. Moderate tolerance levels to both phylloxera strains were exhibited by 140 Ruggeri while Dogridge and Freedom has low to moderate phylloxera tolerances. Schwarzmann show no tolerance of the Rutherglen (G30) strain but had high low tolerance against the King Valley (G4) strain of phylloxera. Ramsey had high tolerance against the only strain tested, ie. the King Valley (G4) strain. Three of the new low-medium vigour selections (A, B & D) and also selection E had high tolerance against all phylloxera strains, including the Nagambie (G1) strain when used (ie. for selections A,C,E). Of the remaining selections (ie. 1-7) only selection 4 had high tolerance against both the Rutherglen and King Valley strains of phylloxera.

Results 4. Genotype and Environmental interactions (GXE)

An investigation into genotype x environment interaction effects on grapevine rootstock performance has been conducted by re-visiting data collected in 1996 and 1997 as part of GWRDC project no. CSH 8GW (Use of rootstocks to reduce chloride and sodium levels in export wines). The raw data collected from trials at four sites (Merbein and Koorlong in the Sunraysia region, The Barossa Valley and Padthaway) have been analysed to investigate whether there were any significant genotype x site effects. The data used were for Chardonnay vines growing either on their own roots or grafted to one of eight rootstocks common across all sites. The data were subjected to analysis of variance and the performance of each rootstock/scion combination with regard to a wide range of characteristics was assessed using regression analyses as described by Finlay and Wilkinson (1963). Vine performance across sites was measured in terms of yield, pruning weight, inflorescence numbers, annual shoot production, concentration of a range of ions (B, Ca, K, Mg, Na, P, S and Cl) in leaf petioles collected at full-bloom, berry weight at harvest, concentration of Na, K, and Cl in juice extracted from berries at harvest, as well as total soluble solids (^{0}Brix) and total titratable acidity.

There were significant site x rootstock interactions for most characteristics. However, there was no evidence for site x rootstock interaction effects for juice pH and total titratable acidity. Regression analyses comparing site means with individual scion/rootstock means showed that some rootstocks performed better at some sites than others for some characteristics. For example, mean yields across all rootstocks were highest at site 1 and lowest at site 3 (Figure 4.1). This was the case generally for most stock/scion combinations across the sites, but some were more stable with less variation between sites. Thus, yields from own-rooted vines varied little between sites. Vines grafted to 1103 Paulsen on the other hand, had twice the yield of own-rooted vines at site 1, but had very low yields on the lowest yielding site. The data showed that some rootstocks were better adapted to some sites than others while other rootstocks were more consistent across sites. A further example is also provided for total soluble solids (Figure 4.2). It clearly shows that Chardonnay grafted on Kober 5BB, compared to the other rootstocks had very high sugar levels at one site and very low levels at another site.

These results indicate that new rootstocks being released from the breeding program need to be tested at a range of sites to ensure their benefits are maximised across the industry. They further suggest that it is unlikely that a universal rootstock can be developed suited to all regions with varying climates, soils and management practices.

Figure 4.1. Genotype x environment interactions described by regression analyses of mean yield (kg) of 4 individual rootstocks (1103 Paulsen, Ramsey and own roots) against the mean value over all rootstocks, including own roots (n=9) at 4 regional sites (Merbein, Koorlong, Padthaway and the Barossa Valley).

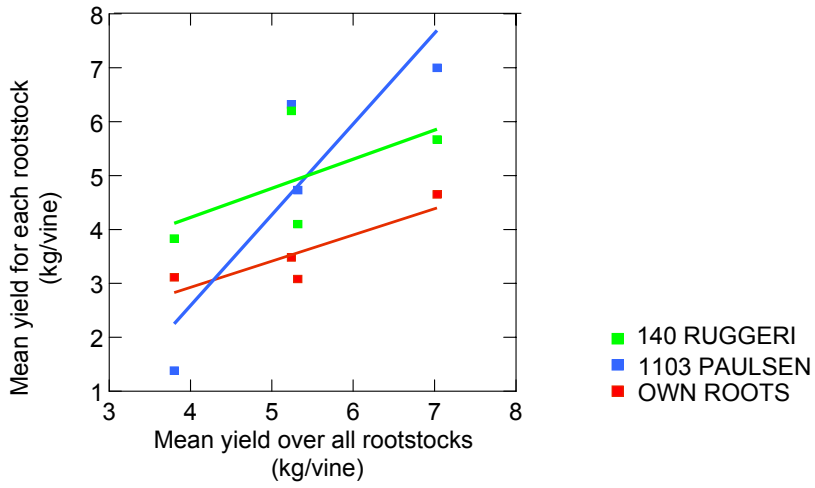
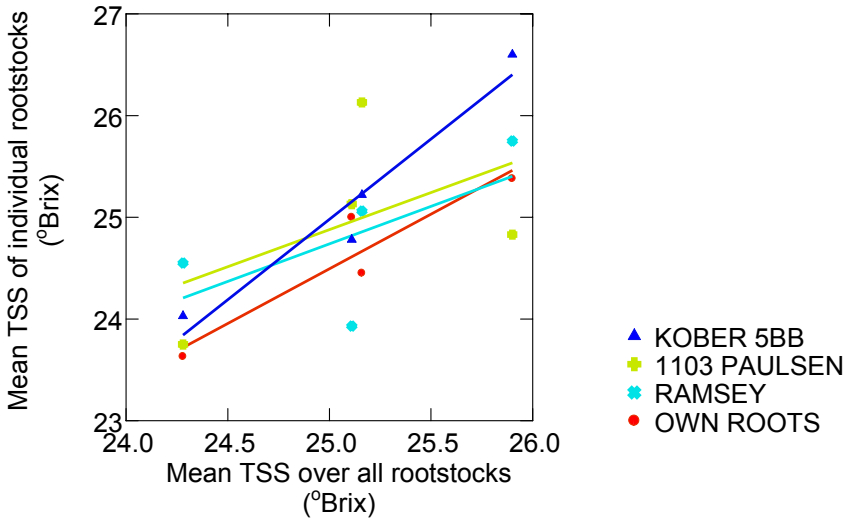


Figure 4.2. Genotype x environment interactions described by regression analyses of mean total soluble solids (^{0}Brix) of 4 individual rootstocks (Kober 5BB, 1103 Paulsen, Ramsey and own roots) against the mean value over all rootstocks, including own roots (n=9) at 4 regional sites (Merbein, Koorlong, Padthaway and the Barossa Valley).



Results 5: Discussion

Research conducted in the project covered the assessment of rootstock material at various stages of development. This included the application of screening techniques to phenotype populations/families of rootstock hybrids established in the field as ungrafted seedlings and the identification of rootstock genotypes meeting key selection criteria for further evaluation as grafted vines. As a result, 20 selections were identified. They were grafted with the main winegrape varieties (Chardonnay, Cabernet Sauvignon and Shiraz) and established in a replicated field trial. The commercial release of four new low-medium vigour rootstocks, in particular collection of data for PBR purposes, was also facilitated.

The project formed part of a larger project which included further funding from Horticulture Australia Limited. A major output of the combined projects has been the commercial release of four new rootstocks with PBR protection. The characteristics of these rootstocks included low to medium vigour, reduced levels of juice potassium, enhanced spectral properties of the wine, moderate to high phylloxera and nematode tolerance. These new low-medium vigour rootstocks have potential to be components of an integrated approach for winegrape management using high density plantings with closer row spacings to increase yields per hectare, and to improve vineyard water use efficiency and sustainability. Further study is required to assess the performance of these rootstocks in other regions when grafted with a range of commercial varieties. The low-medium vigour of these rootstocks may also facilitate adoption of low input pruning systems (eg. mechanical hedging) and minimise canopy management (eg. vertical shoot positioning and summer trimming), and hence the cost of winegrape production in cool regions. This aspect also requires further study.

For most of the characteristics assessed in the screening studies there was significant diversity between and within families indicating very significant potential to breed and select new rootstocks combining beneficial traits across the rootstock gene-pool. Successful propagation (high rooting and grafting percentage) is a basic selection criterion for grapevine rootstocks as genotypes with poor propagation will be more expensive to produce under commercial conditions. In general, across all genotypes a large proportion of the hybrids propagated successfully as there were no major problems with rooting (mean 74%) or grafting (mean 65%). It has been noted by others (Andy Walker, pers. com.) that hybrid rootstocks which callus well and develop good roots systems also graft readily. This requires confirmation in more detailed study across the range of rootstock genotypes in the CSIRO breeding program. If confirmed, this would eliminate the need to use grafting success as a selection criterion and hence, simplify screening processes. The overall results suggest that generalisations about either rooting or grafting success in rootstock species and hybrids are not justified and each specific rootstock and stock/scion combination needs investigation.

With respect to mineral status, potassium concentration was identified of high importance because of established links to winegrape quality when assessed as grafted plants. Previous research had established links between petiole K concentration found in ungrafted rootstocks and K concentration in fruit. In this study there was a four-fold range in petiole K concentrations, with 10% of hybrids having K concentrations considered to be deficient, while a further 3% had excessive concentrations. In particular, it was noted that hybrids containing 0.5 *V. berlandieri* or 0.5 *V. rupestris* had low K concentrations. The replicated trial with 20 hybrids grafted with the three main wine varieties (Chardonnay, Cabernet Sauvignon and Shiraz) will provide a significant resource to confirm relationships between K concentrations of ungrafted and grafted plants in a winegrape context.

Mineral status was determined for a further 11 elements in addition to K. All showed considerable variation in concentrations between and within families. For most elements, sampling of petioles at the end of the season was more appropriate than in spring, when sampling is normally undertaken at flowering based on the significance of correlative relationships within and between seasons. Across the rootstock hybrid populations there were very few significant correlations between the concentrations of most elements. Such results indicate that the control of uptake of each element is generally likely to be independent processes. The main exceptions were the strong positive correlations between Al and Fe, which was most likely due to contamination from dust particles, Na and Cl, Ca and Mn, S and B, Mg and P and a negative association

between K and Mg. For each element, narrow sense heritability was determined across the rootstock hybrid population. For this purpose, end of season samples gave better estimates of heritability compared to spring samples, although there were also significant differences between seasons. The most reliable results over two seasons indicate high heritabilites for B, K, P, and S. In particular, the significant heritabilities measured for K in 2000 and 2001 of 0.82 and 0.43 respectively, and over both seasons (ie. 0.63), indicate potential to develop rootstocks with low potassium uptake by judicious selection of parents. More rapid screening of hybrid populations under controlled glasshouse conditions using individual seedlings or small rooted leaves may provide an opportunity to not only rapidly screen rootstock hybrids for mineral uptake but also minimise variability due to variations at different locations within the field. Further studies are required in this regard.

Rootstock hybrids were screened against three biotypes of root knot nematodes in large sand beds established under glasshouse conditions. Under these conditions Ramsey rootstock was not infected by any biotype while Sultana was infected by all three biotypes. While all species combinations showed evidence of nematode susceptibility, about 30% of the hybrids were not infested by any biotype. Less than 10% were infested by all three biotypes. Testing nematode tolerance in sand beds indicates true susceptible hybrids but does not guarantee that some plants will not "escape" infestation even with thorough mixing of inoculated soil and high inoculum rates. A more stringent duel-culture technique where hybrid selections are grown in pots with tomato plants and infested with nematode biotypes is currently under evaluation. Furthermore, overseas rootstock breeding programs (Peter Cousins, pers. com) are now screening hybrid seedlings against virulent nematode strains using seedlings at a very early stage and eliminating all susceptible types from further evaluation. This approach will also be tested in further studies.

From the population of 350 ungrafted rootstock hybrids, 20 selections have been identified using a number of key criteria (ie. vigour, rooting and grafting ability, nematode tolerance and petiole K status) for evaluation as grafted plants with the three main wine varieties as a scion. A replicated trial has been established to assess vine performance (eg. yield, vigour, berry weight, sugar accumulation, acidity etc), juice composition and aspects of wine quality under modern management practices. Because a high number of genotypes have been included from the population of ungrafted hybrids which are still available for comparative studies, the site will also be useful to assess relationships between grafted and ungrafted plants for vigour, mineral element status, water use efficiency and drought tolerance and root architecture. Furthermore, it is anticipated that new rootstock types meeting key criteria including phylloxera and nematode tolerance will be identified for commercial release.

To the best of our knowledge, this is the first time that a number of the techniques have been applied to assess rootstock hybrid performance in the field. These included the use of carbon isotope discrimination as a measure of transpiration efficiency, the measurement of leaf temperature under well-watered and drought stress conditions to determine drought tolerance and detailed assessment of root architecture.

The carbon isotope discrimination studies indicate a 50% variation in transpiration efficiency across the ungrafted rootstock hybrids. These results suggest that it should be possible to not only identify hybrid selections for further evaluation with high transpiration efficiency but also identify parents for use in further breeding to select for high transpiration efficiency. However, links between high transpiration efficiency of seedling hybrid selections and water use efficiency of mature vines in the field, as measured by crop water use index, needs to be established. If this can be achieved it may be possible to develop the technique as a rapid screening tool for young plants established under glasshouse conditions. If the observed very large differences between hybrids reflect differences in water uptake and transport by the roots system and trunk, as distinct from leaf based canopy responses, then carbon isotope discrimination techniques may offer considerable potential to rapidly screen rootstocks with respect to water use efficiency, at least under field conditions. Verification using grafted plants is required.

The measurement of leaf temperature under well watered and drought conditions showed very large differences across the hybrid populations, both between and within families. The technique, which indicates the level of canopy cooling through transpiration shows considerable promise for assessing the capacity of the root system of rootstock hybrids to maintain an adequate water supply and hence transpiration. Canopy size could influence results under drought conditions due to larger canopies depleting soil moisture at a faster rate. Hence measurements were taken on a number of dates during the onset of 'drought' to minimise this

error. Under well-watered conditions effects of canopy size should be minimal. Very large differences in leaf temperature, from 2 ^{0}C to 11 ^{0}C below ambient, were recorded under well-watered conditions across the population, indicating large differences in the ability of root systems to supply water to the canopy. A similar range in leaf temperature from 2 ^{0}C below ambient to 11 ^{0}C above ambient was also recorded under drought stress conditions. Under such conditions it would be expected that hybrids with the lowest temperature are potentially the most drought tolerant and able to maintain canopy function when droughted. Across the rootstock family means there was a strong correlation between leaf temperature under well-watered and drought stress conditions. This indicates that rootstock hybrids which produce a significant drop in leaf temperature compared to ambient under well-watered conditions are likely to be the most drought tolerant. Verification of these responses with grafted plants is required. The planting of wine varieties with 20 rootstock hybrids will provide material to undertake such a study.

The application of soil trenching techniques to assess root architecture of individual hybrids has provided an opportunity to enhance the understanding of root numbers and distribution within the soil profile. There was a 7.5 fold difference in the number of roots intercepted in a vertical plane located in the inter-row space, 0.3 m from the vine trunk across the hybrid population with significant ranges within and between the families tested. Further analysis of the data will be undertaken to elucidate relationships between root number, root distribution, vine vigour, mineral element status, transpiration efficiency (determined by carbon isotope discrimination) and drought tolerance (determined by leaf temperature techniques). Furthermore, the replicated trial with the main winegrape varieties grafted on 20 hybrid selections will provide material to undertake further studies to assess effects of root architecture on vine performance.

The rootstock hybrids were fully established as individual, mature vines in the vineyard. This has enabled the collection of propagation material to conduct replicated trials for the rooting, grafting and nematode studies whereas assessments of mineral status, transpiration efficiency, drought tolerance and root architecture were undertaken on individual vines. Integration of the outputs from these studies will be used in the selection of parents for ongoing breeding. It is clear that use of whole populations is very beneficial where there is very little information on the inheritance of multiple traits and for the development of new screening tools such as those used for water use efficiency, drought tolerance and root distribution. Such approaches would not have been possible if a large percentage of the population had been eliminated through screening for a key characteristic at an early stage of seedling development.

Currently, phylloxera tolerance /resistance of hybrids is measured on selections at the latter stages of evaluation, generally during their evaluation as grafted plants. This is because it must be done outside of the Mildura region and involves extensive use of expensive in-vitro techniques. The development of molecular markers for phylloxera tolerance/resistance could be used to increase rootstock breeding efficiency. Three of the four, new low-medium vigour selections released for further industry evaluation showed high tolerance against all strains of phylloxera. As part of the phylloxera studies, the tolerance of six standard rootstocks was assessed. Of the standard rootstocks, only 1103 Paulsen had high phylloxera tolerance against the strains tested while moderate tolerance was reported for 140 Ruggeri. Ramsey had high tolerance against the only strain tested. The overall low to moderate tolerance of Dog Ridge and Freedom and the susceptibility of Schwarzmann to the Rutherglen (G30) strain of phylloxera is a matter of concern concern to industry, particularly as Schwarzmann has been widely planted in some regions.

The screening studies have identified a number of potential areas where considerable gains in rootstock breeding efficiency could be made, including reducing the number of plants required to be maintained under field conditions. However, such an approach would limit the availability of complete populations for genetic studies or for the development of improved screening techniques. Because nematode tolerance is considered of very high importance to the program, seedlings with tolerance /resistance could be identified at a very early developmental stage using techniques developed by Peter Cousins, USDA. In such cases only those showing tolerance or resistance would be retained for ongoing evaluation. Furthermore, it is likely that hybrid selections with good callus and root development will also graft readily, hence grafting studies could be eliminated as part of the seedling screening process. It is also possible that the techniques for assessment of transpiration efficiency and drought tolerance using carbon isotope discrimination and leaf temperature could be further developed for application under controlled conditions in the glasshouse or shadehouse and used to identify hybrids for ongoing evaluation under field conditions. However, further work is required to investigate whether transpiration efficiency is an indicator of crop water use index. Similarly it is likely that screening tests to assess mineral element status could be developed for application under controlled conditions and used in selective screening processes.

The root architecture studies conducted as part of the Shiraz study have provided new insights into root distribution and root number of the new selections, but also the standard rootstocks in the trial. Across the five standard rootstocks and four new rootstocks 3 very different patterns of root distribution were recorded. Firstly, 6 rootstocks (1103 Paulsen, Ramsey, 140 Ruggeri and 3 new selections A,B,C) had similar patterns with the most roots in the top 10 cm reducing progressively with each layer, with fewest roots in the 70-80 cm layer. Of these six rootstocks, Ramsey had the most uniform distribution while 1103 Paulsen had the highest number of roots in the surface layer. Secondly, with Dog Ridge root numbers increased with depth reaching a maximum in the 30-40 cm layer and then reduced progressively with lowest numbers in the 70-80cm layer. Root distribution of Freedom and selection A was more uniform at all layers, although selection A had fewer roots in all layers with very few roots in the 70-80 cm layer. The relevance of the significant differences in root distribution to water use efficiency, drought tolerance, mineral element uptake from the profile and vine vigour require considerable further study. Interestingly, the *V. champini* rootstocks Dog Ridge and Ramsey had the lowest and highest root numbers of the 5 standard rootstocks when assessed during dormancy. Further detailed studies of root growth dynamics during the annual growth cycle is required to fully understand the relationships with root architecture as shown in this study and the development and maintenance of fine feeder roots and their impact on scion performance.

The analysis of genotype x environment interactions (GxE) showed significant effects for most variables measured across four sites in Sunraysia, the Barossa Valley and Padthaway, ie. a diverse range of environments. The results suggest it is unlikely that a universal rootstock can be developed suited to all regions with varying climates, soils and management practices. They also indicate that new rootstocks being released from the breeding program need to be tested at a range of sites to ensure their benefits are maximised across the industry.

In summary, key outputs from the project included:-

- Phenotyping of ungrafted populations/families of rootstock hybrids for rooting ability, grafting ability, nematode tolerance, mineral element discrimination, transpiration efficiency and drought tolerance.
- Identification of rootstock genotypes meeting key selection criteria for further evaluation as grafted vines.
- Establishment of a replicated trial with 20 rootstock hybrids identified above, together with a number of standard rootstocks grafted with the three main wine grape varieties.
- Facilitation of the commercial release of the new rootstocks, in particular collection of data for PBR purposes.

7. Outcome/Conclusion:

Over the course of the project the original objectives, outputs and performance targets have been met. Key outputs from the project included phenotyping of ungrafted populations of rootstock hybrids for rooting ability, grafting ability, nematode tolerance, mineral element discrimination, transpiration efficiency, drought tolerance and root architecture; identification of 20 rootstock genotypes for further evaluation as grafted vines; identification of 4 new, low-medium vigour rootstock genotypes for commercial release with PBR protection; and preliminary analyses of genotype x environment interactions using existing data sets. Quantitative genetic techniques were applied to the population data to investigate the modes of inheritance of individual traits across breeding families. Furthermore, over the course of the project more than 1000 new inter- and intra-specific hybrids were produced, grown as seedlings, planted in the field and trained onto the trellis for ongoing evaluation in new studies.

To the best of our knowledge, this is the first time that a number of the techniques have been applied to assess rootstock hybrid performance in the field. These included the use of carbon isotope discrimination as a measure of transpiration efficiency, the measurement of leaf temperature under well-watered and drought stress conditions to determine drought tolerance and detailed assessment of root architecture. The project has also identified further opportunities to increase the efficiency of rootstock breeding through the application of rapid screening techniques at an early seedling stage for nematode tolerance/resistance, mineral element uptake, transpiration efficiency and drought tolerance under controlled conditions in either the glasshouse or shade-house. Further work is required to verify these techniques in relation to field performance. Outputs from this project, such as established trials with grafted plants, are a significant resource for future physiological studies aiming to enhance understanding of the relationships between root architecture, vine vigour, yield, mineral element uptake, water use efficiency and drought tolerance. It is clear that further knowledge of root growth dynamics during the annual growth cycle is required to fully understand the relationships with the genotype, root architecture and the development and maintenance of fine feeder roots and their impact on scion performance.

From an industry perspective, the project has demonstrated that the development and adoption of new rootstocks offers significant opportunities to overcome problems associated with adoption of high vigour rootstocks. A key output from the project has been the release of four, new low to medium vigour rootstocks which have significant advantages over existing rootstocks used in the warmer irrigated regions. These advantages included lower juice potassium and pH, reduced wine production costs associated with a lower requirement for tartaric acid addition for pH adjustment, enhanced spectral properties of wine, and moderate to high phylloxera and nematode tolerance. It is important that the performance of these new rootstocks be assessed in the main wine grape production regions for a range of commercial varieties. There is considerable potential to enhance productivity (yields per hectare), crop water use index and sustainability through the integration of the low to medium vigour rootstocks with high density plantings and closer row spacings. Furthermore, in cooler regions the reduction in shoot biomass production may facilitate adoption of low input pruning systems (eg. mechanical hedging) and minimise canopy management inputs (eg. for vertical shoot positioning and summer trimming) and hence, production costs.

8. Recommendations:

Industry

A key output from the combined project involving funding from GWRDC and Horticulture Australia Ltd. has been the release of four, new rootstocks with PBR protection. The characteristics of these rootstocks included low to medium vigour, reduced levels of juice potassium, enhanced spectral properties of the wine, and moderate to high phylloxera and nematode tolerance. It is recommended that these new rootstocks be assessed by industry in the main wine production regions when grafted with a range of commercial varieties. Consideration should be given to integration of these low to medium rootstocks with high density plantings and closer row spacings to increase yields per hectare, crop water use index and sustainability. In cooler regions, the potential use of the rootstocks which reduce shoot biomass to facilitate adoption of low input pruning systems (eg. mechanical hedging) and minimise canopy management inputs (eg. for vertical shoot positioning and summer trimming), and hence the cost of production should be investigated.

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Further research, development and extension

- Rapid screening techniques for nematode tolerance/resistance be applied at an early seedling stage to facilitate a smarter approach to rootstock selection. This will increase the efficiency of the rootstock breeding program as susceptible types may be discarded prior to field establishment.
- Develop and implement rapid screening techniques for mineral element uptake, water use efficiency and drought tolerance. The development studies should include correlative studies with ungrafted and grafted field grown plants and in particular investigate possible links between transpiration efficiency measured at the seedling level with crop water use index of mature vines in the field.
- The trial site, established with 20 new rootstock selections and a number of standard rootstocks grafted with the main industry varieties as scions (Chardonnay, Cabernet Sauvignon and Shiraz) be used for physiological studies with the aim to enhance understanding of the relationships between root architecture, vine vigour, yield, mineral element uptake, water use efficiency and drought tolerance.
- Detailed studies of root growth dynamics during the annual growth cycle be undertaken to fully understand the relationships with the genotype, root architecture and the development and maintenance of fine feeder roots and their impact on scion performance.

9. Appendix 1: Communication:

Relevant communication over the course of the project involved presentations to the project steering committee involving participants from major companies, the Phylloxera Board of South Australia and GWRDC; to major wine company delegates in November 2003; to the rootstock workshop held as part of the 12th Australian Wine Industry Technical Conference in Melbourne in 2004, to the Viticulture '04: Growing our Future Conference in Mildura in October 2004; the Sunraysia Nurserymen Association in 2005 and all reporting requirements to GWRDC. A pamphlet has been prepared containing relevant information to facilitate commercial release of the new, low to medium vigour rootstocks by licensed nurseries. Furthermore, there has been input into articles for industry prepared by GWRDC and industry journals (see below). Plant Breeders Rights (PBR) applications for the 4 low-medium vigour rootstocks were filed and published in April 2005, Plant Varieties Journal 18 (2): 40-41 (http://pbr.ipaustralia.plantbreeders.gov.au/).

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The project has also been featured in other articles:

Roots of efficiency are found at Merbein. R&D at Work, GWRDC, October 2006, p12.
(http://www.gwrdc.com.au/downloads/r&d@work/merbein.pdf)

Same vigour, less water possible with new CSIRO developed rootstocks, Australian Viticulture (1) 2007.

10. Appendix 2: Intellectual Property:

The key IP issue relates to the release of 4 new rootstock selections for large-scale commercial evaluation with PBR protection. Provisional PBR application has been granted. A licensing arrangement for the supply and sale of grafted material with PBR protection has been finalised with nurseries in Victoria and Western Australia. Issues of PBR protection and the proposed commercialisation strategy have been discussed with GWRDC and HAL and each has approved the strategy. In the longer term, ongoing evaluation and selection of hybrid populations may identify new selections for commercial release.

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12. Appendix 4: Staff

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13. Appendix 5: Budget reconciliation

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