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Rootstock Genetics & Improvement: New Improved Rootstocks with Durable Resistance to Root Knot Nematode and Phylloxera



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Wine Australia

Wine Australia for Australian Wine

Rootstock Genetics & Improvement: New Improved Rootstocks with Durable Resistance to Root Knot Nematode and Phylloxera

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Front Cover Image: First-generation vines

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1. Abstract

Plant Breeders Rights have been lodged for four new CSIRO rootstocks targeted for release. These C-series rootstocks that are adapted to Australian conditions serve as an alternative to major commercial rootstocks bred overseas over 100 years ago. Experimental studies showed that the CSIRO germplasm harbours key traits to develop new rootstocks for a changing climate. Marker-assisted breeding was used to select first-generation rootstocks containing two resistance loci against grape phylloxera and one resistance locus for root knot nematode. Identification of molecular markers linked to the second root knot nematode resistance locus in *V. champinii* will provide the means to develop second-generation rootstocks with durable resistance to grape phylloxera and root knot nematode for a changing Australian climate.

2. Executive Summary

The Australian Wine Grape Industry is dependent on commercial rootstocks bred and selected in Europe and California over 100 years ago that are poorly adapted to the major growing climates in Australia. Developing next generation rootstocks adapted to Australian conditions with durable resistance to major soil pests will have a significant impact on safeguarding vineyards from grape phylloxera and root knot nematode. The development of next generation rootstocks aligns with the sustainability and biosecurity risk management strategies for Wine Australia.

The impact of rootstocks on vine performance in two regions with different environments and management practices was stable over time and with vine age. However, the impact of rootstocks on vine performance was not consistent between regions or when grafted with different scions. As a result, rootstock selection will be critical for optimising scion performance in different regions or when grafted with different scions. Further, different management practices may be necessary to optimise the performance of different scion cultivars, particularly in a hot climate. Therefore, developing a single universal rootstock suited for all wine grape varieties under different climate conditions is not feasible. To better equip the wine grape industry with rootstocks adapted to Australian conditions, four rootstocks were targeted for Plant Breeders Rights protection. These genetically diverse rootstocks provide the Wine Grape Industry with alternatives to major commercial rootstocks used in production.

Evaluation of germplasm and experimental rootstock breeding lines showed that the CSIRO *Vitis* collection has sufficient genetic variation to develop rootstocks for a changing climate, including traits associated with favourable production under deficit irrigation and a limited supply of moderately saline water. Evaluation of commercial rootstocks and new breeding lines also showed that the CSIRO *Vitis* collection contains traits that maintain root knot nematode resistance at high soil temperatures. Lastly, new sources of grape phylloxera and root knot nematode resistance were identified for future germplasm development and rootstock breeding.

A pathway for the development of next generation rootstocks with durable resistance to grape phylloxera and root knot nematode was generated. First-generation rootstocks with durable resistance to grape phylloxera were developed by marker-assisted selection using DNA-markers tightly linked to *RESISTANCE TO DAKTULOSPHAIRA VITIFOLIAE 1* (*RDV1*) and *RDV2* from Börner and *Vitis cinerea* C2-50, respectively. These experimental rootstocks also contained the root knot nematode resistance locus, *MELOIDOGYNE JAVANICA RESISTANCE 1* (*MJR1*). First-generation rootstocks containing *RDV2*, *RDV2* and *MJR1* were established in the field to set up crosses in order to generate second-generation rootstocks with durable resistance to grape phylloxera and root knot nematode. To achieve this goal, we also performed genetic mapping and identified DNA-markers linked the *Vitis champinii* root knot

nematode resistance locus termed *MJR2*. By crossing first-generation rootstocks harbouring *RDV1*, *RDV2* and *MJR1* with *Vitis champinii* material containing *MJR2*, the CSIRO Rootstock Breeding Team will be in position to deliver second-generation rootstocks with durable resistance to grape phylloxera and root knot nematode. Interestingly, preliminary results indicate that *Vitis cinerea* harbours a new grape phylloxera resistance trait specific to an aggressive genotype. In addition, a third root knot nematode resistance trait was identified in *Vitis candicans*. Future genetic studies aimed at identifying molecular markers linked to the new grape phylloxera and root knot nematode resistance traits from *Vitis cinerea* and *Vitis candicans*, respectively, will provide the means to increase the durable resistance in second- and third-generation rootstocks. Moreover, the breeding pathways developed for next generation rootstocks will increase the genetic diversity of future durable pest resistance rootstocks to further safeguard vineyards from these soil pests.

During the project, there has been significant interaction with industry through presentations at conferences and forums, some of which were supported by the tasting of wines produced from new rootstock selections, including the C-series rootstocks. Mother vine plantings of PBR selected C-series rootstocks have been established to ensure that adoption is not limited by the supply of propagation material.

3. Background

Rootstocks for Australian conditions

In the previous project (CSP 1304, Clingeleffer and Smith 2018), three promising rootstocks were selected from a group of 26 CSIRO selections embedded in two rootstock trials. Both trials included key commercial rootstocks as comparators. The evaluation code for the three selected rootstock genotypes was C113, C114 and C20. The commercial rootstocks were 1103 Paulsen, 140 Ruggeri and Ramsey. The first trial, planted in 2005 and located in a hot climate region at the CSIRO Irymple farm and irrigated each season with about 6 ML/ha of water sourced from the Murray River, was grafted with Chardonnay, Cabernet Sauvignon and Shiraz as scions in a fully replicated randomised block design. This allowed both the performance of individual rootstocks to be assessed across the scions but also the ability to evaluate the performance of each rootstock with individual scions and test potential interactions between scion and rootstock. The vines, trained to a two-wire vertical trellis with four quadrilateral cordons, were pruned by simulating mechanical hedging, the standard practice in the region. The second trial, planted in 2010 in Padthaway with Shiraz as the scion was irrigated with a limited supply (supplementary irrigation of approximately 1 ML/ha per annum) of saline bore water (approx. EC_{iw} 2.8 dS/m) also included 101-14 Mgt., a popular rootstock used in that region. The vines, trained with four quadrilateral cordons on a wide T trellis, were spur pruned by hand.

The original rootstock genotypes had been selected for low potassium uptake of ungrafted vines, high rate of root strike and grafting success, and moderate to high vigour of ungrafted vines. Based on their performance in the previous project, C113, C114 and C20 were identified with potential for release. They were included in a Plant Breeders Rights (PBR) comparator trial to establish distinctness, uniformity and stability (DUS) in the shade house at the CSIRO Irymple farm from which key ampelographic trait data have been collected to support their PBR application (see below). C113, which had displayed root knot nematode resistance, was selected as a high vigour rootstock with performance traits similar Ramsey in a hot climate and a moderate vigour, salt tolerant alternative to the drought sensitive 101-14 Mgt rootstock (CSP 1304, Clingeleffer and Smith 2018). In addition, C113 offered distinct advantages over the lower yielding 1103 Paulsen and 140 Ruggeri, in a hot climate study. C114, which displays root knot nematode resistance was selected as an easy to propagate, high vigour, high yielding, salt and drought tolerant rootstock with performance and fruit composition traits similar Ramsey. Chardonnay wine produced from C114 was preferred over other rootstocks. CSP 1304 also noted C114 maintained high productivity under deficit irrigation in dried grape trials and was likely to be adopted by both the wine and dried grape industries. The root knot nematode and grape phylloxera resistant C20 selection had displayed moderate vigour but it was recommended that further data be acquired before a decision to release for industry adoption could be made. It was however included in the PBR comparator trial with 101-14 Mgt as its main comparator also included.

The previous project also identified significant scion x rootstock interactions for production and growth traits and some fruit composition traits (CSP 1304, Clingeleffer and Smith 2018) which have also been published in detail (Clingeleffer et al., 2019). Such results indicated that different rootstocks may be required for different scions to optimise their performance with respect to conferred vigour, production, fruit and wine composition. Such results require confirmation in the longer term for the three scion varieties in the hot climate study with the limited number of rootstock genotypes retained, particularly for the new rootstock selections (i.e. three commercial rootstocks and selections C113, C114 and C20).

Rootstocks – safeguarding vineyards from soil pests

Rootstocks derived from North American *Vitis* species have the potential to safeguard vineyards and manage yield losses caused by grape phylloxera and root knot nematode, as well as other soil pests (Dunlevy et al., 2019). Grapevine rootstock breeding was initiated in the late 1800s after grape phylloxera was unknowingly introduced into Europe from its native habitat in North America (Forneck and Huber, 2009; Powell, 2012). The introduction of this foreign insect pest nearly destroyed the European wine industry, as grape phylloxera is well adapted to feeding and reproducing on the roots of domesticated grapevine varieties derived from *Vitis vinifera*. Grape phylloxera feeding not only diverts nutrients away from the canopy but also induces root swellings or galls on young and lignified roots of *Vitis vinifera*. Galls that develop on lignified roots often crack allowing pathogenic soil fungi to enter into and infect the root system, which eventually kills the vine. In contrast to domesticated grapevine cultivars derived from Eurasia, North American grapevine species that co-evolved with grape phylloxera provide resistance or tolerance to this pest. While resistance prevents grape phylloxera from establishing a permanent feeding site required for reproduction, tolerance mechanisms allow a low level of feeding and reproduction on young roots. As a result of European rootstock breeding efforts, a set of grape phylloxera resistant and tolerant rootstocks derived primarily from the hybridisation of North American *Vitis* species were developed and released to replant vineyards across the continent (Powell, 2012).

In Australia, grape phylloxera is currently restricted to seven grape Phylloxera Infested Zones (PIZs) across Victoria and New South Wales (https://maps.vinehealth.com.au/mapguide/virtual/pmz/). Despite efforts to quarantine this insect pest to PIZs, grape phylloxera continues to spread. For example, since the initial introduction of grape phylloxera into the Yarra Valley in 2006, the boundaries of the Maroondah PIZ have expanded eight times due to new detections in close proximity to the PIZ boundary. In fact, Agriculture Victoria announced two grape phylloxera detections in March 2019, one of which occurred outside of the existing PIZ boundary (https://vinehealth.com.au/2019/03/new-phylloxera-detection-in-victorian-prz/). Further, as of 2019, the estimated cost resulting from the introduction and spread of grape phylloxera

in the Yarra Valley is approximately $1 billion dollars (https://vinehealth.com.au/2019/08/phylloxera-yarra-financial-impact/).

As there are no effective soil treatments for the eradication of grape phylloxera from vineyards, the only sustainable means to maintain grapevine production is to utilise resistant or tolerant rootstocks (Powell, 2012). While tolerant rootstocks may not show a significant decline in vine production under grape phylloxera infestation compared to susceptible *Vitis vinifera* varieties, tolerant rootstocks still permit a low level of grape phylloxera reproduction, which contributes to the spread of this insect pest. In Australia, 83 genetic strains of grape phylloxera are classified into six major clonal lineages (Powell and Korosi, 2014). The two most prevalent strains of grape phylloxera are the G1 and G4 biotype-A superclones, which are highly adapted to feeding and reproducing on *Vitis vinifera* varieties. Other less abundant grape phylloxera genotypes are classified as biotype-C due to their ability to feed and reproduce at low levels on domesticate grapevines and commercial rootstocks with North American *Vitis* species parentage.

Commercial rootstocks display differences in resistance or tolerance to representative strains from each of the six grape phylloxera lineages (Powell and Korosi, 2014). For example, in a glasshouse screen, 1103 Paulsen is rated as tolerant to G1 grape phylloxera but resistant to G4, while 140 Ruggeri is rated as resistant to G1 but tolerant to G4. As tolerant rootstocks support a low level of grape phylloxera feeding and reproduction, there is concern that over time these grape phylloxera strains will continue to adapt resulting in the proliferation of new aggressive populations (Dunlevy et al., 2019). In Europe, the increase in susceptibility of grape phylloxera tolerant rootstocks derived from *Vitis berlandieri* x *Vitis riparia*, including the Teleki series, indicates that grape phylloxera populations are adapting to feeding and reproducing on these rootstocks (Kocsis et al., 1999). In California and Australia, biotype-C grape phylloxera strains that effectively feed and reproduce on 101-14 Mgt have also been identified (https://cenapa.ucanr.edu/newsletters/Vineyard_Views_Newsletter_-_Events43564.pdf; https://vinehealth.com.au/pests/pests-and-diseases/phylloxera/phylloxera-strains/). The breakdown in tolerance by the recent emergence of aggressive grape phylloxera biotype-C strains illustrates that commercial rootstocks are not a long-term and viable solution to manage this insect pest. Further, little is known about how climate change will affect the adaptation of other grape phylloxera populations to tolerant rootstocks. In a recent publication, parentage analysis demonstrates that the genetic diversity of current commercial rootstocks is narrow, as they are derived from a limited number of *Vitis berlandieri*, *Vitis riparia* and *Vitis rupestris* accessions (Riaz et al., 2019). Thus, if a new grape phylloxera population emerged that is better adapted to feeding and reproducing on one rootstock variety; tolerance or resistance in other rootstocks with the same pedigree will likely be compromised. As a result, this would limit rootstock options for replanting in grape phylloxera infested vineyards.

In addition to grape phylloxera, root knot nematodes are another major economically damaging soil pest for grape production primarily in Mediterranean environments in North American, Europe, South Africa and Australia, as these sedentary endoparasitic nematodes readily feed and reproduce on the roots of *Vitis vinifera* cultivars, as well as susceptible rootstocks used in production (Walker and Stirling, 2008; Saucet et al., 2016; Dunlevy et al., 2019). It has been estimated that damage caused by root knot nematodes to susceptible grapevine material decreases production up to 20% (Nicol et al., 1999).

Root knot nematodes are highly adapted to sandy soil environments where a majority of Australian vineyards are planted (Walker and Stirling, 2008). While effective root knot nematode resistance is conferred by the Ramsey rootstock, this variety displays a vigorous growth habit in hot climates, which negatively impacts wine quality for red grape cultivars (Walker and Stirling, 2008). In the past decade, 1103 Paulsen has become the most popular rootstock used in Australian vineyards. Since this rootstock is characterised as having moderate to high resistance to root knot nematode with moderate vigour (Whiting, 2004), 1103 Paulsen has served as an alternative rootstock to Ramsey for grape production in hot climates. However, over the last 7-8 years, new aggressive populations of root knot nematodes have emerged that break resistance in 1103 Paulsen. These aggressive populations have arisen in major Australian wine grape growing regions, including McLaren Vale, Barossa Valley and Sunraysia (Smith et al., 2016; Dunlevy et al., 2019). The potential spread and emergence of additional aggressive root knot nematode populations could further impact grapevine production in vineyards planted on 1103 Paulsen. Further, this example of a potential break in root knot nematode resistance demonstrates that utilising rootstocks with a single resistance gene is not sustainable for long-term management of these soil pests.

Identification of grape phylloxera and root knot nematode resistance loci for marker-assisted breeding of next generation rootstocks

North American *Vitis* species that provide resistance to grape phylloxera and root knot nematode must be viewed as valuable and limited resource that must be preserved through appropriate breeding methods (Dunlevy et al., 2019). To preserve pest resistance, non-redundant function resistant genes must be combined into individual rootstock selections, as this significantly minimises the chance of a pest to overcome and break two non-redundant resistance mechanisms simultaneously (Pilet-Nayel et al., 2017). Yet, combining multiple resistance traits into a new rootstock is not feasible using traditional approaches; however, recent advances in DNA sequencing technologies effectively permit this challenging task.

In the past, traditional breeding programs have relied on screening resistance material with populations of grape phylloxera and/or root knot nematode and quantifying the number of feeding sites (galls) and progeny derived from reproduction. While this is an effective approach for classifying resistance, tolerance and susceptibility, these methods cannot differentiate between breeding material with one or multiple resistance genes against the

same pest. Alternatively, modern DNA sequencing technologies and genetic mapping approaches are used to identify DNA markers linked to resistance genes and loci. DNA markers linked to specific resistance genes and loci can be used to identify and select rootstocks with multiple resistance genes and loci. This breeding approach is called marker-assisted selection, which is frequently performed during seedling development. Therefore, marker-assisted selection allows for thousands of individuals to be screened rapidly compared to conventional screening, which requires plant propagation followed by a three- month pest-screening assay. Further, marker-assisted selection enables the rapid identification of numerous resistant genotypes, for nursery, field and wine trial evaluations. This greatly increases the success of identifying elite rootstock varieties with optimal combinations of key viticulture traits for superior vine performance and berry quality of grafted scions.

Börner, a *V. riparia* 'Gm183' x *V. cinerea* 'Arnold' hybrid, is a rootstock that provides complete resistance to six representative Australian grape phylloxera strains using a glasshouse screening assay (Powell and Korosi, 2014). However, G38 grape phylloxera is able to reproduce on the roots of Börner at low levels (https://vinehealth.com.au/pests/pests-and-diseases/phylloxera/phylloxera-strains/). In 2009, a team of grapevine breeders from Germany identified a major grape phylloxera resistance locus from Börner, called *RESISTANCE TO DAKTULOSPHAIRA VITIFOLIAE 1* (*RDV1*), which they genetically mapped to chromosome 13 (Zhang et al., 2009). Genetic evaluation indicates that *RDV1* is derived from the *V. cinerea* 'Arnold' parent and provides resistance to biotype-C strains isolated from the roots of the SO4 and 125 AA rootstocks in Germany. Given that Börner also contains grape phylloxera resistant determinants from *V. riparia* 'Gm183', it is not clear whether *RDV1* provides resistance to biotype-A and other biotype-C populations found in Australia. A better understanding of *RDV1* mediated resistance will help guide breeding programs, as well as help growers make decisions about the usage of rootstocks containing this resistance locus.

In CSP 1304, Clingeleffer and Smith 2018, a second grape phylloxera resistance locus, *RDV2*, was identified on chromosome 14 in *V. cinerea* C2-50 (Smith et al., 2018) (CSP1304, Clingeleffer and Smith 2018). DNA markers tightly linked to *RDV2* were identified and validated for marker-assisted selection. Interestingly, *RDV1* and *RDV2*, are both derived from *V. cinerea*, albeit from different accessions indicating that this *Vitis* species may be valuable source of grape phylloxera resistance for breeding (Zhang et al., 2009; Smith et al., 2018). Given that *RDV1* and *RDV2* reside on different chromosomes, it is likely that the resistance mechanisms that recognise and/or initiate a grape phylloxera immune response are different. Therefore, combining *RDV1* with *RDV2* is expected to confer durable resistance. In addition, *RDV3*, *RDV4* and *RDV5* were identified in a complex North American *Vitis* species hybrid (Clark et al., 2018), while *RDV6*, *RDV7* and *RDV8* were identified in *M. rotundifolia* (Rubio et al., 2020). It should be pointed out that *RDV3* provides resistance to leaf-galling grape phylloxera

(Clark et al., 2018), while the other *RDV* loci provide resistance to root galling grape phylloxera (Zhang et al., 2009; Clark et al., 2018; Smith et al., 2018; Rubio et al., 2020).

Heritability studies show that *Vitis* species accessions derived from *V. cinerea*, *V. champinii*, and a small subset of rootstocks derived from North American *Vitis* species contain genetic determinants that mediate root knot nematode resistance (Lider, 1954; Cousins and Walker, 2002; Smith et al., 2018). DNA markers linked to *MELOIDOGYNE JAVANICA RESISTANCE 1* (*MJR1*) were identified in *V. cinerea* C2-50 (Smith et al., 2018) (CSP 1304, Clingeleffer and Smith 2018). This root knot nematode resistance locus located on chromosome 18 provides complete resistance to the aggressive *M. javanica* 'pt 1103P' population, which effectively parasitises the popular 1103 Paulsen rootstock. The resistance mechanism mediated by *MJR1* involves localised cell necrosis in the root meristem and vascular initial cells, which likely functions to trap and prevent invading root knot nematodes from establishing a feeding-site. DNA-markers tightly linked to *MJR1* were validated for the development of new durable resistant root knot nematode resistant rootstocks using marker-assisted selection.

V. champinii is a North American grapevine species known to confer resistance to major root knot nematode species (Walker et al., 1994). Heritability studies indicate that two *V. x champinii* rootstocks, Ramsey and Dogridge, are heterozygous for single root knot nematode resistance allele (Cousins and Walker, 2002). Further, the mode of root knot nematode resistance in *V. champinii* appears to be different from resistance mechanism in *V. cinerea* (Cousins et al., 2007). Thus, durable resistance against root knot nematode can be achieved by combining the *MJR1* from *Vitis cinerea* C2-50 with the resistance gene or locus from *V. champinii*. Therefore, identification of the root knot nematode resistance locus in *V. x champinii* rootstocks will provide the means to develop durable root knot nematode resistance rootstocks.

In conclusion, combining multiple root knot nematode and grape phylloxera resistances genes and loci into the same rootstock via marker-assisted selection is the ideal approach to ensure vineyard performance is maintained in the presence of grape phylloxera and root knot nematode. Further, this approach is predicted to effectively safeguard vines from the emergence and spread of new aggressive and virulent grape phylloxera and root knot nematode populations. The identification of DNA markers linked to *RDV2* and *MJR1* from research performed in CSP 1304, Clingeleffer and Smith 2018, provide the basis to develop durable grape phylloxera and root knot nematode resistant rootstocks via marker-assisted selection.

4. Project Aims

The primary objectives were:

1. Evaluate long-term performance of C-series rootstocks developed in the CSP 1304 (Clingeleffer and Smith 2018), CSP 1101 and CSP 1002 projects, as well as widely adopted commercial rootstocks, in two different environments and management practices. Perform genotype x environment analyses to better understand how environment and management impacts rootstock performance.
2. Identify rootstock breeding lines and selections that perform well under deficit irrigation to further deepen our understanding of how the environment impacts rootstock performance and determine if the *Vitis* collection has sufficient genetic variation to develop rootstocks for a changing climate.
3. Provide underpinning information to support the release of selected C-series rootstocks targeted for Plant Breeders Rights (PBR) protection. Provide performance data to develop information packages to support the release and adoption of these new rootstocks by industry.
4. Evaluate rootstock germplasm to identify new *Vitis* species and hybrids that provide resistance to root knot nematode and/or grape phylloxera resistance for future breeding. Propagate and screen new *Vitis* germplasm for root knot nematode and grape phylloxera resistance. Target *Vitis* germplasm harbouring root knot nematode and grape phylloxera resistance for future breeding.
5. Identify molecular markers linked to the *Vitis champinii* root knot nematode resistance locus, *MJR2*, for developing second-generation rootstocks with durable resistance. Perform *Vitis champinii* x *Vitis vinifera* 'Riesling' crosses to establish F_1 populations for the genetic mapping of *MJR2*. Utilise the root knot nematode in vitro and glasshouse screening systems to phenotype F_1 individuals from each mapping populations. Genotype F_1 individuals, perform genetic mapping and validate DNA markers tightly linked to *MJR2* for marker-assisted selection.
6. Utilise DNA marker-assisted selection to identify first-generation rootstocks. Perform *Vitis cinerea* C2-50 x Börner crosses to combine *RDV2* and *MJR1* with *RDV1*, respectively. Utilise DNA-markers to select for F_1 individuals harbouring all three resistance loci, which are referred to as first-generation rootstocks. Validate root knot nematode and grape phylloxera resistance in first-generation rootstocks. Establish first-generation rootstocks field plantings in order to cross this material with *V. champinii* breeding lines harbouring *MJR2*.
7. Determine the biological basis of root knot nematode resistance in commercial rootstocks and evaluate the impact of increased soil temperature on root knot nematode resistance in commercial rootstocks and select breeding lines. Utilise, in parallel, glasshouse and in vitro screening systems to evaluate the impact of increased

temperature on root knot nematode resistance and susceptibility in commercial rootstocks and breeding lines. Utilise this knowledge to develop a system to select second-generation rootstocks with heat-stable resistance to root knot nematode.

5. Method

5.1 Evaluation of rootstock selections and breeding lines

The established field trials are described in CSP 1304, Clingeleffer and Smith 2018. To evaluate the impact of a rootstock selection and/or breeding line on vine performance, yield, vine balance and growth determinants, as well as fruit and wine quality, were measured.

- Collection of berry samples prior to harvest to determine berry weight and for fruit composition analyses
- Collection of yield and bunch number, allowing calculation of all yield component data including bunch weight and berries per bunch
- Collection of pruning weight data and calculation of the Ravaz Index (yield/ pruning weight) as a measure of vine balance and a surrogate for carbon efficiency (using pruning weight as an indicator of canopy size) and water use efficiency (using pruning weight as an indicator of canopy size and hence transpiration)
- Analysis of fruit composition including juice Total Soluble Sugars (TSS) by refractometer, pH and titratable acidity (TA) by titration (Metrohm Auto-titrator) and organic acids (malic and tartaric), volatile acidity (VA), yeast assimilable nitrogen (YAN), alpha amino acids and ammonium by FTIR (Oenphos)
- Conduct PBR comparator trials to establish Distinctness Uniformity and Stability for rootstocks identified with potential for release

5.2 Glasshouse root knot nematode phenotyping

To phenotype grapevine material in the glasshouse, dormant cuttings containing 1-3 buds derived from individual genotypes were propagated in triplicate at the Yalumba Nursery. After adventitious root formation, the propagated vines were transported to the Waite Campus for replanting in rectangular plastic pots (90 x 90 x 180 mm) containing steam-sterilised potting soil (3:1 Bio Gro:Perlite). To propagate material from green cuttings, stem segments containing 1-3 axillary buds were harvested and rooting medium (Yates Clonex, AUS) was applied to the basal section of the stem. Next, green cuttings were potted-up in peat moss containers containing wet perlite and placed in a humid chamber for four weeks to stimulate root formation. After roots formed, plants were re-planted in rectangular plastic pots (90 x 90 x 180 mm) containing steam-sterilised potting soil (3:1 Bio Gro:Perlite). After re-planting rooted dormant and green cuttings, the grapevine genotypes were placed in the glasshouse for 4-5 weeks before root knot nematode screening was performed (Smith et al., 2018).

For glasshouse screening, root knot nematode populations, including *Meloidogyne javanica* 'pt 1103P', *M. javanica* 'Robinvale', *M. arenaria* 'Barossa' and *M. incognita* 'SARDI', were maintained on susceptible roots of tomato, *Solanum lycopersicum* 'cv California Red Cherry', at 22-25°C in a glasshouse. Two months after inoculation, egg masses were dissected from the tomato roots and infectious juvenile root knot nematodes were harvested by incubating egg masses at 30°C in sterile water for 72 hrs to induce hatching. To phenotype for resistance or susceptibility, potted grapevine genotypes were inoculated with 1,000 infectious

nematodes. Eight weeks after inoculation, the roots from each replicated individual were gently washed in water to remove the soil. Egg masses were visualised by incubating washed roots in 0.01 g/L of erioglaucine disodium salt (Sigma, Sydney, AUS), for 1 hr, which stains egg masses blue. Next, egg masses were counted under a dissecting microscope (Leica A60, Leica Microsystems Pty Ltd A60, Australia) to determine the number of egg masses per root system. The root system of individual genotypes devoid of egg masses was classified as resistant (Figure 5.2.1A). Replicated genotypes containing egg masses were classified as susceptible (Figure 5.2.1B). After phenotyping, the root system from each genotype was dried in an oven at 60°C for 24 hrs and weighed to determine the dry mass (g) for each root system. The total number of egg masses per root system and egg masses per dry root weight (g) were calculated for each genotype based on phenotype of each replicate. The inheritance pattern was determined using the chi-squared statistical test, X^2 = total of $(O-E)^2/E$, for resistance and susceptible genotypes (Smith et al., 2018).

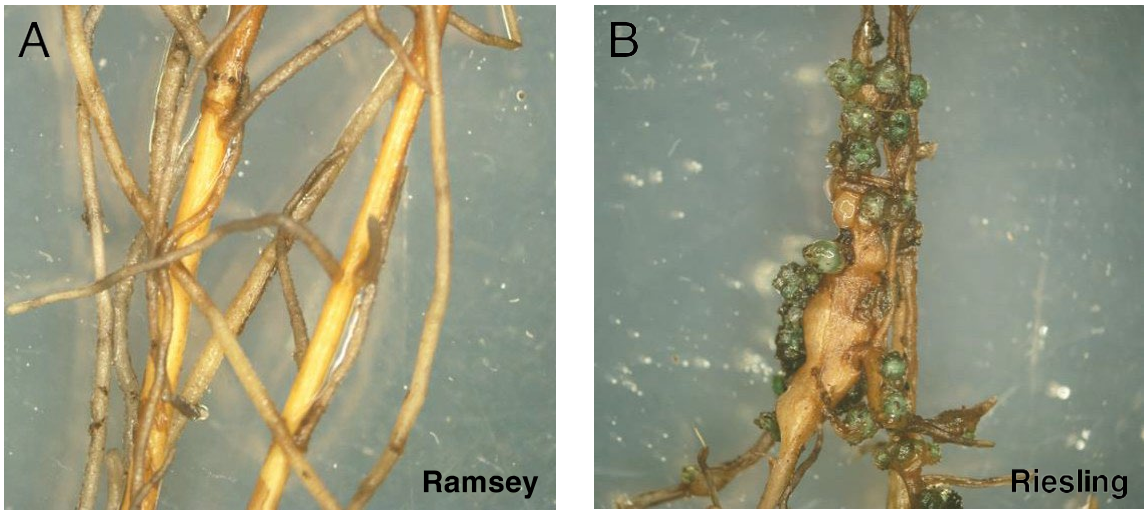


Figure 5.2.1. Resistant and susceptible phenotypes
In glasshouse screening trials, (A) Ramsey displays a resistant phenotype in which the roots are devoid of egg masses after inoculation with a root knot nematode population. (B) In contrast, after inoculation with a root knot nematode population, egg masses developed in the roots of Riesling. The roots of (A) Ramsey and (B) Riesling were stained treated with 0.01 g/L erioglaucine, which stains egg masses blue.

5.3 Grape phylloxera phenotyping

After transport of rootstock breeding material to Rutherglen, VIC, the replicated breeding lines were maintained in the glasshouse for 3 weeks at 22°C ±2°C prior to infestation with the G4 or G38 grape phylloxera genotypes. For each grape phylloxera screening trial, the potted vines were randomised into blocks on a single bench based on the number of replicates for each breeding line. The G4 and G38 trials were performed in separate glasshouses to prevent cross contamination.

The G4 and G38 grape phylloxera genotypes were mass reared under quarantine for three months prior to infestation. Initial founders of G4 grape phylloxera were collected from roots of ungrafted *V. vinifera* in commercial vineyards in the King Valley, Northeast Phylloxera Infested Zone (PIZ) in VIC. The G38 grape phylloxera was collected from the roots of grafted vines in a commercial vineyard in the Northeast PIZ, VIC. Insects were cultured at 25°C ± 2°C on *V. vinifera* cv. Chardonnay roots, sourced from an uninfested vineyard in Northeast Victoria using a modified method as previously described (Kingston, 2007). Cultures were grown in bioassay plates and prepared by washing roots with a soft brush under running water to remove soils particles. Next, roots were excised into 8-10 cm pieces and soaked in Axiom® Plus fungicide solution (7.5 g/L; ADAMA, Australia) for 15 minutes. After the roots were rinsed four times in ultra-pure water and air dried under UV in a laminar flow for three hours, individual roots were wrapped with cotton wool, moistened with three drops of sterile ultra-pure water and placed in a Petri dish (90 × 25 mm). The bioassay plates containing the sterile and moistened roots were stored at 4°C prior to inoculation. To mass rear G4 and G38 grape phylloxera for phenotyping, the excised roots were inoculated with 20 phylloxera eggs (1-4 days old) and maintained for three months at 25°C ± 2°C. The viability of the root pieces was sustained by adding four drops of water to the cotton wool wrapped around the ends of excised roots, once every week. In addition, fungal infection was managed by painting 80% ethanol onto the roots using a moistened artist's sable-haired paintbrush (Art Basics Sable spotter 300-R 2/0, Japan).

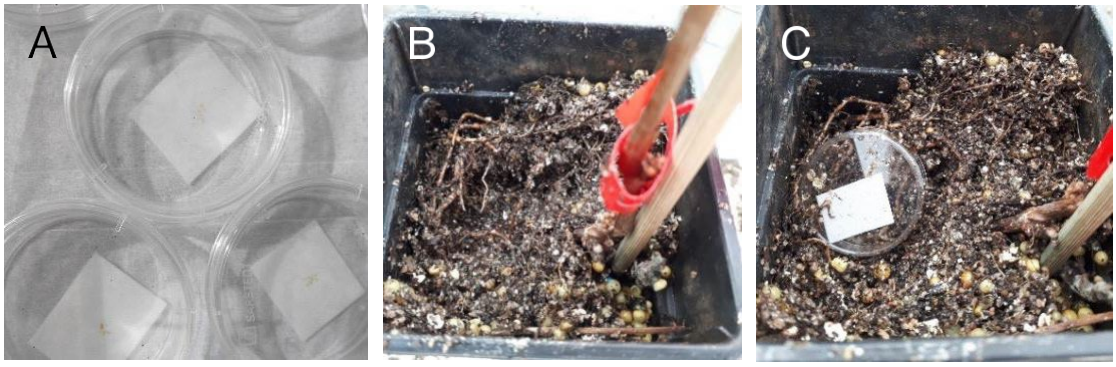


Figure 5.3.1. Inoculation of grape phylloxera onto the roots of replicated breeding lines.
(A) Cell culture dishes containing eggs on wet filter paper. (B) Exposed roots prior to inoculation. (C) Inoculation of roots with grape phylloxera. Dr. Catherine Clarke at AgVIC provided images.

Genotype determination using molecular markers previously described was used to validate the G4 and G38 grape phylloxera genotypes (Umina et al., 2007). The roots of the replicated breeding line were inoculated with 20 eggs of G4 or G38 grape phylloxera. In this procedure, eggs were collected from stock cultures using an artist's sable-haired paintbrush and placed

onto a cut piece of wet filter paper in a 25 x 10 mm cell culture dish (Figure 5.3.1A). Next, soil was gently removed to expose a section of roots (Figure 5.3.1B). The cell culture dish containing the 20 eggs was placed face down onto the exposed roots and covered with fresh sterile soil mixture (80% potting mix sourced from Yarra Valley Garden supplies and 20% Perlite; Figure 5.3.1C). Tanglefoot^{TM} was applied along the rim of the pot and around the base of the replicated breeding line above soil surface to avoid cross contamination between vines.

Eight weeks after inoculation with G4 or G38 grape phylloxera, each replicated breeding line was removed from the pot and roots were washed under running rainwater through a 2 mm sieve to filter soil and silt. The filtrate was passed through a 125 µm filter to collect the G4 or G38 grape phylloxera that detached from roots. The G4 or G38 grape phylloxera filtrate was labelled and poured into a 25-well scoring tray to count and record the number of insects, including eggs, 1st to 3rd instars, adults and alates. Each root system was examined under a light microscope and the number of nodosities and insects were scored and recorded. Roots devoid of insects and nodosities were rated as 'resistant (R)' (Figure 5.3.2A). The roots of 'susceptible (S)' replicated genotypes produced nodosities (Figure 5.3.2B), as well as eggs and insects at various stages of development. Roots that produced a small number of nodosities, <10, with no visual signs of insect development, were labelled as 'incomplete resistant (IR)'.

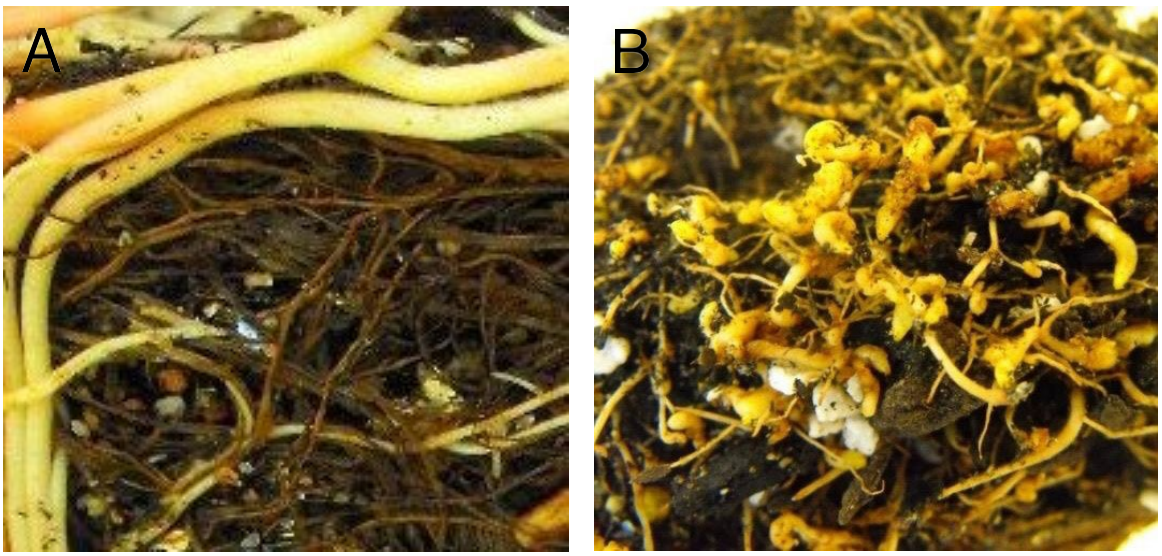


Figure 5.3.2. Phenotype of resistant and susceptible replicated breeding lines.
(A) Resistant roots devoid of nodosities. (B) Susceptible roots with extensive nodosity formation. Dr. Catherine Clarke at AgVIC provided images.

5.4 In vitro root knot nematode screening

Green cuttings isolated from rootstock cultivars and breeding lines containing 1-2 buds were sterilised in 5% chlorine and 0.1% Tween 20 for 12 hrs at 22^{0}C. After rinsing with water, the

sterilised cuttings were placed in callus-inducing media to initiate bud development (Franks et al., 1998). After a period of growth, the axillary shoots were cut from the primary stem and placed in root initiation medium to promote the formation and development of adventitious roots (Torregrosa et al., 2015).

Sterilisation of root knot nematodes was performed as previously described (Diaz-Manzano et al., 2016; Smith et al., 2018). In this procedure, 30 *M. javanica* 'pt 1103P' egg masses were isolated from infested and sterilised with 0.5% chlorine solution for 5 min at 20^0C. Next, the eggs were rinsed five times in sterile water and incubated to at 30°C to promote hatching. The roots from sterile cucumber and du Lot plants were inoculated with the sterilised infectious *M. javanica* 'pt 1103P' nematodes. After the development of egg masses, infectious *M. javanica* 'pt 1103P' nematodes were harvested from the infected cucumber and du Lot plants approximately 30-50 days after inoculation.

In vitro root knot nematode screening was performed with a modification to the previously described procedure (Smith et al., 2018). First, replicated plantlets were placed in nematode screening media (NSM), which allowed roots to grow after this transfer. Next, 50-100 *M. javanica* 'pt 1103P' infectious nematodes were used to inoculate replicated breeding lines at 25°C. After 72 hrs, roots were scored every day for resistance and susceptible phenotypes. The induction of necrosis in the cell layers of the roots is a phenotype associated with resistance, as localised programmed cell death acts in part to trap and prevent the infectious root knot nematodes from establishing a feed site. In contrast, a susceptible phenotype is associated with gall formation followed by a protrusion of callus-like tissue from the gall. Images of resistant and susceptible phenotypes are shown in the results section in Figures 6.5.1, 6.7.2 and 6.7.3.

5.5 Genotyping and genetic mapping

5.5.1 Generation of F_1 mapping populations segregating for the *V. champinii* root knot nematode resistance locus, *MJR2*

To genetically map *MELOIDOGYNE JAVANICA RESISTANCE 2 (MJR2)* from *V. champinii,* two F_1 mapping populations were generated by performing the following crosses: Ramsey x Riesling and Dogridge x Riesling (Table 5.1). As K51-40 likely inherited *MJR2* from Dogridge, we also utilised K51-40 x 140 Ruggeri, which originated from Rob Walker and used by Mandy Walker to identify salinity tolerant genes in grapevine (Gong et al., 2011; Henderson et al., 2018) (Table 5.1).

Table 5.1 F_1 populations used to identify DNA-markers linked to *MJR2* from *V. champinii*.

Female parent	Male parent	Crossing location	Population size
Ramsey (*V. x champinii*)	Riesling (*V. vinifera*)	Nuriootpa	150
Dog Ridge (*V. x champinii*)	Riesling (*V. vinifera*)	Nuriootpa	156
K51-40 (Dogridge × *V. riparia* 'Gloire')	140-Ruggeri (*V. berlandieri* × *V. rupestris*)	Merbein	40

5.5.2 DNA extraction

DNA was extracted and quantified from emerging leaves by the Australia Genome Research Facility (AGRF, Adelaide, Australia) using automated DNA extraction and quantitation platforms. DNA quality was assessed on 0.8% agarose gel run in 1% TAE buffer at 2 V/cm. The DNA concentration was normalised to 50 ng/µL in a final volume of 20 µL.

5.5.3 Genotyping

Genome-wide single nucleotide polymorphisms (SNPs) were identified using the DArTSeq™ genotype by sequencing (GBS) platform by Diversity Arrays Technology Pty Ltd (Canberra, Australia). The DArTseq platform utilises restriction enzyme digestions to reduce genome complexity, followed by next generation (HiSeq2500; Illumina, San Diego, CA, USA) sequencing of residual genome representations to identify thousands of polymorphic loci in a single assay. The DArTSeq methodology was previously described (Courtois et al., 2013) with the exception of alignment of sequence reads to both the PN40024 (version 12x.v2) grapevine reference genome derived from *V. vinifera* (cv. 'Pinot Noir') (Jaillon et al., 2007; Canaguier et al., 2017), and the phased genome of the *V. riparia* rootstock Gloire de Montpellier (version EGFV_Vitrip_1.0) (Girollet et al., 2019).

5.5.4 Filtering and processing of GBS data

The DartR package for R (version 4.0.2) was used to separate the Dartseq data for each population. Population datasets were filtered based on the following criteria: Monomorphs removed; reproducibility = 1; call rate ≥ 95%; read depth minimum of 10 and maximum of 500; minor allele frequency <1%. Variation within the datasets was assessed with Pearson Principal Component Analysis.

5.5.5. Identification of SNPs linked to RKN resistance

Identification of SNPs linked to *MJR2* using the advanced filtering function in Excel to identify deviations from the expected Mendelian segregation frequencies in the F_1 mapping populations for SNPs unlinked to this root knot nematode resistance trait. The threshold

percentages for filtering were empirically selected to identify SNPs extending out to ~14cM from *MJR2* with various zygosity models investigated to maximise the number of potentially informative SNP markers as shown in Table 5.2.

Table 5.2 Filtering parameters for SNP identification linked to *MJR2* in the segregating F_1 mapping populations.

Zygosity model	RKN phenotype	Predicted segregation for SNPs unlinked to phenotype			Threshold genotype frequencies used to filter for SNPs linked to RKN resistance		
		Progeny Genotype	Freq	DartR code	Heterozygous (Code 1)	Homozygous (Code 0 or 2)	Nullizygous (Code NA)
Rr x rr	Resistant	Rr	50%	1	≥85%		
	Susceptible	rr	50%	0 or 2		≥85%	
R- x rr	Resistant	Rr	50%	1	≥85%		
	Susceptible	-r	50%	0 or 2		≥85%	
Rr x r-	Resistant	Rr R-	25% 25%	1 0 or 2	≥40%; ≤60%	≥40%; ≤60%	
	Susceptible	rr r-	25% 25%	2 or 0		≥ 85%	
R- x r-	Resistant	Rr R-	25% 25%	1 0 or 2	≥40%; ≤60%	≥40%; ≤60%	
	Susceptible	-r --	25% 25%	2 or 0 NA		≥40%; ≤60%	≥40%; ≤60%
Rr x --	Resistant	R-	50%	0 or 2		≥85%	
	Susceptible	r-	50%	2 or 0		≥85%	

The predicted genotype frequencies for loci unlinked to *MJR2* within the F_1 mapping populations are shown for various parental zygosity models. Genotypes are shown with 'R' representing the dominant *MJR1* resistance allele; 'r' representing the recessive susceptibility allele, *mjr2*; '-' representing a null allele. Dartseq genotype data processed through DartR were coded as 1 (heterozygous), 0 (homozygous for reference allele), 2 (homozygous for non-reference allele), or NA (null allele(s)). SNPs linked to *MJR2* were identified by filtering for the specified genotype frequencies (based on DartR codes) within the resistant and susceptible classes of each population. The nominated filter frequencies for each zygotic model were applied together with a logical 'AND' function in Microsoft Excel.

5.7 Evaluation of root knot nematode resistance at 32°C

Glasshouse and in vitro trials were used to evaluate the impact of increased temperature on root knot nematode resistance and susceptibility. In the glasshouse trial, propagated commercial rootstocks and breeding lines were placed into a heating block that warmed the soil in the pots to 32°C. After incubating the rootstock material at 32°C for one week, each replicate was inoculated with 1000 infectious *M. javanica* 'pt 1103P' juvenile nematodes. In addition, replicated commercial rootstocks and breeding lines were inoculated with 1000 infectious *M. javanica* 'pt 1103P' at 24°C for comparison with results from the material screened at 32°C. Eight weeks after inoculation, the roots from each replicated rootstock were scored for egg mass development as described in 5.2. After scoring egg masses per plant, the dry mass (g) for each root system was determined. The total number of egg masses per root system and egg masses per dry root weight (g) were calculated for each genotype.

In vitro screening trials, replicated commercial rootstocks and breeding lines were inoculated with 50-100 infectious *M. javanica* 'pt 1103P' at 27°C and 32°C. In addition, replicated commercial rootstocks and breeding lines were incubated at 27°C and 32°C, without the addition of root knot nematodes to examine the phenotype of uninoculated roots at these temperatures. Over the course of the trial, the number of galls, callus-like protrusions and localised cell necrotic events were scored. Approximately 30-40 days after inoculation, the presence or absence of infectious *M. javanica* 'pt 1103P' nematodes in the media was determined. Roots from each replicate were isolated, dried at 65°C for 24 hrs and weighed to determine root mass. The total number of egg masses per root system and egg masses per dry root weight (g) were calculated for each genotype.

6. Results

6.1 Long-term performance of C113, C114, C20 in established field trials

Hot climate site Sunraysia

The long-term productivity of the three scion varieties across the six rootstocks (1103 Paulsen, 140 Ruggeri, Ramsey, C113, C114 and C20) is shown in Figure 6.1.1. The general trends show that the variation in yield across seasons was consistent for the varieties, an indication that responses to environmental cues impacting crop development were similar. The results show that the yield of Cabernet Sauvignon was lower than those of Chardonnay or Shiraz, which were similar in productivity.

Figure 6.1.1. Mean yield (kg/vine) of Chardonnay, Cabernet Sauvignon and Shiraz across six rootstocks genotypes over 10 seasons (2013-22).

All data for yield components, growth and fruit composition were analysed by ANOVA for each individual variety (data not shown). While there were significant effects of season and rootstock on almost all traits for each variety, the interaction between season and rootstock were, with one exception (i.e. VA of Chardonnay), not significant with *p*-values close or equal to 1. These results indicate that effects of rootstocks on all traits measured were highly stable across seasons and did not change with vine age. Consequently, a combined GLM analysis was conducted to test the effects of Block (not shown), Scion, Rootstock and the Scion x Rootstock interaction (Tables 6.1.1, 6.1.2). Where scion x rootstock interactions were significant, the effect of rootstock was determined for each variety. Tukey's HSD was used to identify significant differences between scion and rootstock means in pairwise comparisons. The results for yield and its components and growth traits are shown in Table 6.1.1 and for fruit composition in Table 6.1.2.

There were highly significant (p<0.001) effects of scion on yield, yield components and growth traits and of rootstock on yield, bunch number, pruning weight and Ravaz Index (Table 6.1.1). Highly significant scion x rootstock interactions for yield, bunch number, pruning weight and Ravaz Index and a weak interaction for berries per bunch indicate that rootstock responses were not consistent across scions. In contrast, interactions for bunch weight and berry weight were not significant, an indication that the rootstock response was consistent across the scions. The highly significant effects of scion show that Cabernet Sauvignon had the lowest yield, bunch number, bunch weight, berry weight and Ravaz Index and the highest pruning weight. Shiraz had higher yield, berry weight, pruning weight and lower Ravaz Index than Chardonnay.

The highly significant rootstock effect on yield show that 1103 Paulsen, 140 Ruggeri and C20 had lowest yield, C113 an intermediate yield while Ramsey and C114 were most productive (Table 6.1.1). The highly significant scion x rootstock interaction was associated with the higher yield of Chardonnay on 1103 Paulsen and C113, the higher yield of Cabernet Sauvignon on C20 and C113 and the lower yield of Shiraz on C113. The highly significant effect of rootstock on bunch number shows that 1103 Paulsen, 140 Ruggeri and C20 had the lowest and Ramsey, C114 and C113 the highest numbers. The highly significant scion x rootstock interaction was associated with the low bunch number of Chardonnay on C20 and high bunch numbers of Chardonnay on Ramsey and C113; Cabernet Sauvignon on C20 and Shiraz on 140 Ruggeri. Rootstock had no effect on bunch weight, berry weight and berries per bunch. The weak scion x rootstock interaction for berries per bunch was associated with fewer berries per bunch of Cabernet Sauvignon on 1103 Paulsen and Ramsey and Shiraz on 140 Ruggeri and C113.

The highly significant effect of rootstock on pruning weight shows that C20 and 140 Ruggeri conferred the lowest vigour, 1103 Paulsen and C113 intermediate vigour and, Ramsey and C114 the highest vigour. The highly significant scion x rootstock interaction was associated with the high vigour of Chardonnay on C113, the very high vigour of Cabernet Sauvignon on Ramsey and Shiraz on C114. The highly significant effect of rootstock on Ravaz Index (yield/pruning weight) showed that 140 Ruggeri and C20 had higher values than the other rootstocks. The highly significant scion x rootstock interaction was largely associated with the magnitude of the rootstock responses for scions of different vigour (highest for Chardonnay and lowest for Cabernet Sauvignon) and specifically the higher Ravaz Index values for Chardonnay on 1103 Paulsen and Ramsey.

There were highly significant (p<0.001) effects of scion on all fruit composition traits except TSS. In general rootstock effects on fruit composition were stable across the scions. Highly

significant scion x rootstock interactions were only found for the tartaric to malic ratio, YAN and its components, alpha amino acids and ammonium. Chardonnay had the lowest pH, tartaric to malic ratio and VA and highest TA, malic, YAN, alpha amino acids and ammonia; Cabernet Sauvignon had the lowest YAN and alpha amino acids and highest tartaric, VA, anthocyanins and phenolic substances; while Shiraz had the lowest TA, tartaric, ammonia, anthocyanins and phenolic substances.

Rootstock had no significant effect on juice TA, tartaric, ammonia and phenolic substance concentrations. The weak effect of rootstock on TSS showed that Ramsey and C114 has lower maturity than the other rootstocks. The highly significant effect of rootstock on composition traits showed that C20 had the lowest and C113 the highest pH; C20 had the lowest and 1103 Paulsen, Ramsey and C113 the highest malic concentration; Ramsey and C114 had the lowest and C20 the highest tartaric to malic ratio; C20 had the lowest VA; C20 had the lowest and Ramsey the highest YAN and alpha amino acids; and that C114 had the lowest and C113 the highest anthocyanin concentration. The highly significant scion x rootstock interaction for the tartaric to malic ratio was due entirely to significant effects of rootstock on the ratio with Cabernet Sauvignon, which followed the same trends as the overall effect, as the effects were not significant with Chardonnay or Shiraz. The highly significant scion x rootstock interaction for YAN was associated with the high concentration with Chardonnay and the low concentrations of Cabernet Sauvignon and Shiraz on C113. It should be noted that all rootstocks had acceptable YAN concentrations to meet fermentation requirements (i.e. >150 mg/L) and in the case of Chardonnay, optimum YAN concentrations for flavour development in white wines (200-250 mg/L). The highly significant scion x rootstock interaction for alpha amino acids was associated with the high concentration for Chardonnay on C113, the low concentration of Cabernet Sauvignon on C113 and the low concentrations for Shiraz on C113 and C114. The highly significant scion x rootstock interaction for ammonium was only associated with rootstocks effects with Cabernet Sauvignon which showed that C20 and C113 had lowest and Ramsey highest ammonia concentrations.

A correlation analysis was conducted between all traits using rootstock means to determine relationships that could be attributed to rootstock effects independent of scion (*n*=6, Table 6.1.3). A strong correlation between yield and bunch number (r = 0.96) indicated that bunch number was the major yield determinant. Pruning weight was linked to crop development of the rootstocks, being correlated with yield (r = 0.93) and bunch number (r = 0.83) but not bunch weight, berry weight or berries per bunch. Bunch weight of the rootstocks was positively correlated with both berry weight (r = 0.82) and berries per bunch (r = 0.97). The strong negative correlation between the Ravaz Index and pruning weight (r = -0.91) indicates that pruning weight dominates the calculation. Crop development and vine growth traits across the rootstocks were also linked to fruit composition. Yield was positively correlated with malic acid (weak) and negatively with organic acid balance (tartaric to malic ratio); bunch number was negatively correlated with organic acid balance; bunch weight was negatively correlated with tartaric acid and VA, both weak; berry weight was positively correlated with

ammonia; berries per bunch was negatively correlated with TSS and tartaric acid, both weak; pruning was positively correlated with malic acid, organic acid balance and negatively correlated with phenolics; while the Ravaz Index was negatively correlated with pH and malic acid and positively correlated with the organic acid balance.

There was also a number of significant correlations between fruit composition traits across the rootstocks (Table 6.1.3). TSS was negatively correlated with TA and positively correlated with tartaric acid, which indicates that increases in TSS may at least in part be due to berry wilting as tartaric acid content remains constant post-veraison. Juice pH was positively correlated with malic, tartaric and VA concentrations. Malic acid was negatively correlated with organic acid balance (r = -0.93), an indicator that it was the dominant contributor in the calculation but positively correlated with VA, YAN and both its constituents, alpha amino acids and ammonia. Tartaric acid was positively correlated with VA. YAN was highly correlated with both its constituents, alpha amino acids (r = 1.0) and ammonia (r = 0.98). Alpha amino acids and ammonia were highly correlated (r = 0.97).

Table 6.1.1. Mean yield, bunch number, bunch weight, berry weight, berries per bunch, pruning weight and Ravaz Index of three scion varieties grafted on six rootstocks over 10 seasons (2013-22).

		Scion			Rootstock						Significance		
		Chard.	**Cab.**	**Shiraz**	**1103P**	**140R**	**Ram.**	**C114**	**C113**	**C20**	**S**	**R**	**S x R**
Yield (kg)	**mean**	27.5b	19.4a	30.3c	23.7a	23.8a	28.7c	28.5c	25.9b	22.5a	***	***	***
	Chardonnay				30.6b	27.0ab	32.5c	28.0ab	31.4c	23.4a		**	
	Cabernet				15.4a	15.5a	21.5b	21.5b	21.2b	19.9b		***	
	Shiraz				25.1a	28.9a	33.9b	32.9b	27.5a	27.0a		***	
Bunches	**mean**	273b	254a	290b	245a	259a	303b	289b	282b	236a	***	***	***
	Chardonnay				298b	272b	328c	272b	316c	202a		***	
	Cabernet				207a	215a	279b	263b	278b	275b		***	
	Shiraz				229a	290b	309b	320b	266a	232a		***	
Bunch weight (g)	**mean**	103b	76a	108b	95.8	90.9	94.7	100.7	93.5	98.0	***	ns	ns
Berry weight (g)	**mean**	1.24b	1.03a	1.34c	1.20	1.19	1.19	1.23	1.2	1.22	***	ns	ns
Berries per bunch	**mean**	84.3b	73.7a	81.4b	79.8	76.9	79.9	80.9	78.1	82.0	***	ns	*
	Chardonnay				86.1	83.5	82.8	83.6	83.4	84.6		ns	
	Cabernet				70.2a	68.6a	73.2ab	79.7b	75.0ab	71.2ab		*	
	Shiraz				83.0ab	78.7a	83.8ab	79.3ab	75.9a	90.4b		**	
Pruning wt. (kg)	**mean**	2.18a	6.05c	4.26b	4.43b	3.25a	5.55c	5.86c	4.58b	2.68a	***	***	***
	Chardonnay				2.30b	1.89ab	2.67c	2.81c	2.89c	1.47a		***	
	Cabernet				7.15b	5.02a	8.93c	7.14b	6.84b	3.46a		***	
	Shiraz				3.84b	2.85a	5.31c	7.09d	4.52b	3.12a		***	
Ravaz Index	**mean**	14.7c	4.85a	8.41b	7.90a	10.1b	7.49a	6.24a	6.89a	12.9c	***	***	***
	Chardonnay				14.6b	15.3b	13.1b	10.0a	11.0ab	18.5c		***	
	Cabernet				2.25a	4.26ab	2.76a	3.57ab	3.23ab	10.6d		***	
	Shiraz				7.06a	10.78b	6.19a	5.45a	6.02a	9.61b		***	

The significance of the scion, rootstock and the scion x rootstock interaction is also shown (*** p<0.001, ** p<0.01, * p<0.05). Where scion x rootstock interactions were significant, results for individual scions are also presented. Means followed by different letters are significantly different, Tukeys Honesty Test for pairwise comparisons.

Table 6.1.2. Mean concentrations for fruit composition traits of three scion varieties grafted on six rootstocks over 10 seasons (2013-22).

		Scion			Rootstock						Significance		
		Char.	**Cab.**	**Shir.**	**1103P**	**140R**	**Ram.**	**C114**	**C113**	**C20**	**S**	**R**	**S x R**
TSS (°Brix)	**mean**	24.9	25.3	24.9	25.0ab	25.4ab	24.7a	24.5a	25.7b	24.9ab	ns	*	ns
pH	**mean**	4.00a	4.32b	4.35b	4.27b	4.23b	4.23b	4.24b	4.30c	4.15a	***	***	ns
TA (g/L)	**mean**	4.63c	3.69b	3.26a	3.95	3.82	4.08	3.93	3.80	3.96	***	ns	ns
Malic (g/L)	**mean**	4.25c	3.27b	3.01a	3.77c	3.46b	3.87c	3.66b	3.71c	3.28a	***	***	ns
Tartaric (g/L)	**mean**	4.86a	5.64b	5.11a	5.37	5.28	5.20	5.06	5.40	5.02	***	ns	ns
Tartaric/malic	**mean**	1.20a	1.98b	1.92b	1.55b	1.76b	1.46a	1.50a	1.64b	1.87c	***	***	***
	Chardonnay				1.18	1.18	1.12	1.17	1.13	1.16		ns	
	Cabernet				1.71ab	2.13b	1.64a	1.60a	1.79ab	2.56c		***	
	Shiraz				1.77	1.98	1.60	1.73	2.01	1.86		ns	
VA (g/L)	**mean**	0.16a	0.25c	0.19b	0.22b	0.21b	0.21b	0.19b	0.22b	0.16a	***	***	ns
YAN (mg/L)	**mean**	239c	178a	213b	226b	216b	236c	213b	207b	186a	***	***	***
	Chardonnay				256b	257b	263b	225b	282c	210a		**	
	Cabernet				206b	184b	217b	179ab	161a	153a		***	
	Shiraz				222ab	207ab	228b	183ab	187a	194a		**	
Alpha AA	**mean**	177c	124a	165b	169bc	159b	175c	158b	153b	136a	***	***	***
	Chardonnay				191bc	189bc	190bc	167bc	208c	158a		***	
	Cabernet				144b	128ab	151c	126ab	115a	100a		***	
	Shiraz				177ab	161ab	180b	140a	143a	149a		**	
Ammonia	**mean**	74.4c	65.7b	58.8a	68.9	68.4	72.9	66.5	65.4	60.9	***	ns	***
	Chardonnay				77.9	81.0	78.7	68.3	88.8	68.1		ns	
	Cabernet				75.4ab	67.8ab	79.1b	63.6ab	56.6a	59.9a		**	
	Shiraz				53.5	56.5	60.0	67.8	56.8	54.3		ns	
Antho. (mg/g)	**mean**	-	1.10b	0.83a	0.98ab	1.01ab	0.95ab	0.82a	1.06b	1.04ab	***	**	ns
Phenolics (a.u.)	**mean**	-	1.52b	1.17a	1.33	1.40	1.33	1.23	1.40	1.38	***	ns	ns

The significance of the scion, rootstock and the scion x rootstock interaction are also shown (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$). Where scion x rootstock interactions were significant, results for individual scions are also presented.

Table 6.1.3. Correlation coefficients (r) showing linear relationships between yield, yield components, growth and fruit composition traits (n=6).

	Yield	Bunch number	Bunch weight	Berry weight	Berry/ bunch	Pruning weight	Ravaz	TSS	PH	Mal.	Tart.	Tart./ Mal.	YAN	Alpha
Bunches	**0.96**													
Bunch wt.	-0.14	-0.35												
Berry wt.	0.01	-0.18	**0.82**											
Berry/bunch	-0.01	-0.22	**0.97**	*0.72*										
Pruning	**0.93**	**0.83**	-0.08	0.07	0.05									
Ravaz	*-0.78*	*-0.75*	0.34	0.06	0.27	**-0.91**								
TSS	-0.39	-0.15	-0.62	-0.45	*-0.75*	-0.42	0.05							
PH	0.36	0.44	-0.61	-0.38	-0.60	0.53	**-0.82**	0.46						
TA	0.35	0.20	0.44	0.00	0.64	0.33	0.03	**-0.80**	-0.36					
Malic	*0.70*	0.69	-0.34	-0.37	-0.18	**0.83**	**-0.85**	-0.13	*0.74*					
Tartaric	-0.11	0.05	*-0.72*	-0.67	*-0.72*	0.05	-0.40	*0.72*	**0.84**	0.49				
Tart./Mal.	**-0.83**	*-0.73*	0.16	0.13	0.00	**-0.95**	**0.88**	0.42	-0.58	**-0.93**	-0.20			
VA	0.24	0.33	*-0.73*	-0.63	-0.68	0.41	-0.69	0.46	**0.94**	*0.75*	**0.92**	-0.55		
YAN	0.54	0.54	-0.54	-0.65	-0.35	0.62	-0.62	-0.18	0.55	**0.85**	0.45	**-0.80**		
Alpha AA	0.54	0.53	-0.52	-0.62	-0.33	0.64	-0.64	-0.19	0.57	**0.86**	0.46	**-0.82**	**1.00**	
Ammonia	0.54	0.57	-0.61	*-0.74*	-0.41	0.55	-0.52	-0.13	0.46	*0.78*	0.41	*-0.72*	**0.98**	**0.97**
Antho.	-0.40	-0.27	0.01	-0.49	0.08	-0.52	0.59	0.20	-0.33	-0.13	0.10	0.38	-0.03	-0.06
Phenolics	-0.69	-0.49	-0.43	-0.56	-0.49	**-0.84**	0.70	0.61	-0.26	-0.54	0.24	*0.74*	-0.22	-0.25

Significant r values are highlighted in bold ($p < 0.05$) or shown in italics ($p < 0.1$). For simplicity, columns where r values were not significant have been removed (i.e. TA, VA, ammonium, anthocyanins and phenolics).

Padthaway site (limited supplementary irrigation with saline water)

Harvest of the Padthaway site was conducted just prior to commercial harvest. Management involved the application of less than 100 mm of irrigation of saline bore water. The vines were removed after collection of pruning weights in 2021. Harvest data (yield and bunch number) were not collected in 2020 due to COVID-19 issues, however berry samples were collected and processed. In most seasons, significant berry wilting was noted prior to harvest for most rootstocks. The long-term productivity of Shiraz over all rootstocks is shown on Figure 6.1.2. The results show that the vines,planted in 2009 were not near full production in 2013. Consequently, data for 2013 were not included in statistical analyses. Mean yields generally ranged from 11.4-14.6 kg/vine but were very high in 2021 (17.6 kg/vine). Maturity of samples collected prior to harvest based on commercial decisions ranged from 23.5 ^{o}Brix in 2021 to 28.7 ^{o}Brix in 2021 with most seasons around 25 ^{o}Brix. Data for the 2020 season were left out of the detailed statistical analysis as matching production data were not available and the very high TSS appeared to mask effects of rootstock on some aspects of fruit composition. The season x rootstock interaction was insignificant for all crop, growth and fruit composition traits, except TSS (see below).

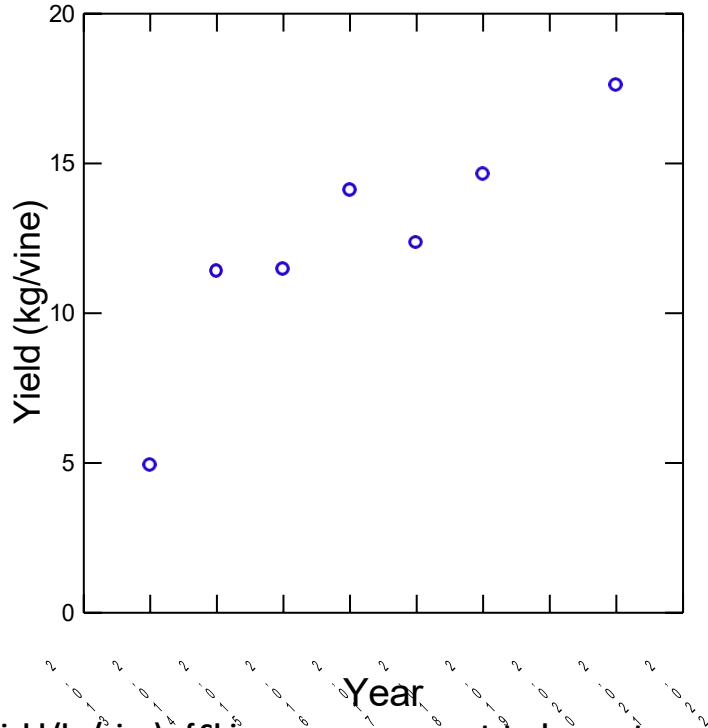


Figure 6.1.2. Mean yield (kg/vine) of Shiraz across seven rootstocks genotypes over eight seasons (2014- 2021).

Results for mean yield, yield components and growth traits of the rootstocks are presented in Table 6.1.4. There was more than a two-fold difference in yield between the rootstocks with 101-14 and Ramsey having the lowest and highest yields respectively. The low yield of 101- 14 (8 .8 kg/vine) was not significantly different to C113 and C20 (10.9 and 10.7 kg/vine). Yields of 1103 Paulsen and 140 Ruggeri were similar (13.9 kg/vine) and lower than C114 (15.6

kg/vine) and Ramsey (18.0 kg/vine). The effect of rootstock on bunch number was not significant although 101-14 had fewer bunches than Ramsey in pairwise comparisons using the Tukey's honesty test. Bunch weight of 101-14 was significantly lower than those of C114 or Ramsey. Smallest berries were produced by 101-14, followed by 1103 Paulsen, 140 Ruggeri and C20 with berries that were smaller than those of C113 while Ramsey and C114 had the largest berries. Berry development studies would be required to determine if the rootstock effects are due to differences in maximum berry size or berry shrivel. There was no effect of rootstock on berries per bunch.

Rootstocks had highly significant effects on pruning weight (Table 6.1.4). Rootstocks 101-14 (0.70 kg), C113 (0.91 kg) and C20 (0.85 kg) had significantly lower pruning weight than 140 Ruggeri (1.41 kg), Ramsey (1.53 kg) and 1103 Paulsen (1.73 kg) while C114 conferred highest vigour (2.26 kg). The highly significant effect of rootstock on Ravaz Index show that C114 and C20 had the lowest and highest Ravaz Index values, compared to the other rootstocks which were similar.

Effects of rootstock on fruit composition are shown in Table 6.1.5 Year x rootstock interactions were not significant, except for a very weak interaction with TSS (P=0.048). This interaction was largely associated with 101-14 rootstock, which had high TSS in 2015, 2019 and 2021 and low TSS in 2017 and 2018 and C113 which generally had high TSS, except in 2016. These results indicate that rootstock effects on fruit composition were stable over time and vine age. There were no significant effects of rootstock on juice pH, tartaric acid or YAN concentrations. YAN values for all rootstocks were well above 150 mg/L required for successful fermentation. There were significant effects of rootstock on fruit maturity with Ramsey having the lowest and 101-14, C113 and C114 the highest TSS. Significant effects of rootstock on TA, although small, showed that C20 had the lowest and 1103 Paulsen the highest TA concentrations. Highly significant rootstock effects on malic acid show that 101-14, 140 Ruggeri and Ramsey had lowest and C20 the highest concentrations. Rootstock effects on organic acid balance, the tartaric to malic acid ratio, were largely due to the effects on malic acid with C20 having lowest and 101-14 highest values. Highly significant effects of rootstock on berry anthocyanin concentrations show that 1103 Paulsen, Ramsey and C114 had low and 101-14 the highest concentrations of anthocyanin. Similarly, C114 and 101-14 had lowest and highest phenolic concentrations.

Table 6.1.4. Mean yield, bunch number, bunch weight, berry weight, berries per bunch, pruning weight and Ravaz Index of Shiraz grafted on seven rootstocks over six seasons (2015-19, 2022), located at two sites (Padthaway and Irymple).

Rootstock	Yield (kg)	Bunches	Bunch Wt. (g)	Berry Wt. (g)	Berry/ Bunch	Pruning (kg)	Ravaz Index
Padthaway							
101-14	9.25a	135a	64.0a	0.74a	88.4	0.72a	12.4ab
1103 P	16.0b	181b	90.8bc	0.93b	99.1	1.74b	10.4ab
140 Ru	15.5b	177b	88.1bc	0.93b	95.0	1.46b	11.3b
Ramsey	17.6c	190b	95.1c	1.06c	93.0	1.58b	11.2ab
C113	11.2ab	179ab	66.9a	1.00bc	70.4	0.97a	12.7ab
C114	17.1c	180b	94.3c	1.10c	90.1	2.26c	8.8a
C20	11.5ab	158a	72.8ab	0.97bc	76.3	0.85a	19.2c
mean	**14.8**	**176**	**84.7**	**1.00**	**87.3**	**1.48**	**12.3**
Significance	***	**	**	***	ns	***	**
Irymple							
1103 P	25.4a	231a	111.1ab	1.34	83.1ab	3.86b	6.9a
140 Ru	28.9ab	290b	102.0a	1.31	78.7a	2.85a	10.8c
Ramsey	32.7b	301c	110.1ab	1.32	83.7ab	5.00c	6.5a
C113	25.2a	254ab	102.0a	1.35	76.1a	4.01b	6.5a
C114	35.0b	325c	107.5ab	1.39	78.6ab	7.40d	5.4a
C20	26.8a	230a	120.1b	1.33	90.4b	3.12a	9.6b
mean	**29.0**	**272**	**108.8**	**1.34**	**81.8**	**4.37**	**7.6**
Significance	***	***	**	ns	**	***	***

The significance of the rootstock is also shown (*** p<0.001, ** p<0.01, ns not significant). Means followed by different letters are significantly different, Tukeys Honesty Test for pairwise comparisons.

A correlation analysis was conducted between all traits using rootstock means to determine relationships that could be attributed to rootstock effects (n=7, Table 6.1.6). Strong correlations between yield and bunch weight (r = 0.99) and to a lesser degree bunch number (r = 0.91) indicate that both bunch development and bunch number contributed to rootstock effects on yield, which was also weakly correlated with berry weight. Pruning weight was closely linked to crop development of the rootstocks, being correlated with yield (r = 0.90), bunch number (r =0.77) and bunch weight (r = 0.90). The associations between bunch number and both bunch weight and berry weight indicate that high vigour rootstock genotypes with high bunch number were also likely to develop larger bunches with larger berries. Furthermore, yield, bunch number, bunch weight and berry weight were all associated with negative rootstock effects on aspects of fruit composition being negatively correlated with tartaric acid, anthocyanin and phenolic concentrations. Berries per bunch was weakly correlated negatively with pH and positively correlated with TA. Pruning weight was also linked to effects of rootstock on aspects of fruit composition being negatively correlated with pH (weak), anthocyanin and phenolic concentrations. The Ravaz Index was positively correlated with pH and malic acid (weak). The negative association between malic

acid and organic acid balance (the tartaric to malic acid ratio) indicates that malic acid was the major factor impacting organic acid balance across the rootstocks. Juice tartaric acid concentration was strongly linked to both anthocyanin and phenolic concentrations across the rootstock genotypes. Berry anthocyanin and berry phenolics were strongly linked across the rootstocks (r = 0.97).

Table 6.1.5. Means for fruit composition traits of Shiraz grafted on seven rootstocks over six seasons (2015- 19, 2022), located at two sites (Padthaway and Irymple).

Rootstock	TSS (°Brix)	pH	TA (g/L)	Malic (g/L)	Tart. (g/L)	Tart. /Mal.	YAN (mg/L)	Anth. mg/g	Phen. a.u.
Padthaway									
101-14	24.7b	4.02	3.48ab	1.69a	6.92	4.35b	223	2.16b	2.06b
1103 P	23.9ab	4.02	3.67b	1.79ab	6.47	3.70ab	221	1.77a	1.75ab
140 Ru	24.4b	4.01	3.33ab	1.64a	6.24	3.94ab	178	1.94ab	1.89ab
Ramsey	23.1a	3.94	3.36ab	1.54a	6.01	3.97ab	207	1.68a	1.59ab
C113	25.0b	4.09	3.12ab	1.69ab	6.26	4.01ab	208	1.93ab	1.82ab
C114	24.9b	3.94	3.43ab	1.78ab	6.29	3.70ab	209	1.68a	1.58a
C20	23.9ab	4.11	3.21a	2.10b	6.55	3.33a	194	1.91ab	1.90ab
Mean[1]	**24.2**	**4.02**	**3.36**	**1.76**	**6.30**	**3.77**	**203**	**1.82**	**1.76**
Significance	**	ns	**	***	ns	***	ns	***	***
Irymple									
1103 P	25.4ab	4.42b	3.26ab	3.28	5.32	1.77	218b	0.83ab	1.18
140 Ru	25.0a	4.36ab	3.12ab	2.85	5.12	1.98	207ab	0.81ab	1.19
Ramsey	24.6a	4.35ab	3.47b	3.34	4.97	1.61	234b	0.86ab	1.19
C113	26.0b	4.44b	3.08a	3.07	5.51	2.02	179a	1.00b	1.28
C114	23.7a	4.36ab	3.35ab	3.06	4.83	1.76	233b	0.73a	1.09
C20	24.6a	4.27a	3.49b	3.01	4.92	1.86	194ab	0.92	1.27
mean	**24.9**	**4.37**	**3.29**	**3.10**	**5.11**	**1.83**	**211**	**0.86**	**1.20**
Significance	*	**	*	ns	ns	ns	***	*	ns

The significance of the rootstock is also shown (*** p<0.001, ** p<0.01, ns not significant. Means followed by different letters are significantly different, Tukeys Honesty Test for pairwise comparisons. TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tart, tartaric acid; YAN, yeast assimilable nitrogen; Anth, anthocyanin; Phen, phenolics.

Table 6.1.6. Correlation coefficients (r) showing linear relationships between yield, yield components, growth and fruit composition traits grown in Padthaway (n=7).

	Yield	Bun.	Bunch wt.	Berry wt.	Berry /Bun	Prun.	Ravaz	Mal.	Tart.
Bunches	**0.91**								
Bunch wt.	**0.99**	**0.86**							
Berry wt.	*0.70*	**0.80**	0.63						
Berry/bun.	*0.65*	0.37	*0.72*	0.09					
Prun. wt.	**0.90**	**0.77**	**0.90**	0.66	0.60				
Ravaz	-0.59	-0.47	-0.57	-0.19	-0.63	*-0.72*			
TSS	-0.42	-0.49	-0.39	-0.33	-0.17	-0.05	-0.15		
pH	*-0.73*	-0.49	*-0.74*	-0.31	*-0.73*	*-0.73*	**0.81**		
TA	0.35	0.09	0.43	-0.30	**0.84**	0.44	-0.54		
Malic	-0.33	-0.33	-0.28	-0.01	-0.39	-0.22	*0.72*		
Tart.	**-0.75**	**-0.90**	*-0.66*	**-0.83**	-0.09	-0.54	0.32	0.43	
Ratio	-0.27	-0.32	-0.30	-0.52	0.14	-0.26	0.44	**-0.78**	0.50
YAN	-0.18	-0.25	-0.17	-0.29	0.10	0.00	-0.46	-0.09	0.19
Anth.	**-0.89**	**-0.89**	**-0.85**	**-0.89**	-0.30	**-0.83**	0.39	0.09	**0.75**
Phen.	**-0.86**	**-0.87**	**-0.81**	**-0.89**	-0.27	**-0.83**	0.50	0.25	**0.78**

Significant r values are highlighted in bold ($p < 0.05$) or shown in italics ($p < 0.1$). For simplicity, columns where r values were not significant have been removed (i.e. TSS, pH, TA, tartaric/malic ratio, anthocyanin and phenolics). TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tart, tartaric acid; YAN, yeast assimilable nitrogen; Anth, anthocyanin; Phen, phenolics; Bun, bunch; Prun, pruning weight.

Comparison of rootstock performance in two regions (i.e. Genotype x Environment interaction).

The evaluation of the new rootstock genotypes involved the evaluation of the performance of grafted Shiraz in two very different environments involving different climate and vine management practices, i.e. a fully irrigated hot climate site in Irymple and a Padthaway site involving supplementary irrigation with a limited supply of saline bore water. The comparisons of rootstock performance at the two sites were used to identify traits that varied and those that were stable across the sites (Tables 6.1.4, Tables 6.1.5). Major differences between the Irymple and Padthaway sites in overall performance across the six rootstocks were found with a three-fold difference in conferred vigour (4.37 vs. 1.48 kg per vine); a two-fold difference in vine yield (29.0 vs. 14.8 kg); a 1.6-fold difference in bunch number (272 vs. 176); a 1.3-fold difference in bunch weight (109 vs 85 g); a 1.3-fold difference in berry weight (1.34 vs. 1.00); a small 6% difference in berries per bunch (81.8 vs. 87.3) and a 40% difference in the Ravaz Index (7.6 vs 12.3). Similarly, there were differences in fruit composition between the Irymple and Padthaway sites with mean juice TSS values of 24.9 and 24.2 ^{o}Brix, pH values of 4.37 and 4.02 pH, TA values of 3.29 and 3.36 g/L, malic acid values of 3.10 and 1.76 g/L, tartaric acid values of 5.11 and 6.30 g/L, organic acid balance (tartaric/malic acid ratio) values of 1.83 and 3.77, YAN values of 211 and 203 mg/L, berry anthocyanin values of 0.86 and 1.82 mg/g and berry phenolics values of 1.20 and 1.76.

Despite the two-fold difference in yield between the sites, the relative response of rootstock genotypes across the sites was reasonably stable (Table 6.1.4). Ramsey and C114 produced high yield, C113 and C20 low yield and 140 Ruggeri a moderate yield at both sites. Yield of 1103 Paulsen was not consistent across the sites as it produced a low yield at Irymple, which was similar to C113 and C20, and an intermediate yield, similar to 140 Ruggeri, at Padthaway. C20 had low bunch number at both sites. 1103 Paulsen and C113 had low bunch number at Irymple but not at Padthaway where all rootstocks, other than C20 had similar bunch numbers. Ramsey and C114, and to a lesser degree 140 Ruggeri, had high bunch numbers at Irymple. Effects of pruning are likely to be associated with these responses as bunch numbers at Padthaway are likely to be controlled by detailed hand spur pruning compared to mechanical hedging at Irymple. Bunch weight responses were not consistent across sites, indicating strong genotype x environment interactions. In particular, C20 and to a lesser degree 140 Ruggeri, developed large bunches at Irymple and small bunches at Padthaway. Ramsey, C114 and 1103 Paulsen had relatively large bunches at both sites. C113 developed small bunches at both sites. There was no significant effect of rootstock on berry weight at Irymple, although C114 tended to produce the largest berries. At Padthaway, 1103 Paulsen and 140 Ruggeri produced small berries, Ramsey and C114 large berries with C113 and C20 having intermediate berry weight. Rootstock response for berries per bunch was not consistent across sites. There was no significant effect of rootstock on berries per bunch at Padthaway. C20 had and small numbers at Padthaway. C113 had a small number of berries per bunch at both sites.

The relative performance of rootstocks on pruning weight was not always consistent between sites (Table 6.1.4). C114, and to a lesser degree Ramsey had high pruning weight at both sites. C20 had low pruning weight at both sites. 140 Ruggeri had low pruning weight at Irymple but high pruning weight at Padthaway. C113 had low pruning weight at Padthaway but an intermediate value at Irymple. Pruning weight of 1103 Paulsen was relatively high at Padthaway and intermediate at Irymple. Vine balance, as determined by the Ravaz index (yield/pruning weight) was reasonably stable across rootstock genotypes at both sites. C114 had low values at both sites while C20 had high values at both sites. 140 Ruggeri had a high Ravaz Index value at Irymple and an intermediate value at Padthaway. 1103 Paulsen, Ramsey and C113 had intermediate Ravaz Index values at both sites.

Effects of rootstock on Shiraz fruit composition traits were not consistent across sites, indicating genotype x environment interactions (Table 6.1.5). C113 consistently produced high TSS at both sites. In contrast, C114 had high TSS at Padthaway and low TSS at Irymple. Ramsey and to a lesser degree C20 produced low TSS at both sites. Effects of rootstock on pH were not significant at Padthaway, whereas at Irymple, C113 and 1103 Paulsen had high pH

and C20 low pH. In the case of juice TA, C113, C114 and 140 Ruggeri had relatively low concentrations at both sites. C20 had high TA at the Irymple site but a low concentration in Padthaway. Similarly, Ramsey had high TA at the Irymple site but a relatively low concentration in Padthaway. In contrast, 1103 Paulsen had relatively low TA at Irymple and high TA at Padthaway. There was no effect of rootstock on malic acid at Irymple. At Padthaway, C20 had highest malic acid and Ramsey the lowest malic acid concentration. Rootstock effects on tartaric acid were not significant at either site. There was no effect of rootstock on organic acid balance at Irymple. At Padthaway C113 had the highest and C20 the lowest tartaric/malic acid ratio. Effects of rootstock on YAN were not significant at Padthaway. At Irymple, Ramsey and C114 had high YAN and C113 and C20 low YAN, although in all cases levels were above 150 mg/L required for successful fermentation. Berries of C113 had high anthocyanin at both sites whereas C114, and to a lesser degree Ramsey had low anthocyanin at both sites. 140 Ruggeri had high berry anthocyanin at Padthaway but relatively low anthocyanin at Irymple. There was no effect of rootstock on phenolic substances at Irymple. At Padthaway 1103 Paulsen and C20 had high phenolics and Ramsey and C114 low phenolics.

Across the two sites (n=12), yield was highly correlated with bunch number (r= 0.97), bunch weight (r = 0.85) and berry weight (r=0.91), but not berries per bunch. This indicates that bunch number was the major yield determinant across the two sites. Also, bunch number was correlated with bunch weight (r = 0.70) and berry weight (r = 0.86) and bunch weight correlated with berry weight (r = 0.82) but not berries per bunch. These results indicate that rootstock genotypes which had high bunch numbers were likely to develop larger bunches with bigger berries independent of site. The multilinear regression equation provided below involving yield determinants, bunch number, berry weight and berries per bunch from both sites, was highly significant (adj. R^2 = 0.99) with all contributing factors highly significant ($p<0.001$).

Yield = -33.52 + 0.102 bunches + 15.54 berry weight + 0.172 berries per bunch

From the results presented in Table 6.1.4, it is apparent that crop development across the two sites could be linked to conferred rootstock vigour. This was confirmed by correlation analyses (n=12) showing that pruning weight was highly correlated with yield (r = 0.91) and bunch number (r = 0.90) and strongly correlated with bunch weight (r = 0.70) and berry weight (r = 0.83). The detailed presentation provided in Figure 6.1.3 describes how these responses are impacted by site and by the magnitude of the rootstock response at each site. Quite clearly, the differences in pruning weight across the genotypes were greater at the fully irrigated, mechanically hedged Irymple site compared to the Padthaway site irrigated with a limited supply of saline water. Yield, bunch number, bunch weight and berry weight were lower at the Padthaway site compared to the Irymple site. The results indicate that both site and rootstock responses at each site were major contributors to the overall responses

described above. Pruning weight was not associated with bunch weight at the Irymple site, compared to strong responses at the Padthaway site. The slope of the regression between berry weight and pruning weight at the individual sites was much lower at the Irymple site compared to the Padthaway site. The difference in rootstock responses for the bunch weight and berry weight to pruning weight at the two sites are most likely due to effects of abiotic stress, associated with the limited supply of saline irrigation water on berry development at the Padthaway site. In contrast, the slope of the relationship between pruning weight and bunch number was similar at both sites. This is somewhat surprising given the very different approaches to pruning at the two sites. A more detailed study would be required to identify if this relationship is associated with differences across the rootstocks in node numbers, budburst or shoot fruitfulness.

Discussion

The main purpose of the studies reported above was to assess the long-term performance of the selected rootstock genotypes (C113, C114 and C20) compared to commercial rootstocks (1103 Paulsen, 140 Ruggeri, Ramsey and 101-14). In doing so, the study has shown that rootstock effects in two regions with very different environment and management practices were stable over time and vine age. In contrast, rootstock effects were not consistent between regions or when grafted with different scions, an indication that different rootstocks may be required to optimise performance in different regions or when grafted with different scions. The Irymple study with different scions across rootstocks grown in a hot climate also indicated that different management practices may be required to optimise the performance of different cultivars. For example, it is clear that the Cabernet Sauvignon vines were excessively vigorous and low cropping compared to the performance of Chardonnay or Shiraz. The response of Cabernet Sauvignon, grafted on the various rootstocks, to a reduced irrigation supply or lighter mechanical hedging or minimal pruning warrants investigation.

The study, together with information collected previously for ungrafted plants (i.e. vigour, K uptake, salt and drought tolerance and root knot nematode and phylloxera tolerance/resistance) has provided strong evidence of field performance to support the naming and release of the new rootstocks for adoption by industry with PBR protection. The summary cases for each of the new rootstock candidates (C113, C114 and C20) compiled for each PBR application is provided in section 1.3 below. C113 has been selected as a moderate vigour alternative rootstock to 140 Ruggeri and 1103 Paulsen; C114 has been selected as a high vigour alternative rootstock to Ramsey; and C20 has been selected as a low vigour rootstock alternative to 101-14 Mgt.

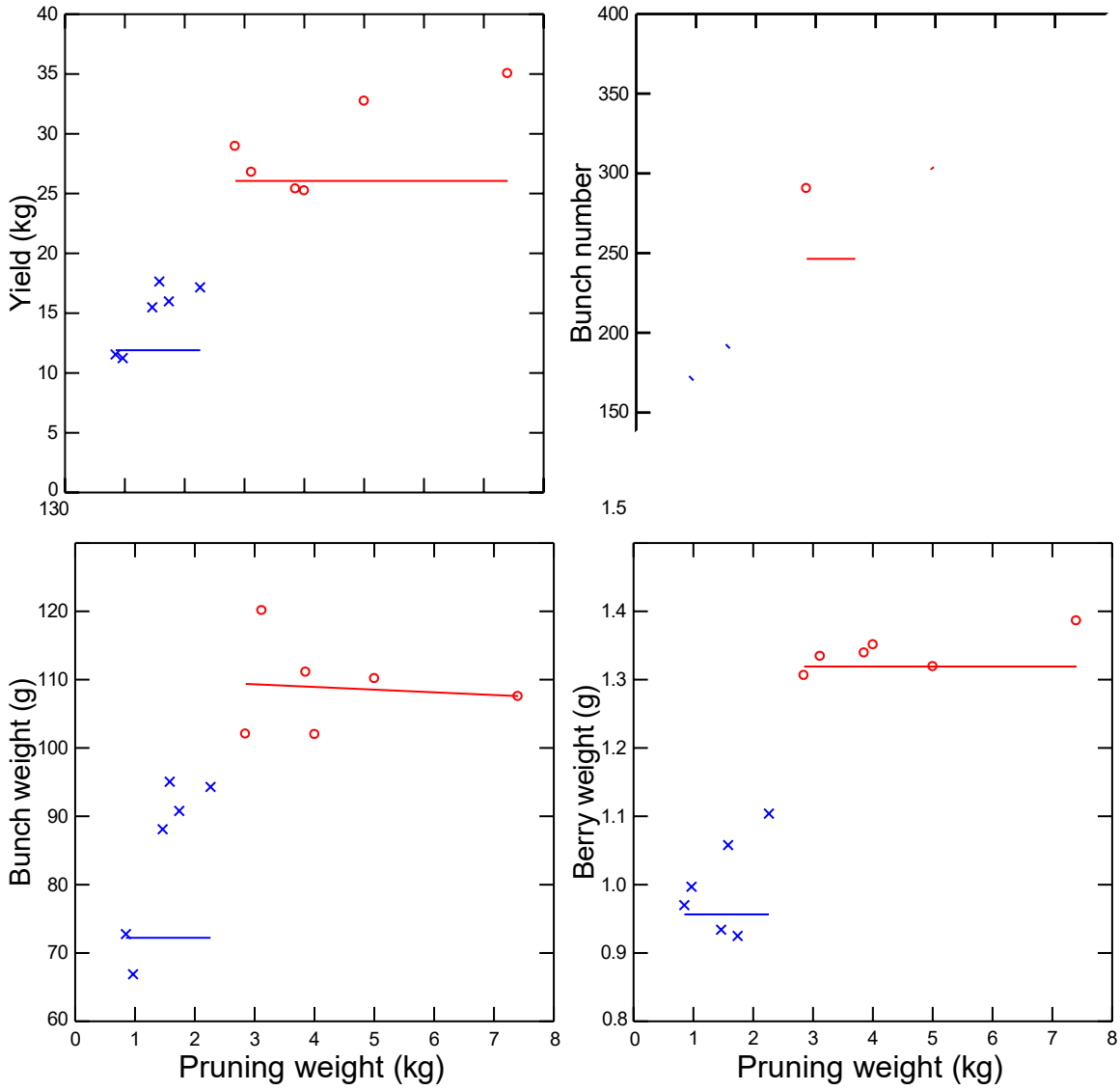


Figure 6.1.3. Relationships between mean conferred vine vigour (pruning weight) and yield (top left), bunch number (top right), bunch weight (bottom left) and berry weight (bottom right) for six rootstock genotypes located at two sites, Irymple (red) and Padthaway (blue).

6.2. Rootstocks that perform well under deficit irrigation (F86).

Shiraz CSIRO Irymple

Background

The Shiraz trial, planted in 2010, was designed to evaluate the field performance of 55 of the most promising drought tolerant genotypes selected under nursery conditions, from 310 genotypes in project (CSP 0503). It included own roots and four commercial rootstocks (Ramsey, 140 Ruggeri, 1103 Paulsen and Merbein 5489). The trial incorporated two irrigation treatments, a full irrigation (control, 5.5 ML/ha per annum) and a deficit irrigation treatment (2.5 Ml/ha per annum), which were applied to individual blocks to minimise potential water movement between irrigation treatments. Volumetric water contents of the control treatment during the growing season at 30 cm was 22.0-22.5% and at 60 cm, 20.0-20.5%. In contrast, the average water contents the deficit treatment at 30 cm was around 15% and at 60 cm, 16.0- 16.5%. (CSP 1304, Clingeleffer and Smith 2018).

Initial results from the trial, conducted for the young vines were reported previously for seasons 2013-17 (CSP 1304, Clingeleffer and Smith 2018). In that study, a systematic approach was used to select the most promising rootstocks genotypes based on their field performance and fruit composition. Key criteria used in this process were ability of rootstock genotypes to maintain acceptable productivity under deficit irrigation with low pH and malate levels and high berry anthocyanins. Based on the results as young vines, poor performing genotypes were identified and not assessed post the 2018 season. In total 26 new rootstock genotypes, own rooted vines and the four commercial rootstocks were assessed, seasons 2018-2022. The 27 new rootstock genotypes included the 12 most promising selections identified previously as young vines (CSP 1304, Clingeleffer and Smith 2018).

The early study with young vines confirmed that rootstocks provide a powerful tool to manipulate vine growth characteristics. Across the 60 rootstock genotypes there were eight-fold differences in conferred vigour (pruning weight), three-fold differences yield and a nine-fold differences in the Ravaz Index (yield to pruning weight ratio). The results also showed significant potential to select rootstocks with lower pH, malate and YAN than standard rootstocks. The study also found large differences in berry mineral concentrations with 2-3-fold differences in B, K, Mg, P and S. The long-term deficit irrigation treatment (45% of control) over all rootstock genotypes reduced yield by only 6%, due in part to an average 9% increase in fruitfulness (bunches per vine), despite significant reductions in bunch weight (13%) associated largely with reduced berry size (9%). The increased fruitfulness was likely to be a result of improved light conditions during fruit bud initiation associated with a 13% reduction in pruning weight, and by inference canopy size. Despite the small differences in yield, the long-term deficit irrigation significantly impacted fruit composition promoting early ripening (0.8 ^{o}Brix), higher pH, tartrate, anthocyanin and phenolic substances and lower TA, malate and YAN. The increases in tartrate, anthocyanin and phenolics were consistent with effects of concentration associated with the reduction in berry size with the deficit irrigation.

The previous study also demonstrated that vine growth characteristics, particularly conferred

vigour, have a major impact on fruit and wine composition. Consequently, rootstocks and deficit irrigation, which have significant effects on vine growth characteristics, provide growers with powerful tools to enhance wine quality attributes. For example, over both irrigation treatments rootstocks which conferred high vigour were likely to have high juice TSS, pH, TA, malate, tartrate, YAN, K, P and S; high must pH and TA; a high requirement for acid addition for pH adjustment during winemaking; and high wine pH, TA and VA. Similarly, high yielding rootstock genotypes were likely to have low TSS, berry anthocyanins, phenolics, Ca and P and high must pH; a high requirement for acid addition for pH adjustment; high wine pH and hue and reduced colour density. Rootstocks with large berries were likely to have high juice TSS, pH, TA, malate, tartrate, YAN, K, P and S but low anthocyanin and phenolics; high must pH; a high requirement for acid addition for pH adjustment; high wine pH, TA, VA and hue (i.e. less bright) and reduced colour density. The study also demonstrated that not all relationships between growth characteristics and fruit composition for rootstock genotypes were stable across irrigation treatments.

In summary it can be concluded that rootstock growth characteristics can be linked to must and wine quality attributes. Rootstocks with good yields but moderate conferred vigour which produce smaller bunches with fewer, small berries are likely to have lower must pH and require less acid addition for pH adjustment, leading to wines without excessive levels of acidity and with enhanced spectral properties. The results show significant potential to identify new rootstocks with enhanced fruit and wine composition and enhanced water use efficiency, capable of maintaining productivity with reduced water supply. Rootstock behaviour and impacts on fruit composition varied across irrigations treatments, indicating a requirement for rootstock evaluation to be conducted under both well-watered and limited water supply conditions. In this project, opportunities have been undertaken to:

- Confirm that the performance of rootstock traits was stable for mature vines in full production
- Confirm that the selection processes applied to young vines were applicable to older vines
- Confirm that rootstock responses to deficit irrigation were stable in the long-term
- Confirm that relationships between rootstock growth traits, fruit composition and wine composition were stable over time
- Identify new rootstock genotypes for release to industry

Results

The long-term performance and fruit composition of the 32 rootstock genotypes, including the five standards, retained for long term evaluation was assessed over 10 seasons (2013-22) for production traits and eight seasons (2014-21) for composition traits. A combined ANOVA analysis was undertaken to assess the significance of season, rootstock genotype and irrigation, the later included as a random variable despite limitations of the experimental design (Table 6.2.1). The analyses show highly significant effects of season on all traits measured; highly significant effects of irrigation treatment on almost all traits except yield

which was just significant ($p<0.05$) and YAN which was not significant; and, highly significant effects of rootstock genotype on all traits except tartaric acid. The absence of significant interactions for season x irrigation effects on yield, yield components, pruning weight and YAN indicates that these traits were stable over time. In contrast, significant season x irrigation interactions indicate that effects of irrigation on fruit composition was not consistent across seasons. Season x rootstock interactions were not significant for almost all traits, indicating stability across seasons for yield, yield components, growth traits, TA, malic acid, YAN and anthocyanins. In contrast, significant season x rootstock interactions were found for TSS, pH and tartaric acid (just significant, $p<0.05$) an indication that the effect of rootstock on these traits varied to varying degrees with season. There were highly significant irrigation x rootstock interactions for all traits except berries per bunch, tartaric acid, anthocyanins and phenolics. There were no significant season x irrigation x rootstock interactions.

Table 6.2.1. Significance values describing effects of season irrigation, rootstock, season x irrigation and irrigation x rootstock interactions on yield, yield component and fruit composition traits for 32 rootstock genotypes over 10 seasons (2013-22). Juice mineral data collected in 2016 are also included.

	Year	Irrigation	Rootstock	Year x Irrigation	Year x Rootstock	Irrigation x Rootstock
Yield	***	*	***	ns	ns	***
Bunches	***	***	***	ns	ns	***
Bunch wt.	***	***	***	ns	ns	***
Berry wt.	***	***	***	ns	ns	***
Berry/bunch	***	***	***	ns	ns	ns
Pruning wt.	***	***	***	ns	ns	***
Ravaz Index	***	***	***	*	ns	***
TSS	***	***	***	**	***	***
pH	***	***	***	***	**	***
TA	***	***	***	**	ns	***
Malic	***	***	***	**	ns	***
Tart	***	***	ns	***	*	ns
YAN	***	ns	***	ns	ns	***
Anthocyanin	***	***	***	***	ns	ns
Phenolics	***	***	***	***	ns	ns
K	-	ns	**	-	-	ns
Ca	-	*	ns	-	-	ns
Mg	-	ns	***	-	-	ns
Na	-	***	***	-	-	ns
P	-	*	ns	-	-	ns
S	-	ns	ns	-	-	ns
B	-	***	**	-	-	ns

*** indicates $p<0.001$, **, $p<0.01$ and* $p< 0.05$, respectively)

TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tart, tartaric acid; YAN, yeast assimilable nitrogen

Effect of season and deficit irrigation

The effects of season on yield, yield components and pruning weight for the two irrigation treatments across the 32 rootstock genotypes is shown in Figure 6.2.1. The significant effects of season on all traits and the absence of significant season x irrigation interactions are clearly shown (Table 6.2.1). Yield results showed that full production of the mechanically hedged vines was not achieved until season 2017 and that deficit irrigation produced minimal loss of yield in most seasons, except in the high yielding 2018 season. While the highest bunch numbers were recorded in 2018, the highly significant effect of irrigation on bunch number (Table 6.2.1) can be attributed to slightly higher bunch numbers in almost all seasons with the deficit treatment, presumably due to higher shoot fruitfulness although effects on bud number, budburst and subsequent higher shoot number cannot be ruled out as a potential cause. Bunch weight was highest in the first season (2013) and reduced by deficit irrigation in all seasons. Berry weight was highest in 2022 and reduced by deficit irrigation in all seasons. The high berry weight in 2022 is most likely associated with the very mild, wet and humid growing conditions and lack of berry wilting in season 2022. Berries per bunch were highest for the young vines in 2013 and reduced by deficit irrigation in all years, except 2021. Pruning weight was highest in the early seasons (2013-15) and reduced in the latter seasons (2018-21) most likely as the vines adapted to long term simulated mechanical pruning and retention of higher bud numbers once the vines were fully established. While the season x irrigation interaction was not significant for pruning weight, the effects of the deficit treatment were reduced in the latter seasons, possibly due to the development of larger root systems over time.

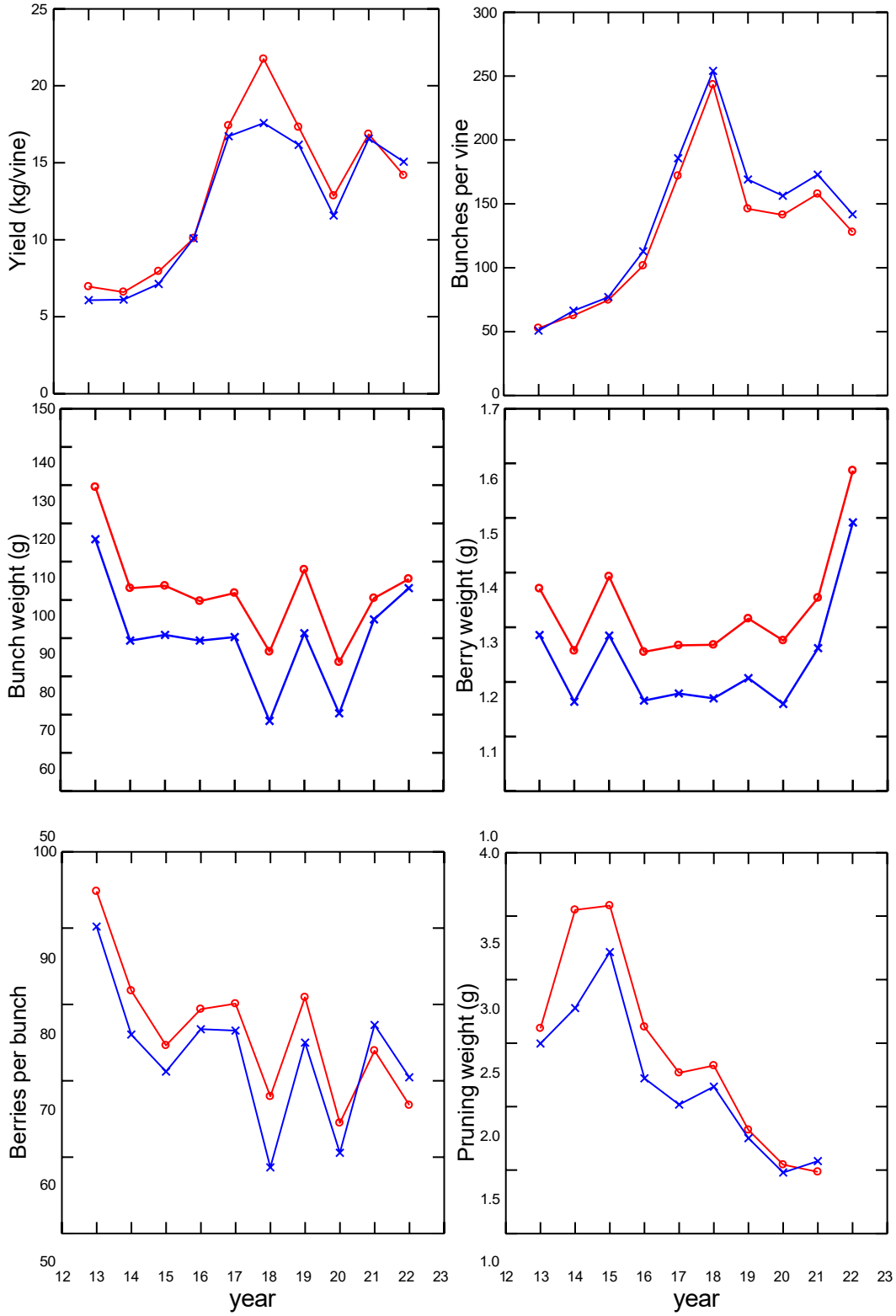


Figure 6.2.1. Effect of season on mean yield, yield components and pruning weight of 32 rootstock genotypes for two irrigation treatments (control red) and sustained deficit (blue), 2013-22.

The effects of season on fruit composition for the two irrigation treatments across the 32 rootstock genotypes is shown in Figure 6.2.2. The highly significant effects of season on all traits are clearly evident (Table 6.2.1). Juice TSS varied from 21.5 to 25 ^{o}Brix for the control treatment and 22.0 to 27.0 ^{o}Brix for the deficit treatment between seasons. Juice TSS was always higher with deficit irrigation than the control treatment, except in 2021. The significant season x irrigation interaction for TSS is largely associated with differences in the magnitude of the response to the deficit treatment between seasons (Table 6.2.1). Deficit irrigation produced higher juice pH than the control in all seasons. The significant season x irrigation interaction for pH is associated with differences in the magnitude of the response to the deficit treatment between seasons. Interestingly, the season to season variation in pH showed a biennial response for both irrigation treatments, which to the author's knowledge has never been reported previously. Juice TA of the deficit irrigation treatment was always lower than the control treatment, with the significant season x irrigation interaction explained by differences in the magnitude of the response. TA also displayed a biennial response that was opposite to the pH response and most likely a contributing factor to differences in the pH. Juice malic acid tended to decrease over time being highest in 2014 and 2015. While deficit irrigation tended to produce lower levels of malic acid than the control, this was not the case in 2019 and 2020. The magnitude of the response also varied between the other seasons contributing to the significant season x irrigation interaction. Juice tartaric acid of the deficit irrigation treatment was generally higher than the control, except in 2013 although the magnitude of the response varied between seasons contributing to the significant season x irrigation interaction. Juice YAN concentration of both irrigation treatments declined over time. Although there was no effect of irrigation on YAN (Table 6.2.1), in latter seasons (2018-21) the deficit treatment produced higher concentrations. The low YAN levels in 2020 and 2021 were close to, or below the 150 mg/L required for successful fermentation. There were 2-3 fold differences in anthocyanins and phenolics between seasons. Deficit irrigation produced higher anthocyanins and total phenolics in almost all seasons, although the magnitude of the responses varied between seasons, contributing to the significant season x irrigation interactions (Table 6.2.1).

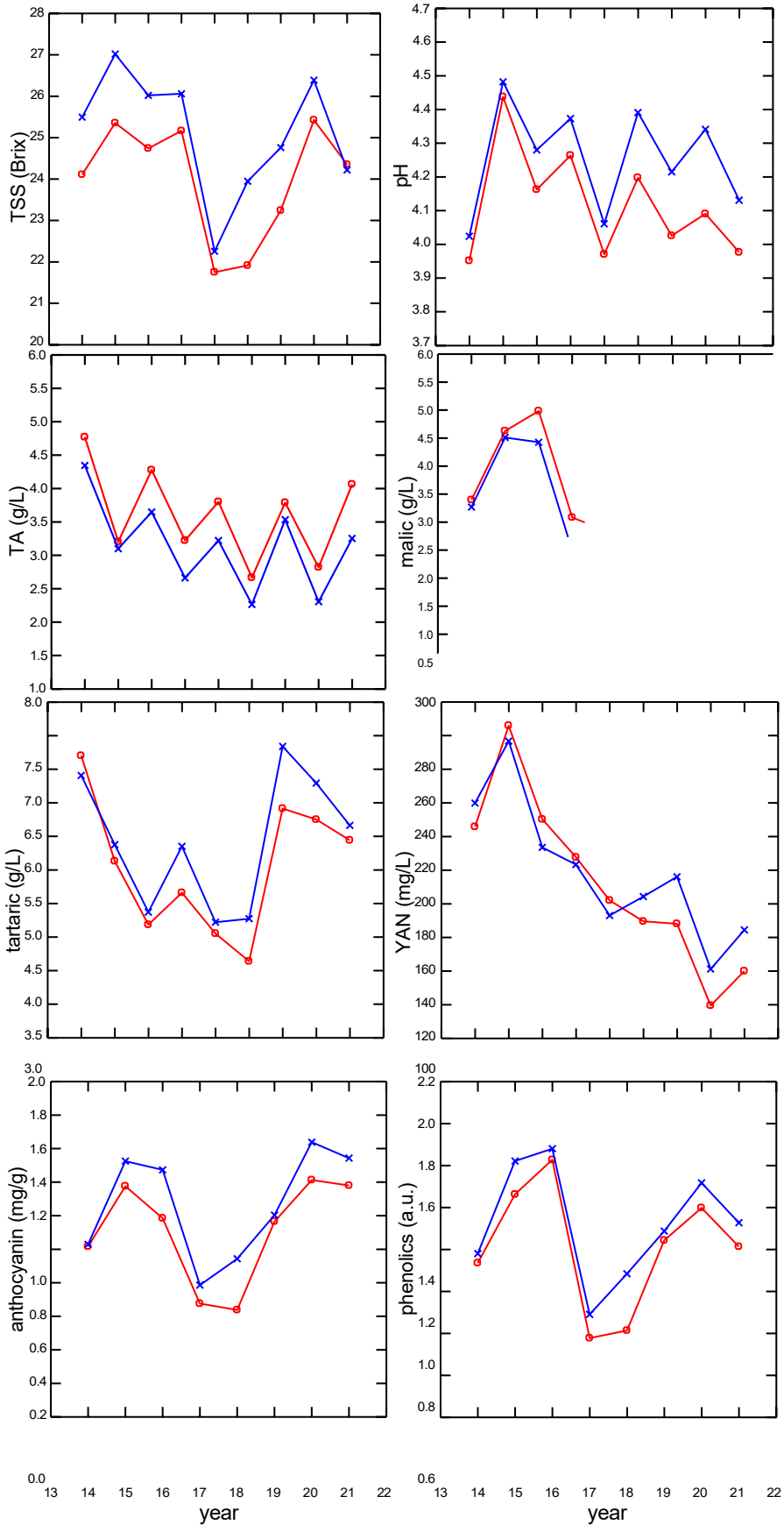


Figure 6.2.2. Effect of season on mean fruit composition traits of 32 rootstock genotypes for two irrigation treatments (control red) and sustained deficit (blue), 2014-21.

A summary of the overall effects of irrigation treatments discussed above on the mean values of measured traits over seasons across the 32 rootstock genotypes is shown in Table 2.1.2. In addition,

the effects of deficit irrigation on juice mineral concentrations measured in 2016 are included in Table 6.2.2. Compared to full irrigation, the deficit treatment produced a significant increase in Na and B and a significant reduction in Ca and P.

Table 6.2.2. Mean values for all measured traits for the full and deficit irrigation treatments over rootstock genotypes and respective seasons, or in the case of juice mineral concentrations measured in 2016.

	Irrigation	
	Full	**Deficit**
Yield (kg/vine)	**13.2**	**12.3**
Bunches/vine	**128**	**139**
Bunch wt. (g)	**102**	**90.5**
Berry wt. (g)	**1.33**	**1.24**
Berries/bun	**76.5**	**73.3**
Pruning wt. (kg/vine)	**2.42**	**2.19**
Ravaz Index	**8.50**	**7.01**
TSS (°Brix)	**24.0**	**25.1**
pH	**4.12**	**4.26**
TA (g/L)	**3.62**	**3.15**
Malic acid (g/L)	**3.04**	**2.72**
Tartaric acid (g/L)	**5.55**	**5.92**
YAN (mg/L)	192	199
Anthocyanin (mg/g)	**1.11**	**1.26**
Phenolics (a.u.)	**1.41**	**1.52**
Ca (mg/L)	**119**	**111**
K (mg/L)	1985	2080
Mg (mg/L)	102	102
Na (mg/L)	**24.2**	**50.2**
S (mg/L)	69.7	67.8
B (mg/L)	**14.5**	**18.1**
P (mg/L)	**122**	**101**

Significant differences ($p < 0.05$) are highlighted in bold.
TSS, total soluble sugars; TA, titratable acid; YAN, yeast assimilable nitrogen

Effect of rootstock genotype

Production and growth traits

The distribution of mean values for yield, yield components and pruning weight for the 32 rootstock genotypes across seasons and irrigation treatments is shown in Figure 6.2.3. There was a 2.6-fold difference in mean yield, range 7.6-20 kg per vine with more than 60% of genotypes were in the 10-15 kg/vine categories. Genotypes with low yield (<10kg/vine) included own roots (9.1 kg/vine) and Merbein 5489 (8.5 kg/vine). Genotypes with high yield (> 17.5 kg/vine) included Ramsey (18.2 kg/vine). There was more than a two-fold difference in bunch number per vine between rootstock genotypes, range 88-181, with more than 50% of genotypes in the 110-140 bunch number categories. Bunch weights ranged from 73.6-116.7 g, with 75 % of genotypes in the 90-110 g categories. Berry weights ranged from 1.15-1.41 g, with more than 80% in the 1.2-1.4 g categories. Berries per bunch ranged from 60-86 with 70% in the 70-80 category. There was almost a five-fold difference in pruning weight conferred by the rootstock genotypes (range 0.9-4.4 kg) with 35% in the 1-2 kg category and 45% in the 2-3 kg category. Own roots (1.5 kg) and Merbein 5489 (1.4 kg) were among rootstocks conferring low vigour while Ramsey (3.5 kg) conferred high vigour. The calculated Ravaz Index (yield/pruning weight), often referred to as vine balance ranged from 4.0 to 13.7 with 80% of genotypes in the normally accepted range between 5-10 (data not shown).

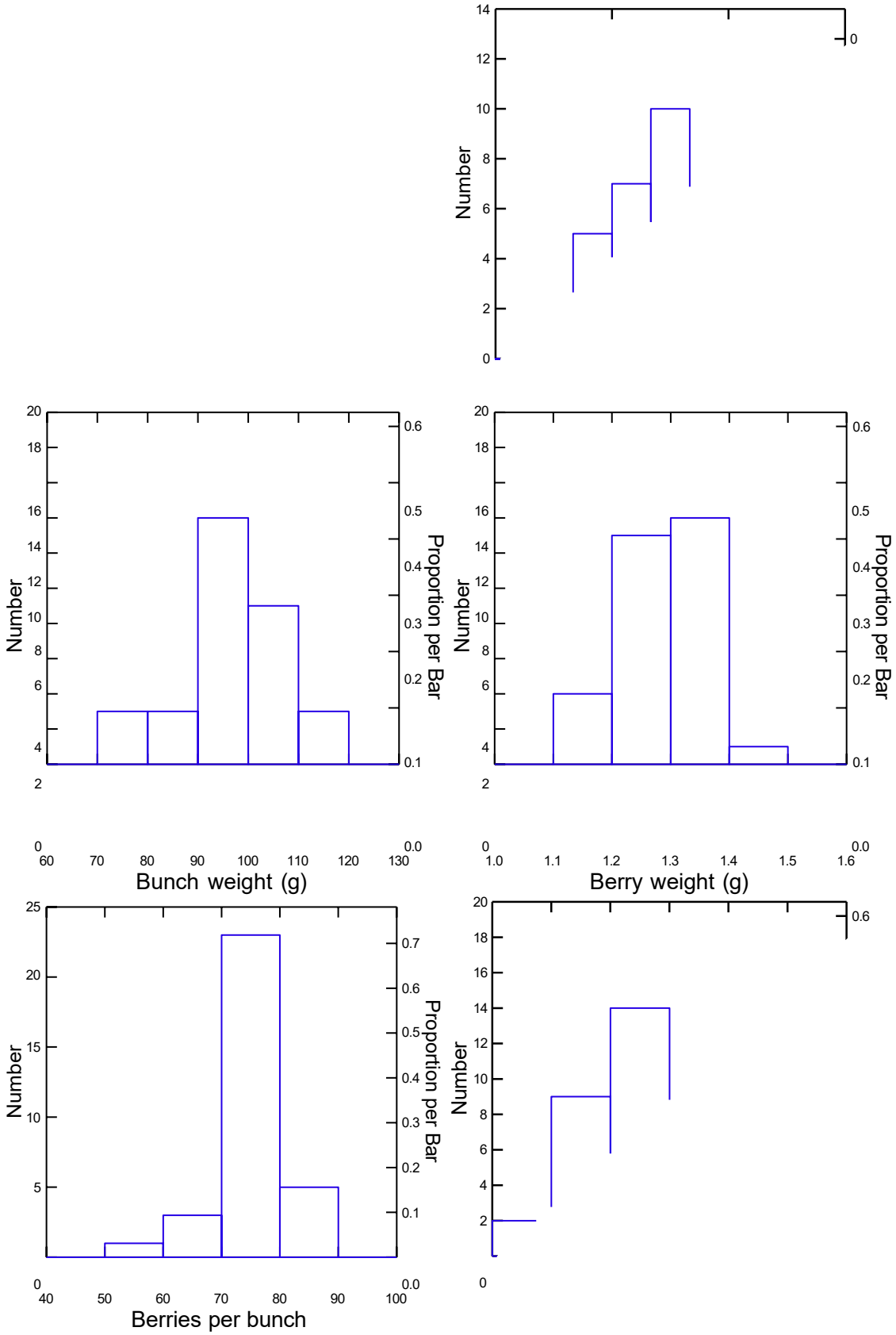


Figure 6.2.3. Distribution of mean yield, yield components and pruning weight across 32 rootstock genotypes over 10 seasons, 2013-2022.

Fruit composition

The distribution of mean values for fruit composition traits for the 32 rootstock genotypes across seasons and irrigation treatments is shown in Figure 6.2.4. Juice TSS ranged from 23.4-25.6 ^{o}Brix across the rootstock genotypes, with 40% in the 24.5-25 ^{o}Brix category. Juice pH across the rootstock genotypes ranged from 4.07-4.32 with 50% in the 4.1 to 4.2 pH category and 30% in the 4.2-4.3 pH category. Juice TA concentrations ranged from 2.89-4.01 g/L with 65% of genotypes in the 3.0-3.5 g/L category. Juice malic acid concentrations ranged from 2.06-3.87 g/L with 30% of genotypes in each of the 2-2.5, 2.5-3.0, 3.0-3.5 g/L categories. Differences in juice tartaric acid between rootstock genotypes, range 5.1-6.1 were not significant (Table 6.2.1). Juice YAN concentrations ranged from 156-259 mg/L with 40% of genotypes in the 175-200 mg/L category. All rootstock genotypes had levels above 150 mg/L required for successful fermentation without N supplementation.

There were 1.7 and 1.6-fold differences in berry anthocyanin concentrations across the rootstock genotypes (Figure 6.2.5). Berry anthocyanin concentrations ranged from 0.89-1.51 mg/g with 45% of genotypes in the 1.0-1.2 mg/g category and 20% in the 1.2-1.3 category. Berry phenolic concentrations ranged from 1.25-2.04 a.u., with 40% of genotypes in the 1.4-1.5 a.u. category.

Mineral element composition

The distribution of mean values for juice mineral elements measured in 2016 for the 32 rootstock genotypes across irrigation treatments is shown in Figure 6.2.6 Juice K was the dominant ion with the distribution strongly skewed to the lower end with a range from 1532 – 2682 mg/L, with 50% in the 1700-2000 mg/L category. Ca ranged from 77-152 mg/L, with 65% in the 110-130 categories. Mg ranged from 75-129 mg/L with 90% in the 80-110 mg/L categories. The distribution of Na was highly skewed to the lower end with a range of 7-118 mg/L with 65% in categories below 30 mg/L. S ranged from 48-98 mg/L with 90% in the 50-80 mg/L categories. B ranged from 11-23 mg/L, with 30% in the 13-15 mg/L category. P distribution was skewed to the lower end, range 74-170 mg/L with 40% in the 60-85 mg/L categories.

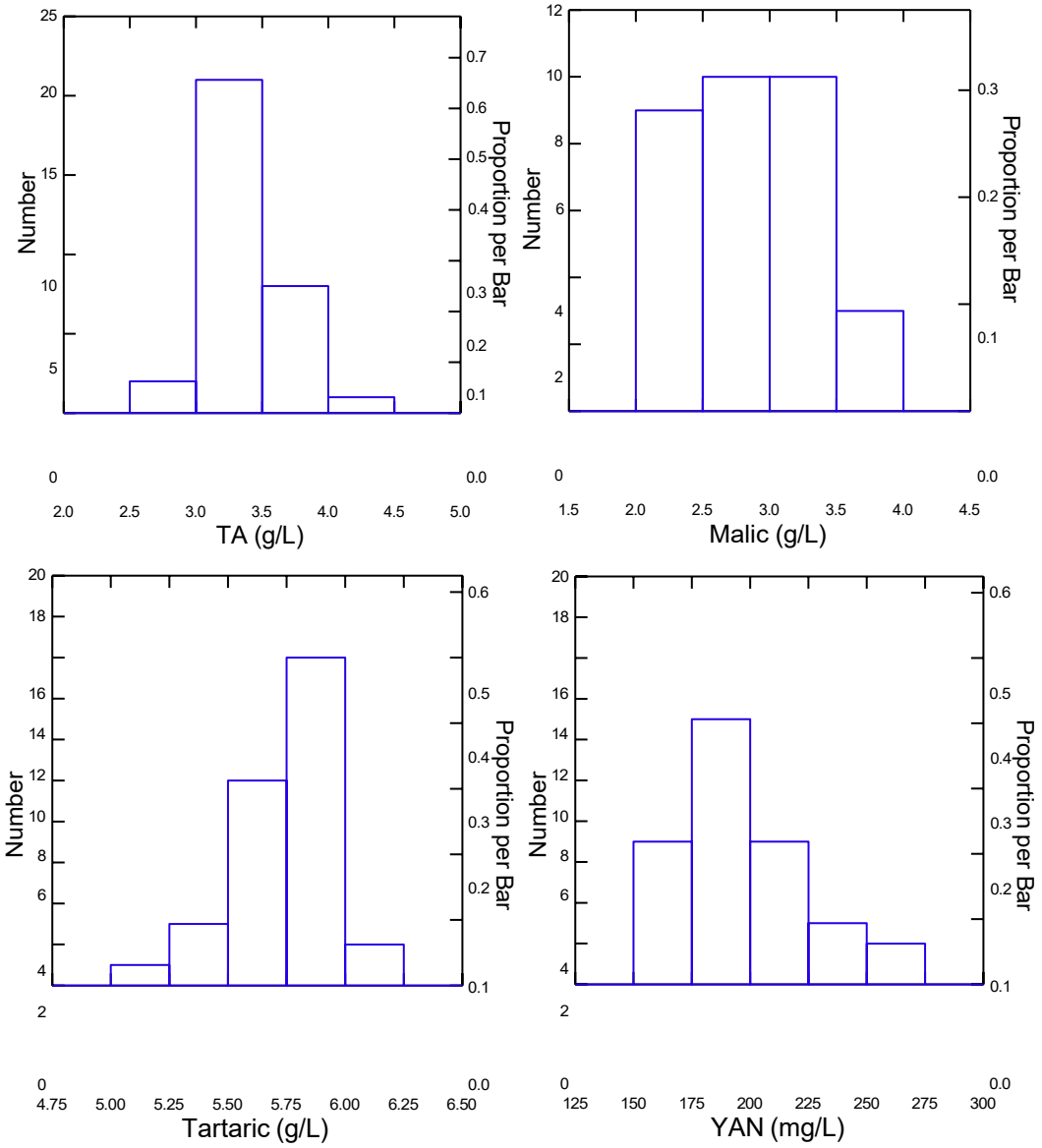


Figure 6.2.4. Distribution of mean fruit composition traits across 32 rootstock genotypes over eight seasons, 2014-2021.

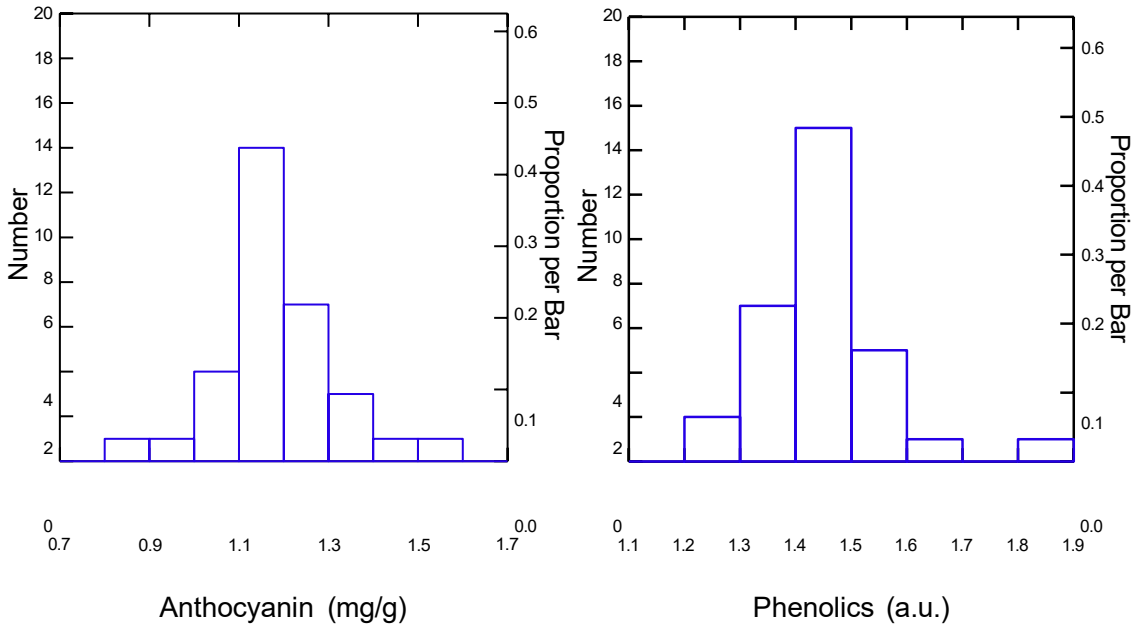
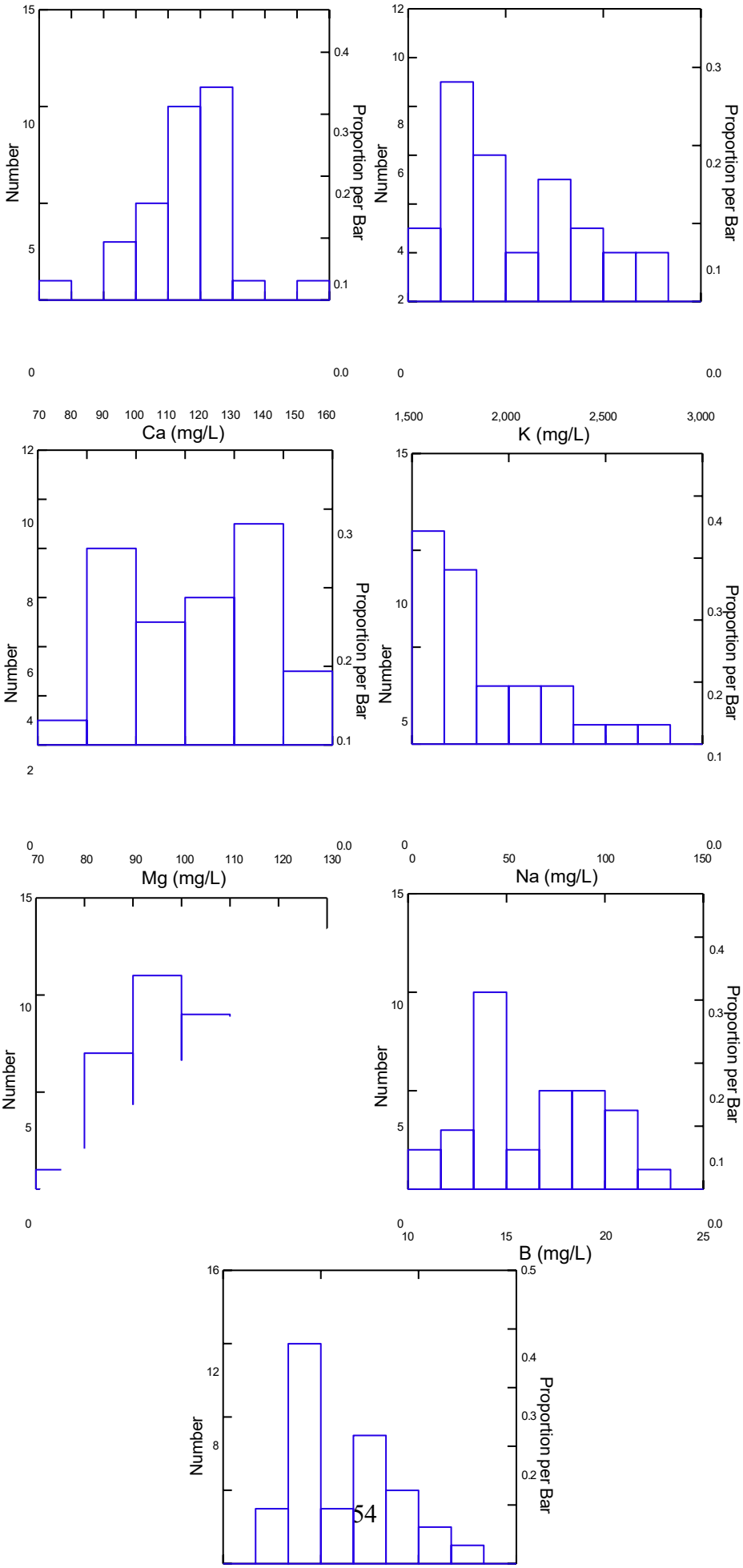


Figure 6.2.5. Distribution of berry anthocyanin and phenolic substances across 32 rootstock genotypes over eight seasons, 2014-2021.


Number
Proportion per Bar
Ca (mg/L)
K (mg/L)
Mg (mg/L)
Na (mg/L)
B (mg/L)

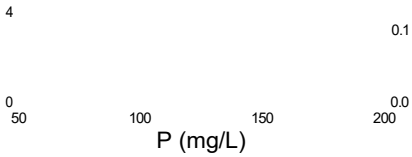


Figure 6.2.6. Distribution of juice mineral elements across 32 rootstock genotypes (2016).

Effect of irrigation on relationships between rootstock performance traits and fruit composition

Correlation coefficients describing relationships between vine field performance traits and fruit composition across the 32 rootstock genotypes for both irrigation treatments are provided in Table 6.2.2. The results show that many of the strong relationships established for fully irrigated rootstock genotypes are not maintained under sustained deficit irrigation. The results indicate that crop development is impacted by irrigation treatments. With the fully irrigated control, there were strong positive correlations between yield and bunch number, bunch weight, berry weight, berries per bunch and pruning weight and a negative association with the Ravaz Index. With the deficit treatment, correlations between yield and both berries per bunch and Ravaz Index were not significant. Multilinear regression models showed that bunch number, berries per bunch and berry weight respectively accounted for 79.0%, 15.2 % and 2.6% of the variation in yield of the rootstocks with the control irrigation and 79.5%, 8.2% and 6.6% of the variation in yield with the sustained deficit treatment. The highly regression equations for these models are provided below.

Irrigation 1 Yield = -24.69 + 0.094 Bunches + 9.45 Berry wt. + 0.173 Berries/bunch (adj. R^2 =0.97)

Irrigation 2 Yield = -30.79 + 0.087 Bunches + 16.02 Berry wt. + 0.153 Berries/bunch (adj. R^2 =0.94)

Significant correlations between yield and fruit composition traits show that higher yielding rootstock genotypes grown under full irrigation were more likely to have a negative impact on fruit composition with low TSS, high pH, high TA, high malic acid, low tartaric acid, high YAN and low anthocyanin and phenolic substances. Yield was also positively correlated with juice K, S and B concentrations with full irrigation. Correlations with yield and fruit composition traits were not significant for TSS, pH and tartaric acid, K, B with deficit irrigation. Significant correlations between bunch number and fruit composition show that rootstock genotypes with higher bunches grown under full irrigation were more likely to have a negative impact on fruit composition with low TSS, high pH, high malic acid, low tartaric acid, high YAN and low anthocyanin and phenolic substances. Bunch number was also positively correlated with juice K and S concentrations with full irrigation. Correlations with bunch number and fruit composition traits were not significant for TSS, pH, tartaric acid and K with deficit irrigation. Significant correlations between bunch weight and fruit composition show that rootstock genotypes with high bunch weights grown under full irrigation were more likely to have a negative impact on fruit composition with low TSS, high TA, high malic acid, low tartaric acid, high YAN and low anthocyanin and phenolic substances. Bunch weight was also positively correlated with juice K and S with full irrigation. Correlations with bunch weight and fruit composition traits were not significant for pH, TA, malic acid, YAN, K and S with deficit irrigation. Significant correlations between berry weight and fruit composition traits show

that rootstock genotypes with large berries grown under full irrigation were more likely to have a negative impact on fruit composition with high TA, high malic acid, high YAN and low anthocyanin and phenolic substances. In the case of berry weight, similar relationships with were found with deficit irrigation. Berry weight was positively correlated with juice K, Mg, S and B concentrations with full irrigation but only with S with deficit irrigation. With full irrigation, responses to berries per bunch were similar to bunch weight as significant correlations between bunch weight and fruit composition traits show that rootstock genotypes with a high number of berries per bunch were more likely to have a negative impact on fruit composition with low TSS, high TA, high malic acid, low tartaric acid, high YAN and low anthocyanin and phenolic substances. Under deficit irrigation correlations with berries per bunch were not significant with pH, TA, malic acid, YAN, anthocyanin and phenolic substances. Berries per bunch was negatively correlated with Ca with full irrigation and negatively correlated with Mg and Na with deficit irrigation.

The significant correlation results for pruning weight show that rootstock which confer high vigour to the Shiraz scion under full irrigation are likely to have high pH, high TA, high malic acid (very strong r = 0.87), high YAN and low anthocyanin and phenolic substances. Similar results were found with deficit irrigation except that the correlation between pruning weight and pH was not significant. With both irrigation treatments pruning weight was positively correlated with K, Mg, S and P concentrations and also B with full irrigation. With full irrigation, the Ravaz Index was negatively correlated with TA, malic acid and YAN but positively correlated with both anthocyanin and phenolics an indication that rootstocks with higher high Ravaz Index values were likely to have enhanced fruit composition. With deficit irrigation there were negative correlations between Ravaz Index and TSS, pH, malic acid, tartaric acid and YAN and a positive correlation with phenolics. The Ravaz index was negatively correlated with juice K, Mg, S and B with full irrigation and K and P with deficit irrigation.

Relationships between fruit composition traits and yield across the rootstock genotypes were impacted by irrigation treatment (Table 6.2.3). In the full irrigation treatment, there were significant positive correlations between TSS and tartaric acid, anthocyanins and phenolic substances. With deficit irrigation, the only positive correlations were between TSS and pH and tartaric acid. TSS was positively correlated with juice Ca and P concentration with full irrigation and K concentration with deficit irrigation. pH was positively correlated with malic acid and YAN with both irrigation treatments and also with tartaric acid and phenolic substances (negative) with deficit irrigation. Juice pH was positively correlated with K, S and P with full irrigation and K, Mg, Na, S, B and P concentration with deficit irrigation. TA was strongly correlated with malic acid and YAN in both irrigation treatments, and negatively with anthocyanin with deficit irrigation. TA was also positively correlated with K, Mg, S and B concentration with full irrigation and Mg, S and P with deficit irrigation. Relationships with malic acid and other fruit composition traits were stable across both irrigation treatments.

Malic acid was positively correlated with YAN and negatively correlated with anthocyanin and phenolic substances in both irrigation treatments. Malic acid was positively correlated with K, Mg, S, B, and P with both irrigation systems. In contrast, tartaric acid was negatively correlated with YAN and positively correlated with anthocyanin and phenolic substances with full irrigation but not with deficit irrigation. Tartaric acid was only positively correlated with Na with full irrigation but positively correlated with most ions including K, Mg, S, B and P with deficit irrigation. YAN was negatively correlated with anthocyanin and phenolic substances in both irrigation treatments. YAN was positively correlated with K, Mg, S, B and P with both irrigation treatments. Anthocyanin was positively correlated with phenolic substances in both irrigation treatments. Anthocyanin was negatively correlated with S with deficit irrigation. Phenolics were negatively linked to K, S and P with deficit irrigation.

Table 6.2.3. Correlation coefficients (r) between yield, its components and growth traits and fruit composition across 32 rootstock genotypes for control and deficit irrigation treatments.

	Yield	Bunches	Bunch wt.	Berry wt.	Berry/ bunch	Pruning wt.	Ravaz Index
Bunches	**0.89**					**Irrigation 1**	
Bunch wt.	**0.85**	**0.55**					
Berry wt.	**0.73**	**0.51**	**0.82**				
Berry/bun	**0.78**	**0.49**	**0.94**	**0.59**			
Pruning wt.	**0.79**	**0.64**	**0.70**	**0.73**	**0.57**		
Ravaz Index	**-0.55**	**-0.49**	**-0.53**	**-0.62**	**-0.41**	**-0.65**	
TSS	**-0.46**	**-0.37**	**-0.49**	-0.32	**-0.48**	-0.16	0.08
pH	**0.42**	**0.52**	0.22	0.15	0.26	**0.45**	-0.15
TA	**0.42**	0.18	**0.55**	**0.69**	**0.37**	**0.65**	**-0.60**
Malic	**0.63**	**0.43**	**0.66**	**0.71**	**0.52**	**0.87**	**-0.57**
Tart	**-0.47**	**-0.43**	**-0.39**	-0.27	**-0.38**	-0.32	-0.02
YAN	**0.61**	**0.47**	**0.55**	**0.54**	**0.46**	**0.82**	**-0.42**
Anthocyanin	**-0.79**	**-0.71**	**-0.72**	**-0.63**	**-0.65**	**-0.50**	**0.38**
Phenolics	**-0.62**	**-0.54**	**-0.62**	**-0.43**	**-0.62**	**-0.39**	**0.53**
Ca	-0.28	-0.19	-0.26	0.05	**-0.40**	-0.20	-0.03
K	**0.48**	**0.37**	**0.45**	**0.55**	0.33	**0.70**	**-0.57**
Mg	0.28	0.31	0.16	**0.46**	-0.04	**0.48**	**-0.61**
Na	-0.10	0.02	-0.20	0.03	-0.31	-0.01	-0.16
S	**0.51**	**0.43**	**0.40**	**0.57**	0.24	**0.77**	**-0.58**
B	**0.38**	0.33	0.27	**0.45**	0.14	**0.58**	**-0.53**
P	0.23	0.11	0.23	0.29	0.16	**0.50**	-0.29
Bunches	**0.89**					**Irrigation 2**	
Bunch wt.	**0.67**	0.35					
Berry wt.	**0.74**	**0.58**	**0.57**				
Berry/bun	0.33	0.05	**0.85**	0.07			
Pruning wt.	**0.82**	**0.67**	**0.45**	**0.79**	0.04		
Ravaz Index	-0.18	-0.16	0.03	**-0.48**	0.30	**-0.56**	
TSS	-0.23	-0.06	**-0.54**	-0.04	**-0.61**	0.05	**-0.56**
pH	0.16	0.22	-0.09	0.23	-0.23	0.31	**-0.57**
TA	**0.48**	0.27	0.28	**0.51**	-0.01	**0.64**	-0.17
Malic	**0.55**	**0.38**	0.27	**0.65**	-0.09	**0.80**	**-0.57**
Tart	-0.29	-0.14	**-0.55**	-0.17	**-0.58**	-0.08	**-0.37**
YAN	**0.63**	**0.46**	0.32	**0.56**	0.02	**0.76**	**-0.38**
Anthocyanin	**-0.68**	**-0.54**	**-0.43**	**-0.67**	-0.08	**-0.61**	0.21
Phenolics	**-0.54**	**-0.43**	**-0.41**	**-0.67**	-0.10	**-0.52**	**0.42**
Ca	-0.06	-0.20	0.01	0.18	-0.08	0.13	-0.23
K	0.19	0.12	-0.02	0.32	-0.22	**0.40**	**-0.50**
Mg	0.23	0.24	-0.17	0.24	**-0.41**	**0.38**	-0.16
Na	-0.07	0.13	-0.38	-0.02	**-0.47**	0.00	-0.10
S	**0.46**	**0.40**	0.12	**0.40**	-0.13	**0.50**	-0.21
B	0.12	0.13	-0.16	0.14	-0.31	0.25	-0.21
P	0.30	0.16	0.13	0.32	-0.04	**0.53**	**-0.42**

Significant correlations ($p < 0.05$) are in bold (n=32). Where the relationships differ between irrigation treatments the correlations coefficients are shown in red.
TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tart, tartaric acid; YAN, yeast assimilable nitrogen; Bun, bunch; Prun, pruning weight

Table 6.2.4. Correlation coefficients between mean fruit composition traits across rootstock genotypes for control and deficit irrigation treatments based.

	TSS	pH	TA	Malic	Tart	YAN	Antho	Phenol
Irrigation 1								
pH	0.18							
TA	-0.22	-0.20						
Malic	-0.15	**0.43**	**0.74**					
Tart	**0.48**	-0.19	-0.15	-0.26				
YAN	-0.11	**0.48**	**0.56**	**0.85**	**-0.45**			
Antho	**0.60**	-0.33	-0.29	**-0.47**	**0.60**	**-0.50**		
Phenolics	**0.54**	-0.13	-0.33	**-0.36**	**0.45**	**-0.39**	**0.81**	
Ca	**0.35**	-0.31	-0.02	-0.21	0.11	-0.17	0.27	0.24
K	0.15	**0.46**	**0.43**	**0.75**	-0.09	**0.60**	-0.28	-0.24
Mg	0.15	0.21	**0.46**	**0.55**	0.07	**0.44**	-0.16	-0.17
Na	0.01	0.08	0.10	0.12	**0.38**	-0.03	0.05	-0.03
S	0.07	**0.39**	**0.55**	**0.76**	-0.18	**0.73**	-0.32	-0.29
B	0.20	**0.41**	**0.38**	**0.61**	0.04	**0.52**	-0.17	-0.15
P	**0.43**	0.26	0.28	**0.42**	-0.11	**0.50**	-0.06	-0.11
Irrigation 2								
pH	**0.50**							
TA	-0.11	-0.21						
Malic	0.20	**0.58**	**0.62**					
Tart	**0.61**	**0.54**	-0.13	0.20				
YAN	0.02	**0.44**	**0.68**	**0.89**	0.17			
Antho	0.06	-0.19	**-0.48**	**-0.58**	0.25	**-0.61**		
Phenolics	-0.06	**-0.49**	-0.25	**-0.57**	0.07	**-0.54**	**0.76**	
Ca	0.00	-0.08	0.18	0.10	0.19	0.02	0.17	0.05
K	**0.37**	**0.76**	0.23	**0.76**	**0.57**	**0.67**	-0.21	**-0.38**
Mg	0.16	**0.35**	**0.35**	**0.61**	**0.43**	**0.52**	-0.30	-0.08
Na	0.24	**0.34**	-0.08	0.19	**0.44**	0.15	-0.07	-0.03
S	0.10	**0.47**	**0.54**	**0.75**	0.24	**0.85**	**-0.50**	**-0.47**
B	0.31	**0.53**	0.24	**0.58**	**0.46**	**0.55**	-0.24	-0.15
P	0.17	**0.49**	**0.53**	**0.75**	0.22	**0.75**	-0.29	**-0.37**

Significant correlations ($p < 0.05$) are shown in bold (n=32). Where the relationships differ between irrigation treatments the correlations coefficients are shown in red.
TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tart, tartaric acid; YAN, yeast assimilable nitrogen; Antho, anthocyanin; Phenol, phenolics;

As reported above, irrigation treatments impacted relationships between field performance traits and fruit composition traits, including mineral elements, and between fruit composition traits across rootstock genotypes (Tables 6.2.3 and 6.2.4). Because response to deficit irrigation is expected to influence the selection of new rootstocks, both in this study and in the future, these relationships are explored in more detail in Figures 6.2.5 - 6.2.15.

The relationships between yield and fruit composition traits of 32 rootstock genotypes for control and deficit irrigation are shown in Figure 6.2.5. The relationships between TSS and yield were impacted by deficit irrigation leading to the significant increase in TSS across the rootstocks (Table 6.2.2) and a decoupling of the significant negative correlation described for full irrigation (Table 6.2.3). The relationships between pH and yield were impacted by deficit irrigation leading to the significant increase in pH across the rootstocks (Table 6.2.2) and a decoupling of the significant positive correlation described for full irrigation (Table 6.2.3). In contrast, deficit irrigation significantly reduced TA across the rootstocks (Table 6.2.2) but had little effect on the significant positive response between yield and TA (Table 6.2.3), as the slopes were similar. Similarly, deficit irrigation significantly reduced malic acid across the rootstocks (Table 6.2.2) but had little effect on the significant positive response between yield and malic acid (Table 6.2.3), as both slopes were similar. Compared to the control irrigation, deficit irrigation significantly increased tartaric acid (Table 6.2.2) but largely decoupled the significant negative relationship described for full irrigation (Table 6.2.3), although slopes for both relationships were similar. Effects of deficit irrigation on YAN were not significant (Table 6.2.2) and did not impact the highly significant positive relationship with yield (Table 6.2.3). Compared to the control irrigation, deficit irrigation significantly increased berry anthocyanin and phenolics (Table 6.2.2). In both cases the slopes of the highly significant negative relationships with yield (Table 6.2.3) were reduced by the deficit treatment, particularly with phenolics.

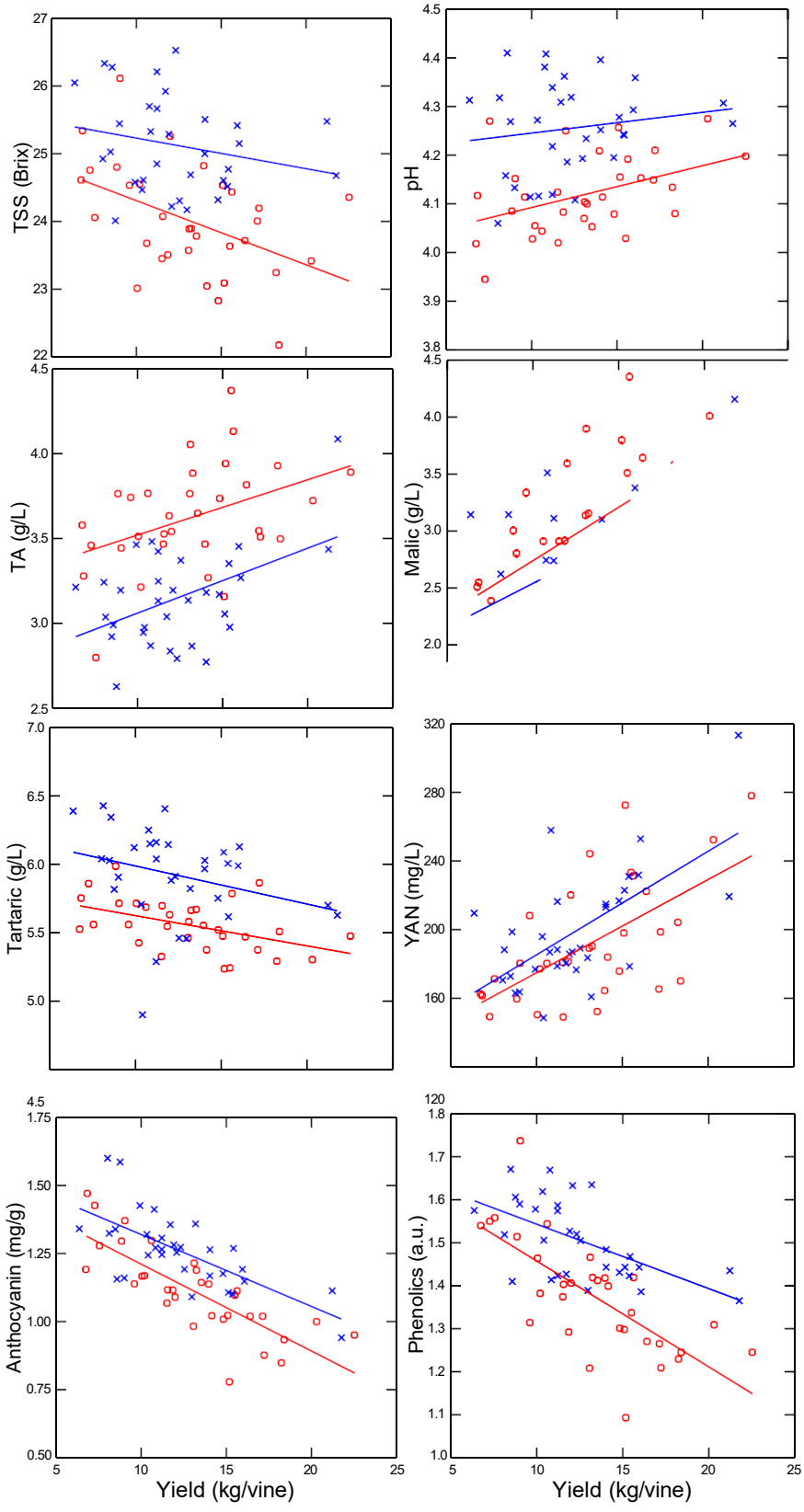


Figure 6.2.5. Linear relationships between means of fruit composition traits and mean yield for control (red) and sustained deficit (blue) irrigation treatments of 32 rootstock genotypes over nine seasons (2013-21). TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tartaric, tartaric acid; YAN, yeast assimilable nitrogen

The relationships between bunch number and fruit composition traits of 32 rootstock genotypes for control and deficit irrigation are shown in Figure 2.1.6. They highlight the significant effects of deficit irrigation on fruit composition compared to the full irrigation, which are provided in Table 6.2.2. The significant negative correlations between bunch number and TSS and tartaric acid and the positive correlation with pH described for full irrigation (Table 6.2.3) were uncoupled by the deficit treatment. With both irrigation treatments, the relationship between TA and bunch number was not significant (Table 6.2.3). The strong significant relationships between bunch number and malic acid (Table 6.2.3) for both irrigation treatments show that deficit irrigation tended to reduce the rate of change, as the slope was lower compared to the full irrigation. For both irrigation treatments, the highly positive significant correlations between bunch number and YAN (Table 6.2.3) were unaffected by deficit irrigation. For both irrigation treatments, there were strong negative relationships between bunch number and berry anthocyanins and phenolics (Table 6.2.3). In both cases, deficit irrigation reduced the slope of the regression, an indication that it reduced the rate of change as bunch number increased.

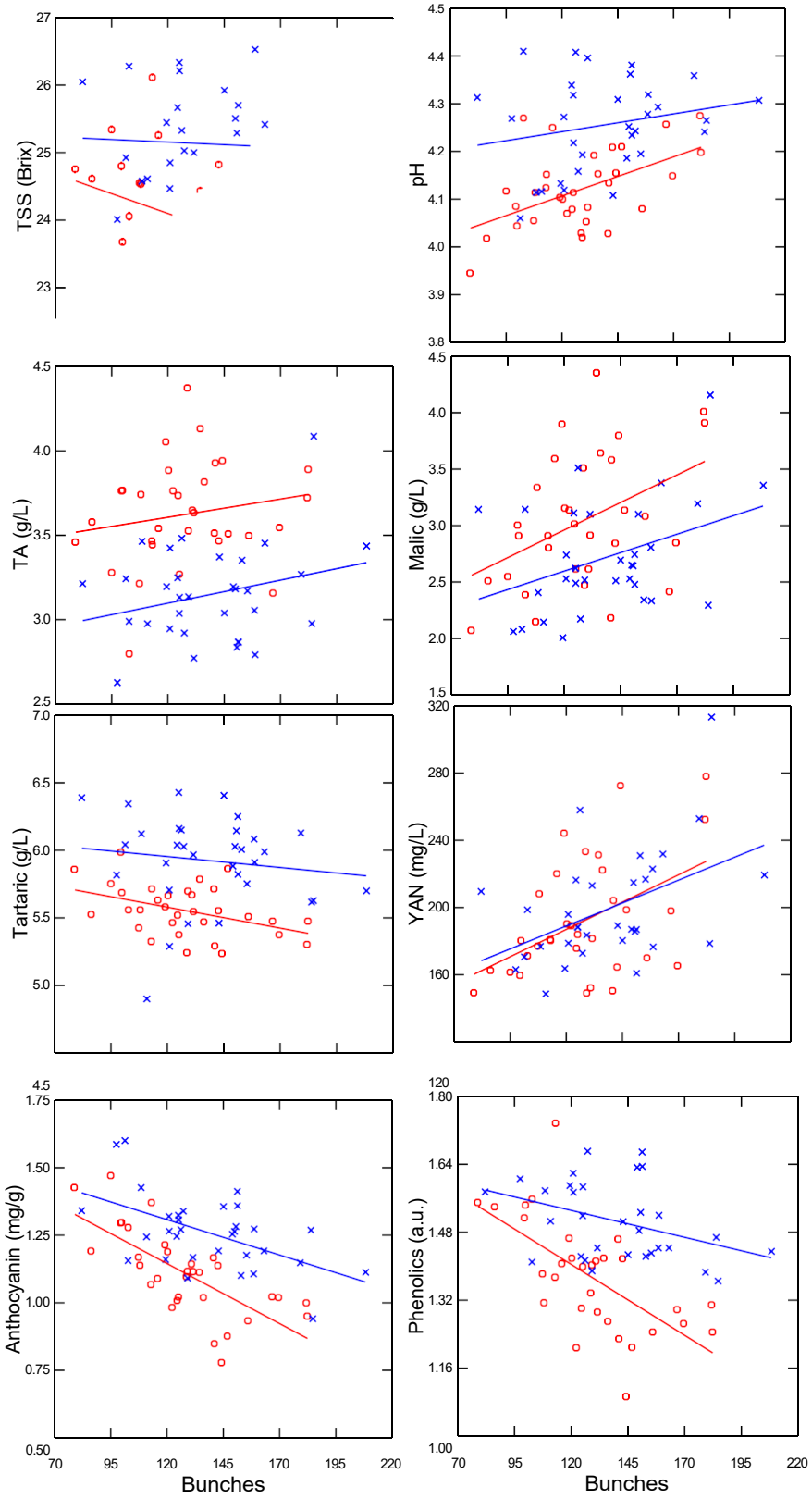


Figure 6.2.6. Linear relationships between means of fruit composition traits and mean bunch number for control (red) and sustained deficit (blue) irrigation treatments of 32 rootstock genotypes over nine seasons (2013-21). TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tartaric, tartaric acid; YAN, yeast assimilable nitrogen

Relationships between bunch weight and fruit composition traits of 32 rootstock genotypes for control and deficit irrigation are shown in Figure 6.2.7. They highlight the significant effects of deficit irrigation on fruit composition compared to the full irrigation, provided in Table 6.2.2. Furthermore, it is apparent that deficit irrigation reduced the range in bunch weights across the rootstock genotypes with a maximum of 105 g compared to 130 g with full irrigation. Both irrigation treatments produced strong negative relationships between TSS and bunch weight, indicating a role for bunch weight in competition for carbohydrates. Relationships between bunch weight and pH were not significant for both irrigation treatments (Table 6.2.3). The strong positive relationships between bunch weight and TA, malic acid concentration and YAN with the full irrigation treatment were uncoupled by deficit irrigation, as the correlations were not significant (Table 6.2.3). For both irrigation treatments, there were strong negative relationships between bunch weight and tartaric acid (Table 6.2.3). The steeper slope with the deficit treatment indicates a more rapid decline in tartaric acid as bunch weight increases. For both irrigation treatments, there were strong negative relationships between bunch weight and berry anthocyanins and phenolics (Table 6.2.3). Deficit irrigation had little impact on these relationships, as the slopes were similar.

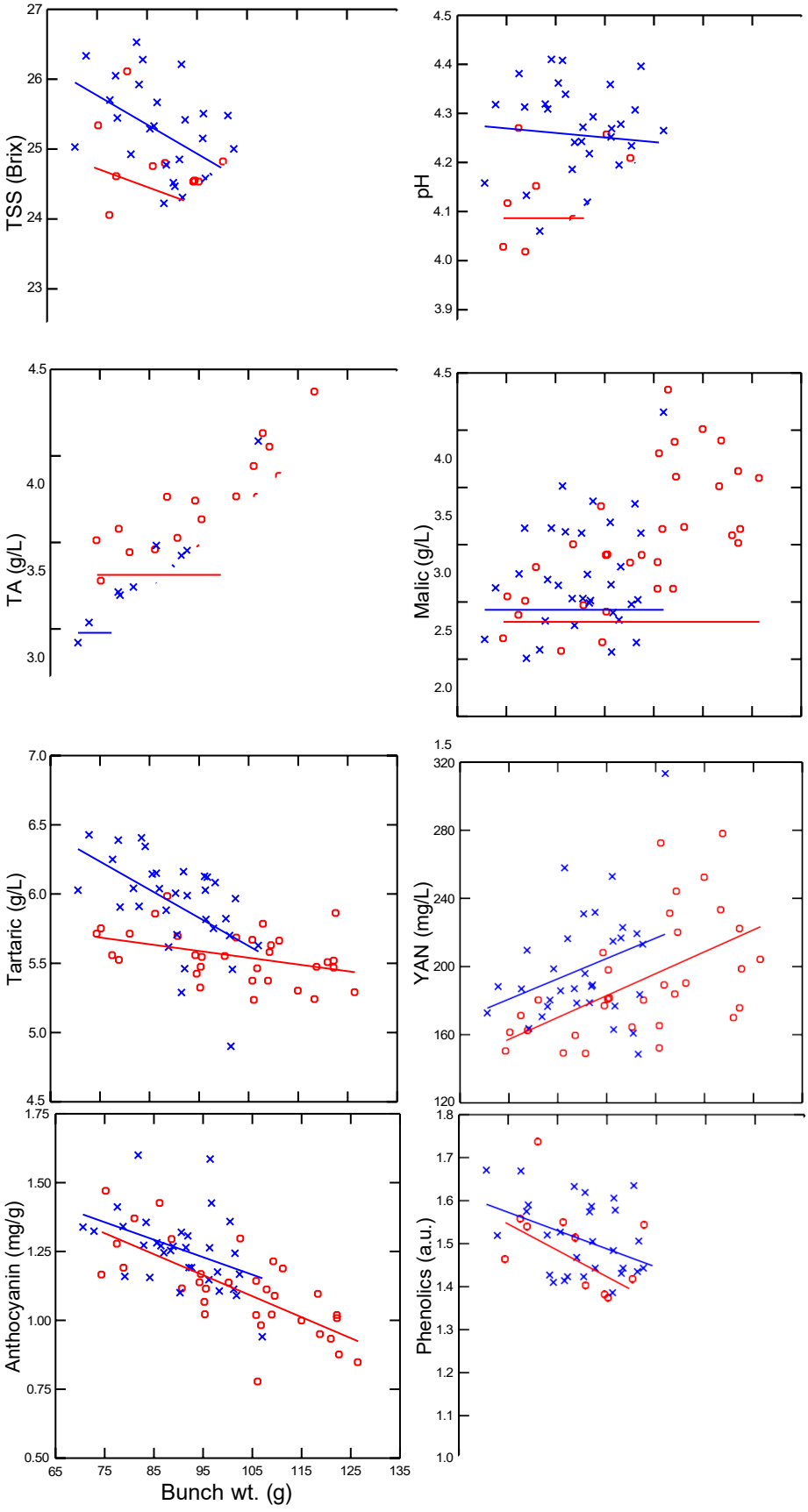

TSS (Brix)
pH
TA (g/L)
Malic (g/L)
Tartaric (g/L)
YAN (mg/L)
Anthocyanin (mg/g)
Phenolics (a.u.)
Bunch wt. (g)

Figure 6.2.7. Linear relationships between means of fruit composition traits and mean bunch weight for control (red) and sustained deficit (blue) irrigation treatments of 32 rootstock genotypes over nine seasons (2013-21). TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tartaric, tartaric acid; YAN, yeast assimilable nitrogen

The relationships between berry weight and fruit composition traits of 32 rootstock genotypes for control and deficit irrigation are shown in Figure 6.2.8. They highlight the significant effects of deficit irrigation on fruit composition compared to the full irrigation, provided in Table 6.2.2. Furthermore, it is apparent that deficit irrigation not only reduced berry weight (Table 6.2.2) but also the range in berry weight across the rootstock genotypes. Relationships between berry weight and TSS, pH and tartaric acid were not significant (Table 6.2.3). For both irrigation treatments, there were strong positive relationships between berry weight and TA, malic acid concentration and YAN (Table 6.2.3). Deficit irrigation had little impact on these relationships, as slopes were similar. In the case of malic acid, the relationships with berry weight were identical. For both irrigation treatments, there were strong negative relationships between berry weight and berry anthocyanins and phenolics (Table 6.2.3). Deficit irrigation had little impact on these relationships. The strong relationships with berry weight and TA, malic acid, YAN, berry anthocyanin (negative) and phenolics (negative) indicate that berry weight is a major driver of both rootstock genotype and irrigation treatment on fruit composition.

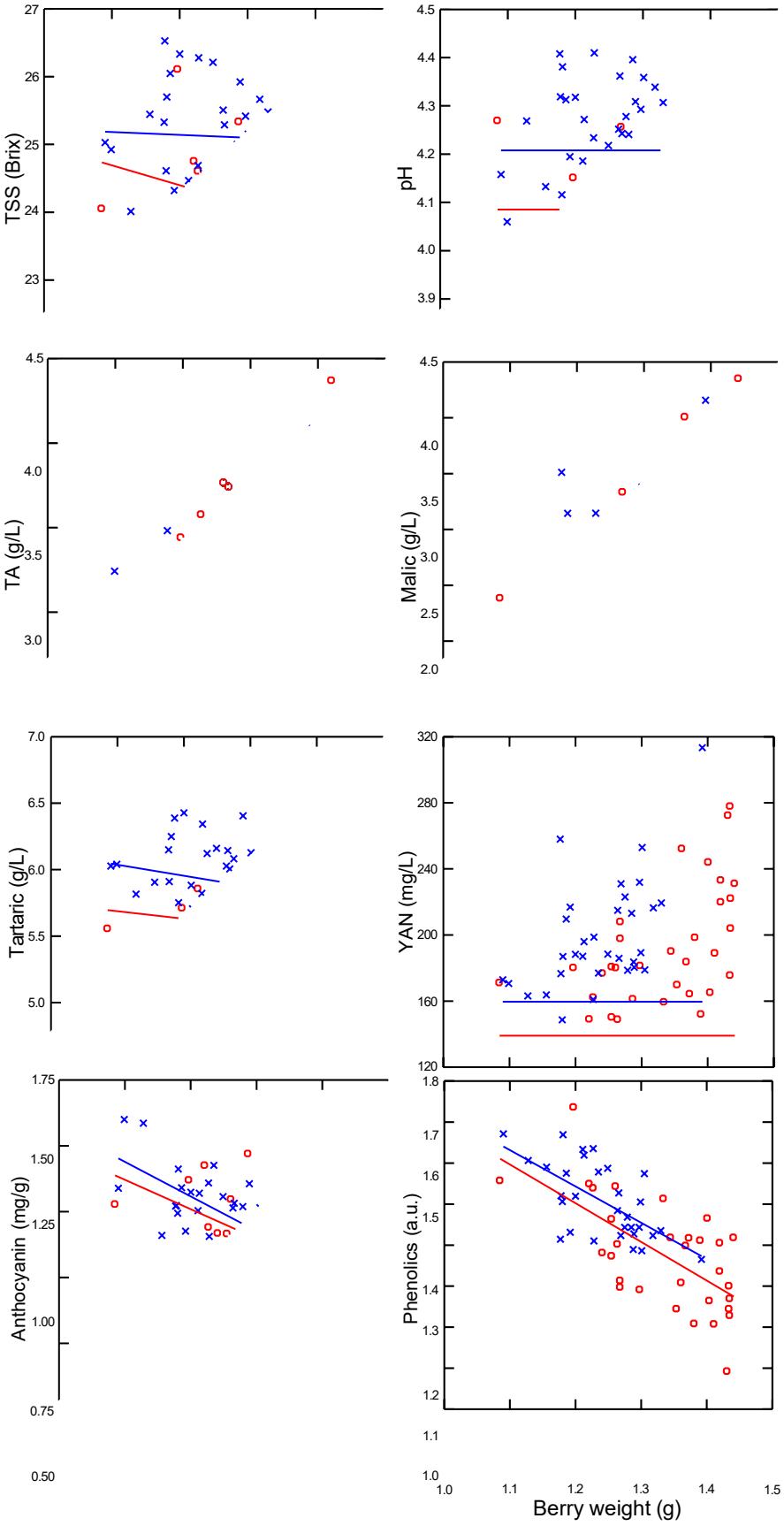


Figure 6.2.8. Linear relationships between means of fruit composition traits and mean berry weight for control (red) and sustained deficit (blue) irrigation treatments of 32 rootstock genotypes over nine seasons (2013-21). TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tartaric,

tartaric acid; YAN, yeast assimilable nitrogen

The relationships between pruning weight and fruit composition traits of 32 rootstock genotypes for control and deficit irrigation are shown in Figure 6.2.9. They highlight the significant effects of deficit irrigation on fruit composition compared to the full irrigation, provided in Table 6.2.2. Relationships between pruning weight and TSS and tartaric acid were not significant for either irrigation (Table 6.2.3). The significant positive relationship between pH and pruning weight found with full irrigation was uncoupled by deficit irrigation, as the correlation coefficient was not significant. For both irrigation treatments, pruning weight was positively correlated with TA, malic acid and YAN. Deficit irrigation not only reduced TA but also reduced the slope of the relationship with pruning weight. In contrast, deficit irrigation had almost no effect on the very strong relationships between pruning weight and malate or YAN. For both irrigation treatments, there were strong negative relationships between pruning weight and berry anthocyanins and phenolics (Table 6.2.3). Although deficit irrigation increased berry anthocyanin and phenolics, it had little impact on the relationships with pruning weight, as slopes were similar. These results indicate that pruning weight associated with either rootstock-conferred vigour or the reduction in pruning weight with deficit irrigation, is a major driver of fruit composition, in particular juice TA, malic acid, YAN and berry anthocyanin and phenolic substances and by inference, negatively linked to negative effects on wine composition.

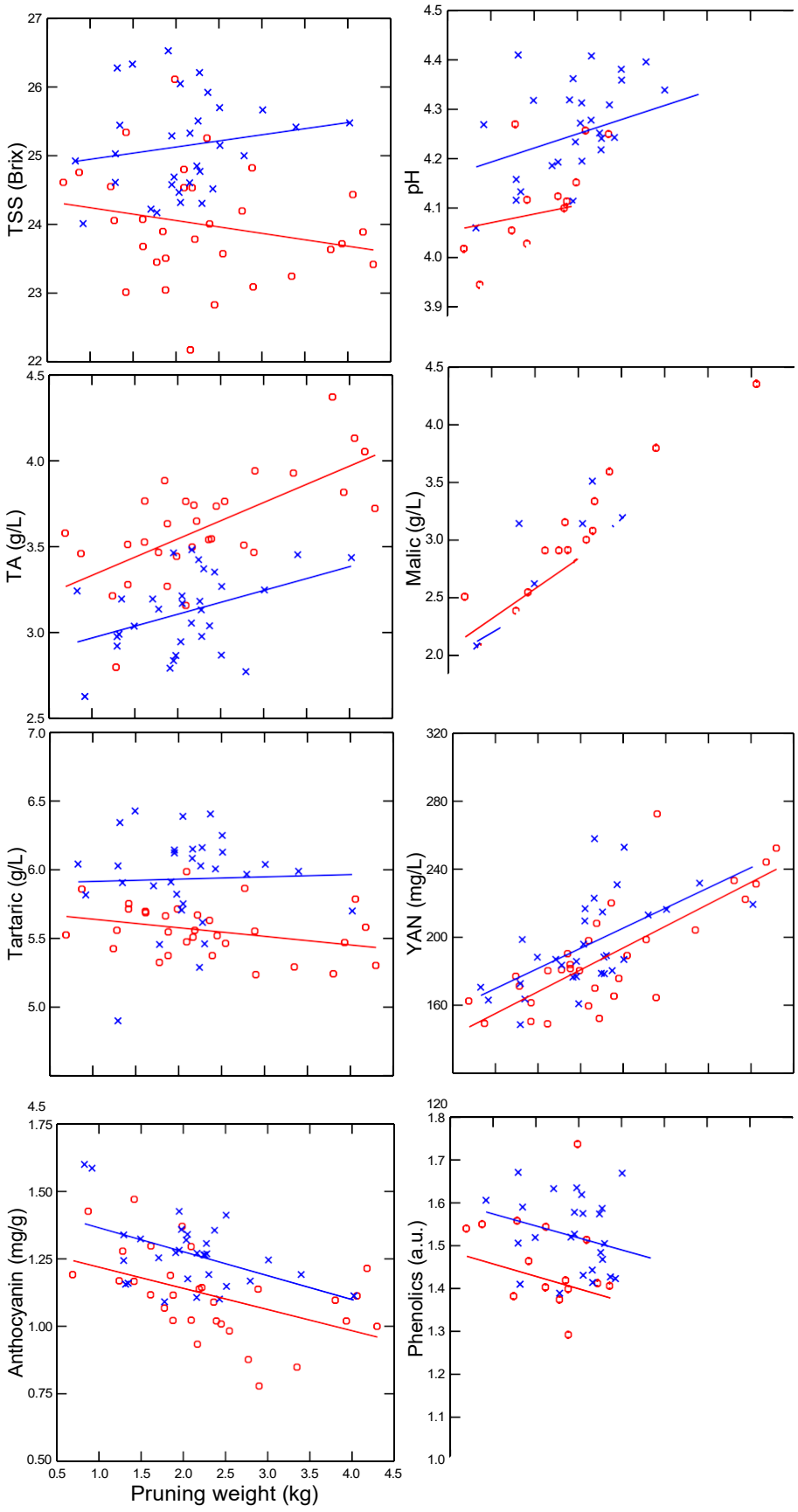


Figure 6.2.9. Linear relationships between means of fruit composition traits and mean pruning weight for control (red) and sustained deficit (blue) irrigation treatments of 32 rootstock genotypes

over nine seasons (2013-21). TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tartaric, tartaric acid; YAN, yeast assimilable nitrogen

As reported above, irrigation treatments impacted relationships between fruit composition traits, including mineral elements across rootstock genotypes (Tables 6.2.4). The most significant of these relationships are explored in more detail in Figures 6.2.10 - 6.2.15. The relationships between TSS and pH, tartaric acid, berry anthocyanins and phenolics are shown in Figure 6.2.10. Deficit irrigation not only increased TSS (Table 6.2.2) but also reduced the range in TSS across the rootstock genotypes. The relationship between TSS and pH was impacted by the deficit irrigation. With full irrigation the relationship between pH and TSS was not significant, whereas with deficit irrigation there was a significant positive relationship (Table 6.2.4), indicating increased pH with increasing TSS across rootstocks. TSS was positively correlated with tartaric acid for both irrigation treatments (Table 6.2.4), although the slope was steeper over a narrower range in TSS with deficit irrigation. These results suggests that higher TSS may be associated with berry wilting, particularly with the deficit irrigation as berry content of tartaric acid is expected to remain constant post veraison. With the full irrigation treatment, berry anthocyanin and phenolics were positively correlated with TSS (Table 6.2.4). In contrast, these relationships were completely decoupled by deficit irrigation, which produced significant increases in both traits (Table 6.2.2). This result is also consistent with responses associated with berry wilting.

The study has identified significant correlations across rootstock genotypes between YAN and other fruit quality traits for both irrigation treatments (Table 6.2.4), which are described in detail in Figure 6.2.11, and to the best of the author's knowledge, have not been reported previously. Overall, deficit irrigation increased pH (Table 6.2.2) but had little impact on the strong relationships with YAN, as the slopes were similar. Deficit irrigation, which reduced TA and malic acid (Table 6.2.4), had little impact on the strong relationships between YAN and TA or malic acid, as the slopes were similar. In contrast, the strong relationship between YAN and tartaric acid with full irrigation was completely decoupled by deficit irrigation. Deficit irrigation increased berry anthocyanin and phenolics (Table 6.2.2) but had little impact on the strong relationships with YAN, as in both cases the slopes were similar.

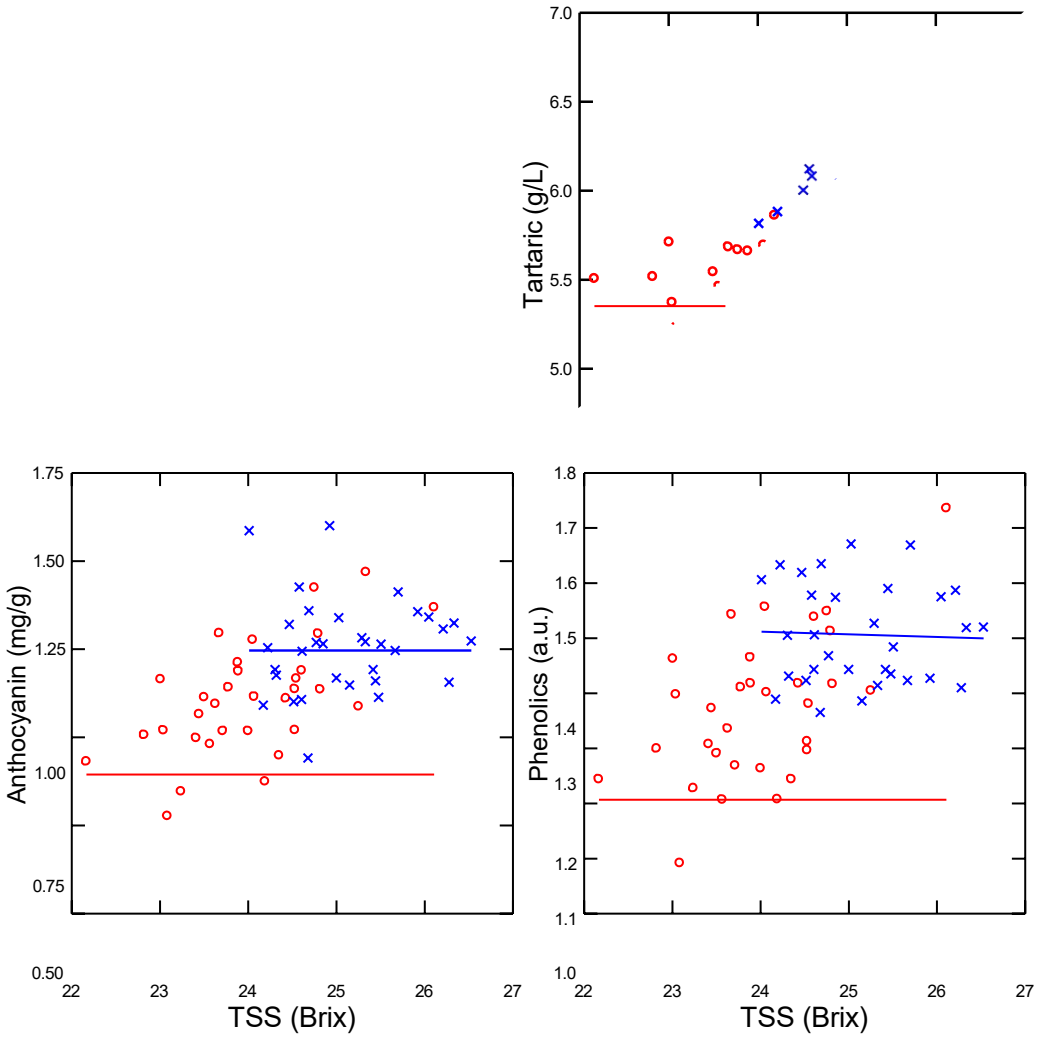


Figure 6.2.10. Linear relationships between means of fruit composition traits and mean TSS for control (red) and sustained deficit (blue) irrigation treatments of 32 rootstock genotypes over nine seasons (2013-21). TSS, total soluble sugars; Tartaric, tartaric acid

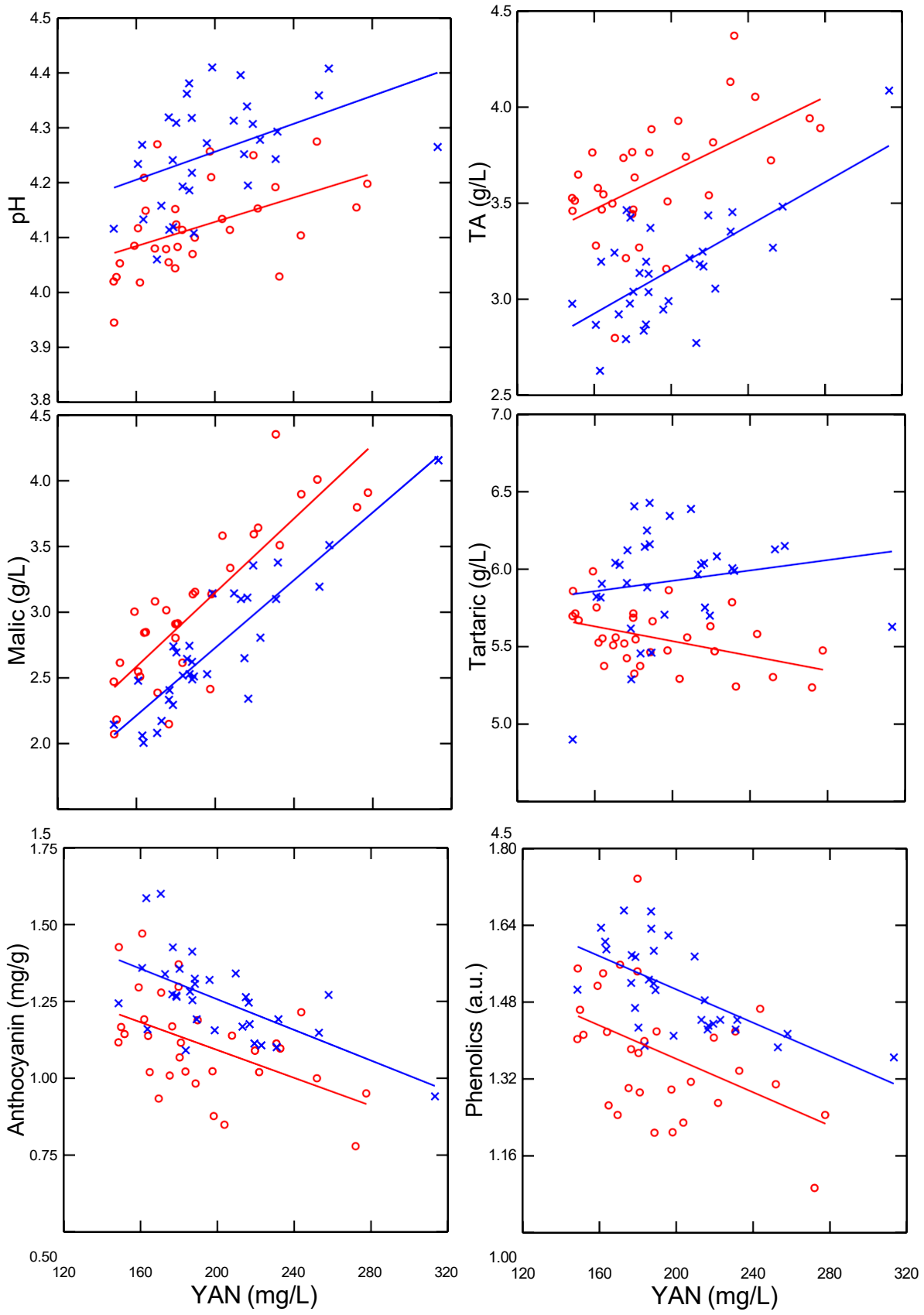


Figure 6.2.11. Linear relationships between means of fruit composition traits and mean YAN for control (red) and sustained deficit (blue) irrigation treatments of 32 rootstock genotypes over nine seasons (2013-21). TA, titratable acid; Malic, malic acid; Tartaric, tartaric acid; YAN, yeast assimilable nitrogen

The study has also identified significant correlations across rootstock genotypes between malic acid and other fruit quality traits for both irrigation treatments (Table 6.2.4), which are shown in Figure 6.2.12. Deficit irrigation not only increased pH (Table 6.2.2) but also impacted on the relationship between pH and malic acid, producing a steeper slope. Deficit irrigation increased TA (Table 6.2.2) but had little impact on the strong relationship with malic acid, as both slopes were similar. Deficit irrigation increased berry anthocyanin and phenolics (Table 6.2.2) but had little impact on the strong negative relationships with malic acid, as slopes were similar.

The significant relationships between tartaric acid identified above (Table 6.2.4) show very interesting results with regard to the response of rootstocks to deficit irrigation (Figure 6.2.13). Compared to full irrigation, the range in tartaric acid concentration was much greater with the deficit treatment. Juice pH was positively correlated with tartaric acid with deficit irrigation whereas there was no relationship with the full irrigation treatment. In contrast, with full irrigation, anthocyanin and phenolics were positively related to tartaric acid, whereas with deficit irrigation these relationships were decoupled and not significant, most likely due to berry wilting and concentration of tartaric acid across the rootstock genotypes. The positive relationship between anthocyanins and phenolics, also shown in Figure 6.2.13, was not impacted to any extent by deficit irrigation.

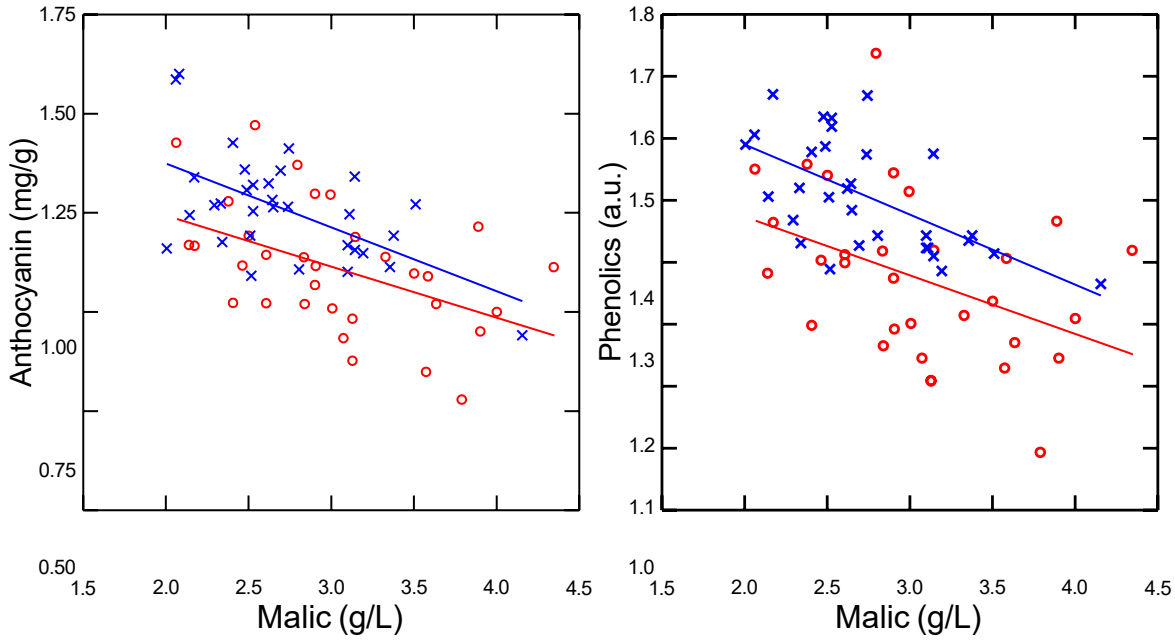


Figure 6.2.12. Linear relationships between means of fruit composition traits and mean malic acid for control (red) and sustained deficit (blue) irrigation treatments of 32 rootstock genotypes over nine seasons (2013-21). TA, titratable acid; Malic, malic acid

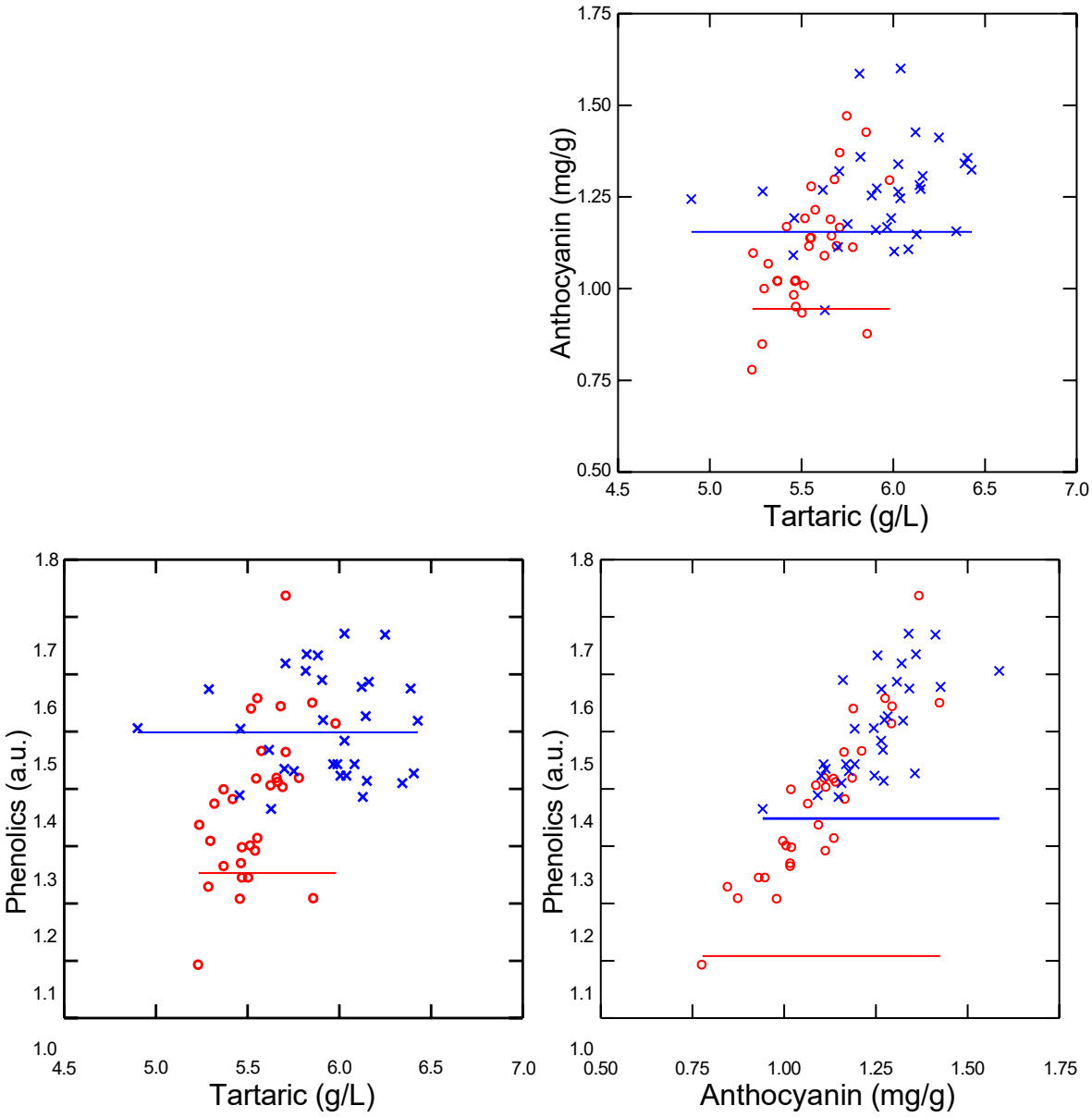


Figure 6.2.13. Linear relationships between means of fruit composition traits and mean tartaric acid for control (red) and sustained deficit (blue) irrigation treatments, and between anthocyanins and phenolic of 32 rootstock genotypes over nine seasons (2013-21). Tartaric, tartaric acid

Juice K, the dominant ion, was strongly correlated across the rootstock genotypes with vine performance and growth traits (Table 6.2.3) and aspects of fruit composition (Table 6.2.4). The relationships between juice K and vine performance traits are shown in Figure 6.2.14. With full irrigation, juice K was positively related to yield, bunch number, bunch weight, berry weight and pruning weight (Table 6.2.3). In contrast, relationships between K and yield and yield components were decoupled by deficit irrigation, as none were significant (Table 6.2.3). The highly significant relationship ($r = 0.70$) between juice K and pruning weight with full irrigation was maintained with the deficit treatment, despite the overall reduction to pruning weight (Table 6.2.2). This result indicates that the effect of deficit irrigation on juice K across the rootstock genotypes was linked to its effect on shoot growth and canopy size and most likely to reported effects of bunch exposure/shade.

The significant relationships between juice K and pH, TA, malic acid and YAN are shown in Figure 6.2.15. In all cases the relationships were significant with both irrigation treatments (Table 6.2.4). Deficit irrigation, which increased overall pH (Table 6.2.2), produced a stronger response between pH and K across the rootstock genotypes, as shown by the increased slope. Deficit irrigation, apart from the shift associated with the overall reduction in TA (Table 6.2.2) had little impact on the relationship, as slopes were similar. Similarly, deficit irrigation had little impact on the relationship between malic acid and K apart from the shift associated with overall reduction (Table 6.2.2). The relationship between YAN and K displayed similar trends with both irrigation treatments.

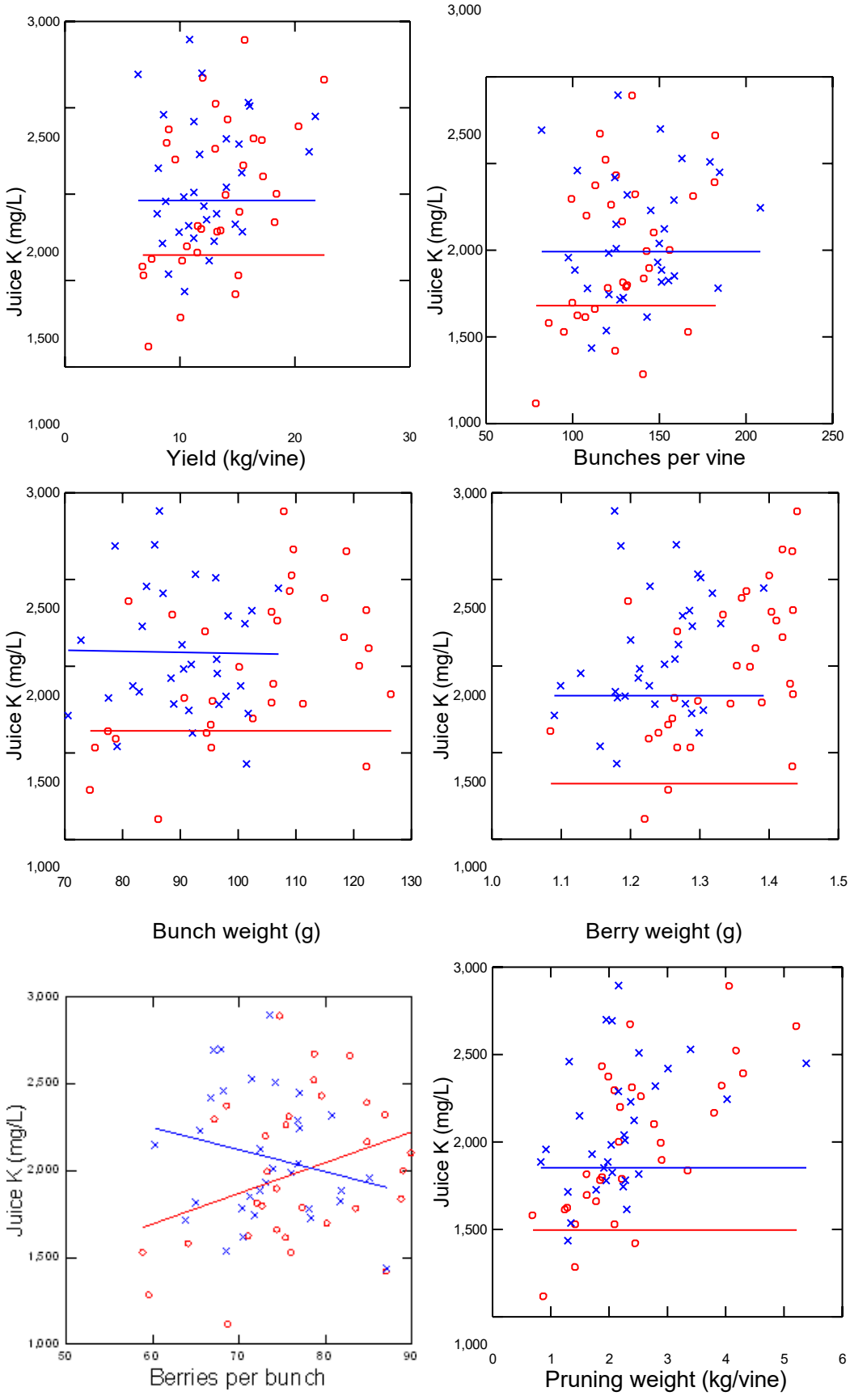
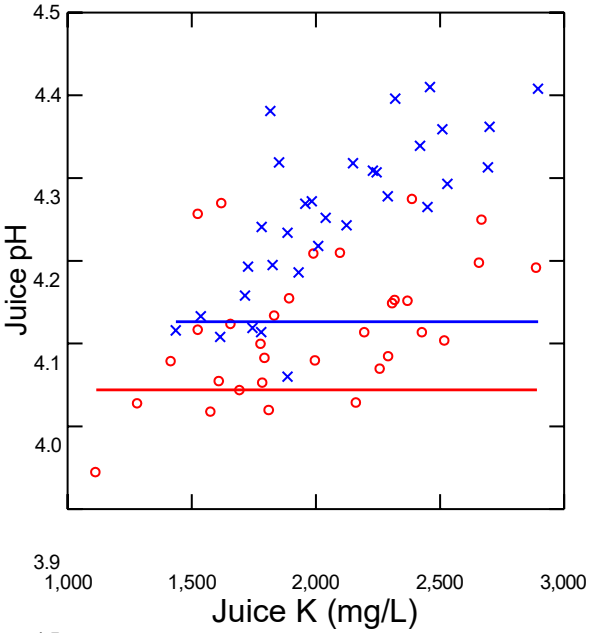
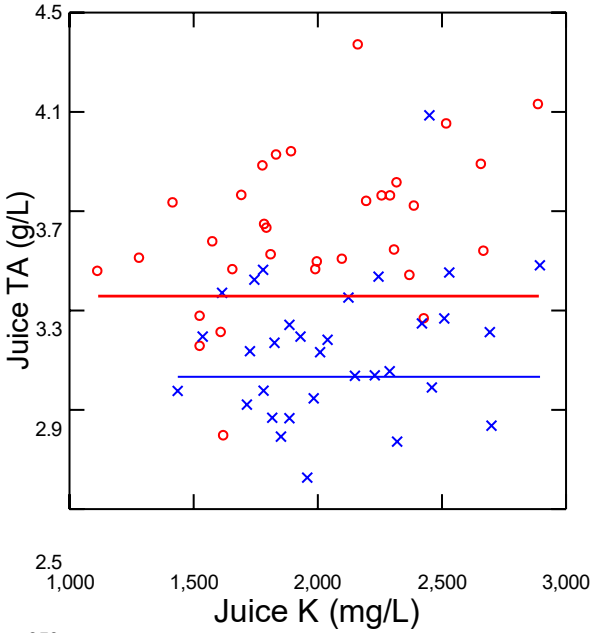
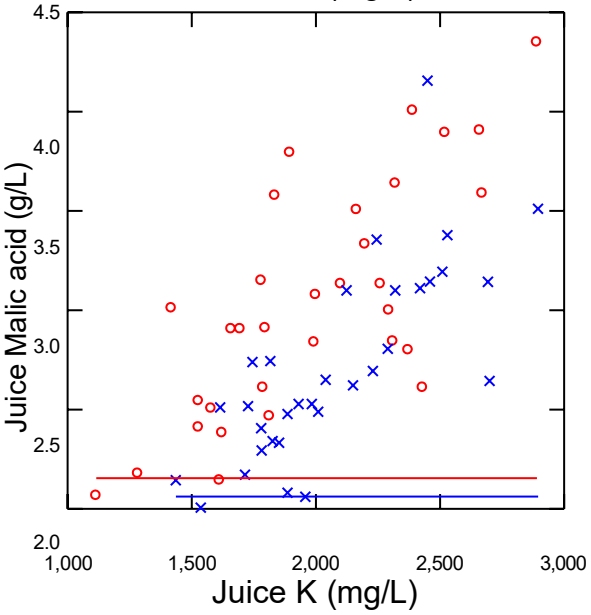


Figure. 6.2.14. Linear relationships between the nine year mean (2013-21) yield, berry weight, berries per bunch and pruning weight and juice K measured in 2016, for control (red) and sustained

deficit (blue) irrigation treatments of 32 rootstock genotypes.


Juice pH
4.5
4.4
4.3
4.2
4.1
4.0
3.9
1,000
1,500
2,000
2,500
3,000
Juice K (mg/L)


Juice TA (g/L)
4.5
4.1
3.7
3.3
2.9
2.5
1,000
1,500
2,000
2,500
3,000
Juice K (mg/L)


Juice Malic acid (g/L)
4.5
4.0
3.5
3.0
2.5
2.0
1,000
1,500
2,000
2,500
3,000
Juice K (mg/L)

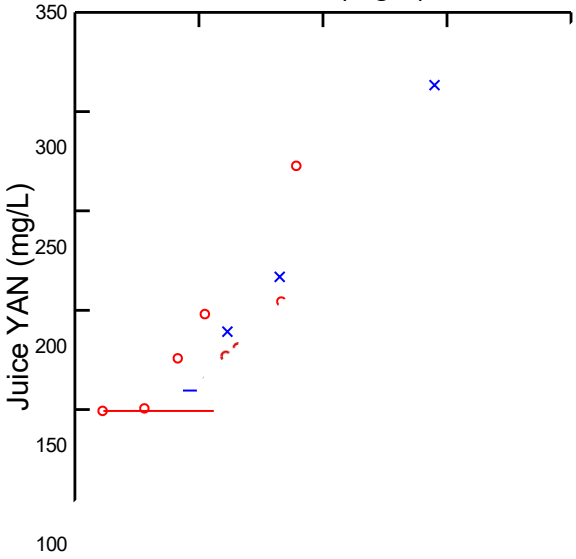

Juice YAN (mg/L)
350
300
250
200
150
100

Figure 6.2.15. Linear relationships between juice K measured in 2016 and the nine year mean (2013-21) and composition traits (juice pH, TA, malic acid and YAN concentration) for control (red) and sustained deficit (blue) irrigation treatments of 32 rootstock genotypes. TA, titratable acid; YAN, yeast assimilable nitrogen

Selection of new rootstock genotypes

The Shiraz trial, planted in 2010, was designed to evaluate the field performance of 55 of the most promising drought tolerant genotypes selected under nursery conditions, from 310 genotypes in project CSP 0503. The trait information shown above (Figures 6.2.3 and 6.2.5), together with the information on the response to deficit irrigation was used to identify a range of genotypes that offer advantages over the commercial rootstocks included in the trial. Key traits used for this purpose were yield, pruning weight, pH, malic acid and anthocyanin concentrations. The number of new rootstock genotypes has been reduced to 15. Over the course of the study, wines have been produced from most of these selections to support decisions with regard to potential release for industry adoption (see section below).

Mean yield, yield component and growth traits of the selections and the standard rootstocks are provided in Table 6.2.5. They are listed in order of rootstock-conferred vigour (pruning weight). Selection 235-74, which conferred low vigour, had similar field performance traits to own roots and Merbein 5489. In contrast, selection 232-80, which confers high vigour, showed similar field performance traits to Ramsey. Conferred vigour of 232-13, 235-01, 230-45, 235-61, 231-51 and 230-30 was greater than own roots but less than 140 Ruggeri. Except for the high yielding selection 230-30, field performance of these selections was more similar to 140 Ruggeri than to own roots. The higher Ravaz Index values indicate that are more likely to be more water efficient than 140 Ruggeri. Despite its lower vigour, yield of selection 230-30 was higher than that produced by 1103 Paulsen. Conferred vigour of 232-11, 235-70, 231-20, 230-46 and 233-80 fell between 140 Ruggeri and 1103 Paulsen. Yields and other field performance traits of 231-20 and 230-46 closely matched 140 Ruggeri. Similarly, field performance of 232-11, 235-70 and 233-80 were more aligned to 1103 Paulsen. Conferred vigour of 230-47 and 232-57 fell between 1103 Paulsen and Ramsey, although their field performance more closely matched 1103 Paulsen.

Table 6.2.5. Mean yield, yield component and growth traits of 15 new rootstock genotypes, own roots and four standard rootstocks over two irrigation treatments (control and deficit) and 10 seasons (2013-22).

Rootstock	Pruning wt. (kg)	Yield (kg)	Bunches	Bunch wt. (g)	Berry wt. (g)	Berry/ Bunch	Ravaz Index
235-74	1.39	7.9	107	77	1.22	63.7	13.7
Merbein 5489	1.41	8.5	107	81	1.15	70.5	8.2
Own	1.46	9.1	133	74	1.23	60.0	6.8
232-13	1.76	10.7	116	93	1.25	74.7	10.6
235-01	1.78	11.5	143	83	1.18	70.7	11.9
230-45	1.79	10.3	104	100	1.25	79.1	9.8
235-61	1.80	12.0	140	92	1.26	72.9	8.8
231-51	1.94	12.8	140	94	1.26	74.5	8.1
230-30	2.08	15.8	154	111	1.29	85.4	7.8
140 Ruggeri	2.11	11.9	124	95	1.33	71.3	7.7
231-11	2.14	14.3	137	101	1.31	78.0	7.7
235-70	2.14	14.2	143	102	1.29	79.5	6.8
231-20	2.20	10.7	121	90	1.32	68.8	7.9
230-46	2.22	10.4	115	93	1.29	72.5	5.9
233-80	2.25	12.6	123	106	1.32	81.6	7.2
1103 Paulsen	2.28	14.0	148	96	1.29	74.7	7.3
230-47	2.46	12.4	134	95	1.35	70.5	6.1
232-57	2.55	13.0	120	110	1.30	86.0	6.8
Ramsey	3.41	18.2	181	106	1.33	79.5	6.1
232-80	4.04	18.4	171	105	1.39	75.9	5.6

Mean values for fruit composition traits for the selections and the standard rootstocks are provided in Table 6.2.6. Merbein 5489 and selections 232-13, 232-18 and 235-74 had highest TSS (i.e. > 25.0 ^{o}Brix). Selections 230-30, 233-80 and 235-61 had lowest TSS (i.e. < 24 ^{o}Brix). The standard high vigour rootstocks, 1103 Paulsen, 140 Ruggeri and in particular Ramsey had the highest juice pH. Juice pH of all selections was lower than the high vigour standard rootstocks, and in many cases was lower than juice of own roots (pH = 4.17), reflecting its use as part of the screening process. In particular, juice pH of selections 230-45, 231-20, 232-57 was lower than Merbein 5489 (pH = 4.11). Own roots (3.27 g/L) and selections 230-30, 232-13, 235-01 and 235-74 had low juice TA concentrations. Selections 230-45, 230-46, 231-11, 231-20, 232-57 and 232-80 had higher TA concentrations than 1103 Paulsen. Own roots, Merbein 5489 and selections 232-13, 235-01 and 235-74 had low juice malic acid concentrations (i.e. < 2.45). The standard rootstocks, 140 Ruggeri, Ramsey and selection 232-80 had highest malic acid concentrations (i.e. > 3.4 g/L). Own roots, Merbein 5489, 1103 Paulsen, 140 Ruggeri and selections, 230-45, 230-47, 231-11, 232-13 and 235-74 had high tartaric acid concentrations (i.e. > 5.75 g/L). Selections 230-46, 232-57 and 235-01 had low tartaric acid concentrations (i.e. < 5.6 g/L). Juice YAN concentrations of all rootstock

genotypes were above the 150 mg/L required for a successful fermentation. Although the effect of rootstock on YAN was not significant (Table 6.2.1), own roots, Merbein 5489 and selections 230-30, 231-20, 235-01 had the lowest YAN concentrations (i.e. < 175 mg/L) while the standard rootstocks, 140 Ruggeri and Ramsey had the highest YAN concentrations (i.e. >230 mg/L). A number of rootstock genotypes had similar or higher berry anthocyanin concentrations than own roots (1.24 mg/g) including Merbein 5489 and selections 230-45, 230-46, 231-20, 232-13, 235-01 and 235-74. All selections had higher berry anthocyanin than the standard rootstocks, 140 Ruggeri, 1103 Paulsen and Ramsey, reflecting its use as part of the screening process. A number of rootstock genotypes had similar or higher berry phenolics concentrations than own roots (1.49 a.u.) including Merbein 5489 and selections 230-45, 231-20, 232-13, 235-01 and 235-74. All selections, except 230-47, had higher phenolics than all standard high vigour rootstocks.

Table 6.2.6. Mean values of juice composition traits of 15 new rootstock genotypes, own roots and four standard rootstocks over two irrigation treatments (control and deficit) and eight seasons (2014-21).

Rootstock	TSS (°Brix)	pH	TA (g/L)	Malic (g/L)	Tartaric (g/L)	YAN (mg/L)	Antho (mg/g)	Phen (a.u.)
235-74	25.4	4.12	3.24	2.28	5.83	162	1.32	2.04
Merbein 5489	25.5	4.11	3.34	2.44	5.88	175	1.49	1.90
Own	24.7	4.17	3.27	2.40	6.07	169	1.24	1.49
232-13	25.4	4.14	3.17	2.32	5.79	183	1.24	1.48
235-01	24.4	4.26	2.89	2.34	5.59	175	1.27	1.51
230-45	24.1	4.08	3.61	2.66	5.90	178	1.36	1.56
235-61	23.9	4.13	3.41	2.72	5.71	184	1.18	1.46
231-51	24.8	4.14	3.35	2.56	5.86	182	1.19	1.44
230-30	23.4	4.16	3.18	2.78	5.66	165	1.15	1.44
140 Ruggeri	24.7	4.28	3.47	3.47	5.79	235	0.97	1.25
231-11	24.2	4.17	3.62	3.13	5.83	210	1.14	1.42
235-70	24.0	4.12	3.41	2.48	5.71	184	1.16	1.42
231-20	24.6	4.10	3.57	2.76	5.72	174	1.24	1.51
230-46	24.7	4.12	3.58	3.04	5.42	193	1.20	1.44
233-80	23.6	4.18	3.34	2.77	5.61	186	1.16	1.46
1103 Paulsen	24.7	4.28	3.51	3.18	5.76	212	1.15	1.34
230-47	24.7	4.19	3.40	2.91	5.93	185	1.17	1.32
232-57	24.1	4.07	3.67	2.83	5.07	191	1.17	1.42
Ramsey	24.3	4.32	3.49	3.60	5.71	253	1.07	1.35
232-80	25.0	4.25	3.78	3.85	5.74	225	1.11	1.43

TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tartaric, tartaric acid; YAN, yeast assimilable nitrogen; Antho, anthocyanin; Phen, phenolics

Juice mineral element ion concentrations were determined in 2016 and reported previously for the full data set (CSP 1304, Clingeleffer and Smith 2018). The data have been reanalysed to include only the 26 new rootstock and the standard commercial rootstocks. The results for the 14 promising selections are provided in Table 6.2.7. Juice K, the dominant ion, ranged from 1,532 - 2,603 mg/L. 1103 Paulsen, Ramsey and the high vigour selection 232-80 had the

highest K concentrations. The low vigour selection, 235-74 had the lowest K concentration. Own roots, 235-01, 233-80 and 230-45 also had low K concentrations. All other selections had K values that were lower than Merbein 5489, which had the lowest K of the commercial rootstocks. Differences in juice Ca were not significant (Table 6.2.1), although selections 235-01 and 230-47 had the lowest and highest Ca, respectively. Selection 235-61 and 232-80 had the highest juice Mg (124-129 mg/L). Own roots and the commercial rootstocks had intermediate Mg concentrations (108-115 mg/L). Selections 235-74, 232-13, 235-01, 230-45, 235-70, 233-80 and 232-57 had low Mg (<90 mg/L). Own roots, selection 235-61 and to a lesser degree Ramsey, had high Na. Selections 232-13, 230-45, 231-51, 230-30, 235-70, 230-46 and 233-80 had low Na concentrations (<14.2 mg/L). While rootstock effects were not significant for S, 1103 Paulsen, Ramsey and 232-80 had high S while 235-74, 230-45, 230-30 and 233-80 had low S concentrations. Juice B was high for all commercial rootstocks including Merbein 5489 and selections 235-61, 231-20 and 232-80 and low in 235-74 and 230-45. While rootstock effects were not significant for P, 1103 Paulsen had the highest and own roots, 235-61 and 230-30 the lowest P concentrations.

Table 6.2.7. Mean values of juice mineral element concentrations of 15 new rootstock genotypes, own roots and four standard rootstocks over two irrigation treatments (control and deficit) measured in season 2016.

Rootstock	Ca	K	Mg	Na	S	B	P
				g/L)			
235-74	121	1532	86	25.1	55.1	12.4	92.6
Merbein 5489	120	2129	112	23.6	69.6	20.0	125.1
Own	106	1717	113	118.2	64.2	17.2	82.9
232-13	125	1811	84	13.4	60.0	13.9	140.0
235-01	78	1702	75	32.0	61.7	13.7	92.9
230-45	119	1738	83	14.1	54.9	11.0	100.3
235-61	120	1864	129	110.1	64.3	19.7	82.1
231-51	123	1927	92	12.5	64.5	13.4	99.4
230-30	103	1943	94	14.1	56.3	13.2	74.3
140 Ruggeri	100	2178	115	38.5	78.6	19.3	97.3
231-11	121	1952	106	57.9	67.6	16.1	93.2
235-70	107	1807	89	9.3	69.2	16.4	89.9
231-20	111	1955	113	70.7	77.6	19.4	94.2
230-46	123	1972	94	8.1	62.5	13.8	122.4
233-80	119	1701	88	9.8	56.6	14.1	87.0
1103 Paulsen	121	2603	112	24.0	85.2	20.6	170.2
230-47	152	2245	103	36.6	72.9	14.4	136.5
232-57	96	1800	81	7.3	69.7	14.3	119.0
Ramsey	98	2450	108	90.1	97.8	19.0	112.1
232-80	117	2568	124	59.9	86.9	20.9	153.5

Small-scale winemaking

To complete the evaluation of the 15 priority selections, small scale-wine batches were produced from fruit of almost all selections, either in 2019 or in 2021. In both cases wines produced from fruit of own rooted vines and 1103 Paulsen rootstock were used as controls. Each rootstock was harvested when berry samples indicated that maturity was in the desired range of 23 - 24 ^{o}Brix. In 2019, harvest dates ranged from 31 January to 21 February. In 2021, harvest dates ranged from 18 to 23 February.

Long term data for key selection criteria (2014- 2021)

The original selection of genotypes for winemaking was, because of the experimental design, based on mean data across both irrigation treatments 2014-18 and 2014-20 for the 2019 and 2021 seasons. The long-term mean data for key selection criteria (pruning weight, yield, juice TSS, organic acid balance and berry anthocyanin), in seasons 2014-21 for these genotypes are provided in Table 6.2.8 for both irrigation treatments. Mean pruning weight covered a range of conferred vigour across the rootstock genotypes, 1.25 - 4.30 kg/vine for control irrigation and 0.83- 4.02 kg/vine for deficit irrigation. Conferred vigour of the commercial rootstocks, Merbein 5489, 140 Ruggeri, 1103 Paulsen and Ramsey was most impacted by deficit irrigation. All selected genotypes maintained vigour with the deficit treatment, an indication that selection, either in 2018 or 2020 based on response to the deficit irrigation had been successful. Mean yield for the control irrigation ranged from 6.9 -20.3 kg/vine and for the deficit treatment from 8-21.2 kg/vine. Yield of all the commercial rootstocks, own roots and genotype 230-47 were most impacted by deficit irrigation. The increase in yield for some selections may reflect higher bunch numbers reported above, either associated with higher shoot fruitfulness, higher budburst or retention of higher bud numbers with mechanical hedging.

Fruit maturity (TSS) at sampling prior to harvest, ranged from 23.0-26.1 ^{o}Brix with control irrigation and 24.3-26.3 ^{o}Brix with deficit irrigation (Table 6.2.8). Except for Merbein 5489 and genotype 231-20, deficit irrigation enhanced fruit maturity with increased TSS of all rootstocks and own roots. This was particularly noticeable for own roots with an increase from 23.0 to 26.3 ^{o}Brix. The increase in TSS with deficit irrigation was associated with development of smaller berries in all cases with deficit irrigation, except for genotypes 230-46 and 235-01 (data not shown), most likely due to increased berry wilting at the latter stages of ripening. Juice pH ranged from 4.03 (own roots) to 4.27 (Ramsey) with control irrigation and from 4.06 (Merbein 5489) to 4.36 (Ramsey) with deficit irrigation. The pH of all genotypes increased with deficit irrigation, except Merbein 5489 and 235-01. Organic acid balance, the ratio of tartrate to malate, ranged from 1.32 (Ramsey) to 2.62 (own roots) with control irrigation and from 1.70 (232-80) to 2.94 (235-74) with deficit irrigation. In most cases organic acid balance was enhanced by deficit irrigation except for own roots and 1103 Paulsen and to a very minor degree, genotypes 232-13 and 231-51. In almost all cases, the enhanced organic acid balance was associated with lower malic acid concentration, except own roots, 1103 Paulsen, 232-13 and 231-51 and higher tartaric acid concentration, except genotypes 231-20

and 230-46 (data not shown). The lower malic acid levels with deficit irrigation are most likely due to high rates of respiration post veraison. The higher tartaric acid concentrations are most likely associated with berry shrivel at the later stages of ripening, as tartaric acid content is stable post veraison.

Berry anthocyanin at sampling prior to harvest ranged from 0.78 mg/g (140 Ruggeri) to 1.47 mg/g (235-74) for control irrigation and 1.10 mg/g (231-11) to 1.60 mg/g (Merbein 5489). In most cases, berry anthocyanin was enhanced by deficit irrigation, except for genotypes 235-01 and 232-80 where it remained unchanged and 235-74, 231-11 and 231-20. These selections had substantial increases in yield with the deficit treatment. Highly significant negative correlations between yield and berry anthocyanin were found for both irrigation treatments (Table 6.2.3). The enhancement of berry anthocyanin with deficit irrigation for most genotypes was most likely associated with development of smaller berries, with the possibility of berry shrivel at the latter stages of ripening leading to changes in the skin to flesh ratio. Highly significant negative correlations were found between berry weight and berry anthocyanin, for both treatments.

Table 6.2.8. Mean values for key selection criteria including pruning weight, yield, juice TSS, pH, organic acid balance (the tartrate to malate ratio) and berry anthocyanin for genotypes identified for winemaking and commercial rootstocks, managed under two irrigation strategies, a well-watered control and a deficit treatment, seasons 2014-21. Data are sorted by the mean pruning weight across irrigations.

Root-stock	Pruning wt. (kg/vine)		Yield (kg/vine)		TSS (°Brix)		pH		T/M		Berry Anth (mg/g)	
	Con.	Def.	Con.	Def.	Con.	Def.	Con.	Def.	Con.	Def.	Con.	Def.
235-74	1.42	1.35	6.9	9.0	25.3	25.4	4.12	4.13	2.26	2.94	1.47	1.16
M5489	1.99	0.83	9.1	8.0	26.1	24.9	4.15	4.06	2.04	2.90	1.37	1.60
own	1.42	1.49	10.1	8.1	23.0	26.3	4.03	4.32	2.62	2.45	1.17	1.32
232-13	1.25	2.27	10.2	11.2	24.5	26.2	4.05	4.22	2.53	2.48	1.17	1.31
235-01	1.29	2.28	7.6	15.4	24.1	24.8	4.27	4.24	2.33	2.45	1.28	1.27
230-45	1.62	1.95	10.6	9.9	23.7	24.6	4.04	4.11	1.96	2.54	1.30	1.43
235-61	1.89	1.71	11.9	12.1	23.5	24.2	4.08	4.19	1.90	2.33	1.11	1.25
231-51	1.62	2.26	11.6	14.0	24.1	25.5	4.02	4.25	2.31	2.27	1.12	1.26
140R	2.90	1.32	15.2	8.6	23.1	26.3	4.15	4.41	1.38	2.02	0.78	1.16
231-11	1.85	2.43	13.3	15.4	23.9	24.5	4.10	4.24	1.80	1.94	1.19	1.10
235-70	2.23	2.05	13.6	14.8	23.8	24.3	4.05	4.20	2.17	2.46	1.14	1.18
231-20	2.10	2.30	8.9	12.5	24.8	24.3	4.08	4.11	1.99	2.17	1.29	1.19
230-46	2.19	2.24	9.6	11.2	24.5	24.9	4.11	4.12	1.67	1.93	1.14	1.27
1103P	2.40	2.16	17.2	10.8	24.0	25.3	4.15	4.41	1.89	1.75	1.02	1.27
230-47	2.55	2.37	13.1	11.7	23.6	25.9	4.07	4.31	1.74	2.38	0.98	1.36
Ramsey	4.30	2.51	20.3	16.1	23.4	25.2	4.27	4.36	1.32	1.92	1.00	1.15
232-80	4.07	4.02	15.7	21.2	24.4	25.5	4.19	4.31	1.33	1.70	1.11	1.11

TSS, total soluble sugars; T/M, tartaric acid/malic acid; YAN, yeast assimilable nitrogen

Must data

The results for the must TSS, pH and TA of each rootstock are provided in Table 6.2.9. In addition, the total amount of tartaric acid added to the must or during fermentation for pH adjustment, aiming for a pH 3.6 below prior to bottling, is provided. In general, must TSS was lower in 2019 compared to 2021. Must TSS from deficit irrigation was generally higher than for fruit from the control treatment. In 2019, must TSS for fruit from the control irrigation was highest for own roots, selections 231-51 and 235-74 and lowest for selection 230-45. The low value for TSS of 230-45 is considered to be an error as it does not match other data (e.g. juice TSS and wine alcohol) thus it is not considered as part of the evaluation and was removed as an outlier from the correlation and regression analyses provided below. With deficit irrigation in 2019, selection 231-51 also had the highest must TSS while 231-20 had the lowest TSS. In 2021, with the control irrigation, fruit from own roots had the highest must TSS while 1103 Paulsen and 232- 13 had lowest TSS. In both seasons, must pH of all selections was lower than for 1103 Paulsen in both irrigation treatments, an indication that selection process for low pH had been successful (Table 6.2.9). In 2019, must TA was highest with selection 230-45 in both irrigation treatments and lowest with 235-01 and 1103 Paulsen in the control and deficit treatments, respectively. In 2021, must TA was highest in the control irrigation with 230-46 and 235-61 and lowest with 1103 Paulsen, while with deficit treatment, it was highest with 230-46 and lowest with 235-61. In general, fruit from deficit irrigation had a higher requirement for tartaric acid addition to adjust pH. In both seasons, 1103 Paulsen had a high requirement for

tartaric acid addition with both irrigation treatments. In 2019, selection 231-51 also had a high tartaric acid requirement with both irrigation treatments. In 2021, selection 231-11 from the control irrigation had a high requirement for pH adjustment.

Table 6.2.9. Mean TSS, pH, TA of must and total tartaric acid addition for pH adjustment of own rooted Shiraz or grafted on promising CSIRO rootstock genotypes and 1103 Paulsen, under two irrigation strategies, a well-watered control and a deficit treatment, seasons 2019 and 2021. Mean values across rootstocks are presented with their standard deviation.

Rootstock	TSS (ºBrix)		pH		TA (g/L)		Tartaric added (g/L)	
	Con.	Def.	Con.	Def.	Con.	Def.	Con.	Def.
2019								
Own	23.7	24.0	4.17	3.95	2.93	3.25	4.3	4.6
1103 P	23.5	24.0	4.19	4.45	3.12	2.85	4.4	6.8
230-45	20.2	24.4	3.81	3.82	3.81	3.52	2.4	2.5
231-20	22.8	22.8	3.75	3.88	3.74	3.03	2.4	2.4
231-51	23.9	25.1	4.12	4.32	2.99	2.98	4.4	5.0
235-01	22.6	24.1	4.00	4.01	2.77	3.08	-	4.3
235-70	22.2	24.2	3.71	4.09	3.60	3.02	2.0	3.6
235-74	23.6	22.9	3.82	3.75	3.22	3.47	2.6	1.9
2021								
Own	24.5	24.3	3.88	4.12	3.69	3.35	2.1	3.7
1103P	22.7	22.9	4.01	4.32	3.42	3.40	3.1	5.6
230-46	23.7	23.1	3.84	4.00	3.85	3.64	1.9	3.8
230-47	23.4	23.7	3.91	4.11	3.59	3.27	2.4	4.0
231-11	23.4	23.8	3.88	4.07	3.59	3.35	3.2	3.8
232-13	22.6	24.2	3.90	4.20	3.46	2.93	2.2	4.0
232-80	23.5	24.0	3.91	4.07	3.77	3.25	2.4	4.2
235-61	23.9	24.8	3.87	4.03	3.97	3.08	2.7	3.4
mean	**23.1**	**23.9**	**3.92**	**4.07**	**3.47**	**3.22**	**2.8**	**4.0**
Std. error	1.0	0.7	0.14	0.18	0.35	0.22	0.8	1.27

TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tartaric, tartaric acid

Wine data

Wine analysis data are presented in Table 6.2.9. In general, wine TA was higher in 2021 than in 2019, although there were no cases that would contribute to an excessive acidic taste perception (i.e. > 8.0 g/L). Wine TA was unaffected by irrigation treatment. The wine pH results indicate that tartaric acid addition for pH adjustment was generally successful, with a mean wine pH of 3.61, range 3.53 - 3.69 over both seasons. In general, wine pH was lower in 2019 than in 2021 and unaffected by irrigation treatment. Overall, wine alcohol (range 14.2 - 16.6 %) was similar in both years and unaffected by deficit irrigation. VA concentrations, although higher in 2019 were in general very low and unaffected by irrigation treatment. In 2019, selection 231-20 had high VA with both irrigation treatments.

Table 6.2.10. Wine pH, TA, alcohol and volatile acidity of own rooted Shiraz or grafted on promising CSIRO rootstock genotypes and 1103 Paulsen, under two irrigation strategies, a well-watered control and a deficit treatment, seasons 2019 and 2021. Mean values across rootstocks are presented with their standard deviation.

Rootstock	pH		TA (g/L)		Alcohol (%)		VA (g/L)	
	Con.	Def.	Con.	Def.	Con.	Def.	Con.	Def.
2019								
Own	3.58	3.57	5.77	6.81	16.6	16.0	0.26	0.35
1103 P	3.56	3.58	5.49	5.69	14.4	16.3	0.25	0.32
230-45	3.61	3.53	6.42	6.10	16.0	16.3	0.33	0.29
231-20	3.53	3.56	7.02	5.94	15.1	14.5	0.62	0.42
231-51	3.54	3.56	6.32	5.87	16.1	15.8	0.25	0.10
235-01	-	3.60	-	6.23	-	15.1	-	0.39
235-70	3.60	3.55	6.33	5.38	14.8	15.1	0.52	0.25
235-74	3.56	3.58	6.14	6.42	16.0	14.2	0.46	0.32
2021								
Own	3.69	3.60	7.28	6.99	16.5	15.3	0.15	0.06
1103P	3.62	3.63	6.97	7.84	14.6	-	0.05	0.08
230-46	3.61	3.63	7.29	7.04	15.0	15.1	0.11	0.08
230-47	3.62	3.61	7.32	7.55	15.8	15.4	0.08	0.09
231-11	3.66	3.65	7.25	6.77	15.3	15.6	0.04	0.06
232-13	3.62	3.62	7.40	7.27	15.6	15.5	0.12	0.08
232-80	3.62	3.67	7.25	6.78	15.1	14.8	0.04	0.06
235-61	3.66	3.64	7.38	6.64	15.7	16.2	0.05	0.14
mean	**3.61**	**3.60**	**6.78**	**6.58**	**15.5**	**15.4**	**0.22**	**0.19**
Std. error	0.04	0.04	0.62	0.66	0.7	0.6	0.18	0.13

TA, titratable acid; VA, volatile acidity

Spectral data

Wine spectral data are presented in Table 6.2.9. Over all wines, deficit irrigation increased colour density, total anthocyanin and ionised anthocyanin, but had little effect on hue or total phenolics. Wines produced from fruit of own rooted vines had high colour density and high total and ionised anthocyanin in both years. Wines from 1103 Paulsen had low colour density, low total and ionised anthocyanin and low total phenolics with both irrigation treatments in 2019 and with control irrigation in 2021. In 2019, spectral properties of wines produced from all selected rootstock genotypes were generally better than from 1103 Paulsen with both irrigation treatments. In 2019, genotype 231-51 produced bright wines with a low hue, high colour density and high total and ionised anthocyanin and high total phenolics from both irrigation treatments. Similarly, wines from genotype 235-74, produced wines with high colour density and high total and ionised anthocyanin and high total phenolics from both irrigation treatments. Genotype 230-45 also produced wines with excellent spectral properties. Spectral properties of wines from 231-20, 235-01 (deficit only) and 235-70, although generally better than those from 1103 Paulsen, were less favourable than the other selections listed above or own roots. In 2021, selection 230-46 produced bright wines with low hue, high colour density and high ionised anthocyanins from both irrigation treatments despite having low total anthocyanin and phenolics. Wines of 232-80 had high colour density, low hue (bright), relatively high total and ionised anthocyanin and high total phenolics with both irrigation treatments. Spectral properties of 232-11 and 235-61 were generally acceptable and enhanced by deficit irrigation giving high colour density, a bright wine with low hue and higher total and ionised anthocyanin and total phenolics. Spectral properties of wines from 230-47 and 232-13, although generally better than those from 1103 Paulsen from the control irrigation, were less favourable than the other selections listed above or own roots.

Table 6.2.11. Wine spectral data of own rooted Shiraz or grafted on promising CSIRO rootstock genotypes and 1103 Paulsen, under two irrigation strategies, a well-watered control and a deficit treatment, seasons 2019 and 2021. Mean values across rootstocks are presented with their standard deviation.

Rootstock	Colour density (a.u.)		Hue		Total anthocyanin (mg/L)		Ionised anthocyanin (mg/L)		Total phenolics (a.u.)	
	Con.	Def.	Con.	Def.	Con.	Def.	Con.	Def.	Con.	Def.
2019										
Own	7.12	7.13	0.72	0.78	337	278	39.5	32.7	43.5	37.3
1103 P	3.98	3.60	0.84	0.91	239	229	19.5	17.0	29.4	25.0
230-45	6.32	6.67	0.79	0.76	301	282	27.1	29.3	40.4	38.4
231-20	5.46	5.33	0.77	0.78	225	253	25.7	25.2	29.5	32.5
231-51	7.06	6.29	0.71	0.75	325	323	39.1	37.3	43.0	35.4
235-01	-	5.35	-	0.82	-	245	-	23.7	-	30.7
235-70	5.35	4.71	0.81	0.76	276	298	23.5	28.1	36.4	30.4
235-74	7.46	6.45	0.81	0.74	293	309	32.6	32.7	41.9	38.4
2021										
Own	7.86	7.78	0.74	0.70	250	247	28.1	32.7	41.3	34.7
1103P	5.83	8.97	0.74	0.67	194	310	24.0	36.3	29.5	32.8
230-46	8.43	7.62	0.72	0.73	210	180	57.3	32.3	30.9	35.4
230-47	5.25	5.83	0.76	0.74	203	215	20.2	20.2	31.7	36.1
231-11	6.47	8.23	0.74	0.70	271	245	25.9	35.3	42.4	40.2
232-13	5.28	6.82	0.78	0.77	229	242	18.9	18.5	35.2	35.9
232-80	7.07	7.95	0.72	0.73	219	265	30.2	31.0	37.1	43.1
235-61	6.17	10.22	0.74	0.67	243	270	25.4	39.0	37.8	44.7
mean	**6.34**	**6.81**	**0.76**	**0.75**	**254**	**273**	**28.6**	**30.6**	**36.7**	**37.7**
Std. dev.	1.08	1.50	0.03	0.03	32	82	10.4	6.3	4.4	9.1

Correlation analyses were used to explore the relationships between growth traits and must and wine composition and spectral properties across rootstock genotypes for the different irrigation treatments (Table 6.2.12). In many cases, the responses were different for the two irrigation treatments. Yield did not have a major impact on wine composition for either irrigation treatment. With the control irrigation it was negatively correlated with wine alcohol and with total wine anthocyanin. With deficit irrigation yield was positively correlated with wine pH. Bunch number had a minor impact on must and wine composition with the control irrigation, being positively correlated with must pH and tartaric acid added for pH adjustment but no effect with deficit irrigation. Bunch weight had a significant impact on must and wine composition. With control irrigation it was positively correlated with wine pH and wine TA and negatively correlated with must TSS, wine alcohol, total and ionised anthocyanin and total phenolics. With deficit irrigation, bunch weight was positively correlated with wine pH and negatively correlated with alcohol and wine hue, but not with wine TA, total and ionised anthocyanin and total phenolics. Berry weight also had significant impacts on must and wine composition. With control irrigation it was negatively correlated with alcohol, total and ionised anthocyanin and total phenolics whereas with deficit irrigation the only correlation with berry weight was with wine pH. Berries per bunch were positively correlated with wine TA and negatively correlated with must TSS, alcohol and total anthocyanins with the control

irrigation. With deficit irrigation berries per bunch were positively correlated with wine pH, wine TA and colour density and negatively correlated with wine hue. For both irrigations, there were no significant correlations between pruning weight and must or wine composition traits. The Ravaz Index was positively linked to wine colour density and total phenolics with deficit irrigation.

Table 6.2.12. Correlation coefficients (r) between growth traits and must and wine composition and spectral properties across rootstock genotypes for both irrigation treatments, a well-watered control and a deficit treatment, seasons 2019 and 2021 (n=15 for control irrigation and 16 for deficit irrigation. Significant correlations are shown in bold ($p < 0.05$) or italics ($p < 0.10$). Red lettering is used to show where relationships are different between irrigation treatments.

	Yield	Bunch	Bunch wt.	Berry wt.	Berry/ bunch	Pruning wt.	Ravaz Index
Irrigation 1							
Must TSS	-0.34	-0.16	**-0.46**	0.09	**-0.63**	-0.16	-0.10
Must pH	0.36	**0.59**	-0.11	0.11	-0.23	0.36	-0.17
Must TA	-0.06	-0.41	0.41	0.37	0.25	-0.15	-0.15
Total tartaric added	0.21	*0.50*	-0.28	-0.12	-0.31	0.34	-0.17
Wine pH	0.32	0.13	*0.47*	0.40	0.33	0.13	-0.01
Wine TA	0.14	-0.26	**0.63**	0.39	**0.53**	-0.14	0.05
Alcohol	**-0.52**	-0.30	**-0.57**	*-0.42*	*-0.49*	-0.38	0.07
Wine colour density	-0.30	-0.24	-0.24	-0.33	-0.08	-0.24	0.25
Wine hue	-0.20	-0.10	-0.25	-0.13	-0.16	-0.20	0.21
Total anthocyanin	*-0.47*	-0.11	**-0.51**	**-0.51**	**-0.59**	-0.05	-0.12
Ionised anthocyanin	-0.30	-0.05	**-0.53**	*-0.47*	-0.39	-0.03	0.01
Total phenolics	-0.38	-0.15	**-0.52**	*-0.46*	-0.38	-0.07	0.03
Irrigation 2							
Must TSS	0.12	0.23	-0.23	-0.13	-0.41	0.02	0.07
Must pH	0.10	0.04	0.20	0.21	-0.12	0.19	-0.10
Must TA	-0.23	-0.28	-0.09	0.14	0.26	-0.08	-0.21
Total tartaric added	0.09	0.15	-0.03	0.10	-0.25	0.25	-0.23
Wine pH	*0.49*	0.19	**0.70**	**0.50**	**0.64**	0.37	0.40
Wine TA	-0.08	-0.29	0.35	0.13	*0.44*	0.03	0.03
Alcohol	-0.20	-0.06	*-0.46*	-0.14	**-0.52**	-0.30	0.11
Wine colour density	0.12	-0.09	0.41	0.12	*0.44*	-0.07	*0.45*
Wine hue	-0.10	0.12	*-0.45*	-0.15	*-0.42*	0.05	-0.36
Total anthocyanin	-0.00	0.09	-0.12	*-0.42*	-0.21	-0.05	-0.12
Ionised anthocyanin	0.10	0.03	0.21	-0.05	0.10	-0.08	0.28
Total phenolics	0.25	-0.11	-0.22	0.09	0.32	-0.03	*0.44*

TSS, total soluble sugars; TA, titratable acid

Bunch weight and berries per bunch were identified as factors influencing fruit maturity (i.e. must TSS at harvest) across the rootstock genotypes (Table 6.2.12). For both factors, the negative relationships with both irrigation treatments are shown in Figure (6.2.16). They indicate that must TSS was increased and the range reduced by deficit irrigation compared to the control across the genotypes. The general trends showing decreased must TSS with larger bunches or more berries per bunch had reasonably similar slopes with both irrigation treatments, an indication that the responses were similar. These results indicate that genotypes which produce larger bunches with more berries are likely to have delayed maturity, possibly associated with source/sink carbohydrate relationships at the bunch level, as there were no significant relationships between yield and must TSS. It is likely that the increase in must TSS with deficit irrigation may have been associated with berry wilting and concentration of other components of TSS including minerals, organic acids and amino acids, as the conversion to alcohol was lower with the deficit treatment (Figure 6.2.24 below).

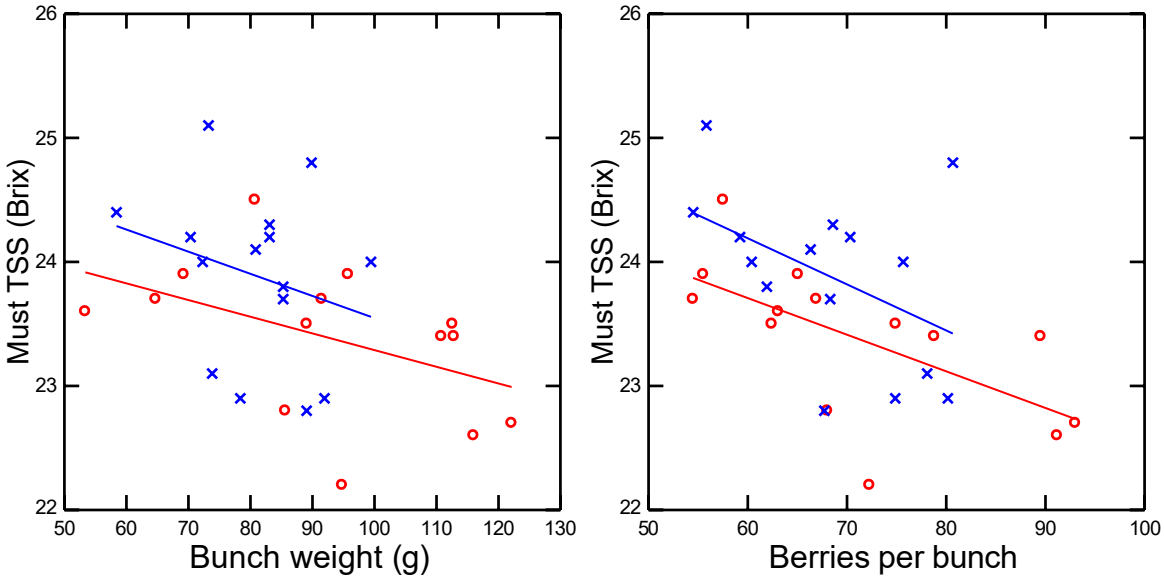


Figure 6.2.16. Linear relationships between bunch weight and berries per bunch and must TSS for control (red, *n*=14) and sustained deficit (blue, *n*=16) irrigation treatments.

Bunch weight and berry weight were identified above as key factors influencing wine composition. The significant relationships for these traits are explored in more detail in Figures 6.2.17 and 6.2.18. Compared to the control irrigation, the range in bunch weight was reduced by deficit irrigation. Compared to the control irrigation, the significant positive linear relationships between bunch weight and both wine pH and wine TA were stronger with steeper slopes with deficit irrigation. The reduction in wine alcohol as bunch weight increased was similar with both irrigation treatments. This suggests that larger bunches have lower sugars available for conversion to alcohol during fermentation. Total wine anthocyanin decreased as bunch weight increased with the control irrigation, however this relationship was uncoupled with deficit irrigation. Similarly, ionised anthocyanin and total phenolics were reduced as bunch weight increased with the control irrigation. In contrast, with deficit irrigation this relationship appeared to be reversed, although in both cases the apparent positive relationships were not significant.

The linear relationships between berry weight and wine composition are shown in Figure 6.2.18. The positive linear relationships between berry weight and wine pH were identical for both irrigation treatments, indicating that the relationship was not affected by irrigation treatment. Similarly, the negative linear relationships between berry weight and wine alcohol followed similar trends with both irrigation treatments. This suggests that larger berries have lower sugars available for conversion to alcohol during fermentation. With control irrigation, berry weight was negatively linked to ionised anthocyanin and total phenolics in wine. This was not the case with deficit irrigation, indicating that relationships between berry weight and both ionised anthocyanin and total phenolics were uncoupled by deficit irrigation.

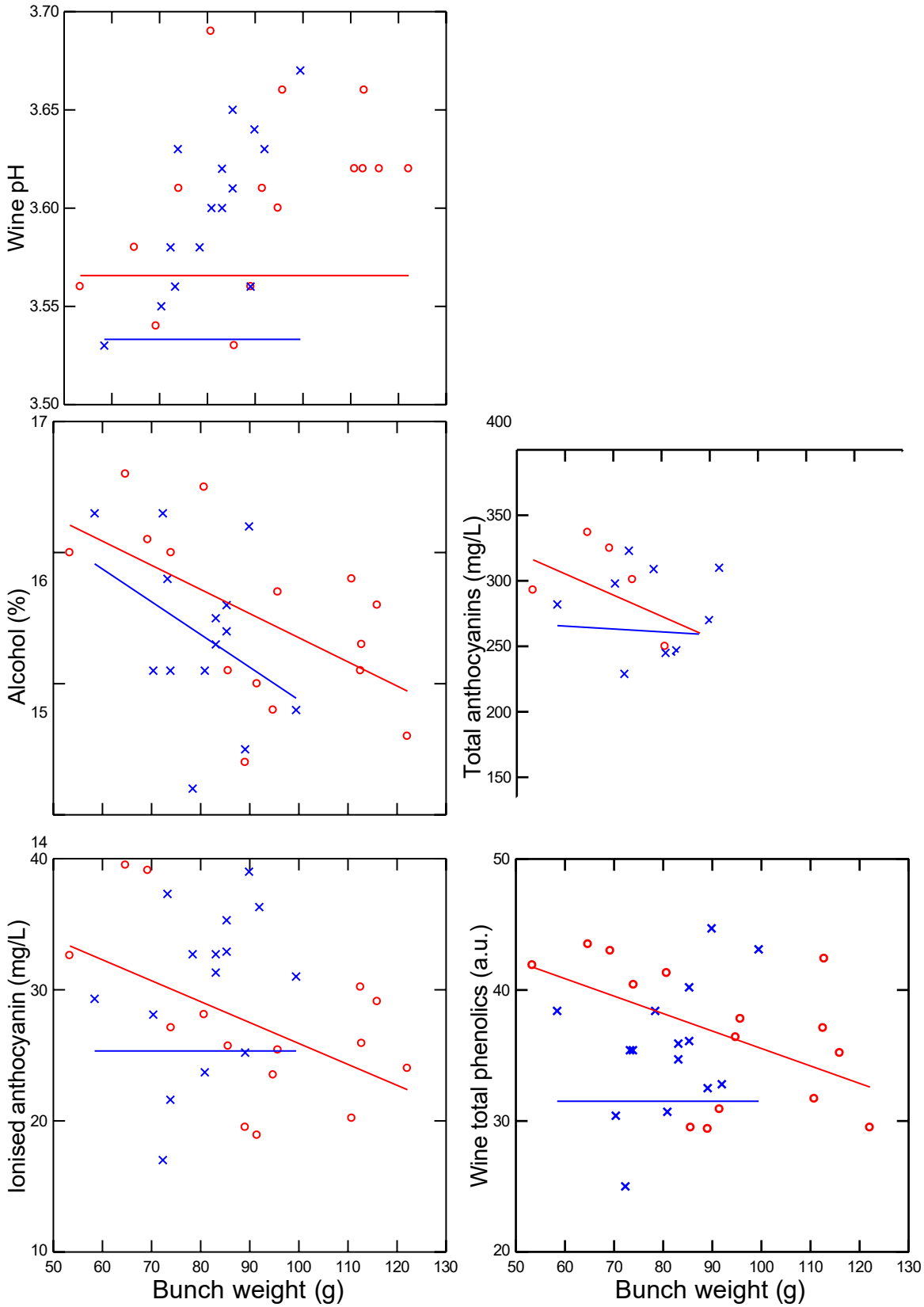


Figure 6.2.17. Linear relationships between bunch weight and wine composition and spectral traits for control (red, *n*=15) and sustained deficit (blue, *n*=16) irrigation treatments. TA; titratable acidity

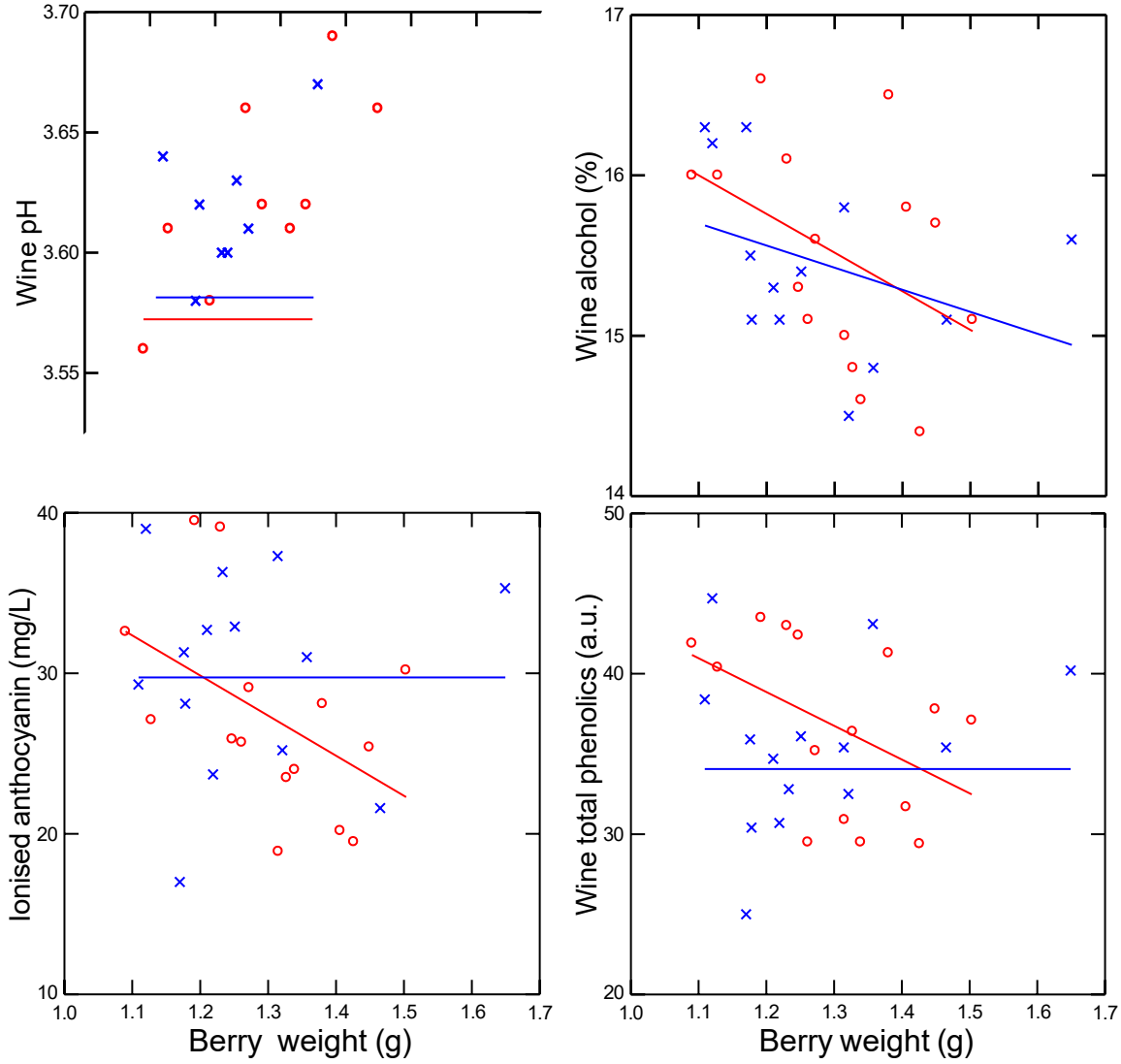


Figure 6.2.18. Linear relationships between berry weight and wine composition and spectral traits for control (red, *n*=15) and sustained deficit (blue, *n*=16) irrigation treatments.

Correlation analyses were used to assess relationships between berry sample data and must and wine composition across the rootstock genotypes (Table 6.2.13). Deficit irrigation had a significant impact on many of the relationships when compared to the full irrigation treatment. With the control irrigation, juice TSS was negatively correlated with must pH and positively correlated with wine colour density and total phenolics. With deficit irrigation, juice TSS was negatively correlated with must pH and positively correlated with must TA. The lack of a correlation between juice TSS and must TSS was not unexpected, as samples for juice analysis were collected on the same date prior to the first harvest, whereas harvest of the fruit of each rootstock genotype for winemaking was delayed until an acceptable level of maturity had been reached. Juice pH was positively correlated with must pH, tartaric acid addition for pH adjustment for both irrigation treatments and with wine alcohol with deficit irrigation. Juice TA was positively correlated with must TA and negatively correlated with must TSS, must pH and tartaric acid addition for pH adjustment with the control irrigation and

must pH with deficit irrigation. Juice malic acid was positively correlated with must TA, wine pH and wine TA with the control irrigation and must pH, tartaric acid addition, wine pH and wine TA with the deficit treatment. Juice tartaric acid was positively correlated with wine hue and negatively correlated with must TSS, must pH and wine pH with control irrigation and with deficit irrigation, was positively correlated with total anthocyanin in wine and negatively with wine pH. There were no significant correlations between juice YAN and must and wine composition with control irrigation, whereas with deficit irrigation, YAN was positively correlated with tartaric acid addition and total anthocyanin in wine. Berry anthocyanin was negatively correlated with must pH and tartaric acid addition and positively correlated with colour density with control irrigation. With deficit irrigation, berry anthocyanin was negatively correlated with must pH and wine hue and positively correlated with must TA, wine TA, colour density, ionised anthocyanins and total phenolics. Juice total phenolics were negatively correlated with must pH and positively correlated with wine colour density, total anthocyanin, ionised anthocyanin and total phenolics with control irrigation. With deficit irrigation juice total phenolics were negatively correlated with must pH, tartaric acid addition and wine hue and positively correlated with must TA, ionised anthocyanin and total phenolics.

Table 6.2.13. Correlation coefficients (r) between berry juice composition and wine composition and spectral properties across rootstock genotypes for both irrigation treatments, a well-watered control and a deficit treatment, seasons 2019 and 2021 (n=15 for control and 16 for deficit irrigation).

	TSS	pH	TA	Malic	Tart	YAN	Antho	Phen
Irrigation 1								
Must TSS	-0.12	0.36	-0.37	-0.06	-0.39	0.22	0.05	0.11
Must pH	**-0.51**	*0.49*	**-0.74**	-0.20	**-0.54**	0.23	**-0.58**	**-0.53**
Must TA	0.28	-0.28	*0.43*	**0.55**	0.05	-0.17	0.31	0.22
Tart. added	-0.41	**0.51**	*-0.48*	-0.19	-0.23	0.28	**-0.54**	-0.37
Wine pH	0.10	0.08	-0.14	*0.50*	*-0.45*	-0.07	-0.04	-0.18
Wine TA	0.31	-0.18	0.01	**0.53**	-0.35	-0.35	0.17	0.00
Alcohol	0.35	0.12	-0.16	-0.31	0.09	0.20	0.37	0.34
Col. density	**0.52**	0.20	-0.13	-0.10	0.00	0.18	**0.55**	*0.48*
Wine hue	-0.13	-0.19	0.41	-0.25	*0.43*	0.04	-0.06	-0.04
Tot. antho	0.24	0.34	0.01	-0.41	0.42	0.40	0.23	*0.43*
Ion. antho	0.26	0.26	-0.16	-0.31	0.17	0.30	0.34	*0.44*
Total phen	*0.46*	0.40	-0.04	-0.10	0.19	0.40	0.39	*0.47*
Irrigation 2								
Must TSS	-0.29	0.17	-0.22	-0.30	0.16	0.11	-0.01	0.02
Must pH	*-0.45*	**0.83**	**-0.62**	*0.45*	-0.26	0.40	**-0.51**	**-0.67**
Must TA	*0.45*	-0.29	0.40	0.18	0.02	-0.12	**0.53**	*0.45*
Tart. added	-0.27	**0.84**	-0.33	**0.62**	-0.05	**0.51**	-0.40	**-0.61**
Wine pH	0.11	0.17	-0.40	**0.56**	**-0.52**	-0.05	0.39	-0.09
Wine TA	0.33	0.19	-0.21	*0.44*	-0.26	-0.31	**0.64**	0.25
Alcohol	-0.41	*0.45*	-0.06	-0.01	0.13	0.27	-0.09	-0.22
Col. density	0.13	0.04	-0.29	0.22	-0.19	-0.29	**0.69**	0.40
Wine hue	-0.09	0.09	0.29	-0.10	0.18	0.24	**-0.56**	*-0.45*
Total antho	0.14	0.10	0.18	0.00	**0.67**	*0.43*	-0.10	0.22
Ion. antho	0.12	0.02	-0.17	0.08	0.17	-0.09	*0.45*	*0.43*
Total phen	0.28	-0.37	-0.02	-0.10	-0.05	-0.32	**0.65**	*0.55*

TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tartaric, tartaric acid; YAN, yeast assimilable nitrogen; Antho, anthocyanin; Phen, phenolics

The effect of deficit irrigation on relationships between juice composition and must and wine data across the genotypes is explored in more detail in Figures 6.2.19 - 6.2.23. The significant negative relationships between juice TSS and must pH show that deficit irrigation increased must pH of most genotypes but that the downward trends were similar (Figure 6.2.19). This result is unexpected, but is most likely due to delayed harvest of genotypes with lower maturity. Wine colour density increased as juice TSS increased with control irrigation, a relationship decoupled by the deficit treatment as the regression was not significant. For most genotypes, wine colour density was higher with deficit irrigation than with the control. The very highly significant positive relationships between juice pH and both must pH and tartaric acid addition for pH adjustment during winemaking were unaffected by deficit irrigation, although the range in juice pH was much broader with the deficit treatment. The negative

trend between juice TA and must pH was unaffected by deficit irrigation except for an increase in must pH for most genotypes. Negative trends showing a reduced requirement for tartaric acid addition with higher juice TA were only significant with the control irrigation. Generally, deficit irrigation produced a higher requirement for tartaric acid addition across the genotypes.

The significant positive relationship between juice malic acid and must TA with control irrigation across genotypes was uncoupled by deficit irrigation (Figure 6.2.20), presumably due to increased malic acid respiration described above leading to lower TA. Relationships describing increases in wine pH and wine TA with juice malic acid concentration, following pH adjustment during the winemaking process, were not impacted by deficit irrigation. There was a strong relationship between juice malic acid and the requirement for tartaric acid addition with deficit irrigation. Such a relationship did not exist with the control irrigation, which in general, had a lower requirement tartaric acid addition for pH adjustment.

The significant negative relationships between juice tartaric acid and wine pH were unaffected by deficit irrigation (Figure 6.2.21). This relationship is most likely associated with reduced tartaric acid addition for genotypes with high juice tartaric acid. The positive relationships describing increased total anthocyanin in wine with increased juice tartaric acid were also unaffected by deficit irrigation. Juice YAN was significantly impacted by deficit irrigation with both an overall increase and a much wider range across the genotypes compared to the control irrigation (Figure 6.2.21). The general trend showing an increased requirement for tartaric addition for pH adjustment as YAN increased, was however not impacted by deficit irrigation. The weak trends, showing increased total anthocyanin in wine as juice YAN increased, largely reflect the impact of deficit irrigation on both the increase and range of juice YAN across the genotypes.

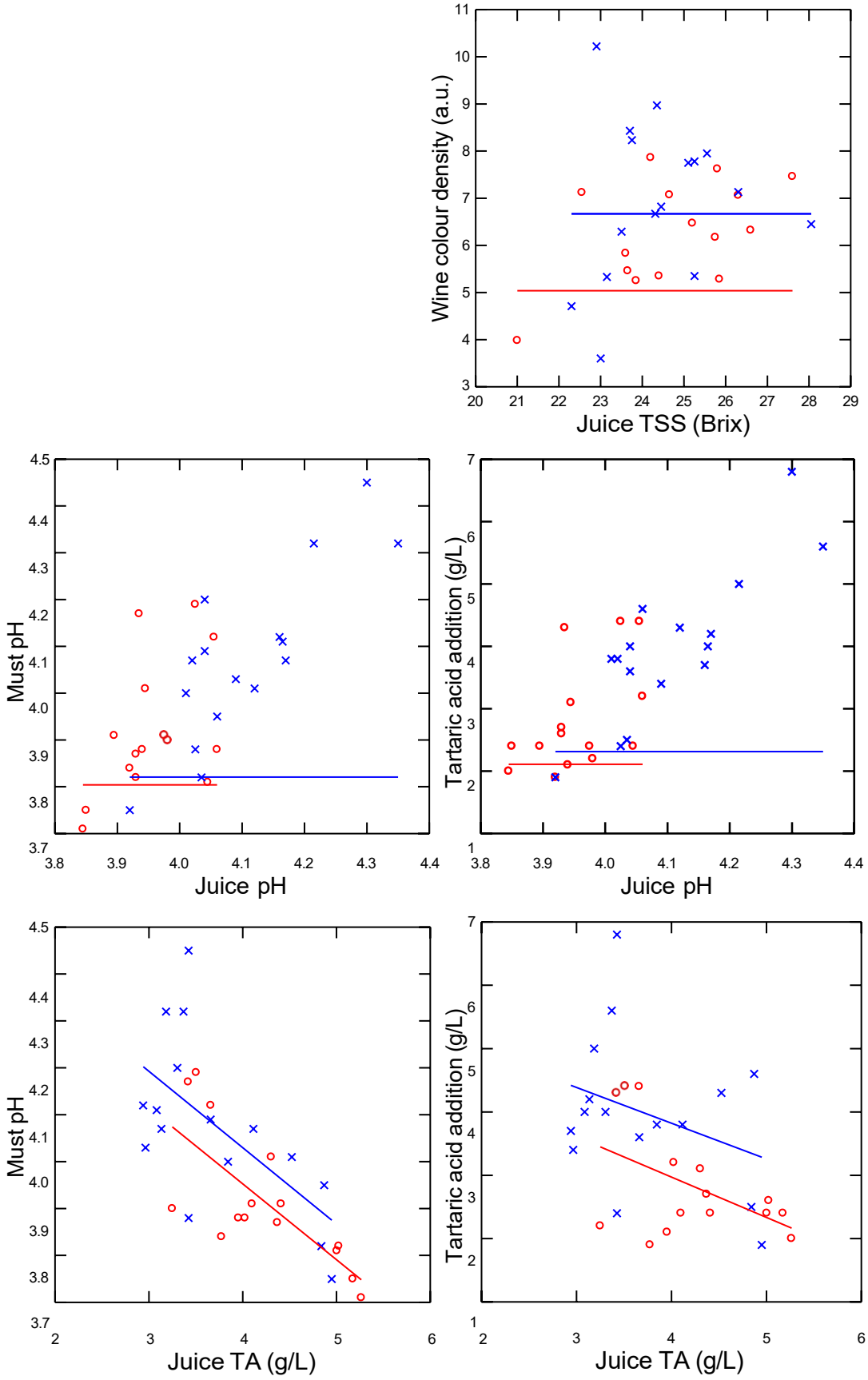


Figure 6.2.19. Linear relationships between juice composition traits (TSS, pH and TA) and must pH or tartaric acid addition for pH adjustment for control (red, *n*=15) and sustained deficit (blue, *n*=16)

irrigation treatments.

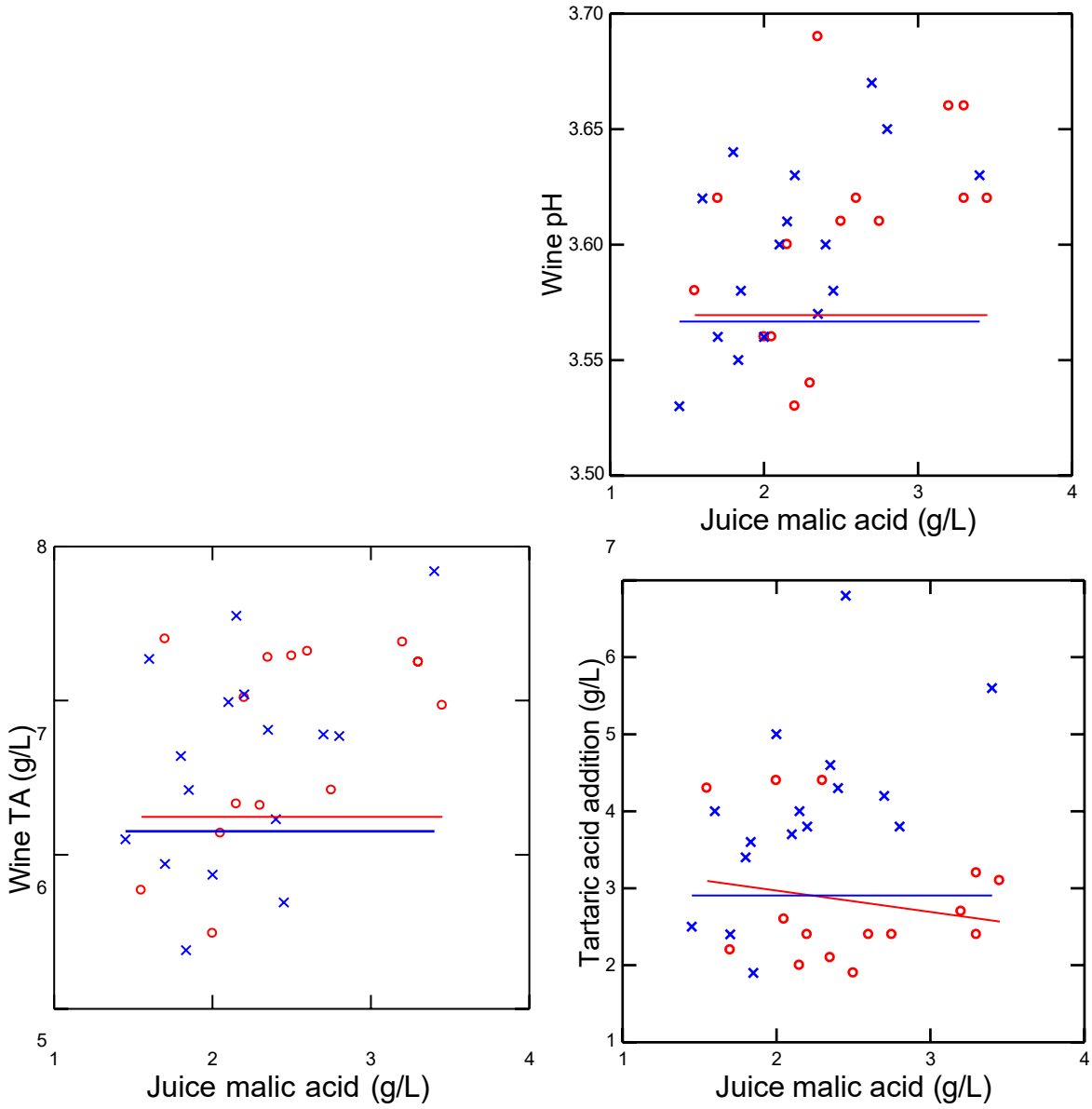


Figure 6.2.20. Linear relationships between juice malic acid and must TA, wine pH, wine TA and tartaric acid addition for pH adjustment for control (red, *n*=15) and sustained deficit (blue, *n*=16) irrigation treatments. TA, titratable acid

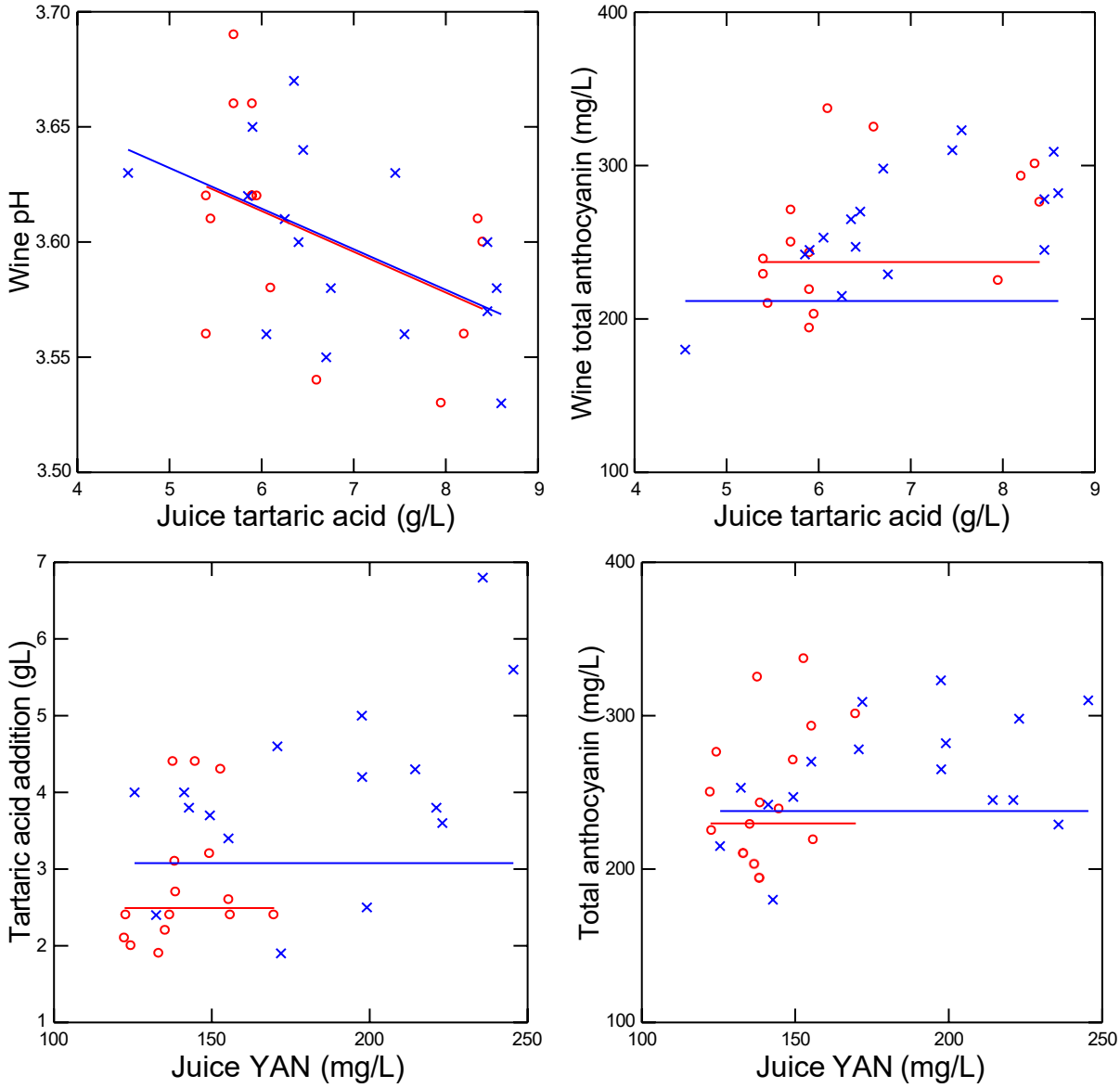


Figure 6.2.21. Linear relationships between juice tartaric acid and wine pH and wine total anthocyanin (top) and juice YAN and tartaric acid addition and wine total anthocyanin (bottom) for control (red, *n*=15) and sustained deficit (blue, *n*=16) irrigation treatments. YAN, yeast assimilable nitrogen

The correlations coefficients presented in Table 6.2.13 show that berry anthocyanin and total phenolic substances were good predictors of must and wine composition and spectral properties. It is likely that these berry traits integrate both the effects of rootstock genotype and environmental conditions on fruit development and subsequent fruit and wine composition. For example, across the genotypes, berry anthocyanin and phenolics were negatively correlated with yield, bunch number, bunch weight, berry weight and pruning weight (Table 6.2.3), positively correlated with juice TSS and tartaric acid, and negatively correlated with malic acid and YAN (Table 6.2.4). The most significant relationships between berry anthocyanins and berry phenolic substances and must and wine composition are presented for both irrigation treatments in Figures 6.2.22 and 6.2.23, respectively. Compared to control irrigation, there was a general increase in must pH with deficit irrigation across the genotypes (Figure 6.2.22). However, the negative relationships between must pH and berry anthocyanins were largely unaffected as the slopes of the regressions for both irrigation treatments were similar. Must TA was positively correlated with berry anthocyanin with both irrigation treatments. In this case, must TA was lower with the deficit treatment, but slopes were similar. Tartaric acid addition for pH adjustment was negatively correlated with berry anthocyanin with both irrigation treatments. In this case tartaric acid addition was higher with the deficit treatment but slopes were similar. Wine TA was positively correlated with berry anthocyanin with the deficit irrigation, but not with the control irrigation.

Not unexpectedly, berry anthocyanin was related to wine spectral properties (Figure 6.2.22). There were highly significant positive relationships between wine colour density and berry anthocyanin with both irrigation treatments, although the slope of this trend was steeper with deficit irrigation. The significant negative relationship between wine hue and berry anthocyanin with deficit irrigation indicates that high berry anthocyanin was linked to development of brighter wines. Such an association was not found with wines from the control irrigation treatment. There were also strong positive relationships between ionised anthocyanin in wines and berry anthocyanin with both irrigation treatments although the slope was slightly steeper with the deficit treatment. Similarly, there were strong positive relationships between total wine phenolics and berry anthocyanin with both irrigation treatments, although the slope was slightly steeper with the deficit treatment.

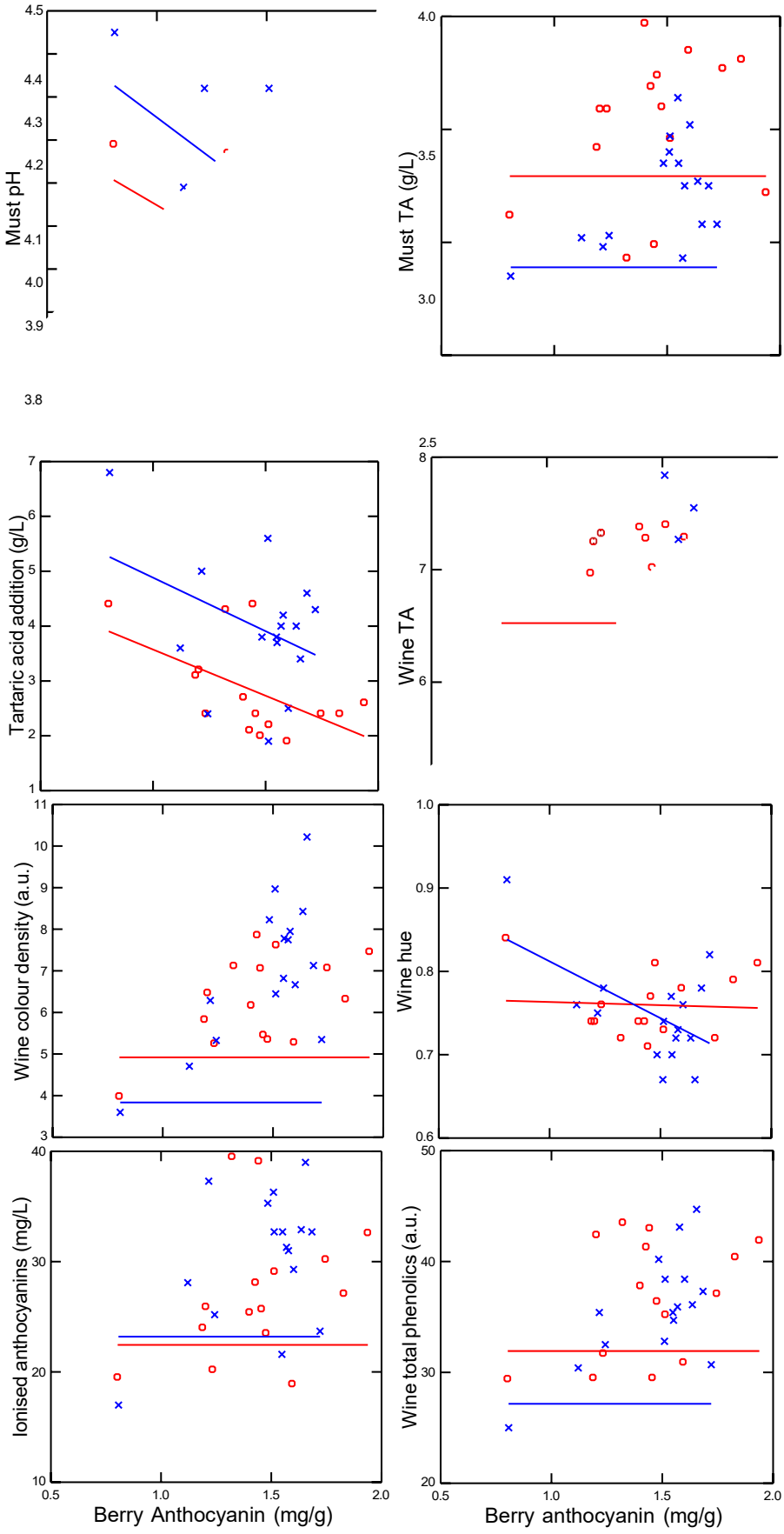


Figure 6.2.22. Linear relationships between berry anthocyanin and must pH and TA, tartaric acid addition and wine TA, colour density, hue, ionised anthocyanin and total phenolics for control

(red, *n*=15) and sustained deficit (blue, *n*=16) irrigation treatments. TA, titratable acid

The most significant relationships between berry phenolics and must and wine composition are shown in Figure 6.2.23. There were strong negative relationships with both irrigation treatments between must pH and berry phenolics, although the slope was much steeper with the deficit treatment. Similarly, there were strong negative relationships with both irrigation treatments between tartaric acid addition for pH adjustment and berry phenolics, although the slope was much steeper with the deficit treatment. Berry phenolics were positively related to the spectral properties of the wine including wine colour density, total anthocyanin, ionised anthocyanin and total phenolics. In all cases, these relationships were similar with both irrigation treatments with only minor deviations from the general trend associated with small differences in slopes.

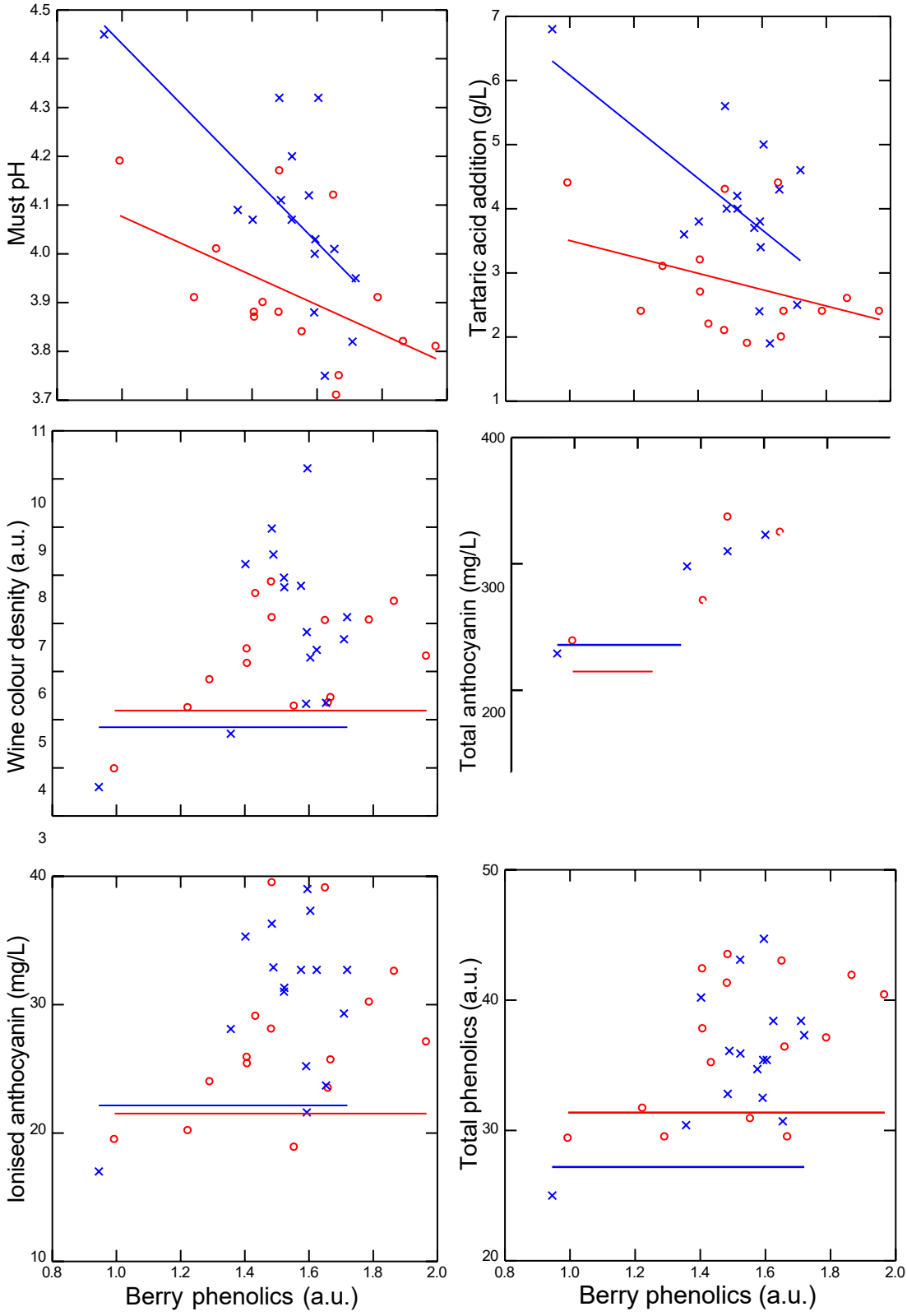


Figure 6.2.23. Linear relationships between berry phenolic substances and must pH, tartaric acid addition and wine colour density, total anthocyanin, ionised anthocyanin and total phenolics for control (red, *n*=15) and sustained deficit (blue, *n*=16) irrigation treatments.

Correlation analyses were also used to explore relationships between must and wine composition traits across the rootstock genotypes (Table 6.2.14). Surprisingly, must TSS was correlated with very few wine composition traits except for significant correlations with wine alcohol with both irrigation treatments; a positive correlation with total phenolics with control irrigation; and a negative correlation with must TA with deficit irrigation. Must pH was negatively correlated with must TA and positively correlated with tartaric acid addition for pH adjustment with both irrigation treatments; negatively correlated wine TA and positively correlated with total wine phenolics with control irrigation; and positively correlated with wine alcohol with deficit irrigation. Must TA was positively correlated with wine pH and wine TA and negatively correlated with tartaric acid addition for pH adjustment and total and ionised anthocyanin in wine. In contrast, with deficit irrigation must TA was only weakly correlated with wine TA and total phenolics. Tartaric addition for pH adjustment was negatively correlated with wine pH and wine TA and positively correlated with total and ionised anthocyanin in wine with control irrigation. The only significant correlation with tartaric acid addition with the deficit treatment was a positive correlation with wine alcohol. Wine pH, following adjustment during the winemaking process was positively correlated with wine TA with both irrigation treatments; positively correlated with wine colour density and total phenolics and negatively correlated with wine hue in the deficit treatment. Wine TA was positively correlated with wine hue and total anthocyanins with control irrigation. With deficit irrigation, wine TA was positively correlated with colour density (very strongly) and total wine phenolics and negatively correlated with hue. With control irrigation, wine alcohol concentration was highly correlated with wine colour density, total and ionised anthocyanin and phenolics and negatively correlated with wine hue. These results indicate that higher alcohol wines were likely to be brighter and highly coloured. This was not the case with deficit irrigation as wine TA was not correlated with wine spectral properties. Wine colour density was high correlated positively with wine anthocyanin and phenolics and negatively correlated with wine hue with both irrigation treatments. Wine hue was negatively correlated with ionised anthocyanins with both irrigation treatments and total wine phenolics with deficit irrigation. Total anthocyanin in wine was positively correlated with wine ionised anthocyanin and phenolics with both irrigation treatments. Ionised anthocyanin was positively correlated with wine phenolics with both irrigation treatments.

Table 6.2.14. Correlation coefficients (r) between wine composition and spectral properties across rootstock genotypes for both irrigation treatments, a well-watered control and a deficit treatment, seasons 2019 and 2021 (*n*=15 for control and 16 for deficit irrigation).

	Must			Wine				Spectral		
	TSS	**pH**	**TA**	**Add**	**pH**	**TA**	**Alc.**	**CD**	**Hue**	**T. An.**	**I. An.**
Irrigation 1											
Must pH	0.34										
Must TA	-0.05	**-0.75**									
Tart. added	0.24	**0.89**	**-0.80**								
Wine pH	0.27	-0.24	**0.55**	*-0.43*							
Wine TA	0.01	*-0.50*	**0.74**	**-0.67**	**0.63**						
Alcohol	**0.60**	0.09	-0.20	0.08	0.15	-0.02					
Col. density	0.33	-0.04	-0.12	-0.10	0.27	0.21	**0.72**				
Wine hue	-0.25	-0.24	0.03	-0.13	-0.31	*-0.48*	*-0.44*	-0.62			
Total antho	0.26	0.24	**-0.54**	*0.49*	-0.29	**-0.63**	**0.60**	0.40	-0.06		
Ion. antho	0.27	0.35	**-0.56**	*0.44*	-0.27	-0.31	**0.68**	**0.75**	**-0.55**	**0.75**	
Total phen	*0.47*	0.07	-0.26	0.22	0.18	-0.18	**0.76**	**0.74**	-0.37	**0.82**	**0.76**
Irrigation 2											
Must pH	0.29										
Must TA	*-0.43*	**-0.51**									
Tart. added	0.21	**0.89**	-0.40								
Wine pH	-0.13	0.21	0.17	0.19							
Wine TA	-0.31	0.09	*0.46*	0.09	**0.65**						
Alcohol	**0.69**	*0.44*	-0.21	**0.52**	-0.13	-0.05					
Col. density	0.08	-0.06	0.37	-0.15	**0.64**	**0.77**	0.14				
Wine hue	-0.02	0.14	-0.37	0.35	*-0.46*	**-0.61**	0.13	**-0.88**			
Total antho	-0.16	0.22	0.08	0.20	-0.06	0.18	-0.02	0.22	-0.38		
Ion. antho	0.28	-0.04	0.14	-0.19	0.24	0.41	0.11	**0.78**	**-0.86**	*0.48*	
Total phen	-0.26	0.07	*0.42*	0.06	*0.45*	**0.65**	0.01	**0.72**	**-0.71**	**0.77**	**0.62**

TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tartaric, tartaric acid; YAN, yeast assimilable nitrogen; Antho, anthocyanin; Phen, phenolics

Key relationships between must composition and wine composition are described in detail in Figures 6.2.24 and 6.2.25. The positive trend between must TSS and wine alcohol were similar with both irrigation treatments having similar slopes although alcohol was generally lower with deficit irrigation. As discussed above, this lower conversion is most likely to be associated with increased concentration of non-fermentable sugar components of TSS such as mineral elements, organic acids and amino acids associated with increased berry wilting. The highly significant relationships between must pH and tartaric acid addition for pH adjustment were unaffected by irrigation treatment.

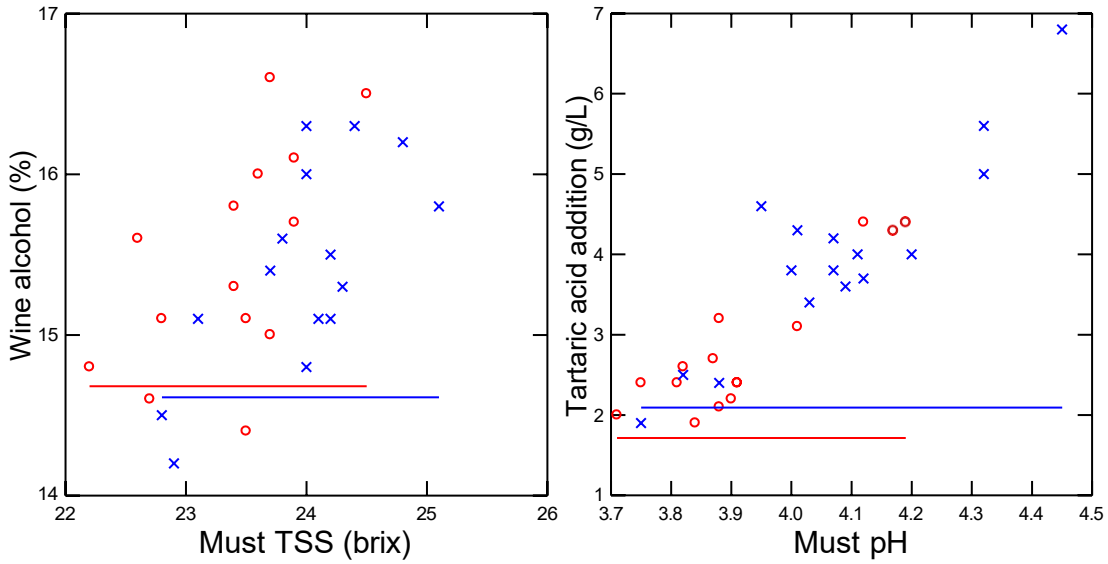
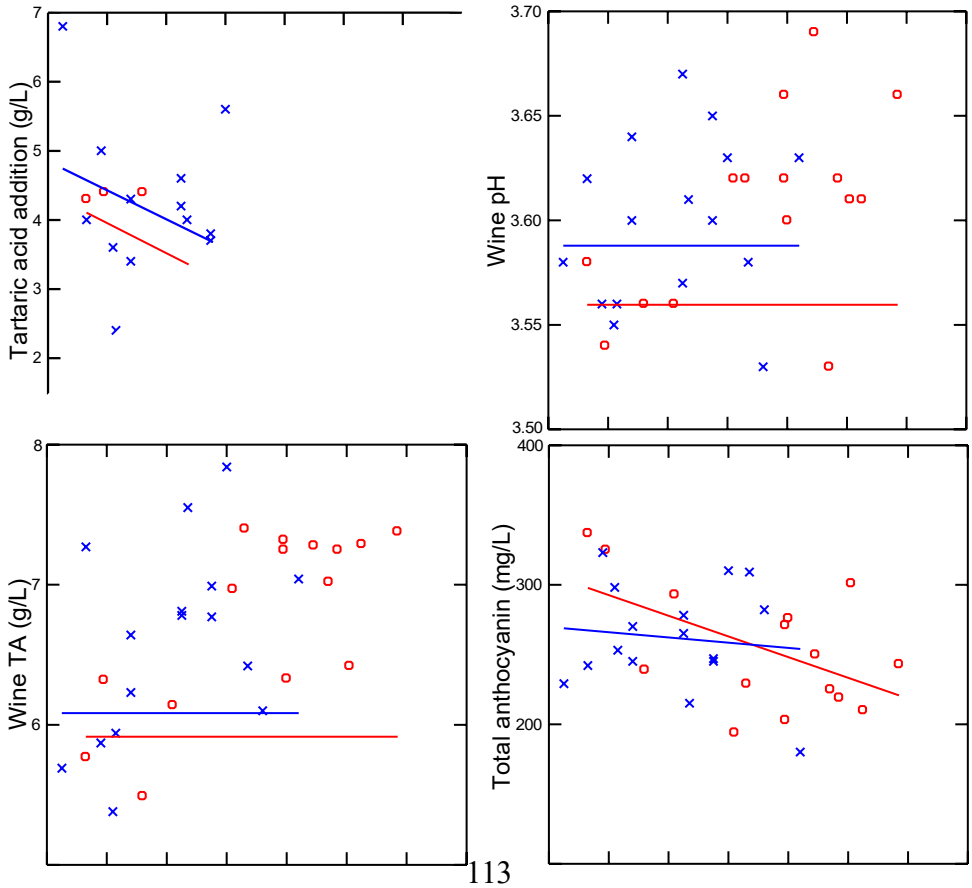


Figure 6.2.24. Linear relationships between must TSS and wine alcohol and must pH and tartaric acid addition for control (red, *n*=15) and sustained deficit (blue, *n*=16) irrigation treatments. TSS, total soluble sugars

Compared to control irrigation, the range in must TA was narrower with deficit irrigation (Figure 6.2.25). The highly significant negative relationships between must TA and tartaric acid addition were largely unaffected by irrigation treatment, except for a slightly higher requirement for pH adjustment with the deficit treatment. There was a positive relationship between wine pH and must TA with control irrigation. This was not the case with deficit irrigation, indicating an uncoupling of the relationship. The positive relationships between wine TA and must TA were similar with both irrigation treatments, except for a small shift to higher wine TA with deficit irrigation. Total anthocyanin in wine was negatively linked to must TA with the control irrigation treatment. This was not the case with deficit irrigation indicating an uncoupling of the relationship. Similarly, ionised anthocyanin in wine was negatively linked to must TA with the control irrigation treatment but not with the deficit treatment. There was a broader range in tartaric acid addition for pH adjustment with deficit irrigation (Figure 6.2.25). There was a significant negative relationship between wine TA and the requirement for tartaric acid addition with the control irrigation treatment. This was not the case with the deficit irrigation treatment.



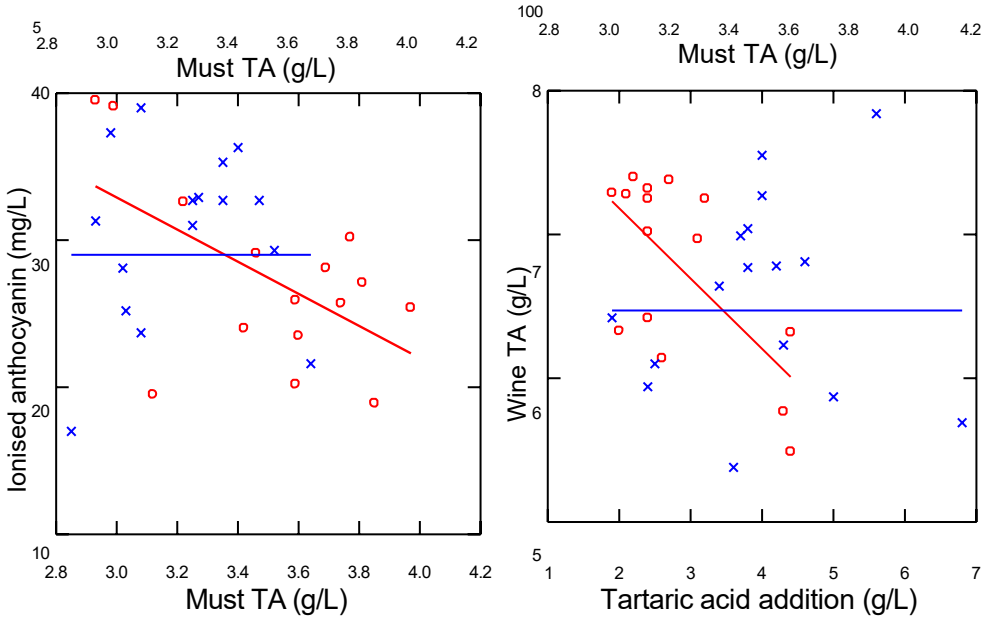


Figure 6.2.25. Linear relationships between must TA and tartaric acid addition, wine pH, TA, total anthocyanin and ionised anthocyanin; and tartaric addition and wine TA for control (red, *n*=15) and sustained deficit (blue, *n*=16) irrigation treatments. TA, titratable acidity

Discussion

A systematic approach over nine years has been used to identify rootstocks with specific attributes for potential release to industry, involving the assessment of field performance and fruit composition of Shiraz grafted on 60 rootstock genotypes including standard commercial rootstocks and own rooted vines grown under two irrigation strategies, a well-watered control and a sustained deficit treatment of 45% of the control. Deficit irrigation not only produced significant changes in most traits across the genotypes, but also produced significant differences in the response of each genotype associated with genotype x environment interactions. This has major implications for the selection of new rootstocks. Vigour conferred by rootstock genotype was used to classify each rootstock genotype to simplify comparisons with commercial rootstocks included in the study. Key criteria used in the selection of genotypes with potential included the ability to maintain performance (pruning weight and yield) under deficit irrigation and effects of irrigation on fruit composition traits that included TSS, pH, malic acid and berry anthocyanin. Small-scale wines were made for the most preferred genotypes on a very limited scale to provide additional support information and to assess effects of deficit irrigation on wine parameters.

Despite significant effects of season on almost all traits, the effect of rootstock genotype on all field performance traits (pruning weight, yield, bunch number, bunch weight, berry weight and berries per bunch) was stable over time. The overall effect of deficit irrigation across the rootstock genotypes over nine seasons, compared to the control irrigation, included a minimal effect on yield, a small but significant increase in bunch number, and reductions in bunch weight, berry weight, berries per bunch and pruning weight accompanied by increased TSS, pH, tartaric acid, anthocyanins and phenolics and reduced TA and malic acid. In meeting this objective, the study has also confirmed for both irrigation treatments that that rootstock-conferred vigour is linked to yield, bunch weight, berry weight and berries per bunch and fruit composition traits. Rootstock yield was a function of both bunch number and bunch weight, the latter correlated with berry weight with both irrigation treatments and with berries per bunch with the control irrigation.

The relationships between field performance traits and fruit composition of the rootstock genotypes varied with irrigation treatment. Rootstock-conferred vigour (pruning weight) was a major driver of fruit composition being positively linked to juice pH, TA, malic acid, YAN, and K and negatively linked to berry anthocyanin and phenolics with control irrigation. With deficit irrigation, pruning weight was positively linked to TA, malic acid, YAN and K and negatively linked to berry anthocyanin and phenolics. There were however, no direct links between pruning weight and must, wine composition or wine spectral properties following addition of tartaric acid for pH adjustment. Across the rootstock genotypes, bunch weight and berry weight were more important drivers of juice and wine composition than yield and bunch number. The study also confirmed that juice pH across the rootstocks was positively correlated with malic acid, YAN and K and that malic acid was positively correlated with YAN and K in both irrigation treatments. The addition of tartaric acid for pH adjustment during

winemaking processes had a significant impact on wine composition and spectral properties. The requirement for pH adjustment was a very useful criterion in the final selection of new rootstocks.

Details on each rootstock genotype selected are provided below. Assessment of phylloxera and nematode tolerance/ resistance has not been completed for these selections.

High Priority Breeding Selections

Genotype 232-80

Genotype 232-80 conferred high vigour to the Shiraz scion. It has been selected as an alternative to the Ramsey rootstock. In this study, it had a similar yield and pruning weight to Ramsey and was more resilient to deficit irrigation, maintaining both its yield and pruning weight. Genotype 232-80 had very favourable juice composition compared to Ramsey, with high TSS, low pH, high TA, high tartaric acid and for its vigour classification, acceptable berry anthocyanins and phenolics. Genotype 232-80 had lower juice Na than Ramsey but a similar, high K concentration. When wine was produced from this genotype in 2021, both irrigation treatments produced acceptable TSS, low must pH, high TA and a low requirement for tartaric acid addition for pH adjustment. With both irrigation treatments, wine spectral properties of 232-80 phenolics were similar to wine from own rooted vines, with high colour density, a bright low hue, high total and ionised anthocyanin and high total phenolics.

Genotype 235-74

Genotype 235-74 conferred low vigour to the Shiraz scion. It has been selected as an alternative to the difficult to propagate Merbein 5489 rootstock and with many characteristics similar to own root rooted vines. In this study, it had similar yield and pruning weight to own rooted vines and Merbein 5489 and was more resilient to deficit irrigation than Merbein 5489 and own roots, maintaining both its yield and pruning weight. Genotype 235-74 had very favourable juice composition with high TSS, low pH, low malic acid, high tartaric acid and high berry anthocyanins (control irrigation only) and phenolics. Genotype 235-74 had very low juice K and Na concentrations. When wine was produced from this genotype in 2019, both irrigation treatments had low must pH, high TA and a low requirement for tartaric acid addition for pH adjustment. With both irrigation treatments, wine spectral properties of 235-74 were similar to wine from own rooted vines with high colour density, high total and ionised anthocyanin and high total phenolics.

Genotype 230-45

Genotype 230-45 conferred low to moderate vigour to the Shiraz scion. Its pruning weight and yield were higher than own roots and Merbein 5489 but lower than 140 Ruggeri. It was selected as an alternative to the difficult to propagate Merbein 5489 rootstock, having many characteristics similar to own root rooted vines. In this study, it was more resilient to deficit irrigation than own roots, Merbein 5489 and 140 Ruggeri. Genotype 230-45 had very favourable juice composition with low pH, high titratable acidity, high tartaric acid and high

berry anthocyanins and phenolics. Genotype 230-45 had low juice K and Na concentrations. When wine was produced from this genotype in 2019, both irrigation treatments had low must pH, high TA and a low requirement for tartaric acid addition for pH adjustment. The wine produced from 230-45 had slightly lower colour density, total and ionised anthocyanins and phenolics than wine from own rooted vines.

Genotype 235-61

Genotype 235-61 conferred moderate vigour to the Shiraz scion. It was selected as an alternative to 140 Ruggeri, which is difficult to propagate. Its pruning weight and yield were slightly less than 140 Ruggeri with control irrigation but higher with deficit irrigation. In this study, it was more resilient to deficit irrigation than own roots or 140 Ruggeri. Compared to 140 Ruggeri, genotype 235-61 had very favourable juice composition with low pH, malic acid, high tartaric acid, and high berry anthocyanin and phenolics. Genotype 235-61 had low juice K but high Na (similar to Ramsey but less than own roots). When wine was produced from genotype 235-61 in 2021, both irrigation treatments had low must pH, high TA and a low requirement for tartaric acid addition for pH adjustment. Wine spectral properties were similar or superior to own roots with high colour density (particularly with deficit irrigation), low hue, high total and ionised anthocyanins and phenolics

Genotype 231-11

Genotype 231-11 conferred moderate vigour to the Shiraz scion. It was selected as an alternative to 140 Ruggeri and 1103 Paulsen. Its pruning weight was similar to 140 Ruggeri but its yield was similar to 1103 Paulsen, particularly with deficit irrigation. In this study, it was more resilient to deficit irrigation than own roots, 140 Ruggeri or 1103 Paulsen. Compared to 140 Ruggeri and 1103 Paulsen, genotype 231-11 had very favourable juice composition with low pH, malic acid, high tartaric acid, and high berry anthocyanin and phenolics. Genotype 231-11 had relatively low juice K and slightly elevated Na. When wine was produced from this genotype in 2021, both irrigation treatments had higher TSS than 1103 Paulsen, low must pH, high TA (control irrigation) and a low requirement for tartaric acid addition for pH adjustment. Wine spectral properties were similar to own roots with high colour density, low hue, high total and ionised anthocyanins and phenolics.

Genotype 235-70

Genotype 235-70 conferred moderate vigour to the Shiraz scion. It was selected as an alternative to 140 Ruggeri and 1103 Paulsen. Its pruning weight was similar to 140 Ruggeri but its yield was similar to 1103 Paulsen, particularly with deficit irrigation. In this study, it was more resilient to deficit irrigation than own roots, 140 Ruggeri or 1103 Paulsen. Compared to 140 Ruggeri and 1103 Paulsen, genotype 235-70 had very favourable juice composition with low pH, malic acid, high tartaric acid, and high berry anthocyanin and phenolics. Genotype 235-70 had relatively low juice K and Na. When wine was produced from this genotype in 2019, it had low must pH, high TA (control irrigation) and a low requirement for tartaric acid addition for pH adjustment. Wine spectral properties were

better than those of 1103 Paulsen wines with high colour density, low hue, high total and ionised anthocyanin and phenolics.

Lower Priority Breeding Selections

Genotype 231-51

Genotype 231-51 conferred moderate vigour to the Shiraz scion. It was selected as an alternative to 140 Ruggeri, which is difficult to propagate. Its pruning weight and yield were slightly less than 140 Ruggeri with control irrigation but higher with deficit irrigation. In this study, it was more resilient to deficit irrigation than own roots or 140 Ruggeri. Compared to 140 Ruggeri, genotype 231-51 had very favourable juice composition with low pH, malic acid, high tartaric acid, and high berry anthocyanin and phenolics. Genotype 231-51 had relatively low juice K and low Na. When wine was produced from this genotype in 2019, both irrigation treatments had low must pH. Unfortunately, the control wine was accidentally lost. For the deficit treatment it had a low requirement for tartaric acid addition for pH adjustment compared to 1103 Paulsen and own roots. Wine spectral properties of 231-51 from the deficit treatment were superior to 1103 Paulsen, but not as good as the wine from own roots with relatively high colour density, total and ionised anthocyanins and phenolics.

Selection 231-51 was not identified as high priority because wine data from the control treatment were not available and spectral properties of the deficit wine, while adequate and superior to 1103 Paulsen, were below those for wine from own roots.

Genotype 232-13

Genotype 232-13 conferred low to moderate vigour to the Shiraz scion. Its pruning weight and yield were higher than own roots and Merbein 5489 but lower than 140 Ruggeri. It was more resilient to deficit irrigation than own roots and 140 Ruggeri. Genotype 232-13 had very favourable juice composition with high TSS, low pH, low malic acid, high tartaric acid and high berry anthocyanins and phenolics. Genotype 232-13 had low juice K and Na concentrations. When wine was produced from this genotype in 2021, both irrigation treatments had low must pH with a low requirement for tartaric acid addition for pH adjustment. The wine produced from 232-13 had poor spectral properties with low colour density, high hue and low total and ionised anthocyanins.

Selection 232-13 was not identified as high priority because of the poor wine spectral properties compared to other selections classified with similar vigour.

Genotype 235-01

Genotype 235-01 conferred low to moderate vigour to the Shiraz scion. Its pruning weight and yield were higher than own roots but lower than 140 Ruggeri. It was more resilient to deficit irrigation than own roots and 140 Ruggeri. Genotype 235-01 juice had high TSS, relatively high pH, low TA, low malic acid and high berry anthocyanins and phenolics. Genotype 235-01 had low juice K and Na concentrations. When wine was produced from this genotype in 2019, both irrigation treatments had low must pH and TA. Winemaking was only completed for the deficit treatment. It had a low requirement for tartaric acid addition for pH adjustment. The wine produced from 235-01 had poor spectral properties with low colour density, high hue and low total and ionised anthocyanins.

Selection 235-01 was not identified as high priority because of its relatively high juice pH and low TA and poor wine spectral properties compared to other selections classified with similar vigour.

Genotype 231-20

Genotype 231-20 conferred low to moderate vigour to the Shiraz scion. Its pruning weight was similar to 140 Ruggeri with yield similar to own roots. It was more resilient to deficit irrigation than own roots and 140 Ruggeri. Genotype 231-20 had favourable juice composition with high TSS, low pH, low malic acid, high tartaric acid and high berry anthocyanins and phenolics. Genotype 231-20 had relatively high juice K and Na concentrations. When wine was produced from this genotype in 2021, both irrigation treatments had low TSS, low must pH, high TA (control) with a low requirement for tartaric acid addition for pH adjustment. The wine produced from 231-20 had poor spectral properties with low colour density, high hue and low total and ionised anthocyanins and phenolics. Spectral properties may have been enhanced if the fruit had been harvested for winemaking when ripe.

Selection 231-20 was not identified as high priority because of its low yield relative to vigour and poor spectral properties when compared to other selections classified with similar vigour.

Genotype 230-46

Genotype 230-46 conferred moderate to high vigour to the Shiraz scion. It was selected as an alternative to 140 Ruggeri, which is difficult to propagate. Its pruning weight was similar to 140 Ruggeri but its yield was slightly lower. In this study, it was more resilient to deficit irrigation than own roots or 140 Ruggeri. Compared to 140 Ruggeri, genotype 230-46 had favourable juice composition with high TSS, low pH, malic acid, high tartaric acid, and high berry anthocyanin and phenolics. Genotype 230-46 had low juice K and low Na. When wine was produced from this genotype in 2021, both irrigation treatments had low must pH, high TA with a low requirement for tartaric acid addition for pH adjustment. Wine spectral properties of 230-46 included a high colour density, low hue and low total anthocyanin, but high ionised anthocyanin and low phenolics.

Selection 230-46 was not identified as high priority because of its low yield and low total anthocyanin in wine when compared to other selections classified with similar vigour.

Genotype 230-47

Genotype 230-47 conferred high vigour to the Shiraz scion. Its pruning weight fell between 1103 Paulsen and Ramsey but it had low yield, similar to the yield of 140 Ruggeri. It was more resilient to deficit irrigation than 1103 Paulsen. Genotype 230-47 had favourable juice composition with high TSS, relatively low pH, low malic acid, high tartaric acid and high berry anthocyanins and phenolics. Genotype 230-47 had relatively high juice K and Na concentrations. When wine was produced from this genotype in 2021, fruit from both irrigation treatments had low must pH with a low requirement for tartaric acid addition for pH adjustment. The wine spectral properties of 230-47 included low colour density, relatively low hue, low total and ionised anthocyanins and phenolics.

Selection 230-47 was not identified as high priority because of its low yield relative to vigour and poor spectral properties.

Genotypes not chosen for winemaking.

Three genotypes included in the best 15 selections were not selected as a high priority for winemaking based on long-term data presented above (Tables 6.2.5, 6.2.6, 6.2.7). These were genotypes 230-30, 233-80 and 232-57.

Genotype 230-30

Genotype 230-30 conferred moderate vigour to the Shiraz scion and was identified as a potential alternative to the 140 Ruggeri rootstock. It had a similar pruning weight but higher yield than 140 Ruggeri. Although better than 140 Ruggeri, it did not display complete resilience to deficit irrigation, as it had a reduction in yield. It had low juice TSS, an indication of a delay in maturity compared to all other genotypes. Despite the low TSS, juice pH of 230-30 was relatively high with low tartaric acid. Berry anthocyanins, although better than 140 Ruggeri were also relatively low, particularly with the control irrigation.

Genotype 233-80

Genotype 233-80 conferred moderate-high vigour to the Shiraz scion with a similar pruning weight to 1103 Paulsen. However, its yield was lower than 1103 Paulsen. Genotype 233-80 was also sensitive to deficit irrigation with significantly lower pruning weight and yield. Compared to 1103 Paulsen it had low juice TSS, low pH (control irrigation only) and low tartaric acid.

Genotype 232-57

Genotype 232-57 conferred high vigour to the Shiraz scion. Its pruning weight was higher than 1103 Paulsen but less than Ramsey. However, its long-term yield was below that of 1103 Paulsen. Genotype 232-57 was also sensitive to deficit irrigation with significantly lower pruning weight and yield. Genotype 232-57 had lower juice pH but lower tartaric acid than 1103 Paulsen or Ramsey.

Shiraz Padthaway (2015-22) (Limited irrigation with moderately saline water)

Background

The Shiraz trial, planted in 2010, was designed to evaluate the field performance of 59 rootstock genotypes from a single family under limited supply of moderately saline water. The drought tolerance of these selections had been assessed previously in a field nursery (CSP 0503). The trial also included own roots and five commercial rootstocks (Dogridge, 140 Ruggeri, 1103 Paulsen, 101-14 Mgt and Merbein 5489). The results from the early study with young vines provided in the final report for CSP 1304, Clingeleffer and Smith 2018, showed 8.5-fold differences in conferred vigour (pruning weight), 6.7-fold differences yield, 3.3-fold differences in the Ravaz Index (yield to pruning weight ratio), 2.2-fold differences in bunch number, 2.8-fold differences in bunch weight and 1.5-fold differences in berry weight across the 59 genotypes. Furthermore, the study confirmed that rootstocks are a useful tool to manipulate berry juice mineral ion concentrations with 1.5-3-fold differences in B, Ca, K, Mg, P, Na and S. The growth traits were linked to must and wine quality attributes, which demonstrated negative effects of berry weight, conferred vigour and bunch weight on fruit composition. For example, rootstocks with large berries are likely to have high juice pH with high malic acid concentration and low tartaric acid, anthocyanin and phenolic concentration and produce dull, high hue wines with low ionised anthocyanin. The results of the study with young vines showed significant potential to identify new rootstocks with acceptable productivity, fruit and wine composition, which had good salt and short-term drought tolerance.

This study aimed to identify selections with high potential when grown in Padthaway with a limited supply of moderately saline water. It also aimed to confirm that the effects of rootstock genotype found for young vines (CSP 1304, Clingeleffer and Smith 2018), were maintained over time as the vines matured. Performance data reported previously (CSP 1304, Clingeleffer and Smith 2018), or collected in subsequent seasons were used to sequentially reduce the number the number of genotypes for ongoing evaluation each season (i.e. 40, 29 and 18 genotypes were evaluated in 2018, 2019, and 2021 respectively including own roots and four commercial rootstocks). Key selection criteria used in this process were yield, conferred vigour, berry weight, pH, malic acid and berry anthocyanins. Production data were not collected in 2020 due to COVID 19 issues. Small-scale wines from the most preferred selections were made in 2019 and 2021. The commercial planting in which the trial was embedded was removed in winter 2021 after pruning weights were collected.

Results

The long-term results for the preferred 13 selections, own roots and the commercial rootstocks 1103 Paulsen, 140 Ruggeri, 101-14 and Merbein 5489 are provided in detail in this report. Harvest dates were 8/03/13, 26/03/14, 25/02/15, 10/03/16, 11/04/17, 13/3/2018, 26/03/2019, 17/03/ 2020 (sample only) and 17/03/2021. The long-term yield and maturity data (TSS) across the 18 rootstock genotypes are provided in Figure 2.2.26. The results show that the vines had not fully matured and were not in full production in 2013 and 2014, and that the vines were sampled at a very low maturity in 2013 and a very high maturity in 2020.

Consequently, for the detailed analysis of performance and fruit composition traits data from seasons 2013 and 2014 and season 2020, in the absence of harvest data and the very high TSS, have been excluded. The harvest date was determined to fit in with the commercial harvest of the vineyard.

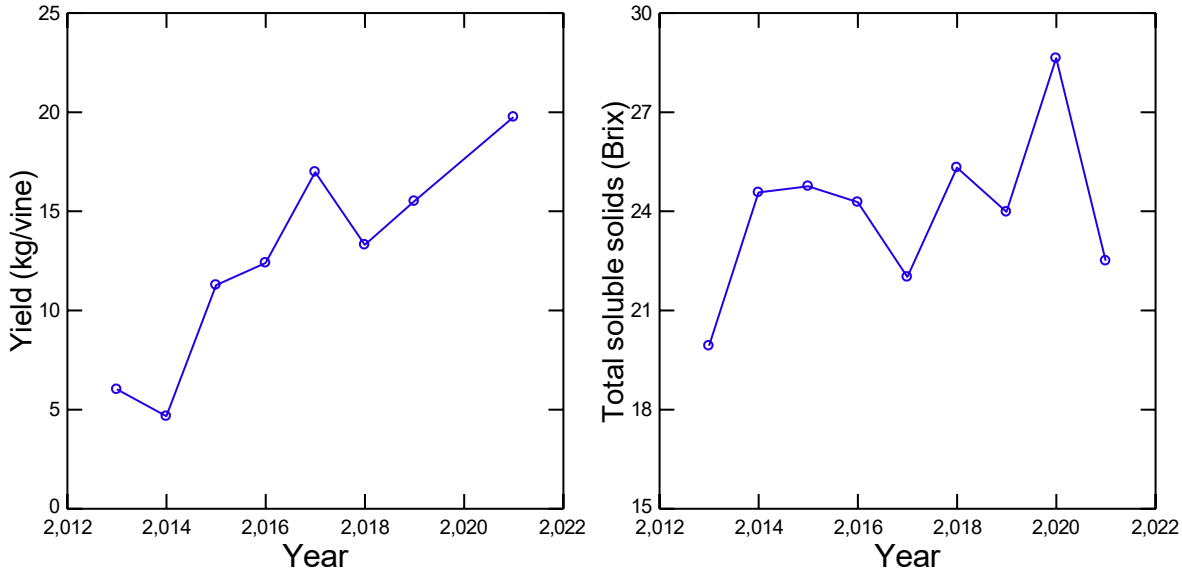


Figure 6.2.26 Mean yield and fruit maturity (TSS) of Shiraz grafted on 18 rootstock genotypes, seasons 2013-2021. Note yield data were not collected in 2020.

Mean Shiraz yield, yield component and growth data for the selected rootstock genotypes and commercial rootstocks are presented in Table 2.2.26. There were highly significant effects of season on all measured traits. Mean yield and bunch numbers were lowest and highest in seasons 2015 and 2021, respectively. Bunch weights were highest in 2017 and smallest in 2018. Berry weight was lowest in 2016 and highest in 2015 and 2021. The calculated value, berries per bunch, was highest in 2017 and lowest in 2021, most probably due to differences in % fruit set although differences between seasons in flower numbers per inflorescence could also be a contributing factor. Pruning weights were lowest in 2015 and highest in 2019. The calculated value for Ravaz Index (yield/pruning weight) was lowest in 2019, due to the high pruning weight and highest in 2017. Interactions between year and rootstock genotype were not significant for any of the measured parameters (yield, bunch number, berry weight and pruning weight), an indication that the effects of rootstock genotype on these parameters was consistent from season to season. The season x rootstock interaction was highly significant for the calculated values, berries per bunch and Ravaz Index. When analysed for the individual years, differences in berries per bunch were only significant in 2017 ($p<0.001$) and 2018 ($p<0.01$). The interaction was largely associated with different rootstock responses in 2017, the season with high berries per bunch and the largest variation, specifically the high values of 101-14, 1103 Paulsen and selections 230-10 and 230-30 and low values of 140 Ruggeri, 230-12 and 230-45. In 2018, differences between rootstock genotypes were much smaller, followed the overall trend and had little impact on the interaction. When the Ravaz Index data were analysed for individual years, there were significant effects of rootstock in 2015 ($p<0.05$), 2016 ($p<0.01$), 2017 ($p<0.001$) and 2018 ($p< 0.01$). The significant season x genotype interaction was largely associated with inconsistent performance of 1103 Paulsen with low values in 2015 and 2018 and high values in 2017; 230-10 with high values in 2017; 230-30 with low values in 2015 and high values in 2017; 230-70 with low values in 2015 and 2016 and high in 2018; 233-72 with high values in 2015; and 23-77 with a high value in 2015 and low value in 2016. These results indicate that rootstock responses to seasonal conditions impacting crop development and yield and shoot growth, measured as pruning weight, are likely to differ.

Rootstock genotype had significant effects on bunch number, berry weight, pruning weight and the Ravaz Index. There was no effect of rootstock on yield, bunch weight or berries per bunch. The lack of a rootstock effect on yield, can at least in part, be attributed to the selection process where low yielding and high yielding genotypes have been eliminated. Yields of all selections, except 234-27, were higher than Merbein 5489, the benchmark used in the selection process. There was a significant, 22% difference in bunch number across the rootstocks with 140 Ruggeri and selections 230-12, 233-77 and 233-80 having the highest number of bunches and 1103 Paulsen, 230-10, 230-30, 232-30 and 321-51 the lowest bunch number. While rootstock effects on bunch weight were not significant, Merbein 5489 tended

to have the smallest bunches and 230-20 and 235-01 the largest bunches. The highly significant effect of rootstock on berry weight shows a 22% difference with own roots, 230-45, 230-70, 231-30, 233-72 and 235-01 producing the largest berries and 101-14, Merbein 5489, 230-30, 231-51 and 234-27 the smallest berries. There was a 2.5-fold difference in rootstock-conferred vigour (pruning weight) across the genotypes, with 101-14, Merbein 5489 and 231-51 having lowest vigour and 230-70, 233-77 and 235-01 the highest vigour. Interestingly, ungrafted own roots had relatively high vigour, 1.33 kg/vine. There was a highly significant effect of rootstock on the Ravaz Index, with rootstocks conferring low vigour (i.e. 101-14, Merbein 5489 and 231-51) producing highest values with own rooted Shiraz, 230-70, 231-30, 234-27 and 235-01 the highest values. It should be noted that almost all Ravaz Index values were above the range generally reported in the literature, range 5 - 10.0. This result is likely to be associated with the impact of the limited supply of moderately saline water on vine growth.

Table 6.2.15. Mean yield, yield components and growth traits of Shiraz grown as own roots or grafted on 17 rootstock genotypes, over six seasons (2015-2019, 2021). Significance values for year, rootstock and the year x rootstock interaction are included. For comparative purposes the rootstocks are presented in order of conferred vigour (pruning weight).

Year	Yield (kg)	Bunches	Bunch wt. (g)	Berry wt.(g)	Berries/ bunch	Pruning wt. (kg)	Ravaz Index
2015	10.9	129	81.9	1.07	76.3	0.82	15.6
2016	12.1	139	87.1	0.73	117.0	1.18	14.1
2017	16.8	151	110.0	0.91	123.0	1.13	16.9
2018	13.1	193	68.1	0.88	76.8	1.26	11.7
2019	15.1	179	84.1	1.01	82.2	1.43	10.6
2021	20.0	257	80.0	1.06	75.8	1.34	16.6
Rootstock							
231-51	12.6	161	80.2	0.83	101.8	0.75	17.7
Merbein 54-89	12.1	174	68.4	0.89	78.7	0.77	18.7
101-14 Mgt.	13.4	171	79.2	0.87	91.2	0.79	19.4
230-30	13.2	156	85.3	0.85	101.3	0.91	15.4
230-10	13.2	159	86.2	0.93	93.0	0.94	15.8
230-45	14.8	179	81.9	1.00	82.6	1.03	16.3
230-69	14.2	168	85.7	0.95	92.8	1.07	13.5
1103 Paulsen	14.9	164	93.9	0.96	97.4	1.13	14.5
230-12	15.3	196	78.3	0.92	86.6	1.13	14.6
140 Ruggeri	15.8	197	80.4	0.97	83.6	1.16	16.0
233-72	15.4	165	94.4	1.01	95.1	1.24	14.1
234-27	11.9	167	70.8	0.86	81.6	1.28	10.7
Shiraz	15.1	182	83.9	0.99	85.8	1.33	11.7
233-80	16.6	193	90.4	0.94	99.0	1.39	12.9
231-30	14.6	163	87.2	1.01	87.7	1.40	10.7
233-77	16.0	194	86.3	0.96	91.5	1.49	13.7
230-70	16.7	167	100.6	1.04	99.6	1.76	11.2
235-01	18.1	185	100.5	0.99	104.1	1.88	9.8
Significance[1]							
Year	***	***	***	***	***	***	***
rootstock	ns	**	ns	***	ns	***	***
Y x R	ns	ns	*	ns	***	ns	***

[1] ***, **, * indicate significance p< 0.001, p<0.01, p<0.05 respectively, ns =not significant

The significant effects of season and rootstock genotype on fruit composition are shown in Table 6.2.16. Interactions between year and rootstock were not significant for any compositional trait, indicating stable rootstock performance across seasons despite the highly significant effects of season on yield, yield components and conferred vigour reported above. TSS ranged from 22.0 - 25.3 ^{o}Brix in 2017 and 2018 respectively; pH ranged from 3.74 - 4.27 in 2021 and 2018; TA ranged from 2.04 - 4.09 g/L in 2018 and 2015; malic acid ranged from 1.01-2.12 g/L in 2019 and 2015; tartaric acid ranged from 4.34 - 7.62 g/L in 2018 and 2015; YAN ranged from 148 - 240 mg/L in 2021 and 2017; anthocyanin ranged from 1.54 -2.00 mg/g in 2017 and 2019; and, total phenolics ranged from 1.62 - 2.12 in 2015 and 2018. The low YAN concentration in 2021 was below 150 mg/L, the concentration required for a successful fermentation without addition of DAP (diammonium phosphate).

There were highly significant effects of rootstock on all compositional traits, except tartaric acid (Table 6.2.16). Over the seasons, Merbein 5489, 231-12, 231-72 and 233-77 had low TSS, whereas 101-14, 1103 Paulsen, 140 Ruggeri, 230-10, 230-69, 230-70 and 235-01 had high TSS. Ungrafted own roots, Merbein 5489, 233-77 and 233-80 had low pH whereas 101-14, 1103 Paulsen, 230-10, 230-12, 230-30 and 235-01 had high pH. Merbein 5489 had the highest TA concentration while 101-14, 140 Ruggeri and 230-12 had low TA. Merbein 5489 and 230-69 had low malic acid concentration whereas 1103 Paulsen, 230-10, 230-30 and 230-70 had high malic acid concentration. Organic acid balance, the tartrate to malate ratio, was superior with Merbein 5489 with the highest value whereas 1103 Paulsen, 230-70 and 235-01 had low values. Mean YAN concentrations of all rootstock genotypes were above 150 mg/L required for successful fermentation. Own rooted Shiraz and 233-77 had the lowest YAN whereas 1103 Paulsen and 234-27 had the highest YAN concentrations. Merbein 5489, 101-14 and 230-30 had the highest berry anthocyanin concentrations whereas 233-72 and 233-77 had the lowest anthocyanin concentrations. Merbein 5489, 101-14 and 230-30 also had high concentrations of total phenolic substances whereas own rooted Shiraz, 230-45, 230-70 and 233-77 had low total phenolics.

Table 6.2.16. Mean data of Shiraz juice composition (TSS, pH, TA, malic and tartaric organic acids the tartrate/malic acid ratio and YAN) and berry anthocyanin and phenolics grown as own roots or grafted on 17 rootstock genotypes, over six seasons (2015-2019, 2021). Significance values for year, rootstock and the year x rootstock interaction are included. For comparative purposes the rootstocks are presented in order of conferred vigour (pruning weight).

Year	TSS (°Brix)	pH	TA (g/L)	malic (g/L)	tart (g/L)	T/M	YAN (mg/L)	antho mg/g	phen (a.u.)
2015	24.8	3.83	4.09	2.12	7.62	3.66	163	1.55	1.62
2016	24.2	4.04	2.97	1.66	5.01	3.15	188	1.93	2.04
2017	22.0	3.93	3.78	1.70	5.74	3.80	240	1.54	1.66
2018	25.3	4.27	2.40	1.19	4.34	3.78	227	1.87	2.12
2019	24.0	4.14	2.84	1.01	6.28	6.58	199	2.00	1.90
2021	22.6	3.74	3.59	1.11	5.27	5.14	148	1.86	1.74
Rootstock									
231-51	23.7	3.99	3.30	1.44	5.82	5.15	205	1.91	2.04
Merbein54-89	23.1	3.76	3.76	1.26	6.06	5.57	194	2.09	2.08
101-14 Mgt.	24.4	4.03	3.17	1.48	5.83	4.33	197	1.95	1.81
230-30	23.3	4.05	3.27	1.62	5.87	3.91	194	1.93	2.07
230-10	24.8	4.12	3.24	1.64	6.01	3.86	202	1.72	1.73
230-45	23.6	4.01	3.25	1.43	5.56	4.09	211	1.58	1.66
230-69	24.5	3.95	3.21	1.25	5.50	5.04	182	1.67	1.76
1103 Paulsen	24.4	4.06	3.20	1.61	5.65	3.76	214	1.83	1.84
230-12	22.8	4.06	3.13	1.46	5.42	3.94	196	1.69	1.84
140 Ruggeri	24.3	4.01	3.10	1.39	5.72	4.38	191	1.73	1.75
233-72	22.6	3.98	3.33	1.52	5.74	5.04	178	1.60	1.74
234-27	23.4	4.02	3.37	1.51	5.96	4.30	218	1.89	2.10
Shiraz	24.1	3.93	3.27	1.33	5.43	4.45	169	1.81	1.71
233-80	23.7	3.92	3.40	1.46	5.79	4.29	200	1.87	1.93
231-30	24.2	3.98	3.31	1.50	5.76	4.23	177	1.78	1.92
233-77	23.0	3.92	3.20	1.36	5.33	4.32	165	1.62	1.65
230-70	24.5	4.01	3.27	1.60	5.64	3.70	198	1.66	1.70
235-01	24.4	4.06	3.21	1.49	5.68	3.99	208	1.90	1.93
Significance[1]									
year	***	***	***	***	***	***	***	***	***
rootstock	***	***	***	***	ns	**	***	***	***
Y x R	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1] ***, **, * indicate significance p< 0.001, p<0.01, p<0.05 respectively, ns =not significant

TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tartaric, tartaric acid; YAN, yeast assimilable nitrogen; T/M, tartaric acid/malic acid; Antho, anthocyanin; Phen, phenolics

In the previous final report for CSP 1304 (Clingeleffer and Smith 2018), the response of the 59 genotypes to short-term drought stress caused by a pre-harvest irrigation malfunction in early February 2017 were provided, based on canopy loss and leaf burn. These data have been reanalysed for the 13 selected genotypes and commercial rootstocks (Table 6.2.17). The results show significant effects of the retained genotypes on their response to short-term drought. The most sensitive genotypes to short-term drought stress with significant canopy loss and leaf burn were 101-14 and selections 231-51, 233-77 and 234-27. The most resilient genotypes to the short-term drought stress were 140 Ruggeri, 230-10, 230-69, 231-30 and 235-01.

In the previous final report (CSP 1304, Clingeleffer and Smith 2018), results for juice mineral composition determined in 2016 for the 59 genotypes were presented. These data have been reanalysed for the 13 selected genotypes and commercial rootstocks (Table 6.2.17). The results show that there are small, but significant differences between the selected genotypes in juice Ca and K but strong or highly significant differences for Mg, Na and P. Differences between genotypes in juice S and B were not significant. The small differences in K are not unexpected as the genotypes have been selected for low pH, which has been shown to be linked to K in previous rootstock research. Juice Ca was high for 140 Ruggeri, Merbein 5489 and 231-51 and low for 230-10 and 234-27. Juice K was high for 230-10 and 235-01 and low for 140 Ruggeri, Merbein 5489, 230-45, 230-70 and 233-80. Juice Mg was high for 101-14, 1103 Paulsen and 140 Ruggeri and low for Merbein 5489, 230-45, 230-69, 231-30, 233-77 and 233-80. Juice Na was high for own rooted Shiraz, 1103 Paulsen, 140 Ruggeri, 230-12 and 235-01 and low for Merbein 5489, 230-30, 230-70, 231-30, 231-51, 233-77 and 234-27. These results indicate that most of the selections have better Na exclusion than own roots, 1103 Paulsen and 140 Ruggeri. Juice P was high in own rooted Shiraz, 230-12, 231-30, 231-51, 233-72 and 235-01 and low for Merbein 5489, 233-80, 101-14 and 230-70.

Table 6.2.17. Mean % canopy loss and % leaf burn in 2017 and juice mineral composition determined in 2016 for Shiraz grown as own roots or grafted on 17 rootstock genotypes. Significance values for rootstock treatment are included. For comparative purposes the rootstocks are presented in order of conferred vigour (pruning weight).

Rootstock	% canopy loss	% leaf burn	Ca	K	Mg	Na mg/L	S	B	P
231-51	50	64	208	1709	112	19.7	97.1	5.90	167
Merbein 54-89	44	58	201	1224	107	20.6	77.9	5.40	116
101-14 Mgt.	58	70	194	1584	127	22.6	81.8	5.88	123
230-30	50	52	184	1741	118	18.1	95.7	5.51	137
230-10	20	33	162	1864	116	25.7	88.8	5.93	140
230-45	28	45	171	1433	108	21.7	88.8	4.51	137
230-69	20	30	174	1579	106	26.4	99.4	6.08	142
1103 Paulsen	30	40	189	1521	127	28.6	92.3	6.04	139
230-12	32	48	178	1617	119	35.5	87.4	4.84	145
140 Ruggeri	21	23	198	1450	131	36.0	95.2	5.40	137
233-72	26	38	196	1766	110	25.9	90.9	5.06	153
234-27	62	61	149	1732	122	17.9	96.5	5.96	147
Shiraz	24	36	190	1583	113	29.7	86.6	5.59	145
233-80	30	42	177	1463	105	27.9	86.1	5.29	117
231-30	15	36	187	1677	109	17.6	86.1	5.25	159
233-77	53	63	177	1602	108	15.5	85.2	6.00	136
230-70	35	35	179	1480	111	12.7	80.6	5.83	121
235-01	27	30	171	1786	110	29.6	95.9	6.39	191
Significance[1]									
rootstock	*	*	*	*	***	**	ns	ns	**

[1] ***, **, * indicate significance p< 0.001, p<0.01, p<0.05 respectively, ns =not significant

Correlation analyses were conducted to determine relationships between the field growth traits and their impact on fruit composition for the selected genotypes (Table 6.2.18). Yield was strongly correlated with bunch weight (r = 0.76) and berry weight (r = 0.73) and to a lesser degree with bunch number (r = 0.58). The linear relationships for these associations are shown in Figure 6.2.27. The multilinear regression equation involving bunch number, berry weight and berries per bunch was highly significant (adj. R^2 = O.97).

Yield = -24.9 + 0.07 bunches + 16.56 berry weight + 0.12 berries per bunch

Table 6.2.18. Correlation coefficients (r) which describe linear relationships between yield, yield components and growth traits and fruit composition, % leaf loss and % leaf burn in 2017 and juice mineral element concentrations in 2016 (n=18). Significant r values ($p<0.05$) are highlighted bold while those with $p<0.10$ are shown in italics.

	Yield	Bunch	Bunch wt.	Berry wt.	Berry/ bunch	Pruning Wt.	Ravaz Index
Bunches	**0.58**						
Bunch wt.	**0.76**	-0.07					
Berry wt.	**0.73**	0.25	**0.64**				
Berry/bunch	0.39	-0.27	**0.75**	-0.02			
Pruning wt.	**0.81**	0.35	**0.68**	**0.67**	0.29		
Ravaz	**-0.52**	-0.10	**-0.52**	**-0.51**	-0.20	**-0.87**	
TSS	0.14	-0.22	0.34	0.22	0.22	0.14	-0.13
pH	0.13	-0.22	0.35	-0.02	0.39	0.08	-0.13
TA	*-0.41*	-0.21	-0.36	-0.21	-0.27	-0.20	0.15
Malic	0.06	*-0.46*	*0.46*	0.01	**0.49**	0.15	-0.17
Tart	**-0.59**	**-0.53**	-0.31	**-0.51**	-0.02	*-0.41*	0.31
T/M	-0.40	-0.08	*-0.45*	-0.26	-0.27	*-0.43*	0.38
YAN	-0.20	-0.20	-0.08	-0.35	0.12	-0.14	0.12
Anthocyanin	*-0.43*	-0.20	-0.38	**-0.66**	0.04	-0.32	0.26
Phenolics	**-0.50**	-0.30	-0.39	**-0.70**	0.07	-0.25	0.04
% canopy	**-0.47**	-0.12	*-0.44*	**-0.71**	0.00	-0.27	0.31
% leaf burn	**-0.56**	-0.12	**-0.55**	**-0.70**	-0.12	*-0.45*	**0.47**
Ca	-0.05	-0.03	-0.06	-0.07	0.08	-0.37	**0.51**
K	-0.04	-0.38	0.29	-0.14	*0.45*	0.14	-0.33
Mg	-0.19	0.00	-0.22	-0.28	-0.14	-0.25	0.25
Na	0.32	**0.52**	0.01	0.12	-0.10	-0.04	0.06
S	-0.06	-0.17	0.08	-0.22	0.29	-0.04	-0.20
B	-0.01	-0.25	0.25	-0.21	*0.45*	0.22	-0.22
P	0.21	-0.09	0.29	0.09	0.32	0.31	*-0.42*

TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tartaric, tartaric acid; YAN, yeast assimilable nitrogen; T/M, tartaric acid/malic acid

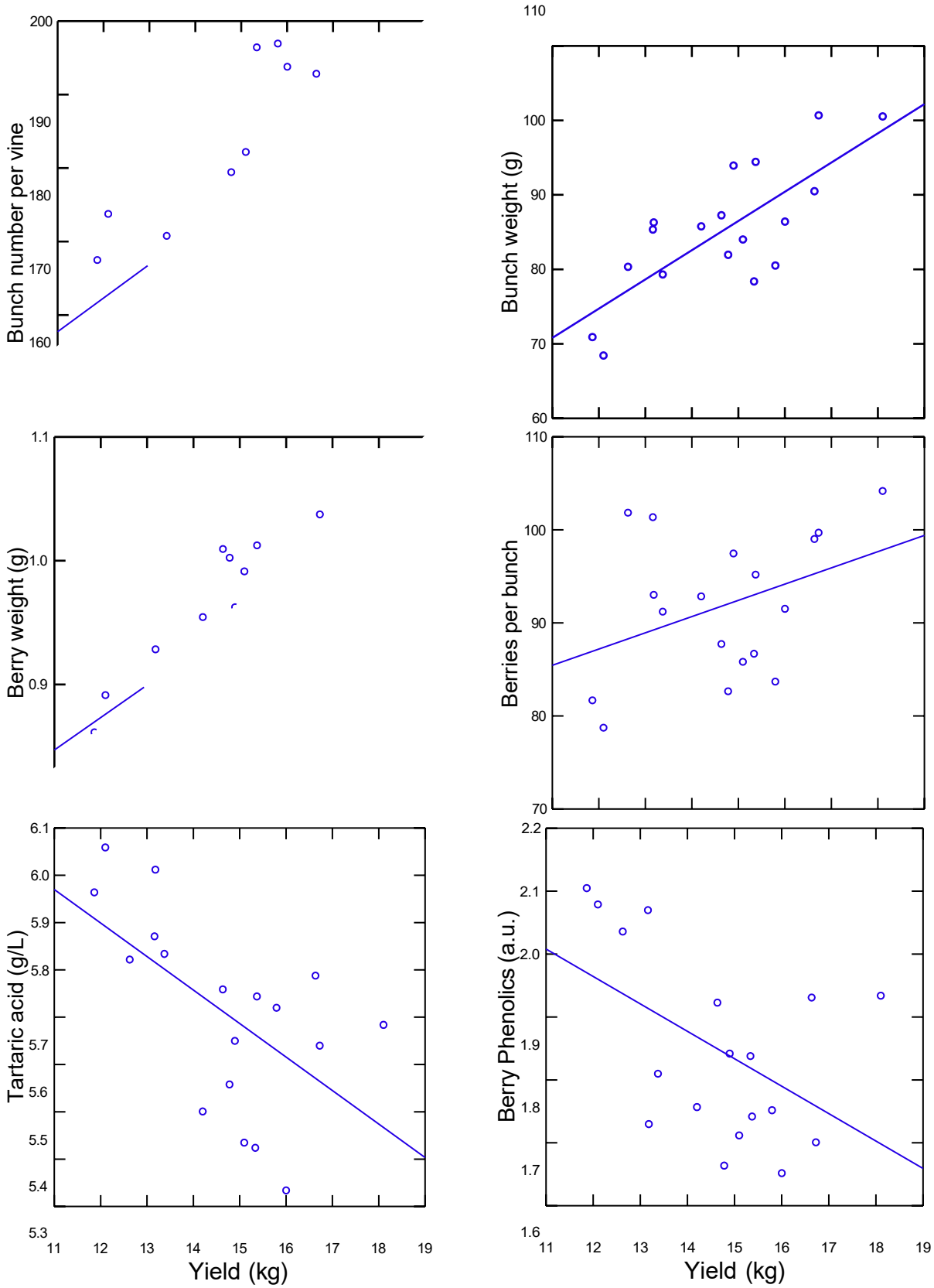


Figure 6.2.27. Linear relationships between mean yield and bunch number (top left), bunch weight (top right), berry weight (middle left), berries per bunch (middle right), tartaric acid concentration (bottom left) and total phenolic substances (bottom right) across 18 selected rootstock genotypes over six seasons (2015-19, 2021).

The correlation analyses showed that yield was also strongly correlated with the rootstock-conferred vigour (pruning weight, r = 0.81) across the rootstocks. Bunch weight was strongly correlated with both berry weight (r = 0.64) and berries per bunch (r = 0.75) and to pruning weight (r = 0.68). Berry weight was also strongly linked to conferred vigour (r = 0.67). The significant correlations between the Ravaz Index and yield (r = - 0.52) and pruning weight (r = - 0.87) indicates that pruning weight with 2.5-fold differences across the genotypes was the major factor contributing to differences in the Ravaz Index values. The significant negative correlations between the Ravaz Index and bunch weight and berry weight indicate that rootstock genotypes with high Ravaz Index values are likely to have smaller bunches with small berries. The linear relationships between pruning weight and yield, bunch number, bunch weight and berry weight across the rootstock genotypes are shown in Figures 6.2.28. They describe the strong relationships between pruning weight and yield, bunch weight and berry weight but a weaker correlation with bunch number.

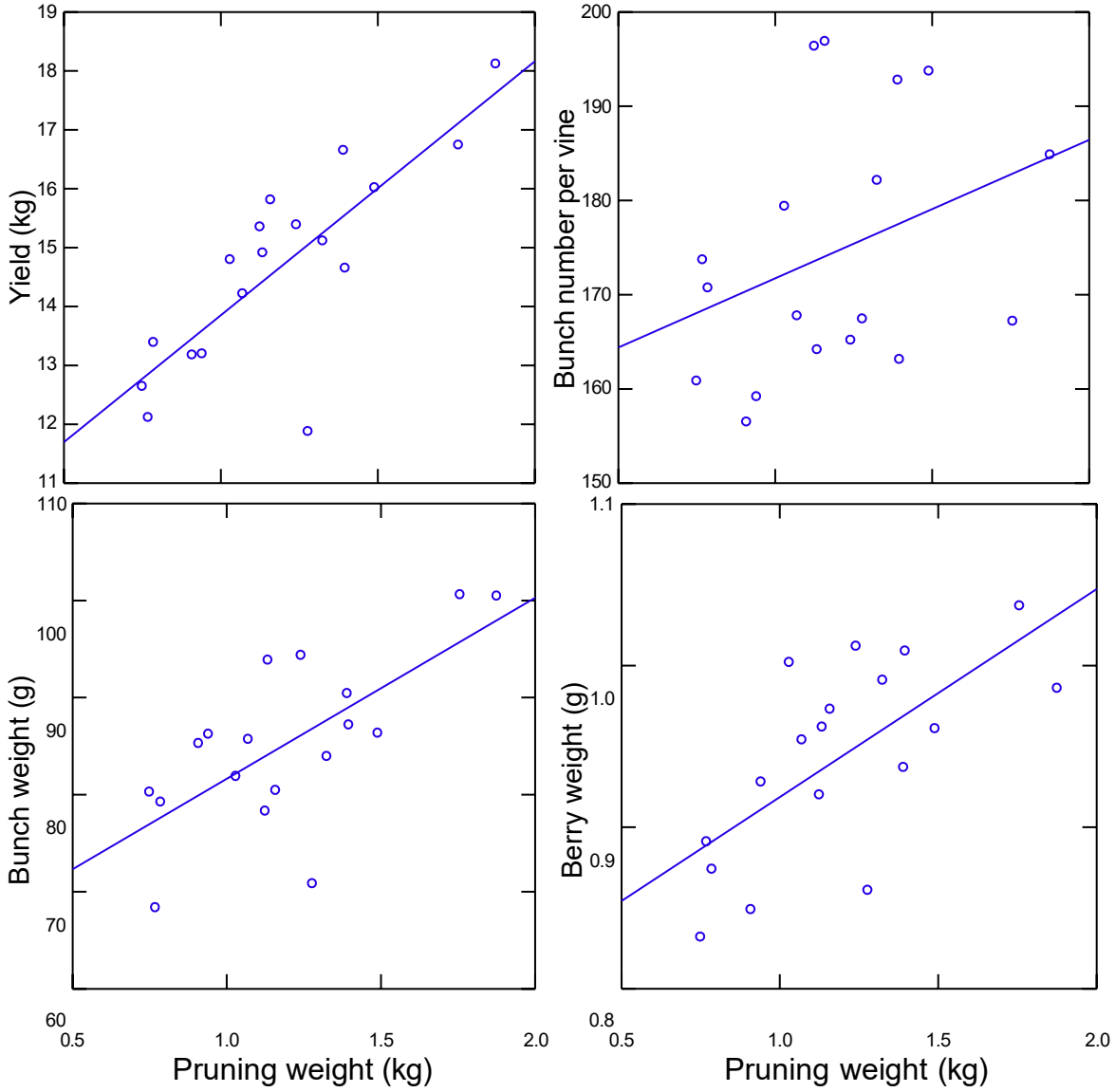


Figure 6.2.28. Linear relationships between conferred vigour (pruning weight) and mean yield (top left), bunch number (top right), bunch weight (bottom left) and berry weight (bottom right) across 18 selected rootstock genotypes over six seasons (2015-19, 2021).

The correlation analyses (Table 6.2.18) show that the small differences in yield across the selected genotypes had little impact on fruit composition, except the negative associations with juice tartaric acid concentrations, berry total phenolics and to a lesser degree with berry anthocyanin concentrations, most likely associated with higher berry weight associated with reduced berry shrivel (see below). The negative associations between yield and short-term drought tolerance (i.e. canopy loss and leaf burn) indicate that the higher yielding rootstocks were more tolerant of drought. Bunch number across the rootstocks was associated with juice tartaric acid (negative) and Na (positive) concentrations. Rootstock bunch weight had little impact on fruit composition, except for a very weak association with malic acid and the tartrate/malate ratio (negative). The negative associations between bunch weight and canopy loss and leaf burn under short-term drought, indicate that the selected genotypes which

develop bigger bunches are less sensitive to drought. Rootstock berry weight was

negatively associated with juice tartaric acid, anthocyanin and phenolics (Figure 6.2.29). These responses are most likely due to differences between the rootstocks in berry shrivel at the latter stages of ripening, as berry tartaric acid content is stable post-veraison, with the concentration impacted by changes in berry size. The strong associations between berry weight and both canopy loss and leaf burn indicate that rootstocks with larger berries and presumably less berry shrivel are less susceptible to short-term drought. Berries per bunch were positively correlated with juice malic acid and to a lesser degree with K and B concentrations.

Despite the highly significant 2.5-fold effect of rootstock on pruning weight, this had little impact on juice composition of the selected genotypes with only very weak negative associations with tartaric acid and the tartrate/malate ratio (Table 6.2.18). While unexpected, based on the previous research that linked higher pruning weight with higher pH, juice malic acid and K concentrations, the result provides strong evidence that the rootstock selection process using these criteria has been successful. There were no significant correlations between Ravaz Index and fruit composition traits, except for a positive correlation with Ca and a very weak negative association with P.

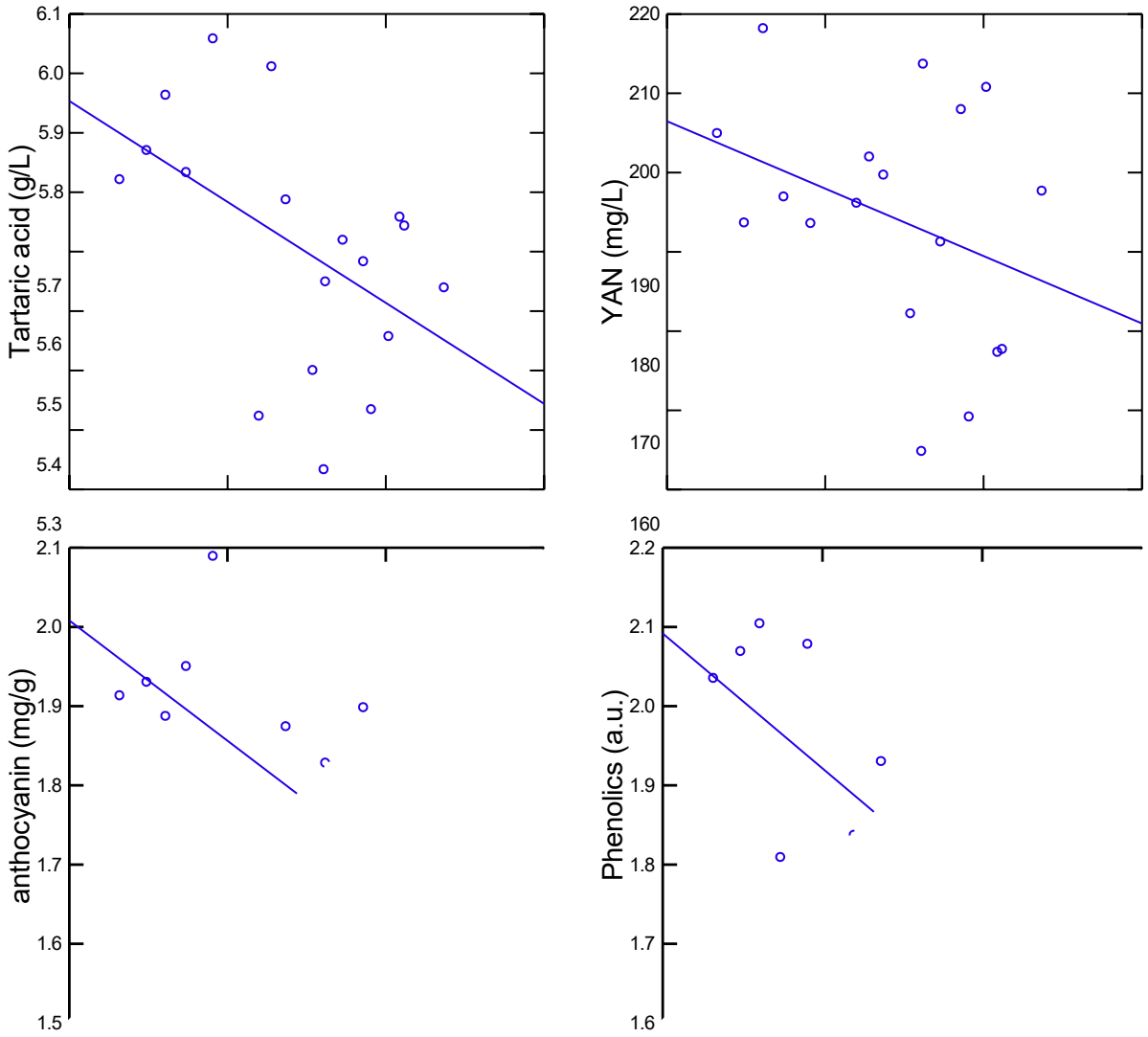


Figure 6.2.29. Linear relationships between mean berry weight and juice tartaric acid concentration (top left), YAN (top right), berry anthocyanin concentration (bottom left) and phenolic substances (bottom right) across 18 selected rootstock genotypes over six seasons (2015-19, 2021).

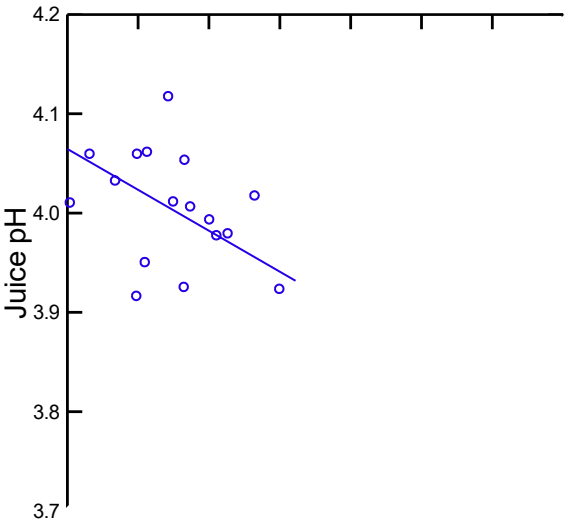
Correlation analyses were used to describe relationships between fruit composition and drought tolerance and berry mineral composition across the rootstock genotypes (Table 6.2.19). TSS was not associated with any other juice composition trait, although it was negatively correlated with leaf burn, which was probably linked to berry shrivel, and B concentration (positive). In contrast, pH was negatively correlated with juice TA and the tartrate/malate ratio and positively correlated with malic acid, K and Mg. These relationships are shown in more detail in Figure 6.2.30. Across the rootstock genotypes, TA was positively associated with tartaric acid, the tartaric acid/malate ratio, berry anthocyanins and phenolic substances and negatively associated with K (weak), Mg and S (weak). The strong negative association between malic acid and the tartaric /malic ratio shows that rootstock malic acid strongly influenced organic acid balance. Juice malic acid was positively correlated with K, and

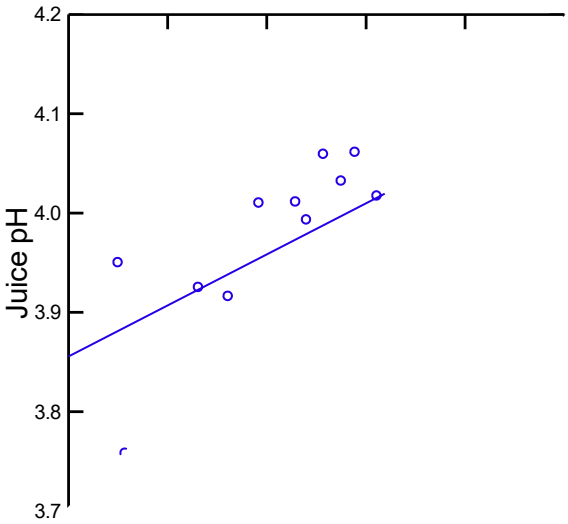
to a lesser degree with YAN concentrations. Juice tartaric acid concentration was weakly associated with YAN but strongly linked to berry anthocyanin and total berry phenolic substances. Organic acid balance, the tartaric/malic acid ratio, was positively correlated with Ca. Berry anthocyanin was strongly correlated with total berry phenolic substances. The weak associations between berry anthocyanin and the indicators of short-term drought tolerance (% canopy loss and % leaf burn) suggest that genotypes that produce high colour are more susceptible to drought. It is possible that this association could be linked to a greater susceptibility to berry shrivel as berry weight was negatively linked to anthocyanin (Table 6.2.18). Total berry phenolic substances also showed weak associations with the indicators of drought tolerance, % canopy loss and leaf burn.

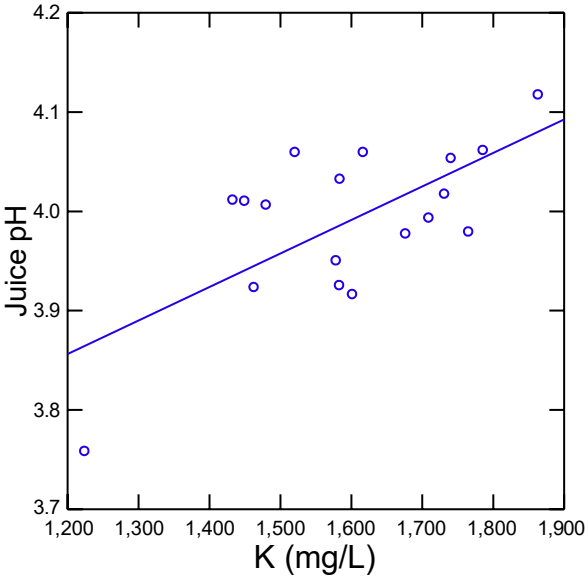
Table 6.2.19. Correlation coefficients (r) which describe linear relationships between fruit composition traits, % leaf loss and % leaf burn in 2017 and mineral element concentrations in 2016 (n=18). Significant r values ($p<0.05$) are highlight bold while those with $p<0.10$ are shown in italics.

	TSS	**pH**	**TA**	**Malic**	**Tart**	**T/M**	**YAN**	**Anth**	**Phen**
pH	0.36								
TA	-0.33	**-0.74**							
Malic	0.19	**0.75**	-0.27						
Tart	0.08	-0.01	**0.56**	0.32					
T/M	-0.35	**-0.73**	**0.58**	**-0.70**	0.21				
YAN	0.19	*0.44*	0.04	*0.43*	*0.46*	-0.30			
Antho.	0.04	-0.27	**0.53**	-0.07	**0.63**	0.27	0.31		
Phenolics	-0.24	-0.15	**0.53**	0.07	**0.65**	0.25	*0.42*	**0.81**	
% canopy loss	*-0.39*	-0.11	0.20	0.03	0.25	0.12	0.22	*0.45*	*0.45*
% leaf burn	**-0.47**	-0.22	0.27	-0.09	0.20	0.25	0.11	*0.42*	*0.39*
Ca	-0.13	-0.37	0.15	-0.25	0.03	**0.50**	-0.33	0.27	0.06
K	0.07	**0.66**	*-0.42*	**0.53**	0.09	-0.27	0.02	-0.15	0.07
Mg	0.22	**0.51**	**-0.47**	0.35	0.16	-0.36	0.32	0.16	0.05
Na	0.10	0.21	-0.35	-0.18	-0.22	-0.04	0.04	-0.08	-0.18
S	0.10	*0.44*	*-0.40*	0.07	-0.03	0.01	0.25	-0.09	0.22
B	**0.51**	0.15	-0.13	0.09	0.11	-0.05	0.10	0.31	0.14
P	0.05	0.38	-0.30	0.10	-0.13	-0.02	0.06	-0.06	0.15

TSS, total soluble sugars; TA, titratable acid; Malic, malic acid; Tartaric, tartaric acid; Malic, malic acid; YAN, yeast assimilable nitrogen; T/M, tartaric acid/malic acid; Anth, anthocyanin; Phen, phenolics


Juice pH
4.2
4.1
4.0
3.9
3.8
3.7


Juice pH
4.2
4.1
4.0
3.9
3.8
3.7


Juice pH
4.2
4.1
4.0
3.9
3.8
3.7
1,200
1,300
1,400
1,500
1,600
1,700
1,800
1,900
K (mg/L)

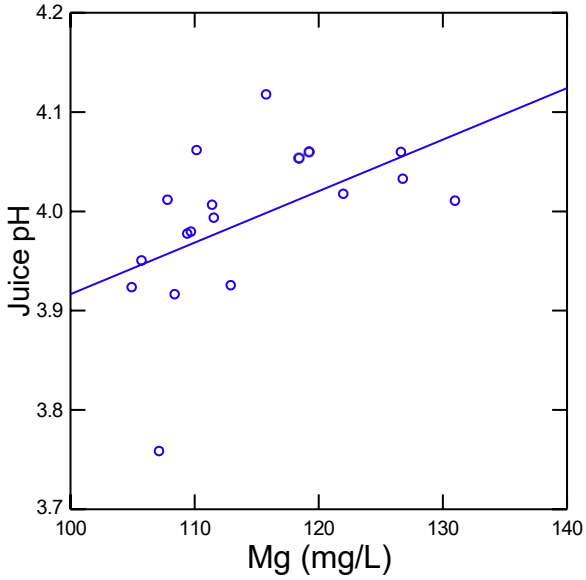

Juice pH
4.2
4.1
4.0
3.9
3.8
3.7
100
110
120
130
140
Mg (mg/L)

Figure 2.2.30. Linear relationships between pH and juice TA (top left), malic acid (top right), juice K (bottom left) and Mg (bottom right) across 18 selected rootstock genotypes over six seasons (2015-19, 2021).

A systematic approach was used to identify selections for winemaking in 2019, based on data recorded from 2015-18. Key criteria used for selection included yield, pruning weight and juice pH, malic acid and berry anthocyanin. This was extended to include 2019 data to identify selections for winemaking in 2021, also taking into account their potential as alternatives to existing rootstock cultivars. Based on their performance, which is summarised for all seasons (2015-19, 2021) in Tables 6.2.15 and 6.2.16., genotype 231-51 with low malic acid, high tartaric acid and high anthocyanin was identified as an alternative rootstock to 101-14 and Merbein 5498, having similar yield and low pruning weight; 230-30 with high tartaric acid and high anthocyanin was also selected as an alternative to 101-14 and Merbein 5489, having similar yield and pruning weight; 234-27 with high tartrate and anthocyanin was selected as an alternative to 1103 Paulsen and 140 Ruggeri, with similar yield but higher pruning weight; 238-80 with low pH, high tartrate and high anthocyanin was selected as an alternative to own roots, with very similar yield and pruning weight; while 235-01 was selected as the best high vigour rootstock, with high yield and high anthocyanin, as an alternative to Ramsey rootstock. Wines were also produced from selections 230-45 and 230-70 in 2019. Selection 230-45 was identified at that time as a potential low K alternative to 1103 Paulsen and 140 Ruggeri. Selection 230-70 was identified as a high vigour alternative rootstock to Ramsey. It had high TSS and low juice K and Na concentration. Wines of all selections identified in either 2019 or 2021 were also produced from the same range of selections in 2017. To assist decision making processes, data for wines produced in 2017 are also included in the assessment of potential rootstocks for release.

Shiraz must and wine data for the three seasons are provided in Table 6.2.20. Fruit maturity was similar in 2017 and 2019, with mean must TSS values of 23.6 and 23.8 ^{o}Brix and low in 2021, mean 21.5 ^{o}Brix. These differences were reflected in mean must pH values of 3.95, 3.94 and 3.58 and TA concentrations of 3.4, 3.7 and 4.3 g/L in 2017, 2019 and 2021 respectively. Consequently, tartaric acid addition for pH adjustment during the winemaking process was lowest in 2021 (mean 0.5 g/L) compared to 2017 (3.6 g/L) and 2019 (3.3 g/L). Mean wine pH values were 3.49, 3.56 and 3.63 in 2017, 2019 and 2021 respectively. Mean wine TA values tend to reflect tartaric acid addition with 6.0, 5.9 and 5.6 g/L in 2017, 2019 and 2021, respectively. Wines from 2019 and 2021 had the lowest and highest levels of alcohol.

Fruit maturity of own rooted Shiraz was inconsistent between seasons with low must TSS (21.8 ^{o}Brix) in 2017 and relatively high TSS in 2019 (24.1 ^{o}Brix) and 2021 (21.6 ^{o}Brix) (Table 6.2.20). Fruit maturity from the 101-14 rootstock was always relatively high. Selection 235-01 consistently had high must TSS in all years. Selection 231-51 tended to have low maturity in all years. Must pH of own rooted Shiraz was always low, whereas it tended to be relatively

high for 101-14. Must pH of 231-51 was consistently low in all seasons. There were no consistent effects of rootstock on must TA, wine pH, wine TA or tartaric acid addition for pH adjustment. Wine from selection 235-01 always had high alcohol as did selection 230-70 in the two years that wines were made (i.e. 2017 and 2019). Selection 231-51 always produced wines with low alcohol.

Table 6.2.20. Must composition (TSS, pH, TA), total acid addition for pH adjustment and wine data (pH, TA, alcohol and Cl) of Shiraz grafted on priority CSIRO rootstock selections or 101-14 and own rooted Shiraz in 2017, 2019 and 2021.

Rootstock	Must °Brix	pH	TA	Wine pH	TA	Alcohol	Tartaric Added (g/L)
2017							
Shiraz	21.8	3.77	3.7	3.45	5.5	12.8	2.0
101-14	23.9	3.82	3.6	3.55	5.7	13.8	2.0
230-30	23.1	3.87	3.6	3.56	5.7	13.7	3.3
230-45	24.0	4.08	3.1	3.48	6.0	14.0	4.5
230-70	23.7	4.06	2.9	3.50	5.9	14.4	4.5
231-51	23.3	3.79	3.7	3.43	6.1	13.5	3.3
233-80	23.8	4.04	2.9	3.48	6.2	14.4	4.5
234-27	23.3	4.00	2.9	3.47	6.5	13.5	4.0
235-01	24.4	4.15	2.9	3.53	6.5	14.3	4.5
2019							
Shiraz	24.1	3.85	3.68	3.60	5.52	15.0	2.5
101-14	23.9	3.93	3.65	3.54	6.22	14.8	3.1
230-30	24.2	3.96	3.48	3.51	6.33	15.1	4.1
230-45	23.1	3.90	3.71	3.59	5.54	13.9	3.5
230-70	24.4	3.96	3.65	3.60	6.02	15.4	4.0
231-51	23.1	3.84	3.82	3.50	6.02	14.1	2.9
233-80	23.5	4.04	3.89	3.59	5.47	14.0	2.2
235-01	24.3	4.02	3.41	3.54	5.67	15.1	3.7
2021							
Shiraz	21.6	3.56	4.18	3.63	5.53	13.9	0.4
101-14	21.6	3.67	3.96	3.62	5.88	13.7	1.3
230-30	21.1	3.54	4.41	3.63	5.54	13.3	0.7
231-51	20.9	3.56	4.25	3.62	5.27	13.1	0.2
233-80	21.7	3.55	4.45	3.63	5.31	13.7	0.0
234-27	21.6	3.61	4.30	3.63	5.48	13.1	0.8
235-01	22.2	3.59	4.22	3.64	6.13	14.3	0.3

TA, titratable acid

All rootstocks produced wines with good colour, bright purple hues and acceptable flavour and aroma. The spectral properties of wines from the different rootstock genotypes are presented in Table 6.2.21. Own rooted wines generally had high colour density (except in 2021), a low hue (i.e. bright hues) and high total and ionised anthocyanins. Spectral properties of wines of 101-14 were not consistent between seasons, having low colour density, total and ionised anthocyanins and phenolics in 2017 but high values in 2019 and 2021. Wines of 101-14 always had bright, low hue values. Selection 230-30 produced wines with high colour density in all seasons, with relatively high total ionised anthocyanins and phenolics in most seasons. Spectral properties of selection 234-27, in the two seasons that wines were made (2017 and 2021), were poor having low colour density, total and ionised anthocyanins and phenolics with a high hue value, indicative of dull wines. Spectral properties of wines from other selections were generally acceptable, but not consistent across the seasons.

Table 6.2.21. Wine spectral properties of Shiraz grafted on priority CSIRO rootstock selections or 101-14 and own rooted Shiraz in 2017, 2019 and 2021.

	Colour Density (a.u.)	Hue	Total anthocyanins (mg/L)	Ionised anthocyanins (mg/L)	Total phenolics (a.u.)
2017					
Shiraz	9.70	0.48	747	101	47.2
101-14	6.05	0.49	629	61	39.2
230-30	9.05	0.52	810	87	60.0
230-45	7.92	0.54	722	71	56.4
230-70	7.47	0.55	658	65	49.5
231-51	9.57	0.48	699	97	50.9
233-80	9.34	0.53	757	83	57.8
234-27	7.96	0.55	676	66	54.0
235-01	7.85	0.57	675	64	53.0
2019					
Shiraz	10.9	0.57	575	91.0	47.8
101-14	12.8	0.56	551	111.6	49.1
230-30	11.4	0.60	520	86.1	50.7
230-45	8.1	0.60	493	64.7	44.8
230-70	10.3	0.61	541	77.2	49.7
231-51	8.5	0.62	430	63.8	42.3
233-80	8.9	0.60	444	69.0	40.9
235-01	9.4	0.61	504	71.5	43.6
2021					
Shiraz	9.25	0.62	335	55.7	43.6
101-14	9.88	0.64	300	54.3	43.7
230-30	10.83	0.66	219	42.5	41.6
231-51	9.15	0.69	229	27.3	41.4
233-80	9.71	0.63	257	31.7	43.6
234-27	8.00	0.71	169	15.6	37.5
235-01	9.61	0.62	319	52.1	46.2

The wine data results suggested that the value in using small-scale wines as part of the evaluation process may have been compromised by differences in fruit maturity between rootstocks and between seasons, with effects on juice pH and the subsequent requirement for acid addition during winemaking to enhance wine stability. This issue was explored using correlation analyses across the complete wine data set from the three years (n=24) and presentation of key relationships for each season in Figures 6.2.31 and 6.2.32. Over all wines, must TSS was positively correlated with pH (r = 0.89), wine TA (r = 0.57), tartaric acid addition (r = 0.85), wine alcohol (r = 0.75), wine total anthocyanin (r = 0.68), ionised anthocyanin (r = 0.62) and total phenolics (r = 0.49) and negatively correlated with must TA (r = -0.76), wine pH (r = 0.50) and hue (r = 0.52). The differences between seasons in must TSS, reported above, are clearly evident in presentations in Figures 6.2.31 and 6.2.32, with a broader range of values in 2017. Despite the differences between seasons in must TSS, the strong relationships showing increased must pH, lower must TA, an increased requirement for tartaric acid addition for pH adjustment, higher wine TA and alcohol with higher must TSS were reasonably consistent across seasons (Figure 6.2.31.). In contrast, the negative association between must TSS and wine pH was due entirely to differences between seasons rather than rootstock genotype.

The relationships between wine spectral properties and must TSS can largely be attributed to the effects of season (i.e. different fruit maturity at harvest) (Figure 6.2.32). The absence of a correlation between must TSS and wine colour density can be attributed to the different positive response in 2019 and the negative response in 2017 across the rootstocks. The positive relationship between must TSS was largely driven by the differences between seasons. The positive relationship between must pH and ionised anthocyanins was reasonably consistent across the seasons, despite the differences in must pH between seasons for grafted vines. The genotype with low TSS and high ionised anthocyanins in 2017 was own roots. The general trend for higher wine phenolics as must pH increased can be attributed to both the effects of season (i.e. lower in 2019) and rootstock genotype. The genotype with low phenolics in 2017 was 101-14.

Must pH was highly correlated with the requirement for tartaric acid addition to adjust the pH during the winemaking process (r = 0.93) (Figure 6.2.33). The outlier in 2019, with a low requirement for pH adjustment was genotype 230-80, a selection with low K (Table 6.2.17) and hence potential for lower precipitation of potassium tartrate. As a consequence of tartaric acid addition, wine pH was positively linked to wine TA (r = 0.57) (Figure 6.2.33). Genotype 230-80 was the main outlier in 2019. Over all wines, tartaric acid addition was negatively correlated with wine pH (r = -0.71), due entirely to differences between seasons (Figure 6.2.33), rather than rootstock genotype. In contrast, wine TA was positively correlated with tartaric acid addition (r = 0.65) due largely to effects of rootstock. The positive relationships between tartaric acid addition and total and ionised anthocyanins in the wines (r = 0.79 and 0.61 respectively) and total phenolics (not shown) were associated entirely with effects of season and not rootstock genotype.

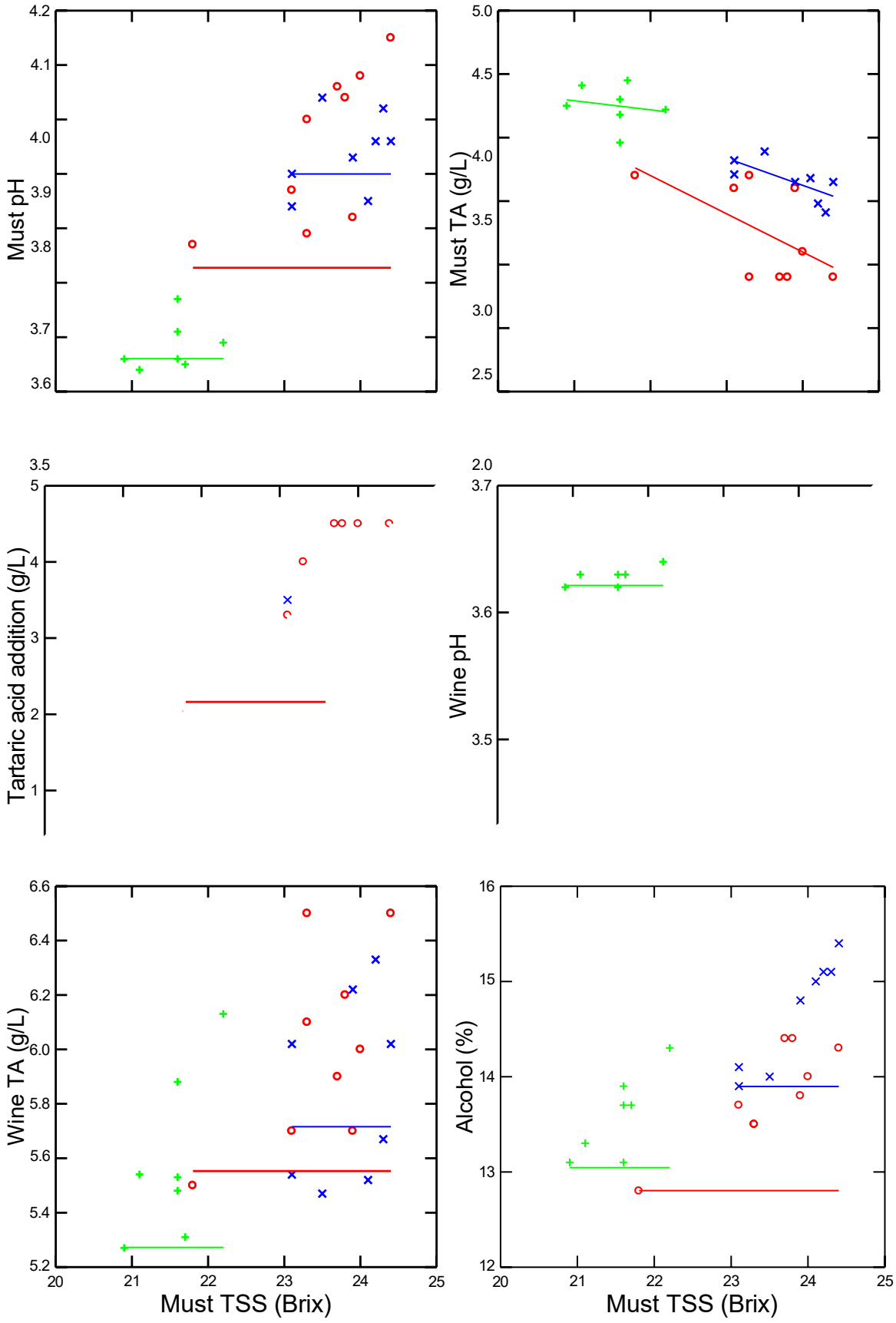


Figure 6.2.31. Relationships between must TSS and must pH (top left), must TA (top right), tartaric acid addition (middle left), wine pH (middle right), wine TA (bottom left) and wine alcohol (bottom

right) for seasons 2017 (red), 2019 (blue) and 2021 (green). TSS, total soluble sugars; TA, titratable acid

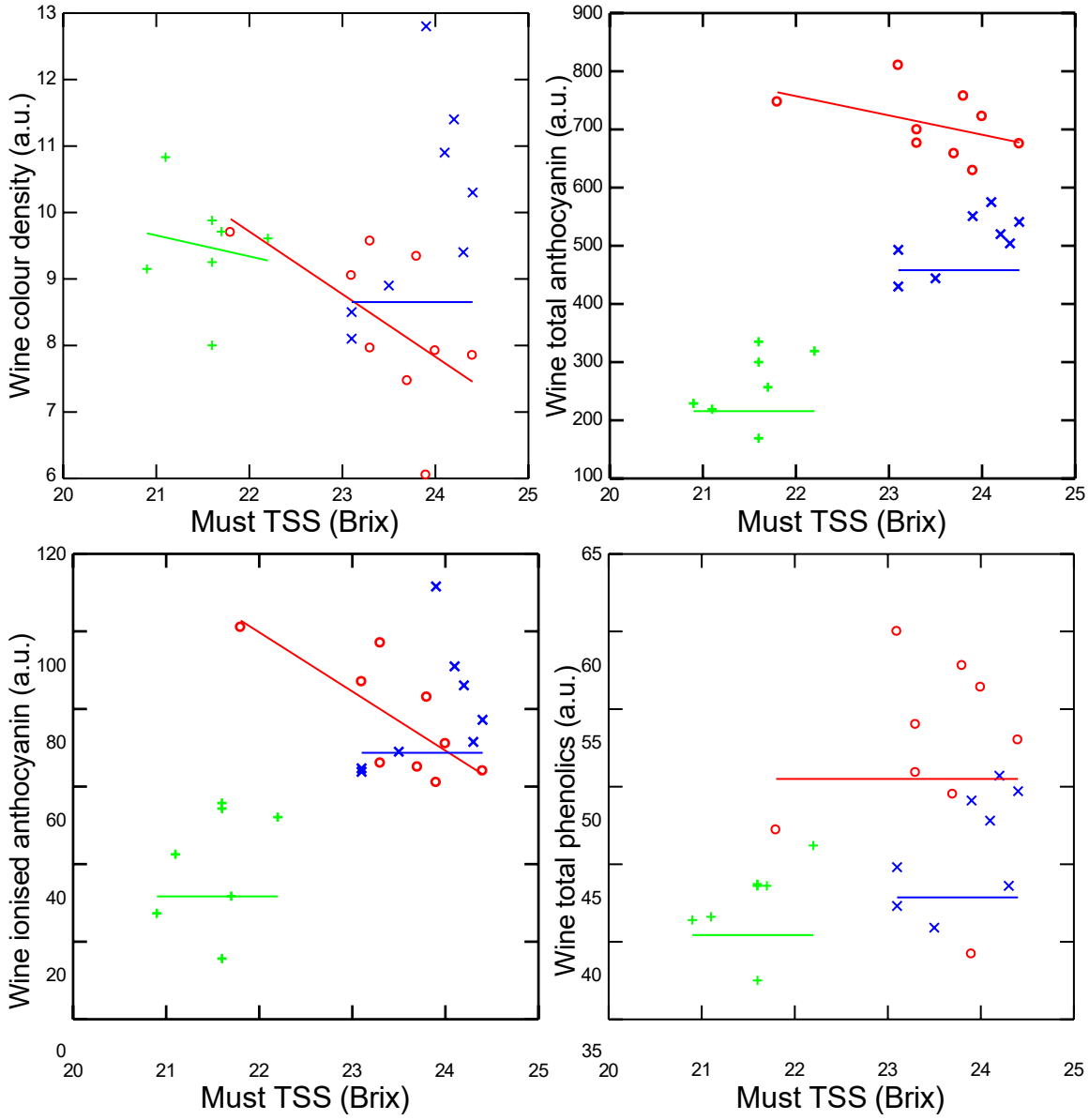


Figure 6.2.32. Relationships between must TSS and wine spectral, colour density (top left), total anthocyanin (top right), ionised anthocyanin (bottom left) and total phenolic substances (bottom right) for seasons 2017 (red), 2019 (blue) and 2021 (green). TSS, total soluble sugars

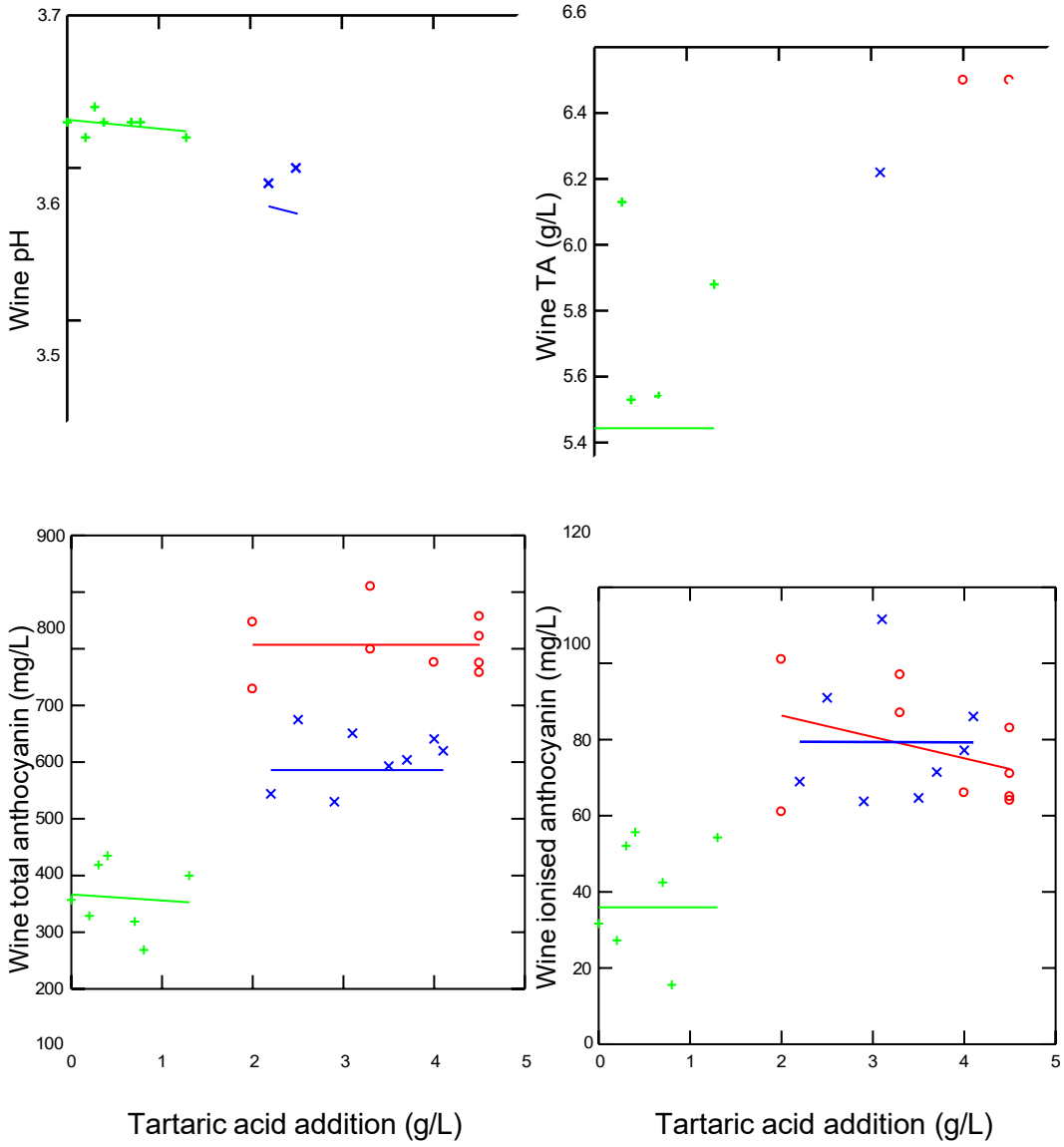


Figure 6.2.33. Relationships between must pH and tartaric acid addition (top left) and wine TA (top right) and tartaric acid addition and wine pH (middle left), wine TA (middle right) and wine total and ionised anthocyanin (bottom left and right) for seasons 2017 (red), 2019 (blue) and 2021 (green).

Discussion

A systematic approach over nine years has been used to identify rootstocks with specific attributes for potential release to industry, involving the assessment of field performance and fruit composition of Shiraz grafted on 59 rootstock genotypes, standard commercial rootstocks and own rooted vines grown with a limited supply with moderately saline water in Padthaway. In meeting this objective, the study has also confirmed that rootstock-conferred vigour is linked to yield, bunch weight, berry weight of the rootstocks. Rootstock yield was a function of both bunch number and bunch weight, the latter correlated with berry weight. The study also confirmed that berry weight was a major driver of fruit composition across the rootstocks being negatively correlated with tartaric acid, anthocyanin and phenolic substance concentrations. The study also confirmed that juice pH across the rootstocks was positively correlated with malic acid, K and Mg concentration. The use of small-scale winemaking techniques in the evaluation of rootstock genotypes was compromised by differences in fruit maturity across the rootstocks and between seasons, as must TSS was linked to must pH, tartaric acid addition for pH adjustment, final wine pH (negative), % alcohol and wine spectral properties.

Details on each rootstock genotype selected are provided below. Assessment of phylloxera and nematode tolerance/ resistance has not been completed for these selections.

High Priority Breeding Selections

Genotype 231-51

Genotype 231-51 conferred low vigour to the Shiraz scion. It has been selected as an alternative to the drought sensitive 101-14 and the difficult to propagate Merbein 5489 rootstock. In this study, it has produced similar yield and pruning weight to these commercial rootstocks. It has favourable fruit composition with low pH, low malic acid, high tartaric acid, high berry anthocyanin, a moderate juice K concentration and low Na uptake. When wines were produced from 231-51 fruit, it had low must pH, high must TA and a low requirement for acid addition for pH adjustment. Spectral properties of 231-51 wine varied across seasons. Wine colour density was similar to that from own rooted Shiraz wines in 2017 and 2019 and greater than 101-14 wine in 2017.

Genotype 230-30

Genotype 230-30 conferred low vigour to the Shiraz scion. It has been selected as an alternative to the drought sensitive 101-14 and the difficult to propagate Merbein 5489 rootstock. In this study, it has produced similar yield and pruning weight to these commercial rootstocks. It has favourable fruit composition with high tartaric acid and high berry anthocyanin, a moderate juice K concentration and low Na uptake. When wines were produced from 230-30 fruit, it had low must pH, high must TA and a relatively low requirement for acid addition for pH adjustment. Wine colour density was greater than own

rooted Shiraz wines in 2019 and 2021 but slightly lower in 2017 and greater than 101-14 wine in 2017.

Genotype 234-27

Genotype 234-27 conferred moderate vigour to the Shiraz scion. It has been selected as an alternative rootstock to 1103 Paulsen and 140 Ruggeri, with slightly higher pruning weight but a lower yield. It has favourable fruit composition with low pH, high tartaric acid and high berry anthocyanin, a moderate juice K concentration and low Na uptake. When wines were produced from 234-27, wine colour density was lower than own rooted Shiraz in 2017 and 2019 but higher than 101-14 in 2017.

Genotype 233-80

Genotype 233-80 was selected as an alternative to own roots with very slightly higher yield and conferred vigour. It has favourable fruit composition with low pH, low malic acid, high tartaric acid, high anthocyanin and low K. When wines were produced from 230-30 fruit, it had low must pH, high must TA and a relatively low requirement for acid addition for pH adjustment. Wine colour density was greater than own rooted Shiraz wines in 2021 and slightly lower in 2017 and greater than 101-14 wines in 2017 and equal to 101-14 in 2021.

Genotype 235-01

Genotype 235-01 was selected as the best higher vigour rootstock, with high yield and high berry anthocyanin, as an alternative to Ramsey rootstock (not included in this study). It has also displayed good drought tolerance. When wines were produced from 230-30 fruit, it had the highest TSS in all years and hence could be considered as an early ripening rootstock. Wine colour density was lower than own rooted Shiraz in 2017 and 2019 but higher in 2021 and higher than 101-14 in 2017.

Selections Identified for Winemaking but given Low Priority

Genotype 230-45

Selection 230-45 conferred moderate vigour to the Shiraz scion and was selected as a potential alternative to 1103 Paulsen and 140 Ruggeri. Drought tolerance of 230-45 was similar to 1103 Paulsen. It has favourable fruit composition with moderately low pH, low malic acid, low K and Na concentrations. However, berry anthocyanin and total phenolics have been shown to be low. Wines were produced from 230-45 in 2017 and 2019. Wine colour density was lower than for Shiraz wines in 2017 and 2019 but higher than for 101-14 wines in 2017.

Genotype 230-70

Selection 230-70, which confers high vigour, was identified as a potential alternative rootstock to Ramsey. Its drought tolerance was similar to 1103 Paulsen. Selection 230-70 has

favourable fruit composition for some traits, including high TSS (early ripening) and low juice K and Na concentrations. However, high malic acid and poor organic acid balance and low berry anthocyanin and total phenolics reduce its potential, compared to 235-01 (see above). Wines were produced from 230-70 in 2017 and 2019. Must of 230-70 had high pH, which may be attributed to high TSS associated with early ripening. Consequently, high additions of tartaric acid were required for pH adjustment. Wine colour density was similar to Shiraz wines in 2019 but lower in 2017 and higher than for 101-14 wines in 2017.
Selection 230-70 could be considered as a potential rootstock if there are any issues with selection 235-01.

Selections Not Given Priority for Breeding

Six genotypes identified previously as having useful traits and included in Tables 6.2.15-6.2.17, have not been given high priority as having potential for release. The reasons for their exclusion, based on long-term performance are given below.

Genotype 230-10.

Genotype 230-10 was included as a low vigour selection with yield and pruning weight similar to Merbein 5489 and 101-14. Compared to the commercial low vigour rootstocks, it displayed significant benefits including high drought resistance, high TSS indicative of early ripening and high juice tartaric acid, but was not selected because of high pH, high K and low berry anthocyanin.

Genotype 230-69

Genotype 230-69 was included as a moderate vigour selection with pruning weight and yield slightly below that of 1103 Paulsen but higher than Merbein 5489 or 101-14. Compared to 1103 Paulsen, it displayed significant benefits including high drought resistance, low pH, low malic acid, favourable organic acid balance but was not selected because of low berry anthocyanin. Genotype 230-69 warrants retention in the moderate vigour rootstock category.

Genotype 230-12

Genotype 230-12 was included as a moderate-high vigour selection, with pruning weight and yield that fell between 1103 Paulsen and 140 Ruggeri. It was not selected because it had low TSS but high pH and low berry anthocyanin.

Genotype 233-72

Genotype 233-72 was included as a high vigour rootstock, with pruning weight and yield that were similar to 140 Ruggeri. It was less tolerant of drought and was lower than 140 Ruggeri, being similar to 1103 Paulsen. It had higher juice K but lower Na than 140 Ruggeri. It was not selected because it had low TSS, high malic acid and low berry anthocyanin.

Genotype 231-30

Genotype 231-30 was included because it displayed very similar traits to own rooted Shiraz, with high vigour, higher drought tolerance (less canopy loss), slightly higher juice pH, malic acid, tartaric acid, K with similar berry anthocyanin. Genotype 231-30 warrants retention in the high vigour rootstock category with similar traits to own rooted vines.

Genotype 233-77

Genotype 233-77 was included as a high vigour alternative rootstock to Ramsey (not included in this study), with high pruning weight and yield. Its favourable traits included low juice pH, low malic acid and low Na. It was not selected because of its low drought tolerance, low TSS, low tartaric acid and low berry anthocyanin.

6.3. Plant Breeders Rights for C113, C114, C20 and C7

PBR provides legal protection for new varieties, in this case four new rootstocks for wine grape production. PBR is administered through IP Australia. The application for PBR is a two-stage process. Stage 1 involves selection of a name, which may also include a synonym; provision of information on the breeding history, including the parents and steps taken to develop the new variety; provision of information of key traits to show differences between the new variety and its parents and common knowledge comparator varieties, which is used to identify varieties to include in a replicated PBR comparator trial to establish Distinctness, Uniformity and Stability (DUS), and provision of photographs to show distinguishing traits. Other parties who have contributed to the development of the new variety, in this case Wine Australia are also identified in the Stage 1 application. In addition, an IP Australia approved qualified person (QP) is nominated to oversee the conduct and analysis of the DUS comparator trial. The case for PBR protection includes data collected from ungrafted material including vigour, K uptake, salt and drought tolerance and root knot nematode and phylloxera tolerance/resistance and the performance in replicated field trials as grafted vines. The final Stage 2 application involves submission of the results of the DUS comparator trial once statistical treatment of the data is completed and confirmed by the QP (in this case Peter Clingeleffer, a CSIRO employee). The case for PBR protection is assessed by an IP Australia examiner and includes an inspection of the DUS comparator trial and field-grown vines used to identify distinguishing characteristics.

In this case, Stage 1 applications for four new rootstocks were lodged. These include the three rootstocks evaluated above (i.e. C113, C114 and C20) and C7, which was also identified in other studies supported by Wine Australia and assessed in the previous project (CSP 1304, Clingeleffer and Smith 2018). The current applications use the full breeding code as the name for each rootstock variety although there is the possibility to add a synonym which can also be protected. In anticipation of potential naming and release, a PBR DUS comparator trial was established in the previous project (CSP 1304, Clingeleffer and Smith 2018). All the ampelographic data required for submission of the Stage 2 applications for each of the candidates have been collected and analysed in readiness to submit the final Stage 2 applications once approval of the Stage 1 applications are given by IP Australia. December 2022 has been nominated as the best time for the IP Australia examiner to inspect the vines.

To facilitate rapid adoption, mother vine source plantings of the new rootstock candidates have already been established by the nurseries licensed by CSIRO to propagate and sell existing rootstock varieties, with appropriate agreements in place.

The summary cases for each of the candidates for PBR, extracted from their Stage 1 application, are provided below.

C113

Vine performance, fruit composition and winemaking data were collected and analysed from the field trials and used to select C113 as a potential new rootstock for the wine industry. The performance of C113 closely matched the high vigour rootstocks, 1103 Paulsen and 140 Ruggeri. Yield and conferred vigour of C113 were slightly higher at the Irymple site but slightly lower at the Padthaway site than 1103 Paulsen and 140 Ruggeri. Specifically, C113 has been selected because of its:

- ease of propagation
- graft compatibility and strong performance with major wine cultivars
- root knot nematode resistance
- ability to exclude chloride from plant tissues, juice and subsequent wine
- ability to promote fruit maturity
- ability to produce high berry anthocyanin concentrations.

C114

Vine performance, fruit composition and winemaking data were collected from the field trials and analysed and used to select C114 as a potential new rootstock for both the wine and dried grape industries. C114 was selected as an alternative rootstock to the widely planted, very high vigour Ramsey rootstock, conferring similar growth, productivity and fruit composition traits. Specifically, C114 has been selected because of its:

- ease of propagation as dormant rootlings
- graft compatibility and strong performance with major wine and dried fruit cultivars
- root knot nematode resistance
- ability to exclude chloride from plant tissues, juice and subsequent wine
- ability to maintain productivity under sustained deficit irrigation compared to other high vigour rootstocks
- drought tolerance
- ability to promote fruit maturity, compared to Ramsey, when grown under moderate salt and water stress

C20

Vine performance, fruit composition and winemaking data were collected and analysed from the field trials and used to select C20 as a potential new rootstock for the wine industry. Under hot climate, full irrigation conditions at CSIRO Irymple, C20 conferred lower vigour to the scions than 1103 Paulsen or 140 Ruggeri but produced similar yields, an indication of enhanced water use efficiency associated with reduced transpiration from a smaller canopy. Compared to 1103 Paulsen or 140 Ruggeri, fruit composition was enhanced by C20, with lower juice pH, lower malic acid and higher berry anthocyanins. At the Padthaway Shiraz site, with limited supply of saline water, the performance of C20 best matched the low vigour rootstock 101-14 Mgt, conferring similar vigour and fruit composition but with a higher yield. Shiraz grafted on C20 also displayed higher drought tolerance and had lower juice chloride than 101-14 Mgt. Specifically, C20 has been selected because of its:

- potential as a water efficient, low vigour rootstock
- drought and salt tolerance compared to 101-14 Mgt.
- root knot nematode and phylloxera resistance
- ability to produce yields equal to 1103 Paulsen and 140 Ruggeri with enhanced fruit composition under hot conditions irrigated.

C7

Vine performance, fruit composition and winemaking data were collected and analysed for the field trials and used to select C7 as a potential new rootstock for both the wine and dried grape industries. In these studies, it has shown similar growth, production and fruit quality traits to 1103 Paulsen and 140 Ruggeri. C7 has shown capacity in a long-term (14 year) field trial to maintain good chloride exclusion when irrigated with saline irrigation water (mean EC_{iw} across all years of 2.0 dS/m) and an ability to maintain high productivity with dried grape scions under sustained deficit irrigation. Specifically, C7 has been selected because of its:

- good capacity to exclude chloride and to maintain acceptable productivity with saline irrigation water over a long-term period
- root knot nematode and phylloxera resistance
- ability to maintain productivity under sustained deficit irrigation with dried grape cultivars
- potential as a rootstock for Sunmuscat, a major dried grape cultivar in Australia

Measurement of vine ampelographic traits provided the basis for showing distinctness, uniformity and stability (DUS) in the PBR comparator trials required for the Stage 2 PBR application. Views of leaves of the candidate rootstocks (C113, C114, C20 and C7) and their main common knowledge comparator varieties, submitted as part of the PBR Stage 1 application are shown in Figures 3.1-3.4. They show both the similarities and distinguishing features of the new varieties compared to their main comparator, identified in the studies reported above.

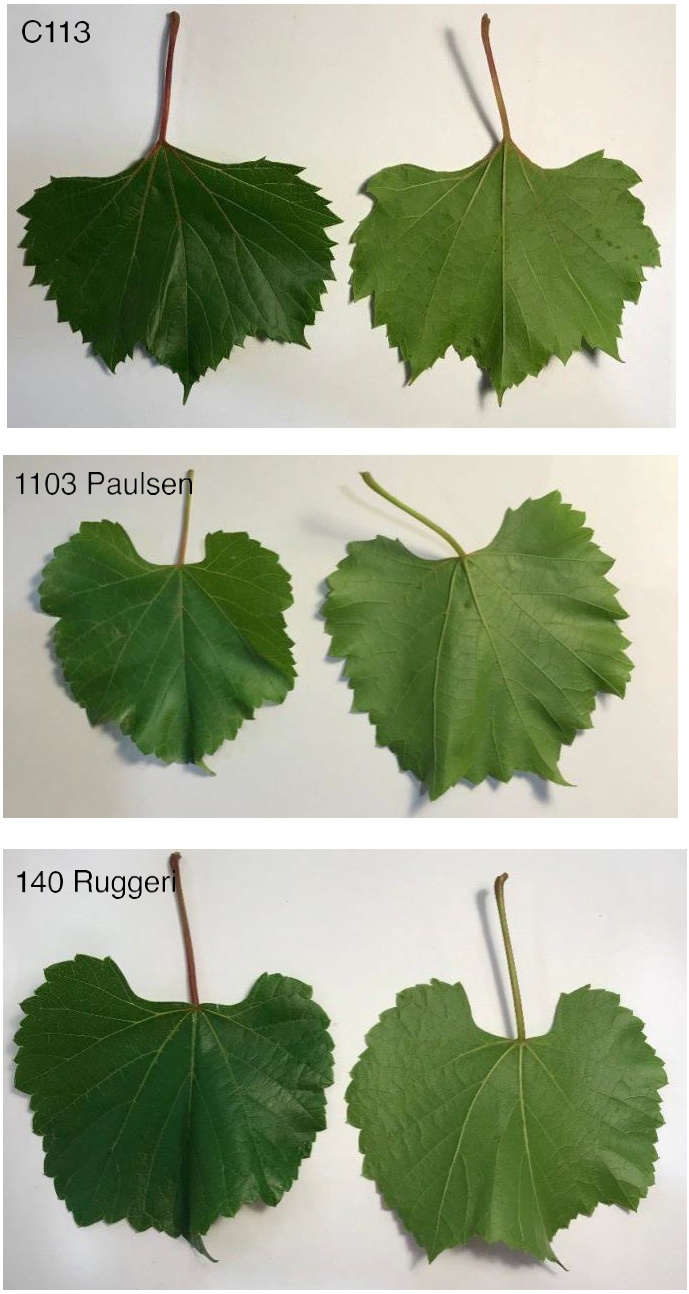


Figure 6.3.1. Views of the upper and lower surfaces of mature leaves of C113, 1103 Paulsen and 140 Ruggeri.

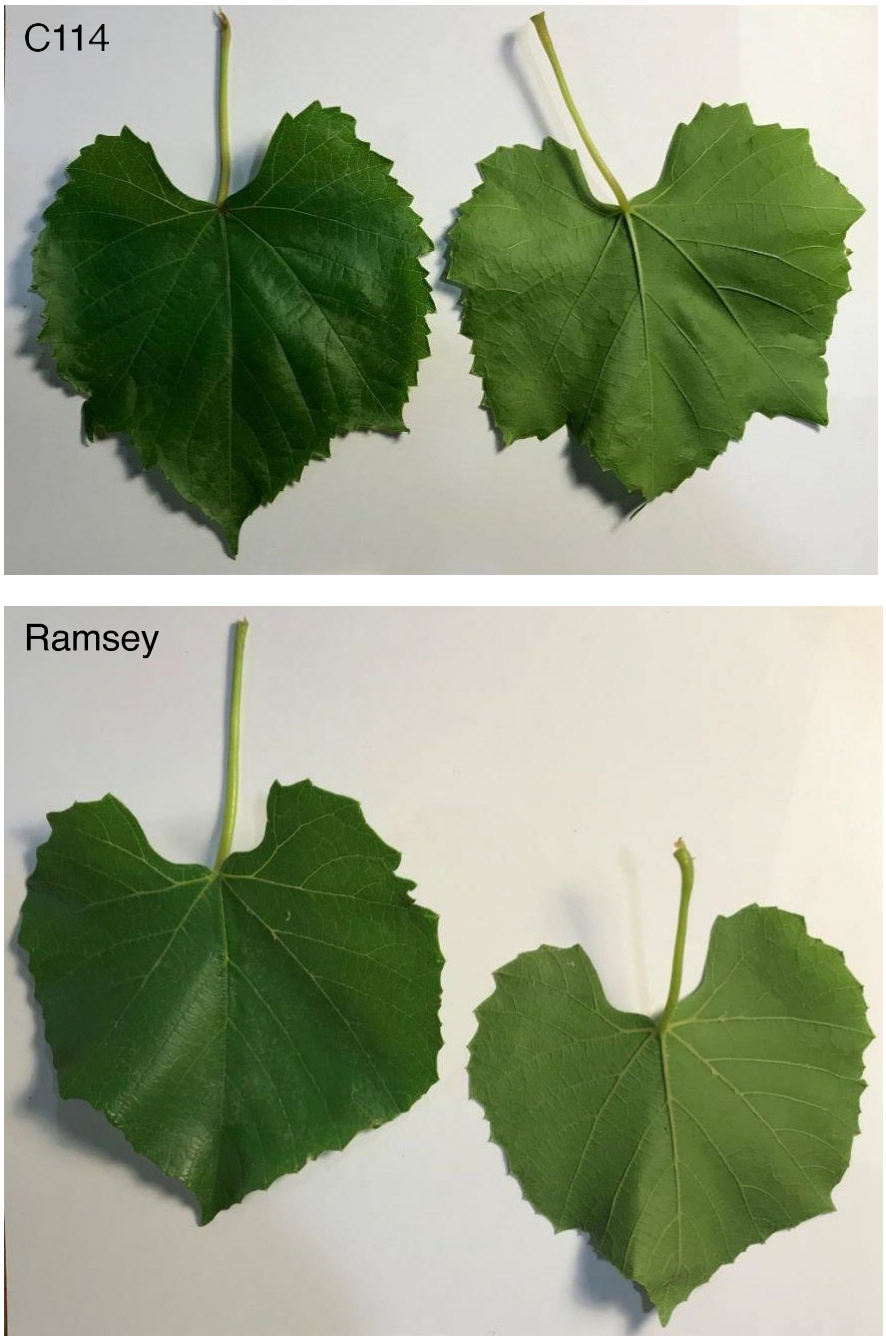


Figure 6.3.2. Views of the upper and lower surfaces of mature leaves of C114 and Ramsey.

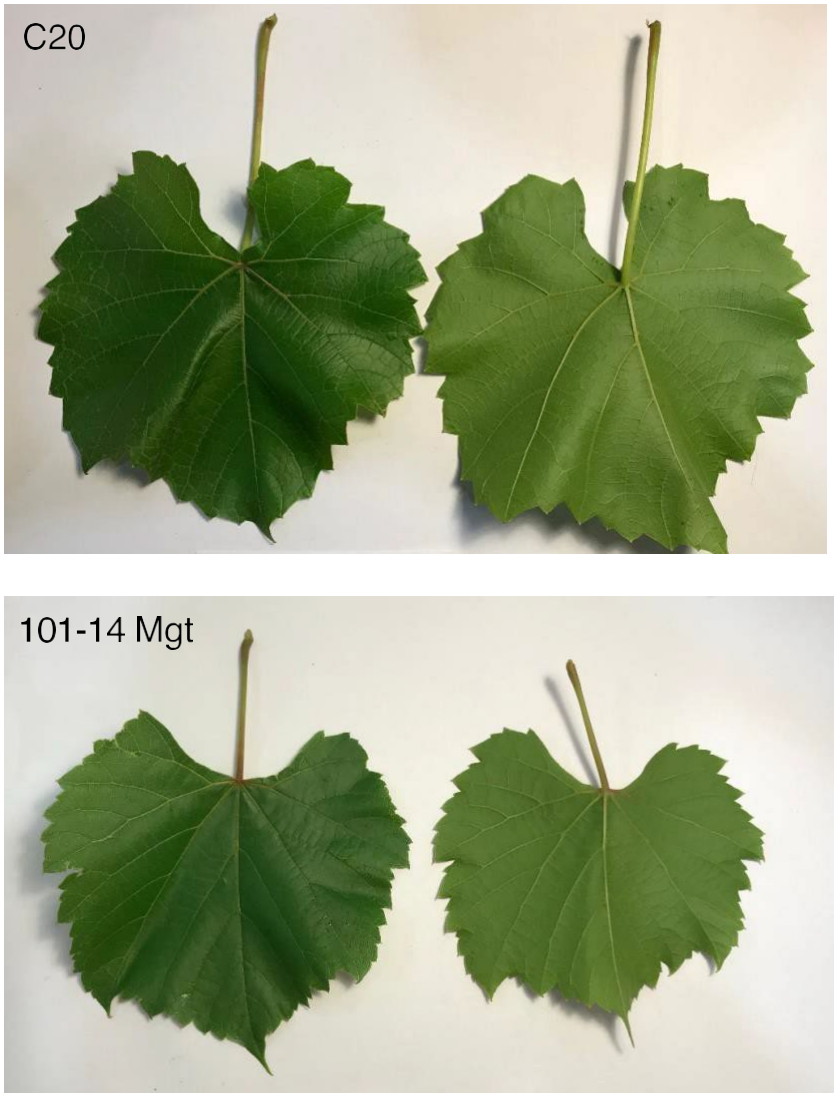


Figure 6.3.3. Views of the upper and lower surfaces of mature leaves of C20 and 101-14 Mgt.

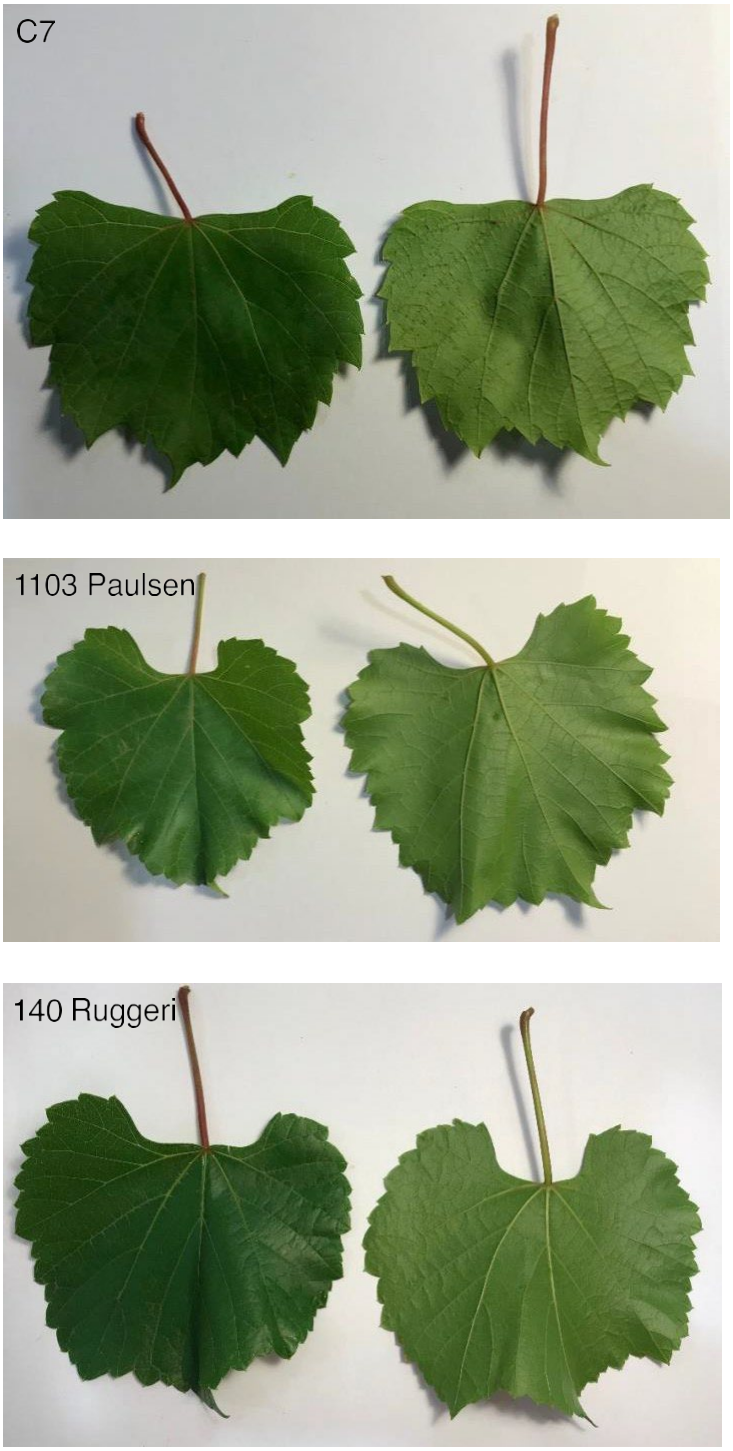


Figure 6.3.4. Views of the upper and lower surfaces of mature leaves of C7, 1103 Paulsen and 140 Ruggeri.

6.4 Evaluation of rootstock germplasm for pest resistance

The domestication of crops has significantly reduced genetic diversity (Zsogon et al., 2022), which negatively impacts plant immunity (Karasov et al., 2020). Commercial rootstocks used in grapevine production were bred over 100 years ago and used throughout the world to primarily safeguard vineyards from grape phylloxera (Ollat et al., 2016). Genetic analysis shows that a limited number of parents, including Rességuier 2 (*V. berlandieri*), Boutin B (*V. berlandieri*), Gloire de Montepllier (*V. riparia*) and du Lot (*V. rupestris*), were used to develop these rootstocks (Riaz et al., 2019). Low genetic diversity is a significant concern, as a breakdown in grape phylloxera resistance in one rootstock will impact selection of an alternate rootstock for replanting. For example, the majority of rootstocks derived from North American *Vitis* species are resistant to biotype-A phylloxera populations (Forneck et al., 2016). However, rootstocks with *V. riparia* parentage support a low level of biotype-C phylloxera feeding and reproduction. Since these rootstocks were deployed, new aggressive populations have emerged with the ability to feed and reproduce at high levels (Kocsis et al., 1999) (https://cenapa.ucanr.edu/newsletters/Vineyard_Views_Newsletter_-_Events43564.pdf; https://vinehealth.com.au/pests/pests-and-diseases/phylloxera/phylloxera-strains/). As a result, the increased performance of these new aggressive biotype-C populations is expected to negatively impact production, particularly when vines experience abiotic stress events. In addition, a breakdown of root knot nematode resistance in Freedom and Harmony occurred from widespread usage in the Central Valley of California (Cain et al., 1984; Anwar and McKenry, 2000; McKenry et al., 2001). As the root knot nematode resistant determinant in Freedom and Harmony was likely derived from *V. x champinii* (Cousins and Walker, 2002), rootstocks such as Ramsey (*V x champinii*) are also susceptible to these virulent root knot nematode populations. In order to increase the genetic diversity of rootstocks, new sources of grape phylloxera and root knot nematode resistance were evaluated for future breeding.

Table 6.4.1 shows the pedigrees of *Vitis* rootstocks and accessions, which were targeted for grape phylloxera and root knot nematode testing and evaluation. In our resistance/susceptibility testing, we used commercial rootstocks and *V. cinerea* C2-50 as a comparator with new hybrid and accessions derived from *V. rupestris*, *V. longii* (*V. acerifolia*), *V. aestivalis*, *V. candicans*, *V. caribaea* and/or *M. rotundifolia* hybrids (Table 6.4.1). In addition, two Riesling clones and one Cabernet Sauvignon were used as susceptible controls.

Table 6.4.1. Pedigree of rootstocks and accessions

Cultivar/Accession	Pedigree
Ramsey	*V. x champinii*
Dogridge	*V. x champinii*
Freedom	1613-59* x *V. x champinii* , open pollinated 'Dogridge 5'
K51-40	Dogridge x Gloire de Montpellier
Schwarzmann	Unknown x Gloire de Montpellier
101-14	*V. riparia* x *V. rupestris*
140 Ruggeri	*V. berlandieri* 'Boutin B' x du Lot
du Lot (St. George)	*V. rupestris*
Börner	*V. riparia* 'Gm183' x *V. cinerea* 'Arnold'
Couderc 1613	*V. acerifolia* x Othello
Gloire de Montpellier	*V. riparia*
Couderc 160-19	*V. rupestris x V. monticola*
Kober 5BB	*V. berlandieri 'Resseguier #2'* x Gloire de Montpellier
Dogridge	*V. x champinii*
C114	Ramsey x *V. berlandieri* 'Mazade'
C113	Ramsey x *V. cinerea* 'C9'
MI 09-07	K51-32 (Dogridge x Gloire de Montpellier) x MG 54-12 (V. *berlandieri*)
MR 04-04	K51-40 x 1103 Paulsen (*V. berlandieri* 'Rességuier 2' x du Lot)
MR 33-31	Kobber 5BB x K49-56 (Dogridge x du Lot)
MR 09-29	Kobber 5BB x K49-56 (Dogridge x du Lot)
188-04 Castel	*V. riparia x V. monticola*
160-19 Couderc	*V. rupestris* x *V. monticola*
62-66 Couderc	(*V. vinifera* x *V. rupestris*) x *V. cordifolia*
125-1 Millardet	*V. rupestris* x *V. cordifolia*
MG 59-01	*V. rupestris* hybrid
MG 59-06	*V. rupestris* hybrid
MG 59-19	*V. rupestris* hybrid
Vitis longii	*V. acerifolia*
MF 47-29	*V. aestivalis*
MF 47-16	*V. aestivalis*
MF51V	*V. aestivalis*
V. candicans	*V. candicans*
MG 60-59	*V. caribaea*
MG 60-55	*V. caribaea*
V. cinerea C2-50	*V. cinerea*
DRX55	*M. rotundifolia* hybrid
MS 27-12	*M. rotundifolia* hybrid
MG 17-10	K51-40 x MS 27-12
MG 17-04	Kobber 5BB x MS 27-12
MG 16-47	Ramsey x MS 27-12
MG 16-37	Ramsey x MS 27-12
MG 16-49	Kobber 5BB x MS 27-12
MG 16-53	Kobber 5BB x MS 27-12
Riesling 140 Nuriootpa	*V. vinifera*
Riesling FVI10V15	*V. vinifera*
Cabernet Sauvignon	*V. vinifera*
Chardonnay	*V. vinifera*

The phylloxera testing trials were performed on new *Vitis* species and hybrids in collaboration with Dr Catherine Clarke at Ag VIC. Overall, the results presented in Table 6.4.2 are preliminary, based of the effectiveness of the screening method (see below). The majority of material testing was carried out in the 2020-2021 season, against the biotype-A grape phylloxera, G4, except for Cabernet Sauvignon[$] and *V. candicans*[$], which were evaluated the following season using the same phylloxera genotype (Table 6.4.2). As expected, *V. vinifera* cultivars, Riesling and Cabernet Sauvignon, were highly susceptible due to the high number of mean nodosities and insects produced. Compared to the *V. vinifera* cultivars, G4 grape phylloxera established more mean feeding sites or nodosities and had a higher rate of reproduction on Dogridge (*V. x champinii*), indicating that this rootstock is highly susceptible

to this biotype-A phylloxera genotype. Couderc 160-19 (*V. rupestris x V. monticola*), MG 60-59 (*V. caribaea*) and MG 59-06 (*V. rupestris* hybrid) were also highly susceptible to G4 grape phylloxera. Based on the results of the G4 grape phylloxera testing, MG 60-114 (Ramsey x *V. berlandieri* 'Mazade') and MG 60-113 (Ramsey x *V. cinerea* 'C9') displayed a moderate level of susceptibility. The low level of feeding site establishment and reproduction, as indicated by nodosity formation and insect production, occurred on the roots of MG 59-19 (*V. rupestris* hybrid), MG 16-37 (Ramsey x MS 27-12), MG 60-55 (*V. caribaea*) and *V. candicans*[$] (screened in 2021-2022). Results from the screening showed that G4 grape phylloxera induced nodosity formation on the roots of K51-40 (Dogridge x Gloire de Montpellier), MG 16-47 (Ramsey x MS 27-12), MF 47-29 (*V. aestivalis*), *Vitis longii*), MG 59-01 (*V. rupestris* hybrid), MF51V (*V. aestivalis*), 188-04 Castel (*V. riparia x V. monticola*), MF 47-16 (*V. aestivalis*) and MR 33-31 (Kober 5BB x MG 54-12 *V. berlandieri*). However, these nodosities did not support G4 grape phylloxera reproduction, as no insects were associated with the roots. Therefore, this rootstock material has been classified as having incomplete resistance to G4 grape phylloxera due to the low level of nodosity formation. *Vitis* material that appeared to be resistant due to the complete absence of nodosities and insect production on three replicates included Ramsey, MG 17-10 (K51-40 x MS 27-12), *V. candicans* (screened in 2020-2021), MS 27-12 (*M. rotundifolia* hybrid), MI 09-07 (K51-32 x MG 54-12 *V. berlandieri*), 101-14 Mgt, Börner and *V. cinerea* C2-50.

As stated above, the results in Table 6.4.2 are preliminary due to the effectiveness of the screening method. For example, Ramsey has been classified as tolerant to G4 grape phylloxera, as this insect is able to feed and reproduce on the roots of this *V. champinii* rootstock at low levels (Korosi et al., 2011). In the above analysis, nodosity formation and insect production were not detected on the roots of Ramsey, therefore, the screening performed in 2020-2021 was not sufficient to differentiate tolerant and resistant rootstocks (Table 6.4.2). In 2020-2021, the roots of *V. candicans* were devoid of nodosities and insects after inoculation with G4 grape phylloxera (Table 6.4.2). However, results from 2021-2022 showed that G4 grape induced nodosity formation on one replicate, while a low number of insects were detected on two replicates. The inconsistency in the presence or absence feeding sites and insect production on the replicates for most of the susceptible accessions and rootstocks is a further indication that the results in this table must be repeated to validate the susceptible, incomplete resistance and resistant ratings.

Root knot nematode resistance was evaluated in *Vitis* species and hybrids with distinct pedigrees from commercial rootstocks used in production. In this research activity, we evaluated *M. javanica* 'pt 1103P' resistance in germplasm with *V. monticola*, *V. cordifolia*, *V. candicans* and *V. longii*, *V. caribaea* and *M. rotundifolia* parentage in a glasshouse trial (Table

6.4.3). Ramsey, Dogridge and *V. cinerea* C2-50 were used as resistant controls, as no *M. javanica* 'pt 1103P' egg masses were observed on the roots of this material (Smith et al., 2016; Smith et al., 2018) (Table 6.4.3). In contrast, *M. javanica* 'pt 1103P' successfully replicated on the roots of the *V. vinifera* Riesling susceptible control. For the two *V. cordifolia* hybrids, 62-66 Couderc was resistant while egg masses were detected on the roots of 125-1 Millardet. The two *V. monticola hybrids*, 188-04 Castel and 160-19 Couderc were susceptible to *M. javanica* 'pt 1103P'. A number of *M. rotundifolia* hybrids were evaluated and only DRX55 and MG 16-47 (MS27-12 x Ramsey) were resistant to *M. javanica* 'pt 1103P'. While *M. javanica* 'pt 1103P' was able to parasitise *V. longii* and the two *V. caribaea* accessions, *V. candicans* was resistant to this root knot nematode population. As root knot nematode resistance derived from Ramsey and Dogridge, *MJR2*, is targeted for the breeding of second-generation rootstocks, we assessed *M. javanica* 'pt 1103P' resistance in a number of hybrids that utilised Dogridge. Results showed that MR 33-31 (Kobber 5BB x K49-56 (Dogridge x du Lot)) and MR 04-04 (K51-40 x 1103 Paulsen (*V. berlandieri* 'Rességuier 2' x du Lot)) were resistant to *M. javanica* 'pt 1103P'. Taken together, new rootstock breeding material with resistance to *M. javanica* 'pt 1103P' was identified. To further increase the genetic diversity in future rootstock development, it is highly recommended to genetically identify molecular markers linked to *M. javanica* 'pt 1103P' resistance in *V. candicans*. Given the plausibility that MG 16-47 (MS27-12 x Ramsey) and MR 04-04 (K51-40 x 1103 Paulsen (*V. berlandieri* 'Rességuier 2' x du Lot) inherited the *V. champinii* based root knot nematode resistance mediated by *MJR2*, these hybrids should be incorporated into the breeding scheme to further increase the genetic diversity of second-generation rootstocks. Lastly, results presented in Table 6.4.3 showed that C114 and MR 33-31, two rootstocks targeted for release to the wine and dried grape industries, displayed complete resistance to *M. javanica* 'pt 1103P'.

Table 6.4.3. Evaluation of *Vitis* material for *M. javanica* 'pt 1103P' resistance and susceptibility

Cultivar/Accession	Mean Egg Masses	SD	Mean Root Mass	SD	Mean EM/RM	SD	Rating
Ramsey	0.0	0.0	3.4	1.7	0.0	0.0	**R**
Dogridge	0.0	0.0	17.5	3.2	0.0	0.0	**R**
K51-40	0.0	0.0	156.5	91.9	0.0	0.0	**R**
Vits cinerea C2-50	0.0	0.0	2.1	0.5	0.0	0.0	**R**
62-66 Couderc	0.0	0.0	10.8	1.9	0.0	0.0	**R**
MR 33-31	0.0	0.0	1.7	1.5	0.0	0.0	**R**
MR 09-29	0.0	0.0	3.5	1.1	0.0	0.0	**R**
MR 04-04	0.0	0.0	7.7	3.6	0.0	0.0	**R**
MG 60-114/C114	0.0	0.0	174.2	12.5	0.0	0.0	**R**
Vitis candicans	0.0	0.0	2.1	0.7	0.0	0.0	**R**
MG 16-47	0.0	0.0	9.7	1.4	0.0	0.0	**R**
DRX55	0.0	0.0	4.3	3.8	0.0	0.0	**R**
5BB Kober	4.7	4.7	27.1	17.1	0.3	0.5	**S**
MG 16-53	6.0	4.0	20.6	11.7	0.4	0.4	**S**
MG 60-55	6.7	7.2	9.4	2.8	0.8	0.8	**S**
188-04 Castel	18.7	15.7	21.5	17.8	0.8	0.7	**S**
MG 16-49	28.0	12.3	8.4	1.3	3.3	1.0	**S**
125-1 Millardet	33.7	29.3	11.6	6.1	4.8	6.1	**S**
MG 17-04	45.3	18.6	10.1	2.2	4.4	1.1	**S**
Vitis longii	48.7	13.5	8.1	2.7	6.2	1.8	**S**
140 Ruggeri	51.3	14.6	4.4	4.3	20.6	16.8	**S**
MG 60-59	52.3	31.5	14.6	2.5	3.4	1.6	**S**
160-19 Couderc	62.3	29.7	37.6	47.5	5.0	6.1	**S**
MG 16-37	75.0	15.7	11.8	1.4	6.3	0.7	**S**
MS 27-12	163.3	61.0	11.3	5.1	17.7	10.9	**S**
Riesling	188.0	68.5	11.4	3.5	16.6	5.4	**S**

EM-Rep, Egg Mass replicate; RM-Rep, root mass replicate

6.5. DNA-markers linked to *MJR2* in *V. x champinii*

6.5.1 Generating mapping populations

Grapevine rootstocks are outbreeding species, which are highly heterozygous, facilitating genetic mapping in the F_1 generation. As stated previously, the *V. champinii* rootstocks, Ramsey and Dogridge, are heterozygous for single root knot nematode resistance locus (Cousins and Walker, 2002). To identify molecular markers linked to this root knot nematode resistant locus, termed *MELOIDOGYNE JAVANICA RESISTANCE 2* (*MJR2*), two F_1 mapping populations, Ramsey x Riesling (*n*=150) and Dogridge x Riesling (*n*=156), were generated by crossing these *V. champinii*-derived female rootstocks with pollen from root knot nematode susceptible plants (as detailed in Table 5.1 in methods). In addition, we also utilise the K51-40 x 140 Ruggeri (*n*=40), which was used to genetically characterise salinity tolerance in grapevine (Gong et al., 2011; Henderson et al., 2018). K51-40 is a rootstock derived from Dogridge x Gloire de Montpellier cross (Riaz et al., 2019), which is resistant to *M. javanica* 'pt 1103P' (Smith et al., 2016). As Gloire de Montpellier is susceptible to root knot nematode (Smith et al., 2016), we reasoned that the root knot nematode resistance displayed by K51-40 is due to the inheritance of *MJR2* from Dogridge. Taken together, the F_1 individuals, as well as the parents, in the three mapping populations described above were phenotyped for root knot nematode resistance and genotyped to facilitate the genetic mapping of *MJR2*.

6.5.2 Phenotyping mapping populations

The roots of resistant and susceptible plants produce distinct phenotypes when infected with endoparasitic root knot (*Meloidogyne* spp.) nematodes (Figure 6.5.1). In essence, root knot nematode-infected susceptible plants such as Riesling and 140-Ruggeri typically form characteristic galls at established nematode feeding sites within the root (Figure 6.5.1., panel B). Completion of the nematode life cycle *in planta* consists of adult females releasing eggs within a gelatinous matrix onto the galled root surface presumably to facilitate dispersion of offspring (Figure 6.5.1., panel C). In contrast, resistant *V. champinii* exhibits an early hypersensitive response upon root knot nematode infection with induced cellular necrosis in the root tip (Figure 6.5.1., panels E and F).

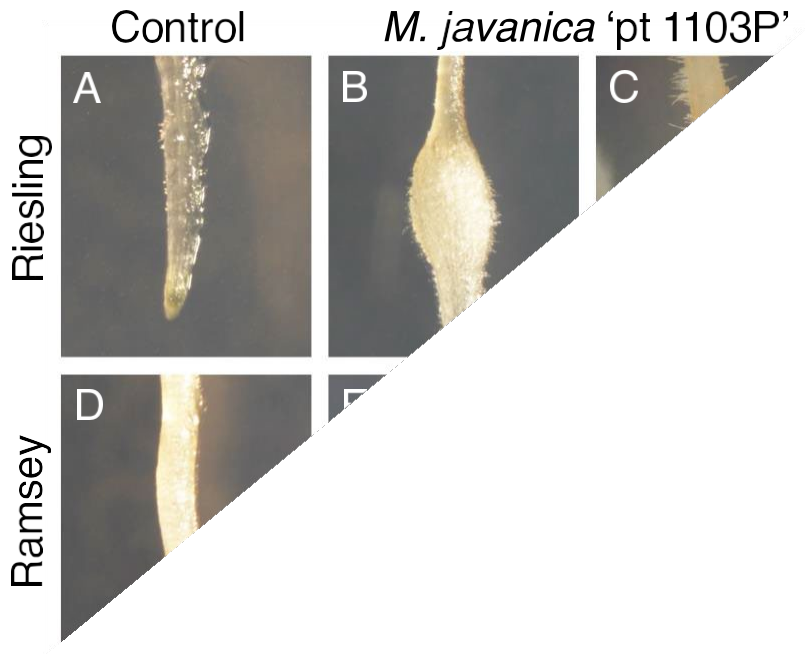


Figure 6.5.1. Susceptible and resistant phenotypes displayed in Riesling and Ramsey, respectively. Morphology and appearance of uninfected (A) Riesling and (D) Ramsey roots. (B) Riesling roots infected with *M. javanica* 'pt 1103P' develop galls with no visible signs of cell necrosis. (C) The galls on Riesling roots produce callus-like protrusions (green arrow) associated with egg mass production (orange arrow). (E and F) After infection with *M. javanica* 'pt 1103P', localised cell necrosis is induced at the tips of Ramsey roots.

The Ramsey x Riesling, Dogridge x Riesling, and K51-40 x 140-Ruggeri mapping populations were infected with *M. javanica* 'pt 1103P'. In addition, the Ramsey x Riesling population was also infected with *M. incognita* and *M. arenaria*. Phenotyping was undertaken with soil-based (Smith et al., 2016) and/or *in-vitro* (Smith et al., 2018) screening assays. Screening infected plants *in-vitro* facilitated tempo-spatial observations of the infection process and provided greater resolution of the host plant's response to infection (e.g. visualisation of the localised HR events in RKN resistant plants) compared to the soil-based assay. Thus, the *in vitro* screening assay was used to determine if other genetic elements affecting the RKN infection response were also segregating within the F_1 mapping populations. In contrast, soil-based screening in the glasshouse provided an additional and less laborious means of determining if plants are resistant or susceptible to root knot nematode infection. To confirm consistency between the results of the two types of screening assays employed for screening root knot nematode-infected F_1 progeny, a subset of 36 and 16 Ramsey x Riesling F_1 plants were infected with *M. javanica* 'pt 1103P' and *M. incognita*, respectively, and assayed both in soil and *in-vitro*. All F_1 progeny with the exception of one individual produced similar results in both assays, indicating that the assays were generally robust, reliable, and consistent (Table 6.5.1).

Root knot nematode resistance segregated within each mapping population in a 1:1 ratio (Table 6.5.1; Chi square *p* value >0.1779), confirming the previous observation that *V.*

champinii-mediated root knot nematode resistance is conferred by a single, dominant, and heterozygous causal locus (Cousins and Walker, 2002). *In-vitro* screening did not identify any F_1 progeny with altered resistance or susceptibility responses suggest there were unlikely to be other segregating background factors significantly influencing the qualitative resistance response.

It has long been recognised that *M. javanica*, *M. incognita*, and *M. arenaria* species are among the most important nematode pests in grapevine and cause significant economic losses (Walker and Stirling, 2008; Saucet et al., 2016). However, resistance to one nematode species does not necessarily confer resistance to other species (Smith et al., 2016). As such, 25 plants from the Ramsey x Riesling population were also screened with *M. incognita* 'SARDI' and *M. arenaria* 'Barossa' in addition to *M. javanica* 'pt 1103P'. Of these, 23 plants showed consistent resistance and susceptibility responses to all three nematode species. Two F_1 plants had inconsistent responses with one being susceptible to *M. javanica* 'pt 1103P' and *M. arenaria* 'Barossa' but resistant to *M. incognita* 'SARDI', and the other was resistant to *M. javanica* 'pt 1103P' and *M. incognita* 'SARDI' but susceptible to *M. arenaria* 'Barossa'. Overall, the results of these screens demonstrated that *MJR2* provides broad resistance to at least these three nematode species (Table 6.5.1).

Population	RKN species	Total plants Phenotyped	RKN resistant plants	RKN susceptible	Chi square p-value for 1:1 segregation	Assayed *in-vitro*		Assayed in soil	
						Resistant plants	Susceptible plants	Resistant plants	Susceptible plants
Ramsey x Riesling	MJ	115	60	55	0.64	60$^{\wedge}$	55$^{\S *}$	15$^{\wedge *}$	21§
	MI	29	13	16	0.58	7*	12†	11*	14†
	MA	27	10	17	0.18	-	-	10	17
Dogridge x Riesling	MJ	95	45	50	0.61	18	30	27	20
K51-40 x 140-Ruggeri	MJ	27	15	12	0.56	-	-	15	12

Table 6.5.1 *MJR2* resistance phenotyping summary for three F_1 mapping populations. Resistance or susceptibility to root knot nematode was determined by assaying plants infected with *M. javanica* 'pt 1103P' (MJ), *M. incognita* 'Barossa' (MI), and/or *M. arenaria* 'SARDI' (MA) using glasshouse and/or *in-vitro* screening systems. Progeny that exhibited localised cell necrosis in the tissues of the root tip and did not form galls were scored as resistant. Infected roots that formed galls were scored as susceptible. Chi-square P-values ≥ 0.05 indicate the data were statistically consistent with 1:1

segregation in the F_1 mapping populations. A varying number of F_1 lines were assayed both *in-vitro* and in soil and are annotated with the following superscripts: ^14, §21, ‡1, *5, and †11.

6.5.3 Genotyping

Marker-assisted selection (MAS) is a useful strategy to expedite selective breeding in perennial plants with have long breeding cycles, including grapevine, and requires DNA-markers tightly linked to the genes underlying the trait of interest. SNPs are a particularly useful type of molecular marker for MAS as they are widespread, abundant, and suitable for cost effective and high-throughput genome-wide analyses with SNP genotyping platforms. The DaRTseq genotype-by-sequencing (GBS) platform facilitates genome-wide SNP discovery through restriction enzyme-mediated genome complexity reduction and next-generation sequencing of the reduced genomic representations, which increases the proportion of unique SNPs within the DArTseq dataset (Barilli et al., 2018). SNPs within the three mapping populations were identified using the DArTSeq GBS platform, which were subsequently filtered and analysed using the dartR package in R with the parameters described in methods. Principal component analysis (PCA) analysis was undertaken to assess variation within the mapping population datasets and identified distinct outlier clusters in the Ramsay x Riesling and Dogridge x Riesling populations consisting of 26 and 13 F_1 plants, respectively. These two mapping populations were generated through field crossings and genotype analysis of the outlying progeny indicated they were likely due to cross-pollination with a non-Riesling parent(s). These individuals were subsequently removed from the SNP datasets and the DartR processing and analysis was repeated. The total number of SNPs obtained in each mapping population after filtering ranged from ~9,000 to ~12,000 (Table 6.5.2). The DArTSeq reads containing the SNPs were aligned against the 12xV2 version of the Pinot noir reference genome (PN40024) and the phased genome of *V. riparia* Gloire de Montpellier (version EGFV_Vitrip_1.0) (refer methods) as a means of identifying the putative chromosomal location of the SNPs.

Identification of SNPs linked to the *MJR2* in *V. champinii* initially involved using a pseudo-testcross strategy. This approach is typically used in linkage mapping in outcrossing species to identify DNA polymorphism present in the parent conferring resistance that is absent in the susceptible parent (i.e. heterozygous and homozygous in the resistant and susceptible parents, respectively) and therefore segregates 1:1 in the F_1 generation (Grattapaglia and Sederoff, 1994; Smith et al., 2018; Smith et al., 2018). For MAS, markers both within and flanking the MJR2 are desirable to ensure the entire root knot nematode resistance locus is inherited. Filtering the DArTseq dataset for testcross configured markers identified 12, 9, & 22 SNPs linked to *MJR2* in the Ramsey x Riesling, Dogridge x Riesling, and K51-40 x 140-Ruggeri F_1 populations, respectively (Table 6.5.2, Table 6.5.3). Previously, it has been shown that successfully adapting GBS-identified SNP markers to other SNP genotyping platforms can be limited (Smith et al., 2018; Smith et al., 2018), presumably due to various factors such as

flanking polymorphism interference at probe/primer binding sites. SNPs present in a pseudo-testcross configuration are relatively simple to analyse but constitute only a subset of selectable variation. As such, we sought to identify additional *MJR2*-linked SNPs in the DArTSeq dataset that could potentially be used as molecular markers.

Mapping population	# Plants genotyped	Total # SNPs (post-filter)	# SNPs linked to RKN resistance	#SNPs linked to RKN resistance in different parental zygotic cross types				
				Rr × rr (pseudo-testcross)	Rr × r-	R- × r-	R- × rr	Rr × --
Ramsey x Riesling	106*	11601	77	12 *(16%)*	14 *(18%)*	2 *(3%)*	*15* *(20%)*	34 *(44%)*
Dogridge x Riesling	99+	12055	51	9 *(18%)*	4 *(8%)*	7 *(14%)*	10 *(20%)*	21 *(41%)*
K51-40 x 140-Ruggeri	40	8925	65	22 (*34%)*	7 *(11%)*	9 *(14%)*	5 *(8%)*	22 *(34%)*

Table 6.5.2 Genotyping summary for three F_1 mapping populations. The number of plants genotyped in the final mapping populations after exclusion of *26 and +19 putative cross-pollinated progeny identified by PCA. The total number of SNPs for each population is shown after filtering the datasets for monomorphs; reproducibility; call rate; read depth; and minor allele frequency. The total number of SNPs linked to RKN resistance is shown along with the subtotals for the five parental zygosity models investigated (percentages are noted in italics and brackets). Genotypes are shown with 'R' representing the dominant RKN resistance allele; 'r' representing the recessive susceptibility allele; '-' representing a null allele.

Structural variants (SV) such as presence/absence variation (PAV) have recently been identified as substantial contributors to the heterozygosity of grapevine lines (Zhou et al., 2019). The relatively recent advent of long-read sequencing technologies, optical mapping, and improvements in assembly software has helped resolve structural variation, particularly PAV (Wellenreuther et al., 2019). SV exists throughout the genome in both non-repetitive and repetitive sequence space and have been estimated to be responsible for the hemizygous state of approximately one in seven genes in Chardonnay and Cabernet Sauvignon cultivars (Zhou et al., 2019). In addition, hemizygosity could arise through substantial sequence divergence between haplotypes, particularly if cultivars are subjected to ongoing clonal propagation. Taken together, it was postulated that there could be additional hemizygous SNPs within the DArTSeq dataset that could be used to increase the density of markers linked to the *MJR2* in the three *V. champinii* mapping populations. However, it should be noted that the restriction enzyme-mediated genome complexity reduction underlying the Dartseq platform could result in apparent hemizygosity within the DArtSeq dataset that may not exist

within the complete plant genome. However, these artefactual hemizygous SNPs are reproducible and therefore valid as potential markers in analyses of the DArTSeq dataset itself. Therefore, the hemizygous DarTseq SNP marker may not transfer to other SNP genotyping platforms and highlights the need for cross-platform validation of all SNP candidates if alternative genotyping platforms are to be utilised. Identification of linked hemizygous SNPs to *MJR2* was undertaken by filtering the DarTSeq dataset for SNPs that segregated in a manner consistent with four additional hemizygous or nullizygous parental configurations as described in methods. Specifically, hemizygosity in the susceptible parent (Rr x r-); hemizygosity in the resistant parent (R- x rr); hemizygosity in both parents (R- × r-); and nullizygosity in the susceptible parent (Rr × --). This analysis identified an additional 65, 42, and 43 SNPs linked to *MJR2* in the Ramsey x Riesling, Dogridge x Riesling, and K51-40 x 140-Ruggeri F_1 populations, respectively (Table 6.5.2, Table 6.5.3). Less than 11% of *MJR2*-linked SNPs were common between any two populations, and only one SNP was common to all three mapping populations (Table 6.5.3).

Ramsey x Riesling			
SNP ID	cM	Group	Cross type
c18_30323825|15	11.1		H2
c18_30659767|39	11.1		H2
c18_30715553|57	9.2		N
c18_30726160|29	10.0		H1
c18_30726724|42	11.0		H1
c18_30726724|8	9.3		N
c18_30773246|21	8.0		H1
c18_30786471|45	8.0		H1
c18_30786471|47	9.0		H1
c18_31087282|59	3.2		H2
c18_31113136|29	6.0	RK	P
c18_31132049|8	7.0		H2
c18_31132115|63	4.3		P
c18_31181720|15	4.2		N
c18_31181720|58	5.1		H1
c18_31271359|15	6.0		H1
c18_31477763|24	5.0		H1
c18_31509239|15	5.0		P
c18_31547661|32	5.1		N
c18_31547661|19	5.0		N
c18_31583250|63	5.2		N
c18_31584545|5	3.1		H2
c18_31662497|9	4.1		N
c18_31687466|21	5.0		H1
c18_31719604|62	7.0	RD	H2
c18_31738047|36	4.0		P
c18_31801009|10	4.1		N
c18_31801009|41	6.0		H1
c18_31826180|41	5.0		H2
c18_31826316|17	6.0		P
c18_31886871|46	8.0		P
c18_31886937|36	4.1		N
c18_31899283|15	7.0	RD	H1
c18_32033782|5	4.1		N
c18_32211546|33	6.0		H1
c18_32217092|59	5.0		H1
c18_32547773|6	5.0	RK	P
c18_32558880|8	4.1		N
c18_32595367|24	4.0		P
c18_32789584|68	4.0		H2
c18_32833275|61	3.1		N
c18_32905638|10	0.0		N
c18_32908795|12	3.0	RDK	H2
c18_33198697|66	4.0	RD	H1
c18_33244517|6	3.0	RD	H2
c18_33335742|68	2.1		N
c18_33335808|60	3.0		P
c18_33367709|36	2.0		H3
c18_33425789|58	3.0		N
c18_33516284|56	3.2		N
c18_33655773|13	7.0		H1
c18_33901094|21	7.1		N
c18_33954143|7	6.3		N
c18_33993215|13	7.2	RK	N
c18_34014870|53	5.3		H2
c18_34384654|22	11.0		N
c18_34411748|43	11.0		N
c18_34454388|55	12.0		P
c18_34467408|25	11.0		N
c18_34469287|15	11.0		N
c5_1961332|56	7.1		H3
c13_13675134|18	5.0		P
cNA_0-19154064|17	11.0		P
cNA_0-19155548|12	2.0		N
cNA_0-19167523|40	5.0		H2
cNA_0-19172760|53	4.0		N
cNA_0-6590920|27	9.2		N
rc15_543229|39	9.4		H2
rc18_32987110|51	9.2		H2
rc18_33502455|65	10.2		N
rc18_33677882|19	9.1		N
rc18_34782602|15	4.1		N
rc18_35608864|25	4.1		N
rc18_38984593|12	7.0		N
rc18_39586824|57	6.0		N
rs460_156909|13	4.1		N
rs460_185044|10	4.0		N

Dogridge x Riesling			
SNP ID	cM	Group	Cross type
c18_30219163|39	10.0		H3
c18_31719604|62	10.7	RD	H2
c18_31899283|15	9.3	RD	H1
c18_31907425|16	8.4	DK	N
c18_32125045|14	10.6		P
c18_32126791|41	9.4		H1
c18_32547773|6	7.0	RD	P
c18_32558849|11	8.1		H1
c18_32595367|24	8.1		P
c18_32851471|62	2.4		N
c18_32908795|12	4.7	RDK	H2
c18_33123278|37	2.4		H3
c18_33198697|66	4.7		H1
c18_33244517|6	2.4	RD	H3
c18_33329611|50	3.5		H2
c18_33335742|35	7.0		H1
c18_33335742|40	4.8		N
c18_33362303|17	4.8		N
c18_33364563|66	4.8		N
c18_33373168|8	4.8		N
c18_33417328|11	5.8		H1
c18_33425789|46	4.7	DK	N
c18_33518124|5	4.8		N
c18_33631425|40	3.7		H3
c18_33651207|24	4.7		N
c18_33774526|40	12.0	DK	H2
c18_33819894|66	7.1		H1
c18_33819894|45	6.0		N
c18_33897397|44	6.0		N
c18_33903322|46	5.9	DK	N
c18_33958833|55	7.1	DK	N
c18_34067250|38	4.8		H3
c18_34109003|46	3.6		H3
c18_34109069|53	8.1		P
c18_34158342|61	10.5		H1
c18_34158342|11	8.4		N
c18_34267926|66	11.6	DK	P
c18_34291291|66	12.8		P
c18_34433331|50	14.0		H1
c18_34433723|29	14.0		P
c18_34435456|11	12.8		P
c18_34455271|5	14.0		P
c0_16811346|45	4.8		N
cNA_0-19155548|12	4.8		N
cNA_0-19165127|25	6.0		N
cNA_0-19168607|6	6.1		N
cNA_0-34189074|58	9.6		N
rc18_35195486|51	7.3		N
rc18_35608798|5	8.1		H1
rc18_39114941|51	3.7		H3
rc18_39664233|64	7.1		N

K51-40 x 140-Ruggeri			
SNP ID	cM	Group	Cross type
c18_26339183|53	11.1		H3
c18_27208879|38	7.4		N
c18_27391308|30	4.0		N
c18_27406449|31	7.4		H3
c18_27819743|22	7.4		P
c18_27841491|50	7.4		P
c18_27845683|29	7.4		H3
c18_27888713|31	4.0		N
c18_28073986|20	7.4		P
c18_28125334|14	7.4		P
c18_28228406|11	7.4		P
c18_28240164|29	7.4		H2
c18_28240230|10	7.4		P
c18_28289551|44	7.4		P
c18_28468247|57	3.8		H1
c18_28469454|52	7.4		P
c18_28474509|54	7.7		N
c18_28499801|13	7.4		P
c18_28556352|30	7.4		P
c18_28605166|18	7.4		H3
c18_28633017|64	7.4		H2
c18_30099763|60	4.0		N
c18_30517858|9	7.4		P
c18_30788176|12	7.4		P
c18_31113136|29	3.7	RK	H2
c18_31172936|64	0.0		H1
c18_31218326|13	3.8		N
c18_31721909|16	3.8		N
c18_31890875|21	3.8		H1
c18_31907425|16	7.4	DK	H3
c18_32547773|6	3.7	RK	H1
c18_32577611|68	3.7		H2
c18_32789584|29	3.7		P
c18_32905572|39	3.8		N
c18_32908795|12	3.7	RDK	P
c18_33329611|50	7.4		P
c18_33360982|51	7.4		H2
c18_33425789|46	7.7	DK	N
c18_33773538|16	7.4		P
c18_33774526|40	7.4	DK	P
c18_33903322|46	7.4	DK	P
c18_33958833|55	7.4	DK	H3
c18_33993215|13	7.4	RK	P
c18_34209193|8	11.1		H2
c18_34291291|66	11.1	DK	P
c17_1989232|26	3.8		N
cNA_0-19155055|13	3.8		N
cNA_0-19170989|24	7.4		P
cNA_0-50022232|44	7.4		H3
cNA_0-50024379|33	4.0		N
cNA_0-6561595|54	4.0		N
cNA_0-6572514|16	7.4		H2
cNA_0-6594216|19	3.7		H3
rc18_29132735|37	7.7		N
rc18_29136721|5	7.7		N
rc18_30818206|11	7.4		H3
rc18_31230364|25	3.8		H1
rc18_31577605|47	7.7		N
rc18_32407907|64	7.7		N
rc18_32639611|18	7.7		N
rc18_32842106|52	4.0		N
rc18_33504875|7	7.4		P
rc18_33677882|16	7.7		N
rc18_9318470|28	7.7		N
rs478_9750|67	7.7		N

Table 6.5.3 SNPs linked to *MJR2* in Ramsey x Riesling, Dogridge x Riesling, and K51-40 x 140-Ruggeri populations.

SNPs aligning to the 12xV2 Pinot noir (PN40024) reference genome have SNP IDs prefixed with a 'c' followed by the chromosome number. SNPs aligning to the *V. riparia* reference genome (EGFV_Vit.rip_1.0) are prefixed with an 'r' followed either by a 'c' and the chromosome number or an 's' and the scaffold number. The physical chromosomal position is noted between underscore and pipe symbols in the SNP ID. SNPs that do not align to either reference genomes are prefixed with 'cNA' followed by a unique identifying number. The genetic distance from the *MJR2* locus to each SNP is expressed in centimorgans (cM). The group code indicates if a SNP is common to two or more populations as follows: RD) Ramsey x Riesling and Dogridge x Riesling; DK) Dogridge x Riesling and K51-40 x 140-Ruggeri; RDK) Ramsey x Riesling, Dogridge x Riesling, and K51-40 x 140-Ruggeri. The cross types denote the putative parental allele configurations as inferred by genotype segregation patterns within the DarTSeq dataset. The cross types are coded as P) Rr x rr (pseudo-testcross); H1) R- x rr; H2) Rr x r-; H3) R- x r-; or N) Rr x -- with R and r representing the RKN resistance and non-resistance conferring alleles, respectively. Absence of alleles is represented by '-' or '--', indicating hemizygosity or nullizygosity, respectively. SNPs within the delineated *MJR2* region in each population are shown in bold.

The genetic distance between *MJR2* and the linked SNPs ranged from 0 to 14cM with 21%, 14%, and 32% of SNPs being within 4cM of the trait in the Ramsey x Riesling, Dogridge x Riesling, and K51-40 x 140-Ruggeri populations, respectively (Table 6.5.3). Markers c18_32905638|10 and C18_31172936|64 were completely linked (0cM) to *MJR2* in the Ramsey x Riesling and K51-40 x 140-Ruggeri populations, respectively. However, the mapping population sizes are relatively small with a correspondingly small number of recombinants, particularly in the K51-40 x 140-Ruggeri population (n=40). As such, confirmation of the very tight linkage of these markers requires analysis in larger segregation mapping populations.

In all three populations, 70% or more of the *MJR2* linked SNPs aligned to a contiguous region in the lower arm of chromosome 18 in the 12xV2 reference genome ranging from ~30.3Mb to ~34.5Mb, ~30.2Mb to ~34.4Mb, and ~26.3Mb to ~34.3Mb in the Ramsey x Riesling, Dogridge x Riesling, and K51-40 x 140-Ruggeri populations, respectively (Table 6.5.3). The majority of linked SNPs that aligned to the Gloire de Montpellier genome were also positioned in the lower arm of chromosome 18 (Table 6.5.3). Given the reference genomes used are of different genetic backgrounds to the mapping populations analysed, the aligned marker order and distances cannot be certain and should be interpreted with caution. However, analysis of recombinant F_1 plants indicated the overall order of marker SNPs generally appeared to be consistent with the Pinot 12xV2 reference genome (Figure 6.5.2). Linkage analyses between all SNPs would be required for a complete understanding of the relative positioning of markers. Recombinant analysis indicated that SNP markers were present on both flanks and within *MJR2*, which is important for MAS to ensure that the entire resistance locus is inherited within progeny. Based on the alignment of SNP markers from all three mapping populations against the 12xV2 reference genome, MJR2 tentatively maps to an ~370kb region between ~32.83Mbp and ~33.20Mbp on chromosome 18 (Table 6.5.3; Figure 6.5.2). The position of

the *MJR2* locus in *V. champinii* corresponds to the *MJR1* locus in *V. cinerea* which mapped to an ~3.24Mbp region between ~30.71Mbp and ~33.95Mbp on chromosome 18 [Smith & friends, 2018]. Analysis of the region 32.83Mbp to 33.20Mbp on chromosome 18 using the Augustus gene prediction algorithm resulted in 40 predicted genes. BlastX of these predicted genes against the *Arabidopsis* TAIR10 protein database enabled 16 of these genes to be annotated and identified a cluster of putative defence response genes (Table 6.5.4). Without isolation of the *MJR1* and *MJR2* genes, it is unknown if these are different genes or alleles of the same gene. However, the resistance response elicited by *MJR1* and *MJR2* display significant differences. While *MJR1* elicits an immune response confined to the root meristem and vascular initials, the resistance response of MJR2 occurs in the cortex as well as inner cell layers for the root. Further, *V. cinerea* is resistant to the *MJR2* resistant breaking root knot nematodes (Boyden and Cousins, 2003). Therefore, this region of chromosome 18 may be analogous to the co-localisation of root knot nematode genes in tomato (El-Sappah et al., 2019). Therefore, we propose that *MJR1* and *MJR2* are tightly linked genes located on chromosome 18.

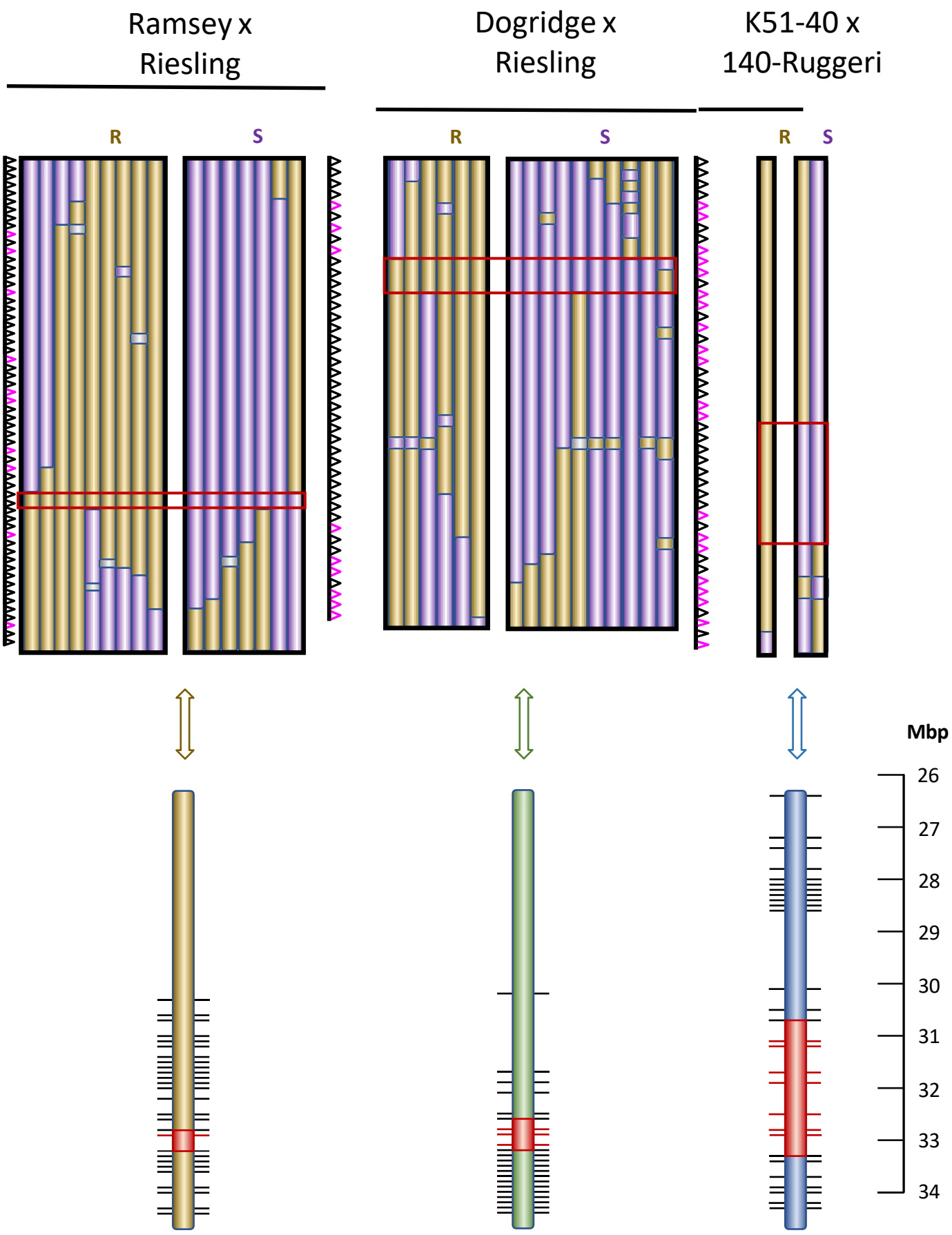

Ramsey x Riesling
Dogridge x Riesling
K51-40 x 140-Ruggeri
R
S
R
S
R
S
Mbp
26
27
28
29
30
31
32
33
34

Figure 6.5.2 Delineation of the *MJR2* locus

(A) Non-scaled representation of informative recombinant progeny in the Ramsey x Riesling, Dogridge x Riesling, and K51-40 x 140-Ruggeri F_1 mapping populations used to delineate the chromosomal location of the *MJR2* locus in *V. champinii*. SNPs identified in this study that align to the lower arm of chromosome 18 are shown in physical order (based on the PN40024 (12v2) reference genome) as triangles to the left of the resistant (R) and susceptible (S) grouped recombinants. Pink triangles show SNPs in a pseudo-testcross configuration. Vertical bars represent the lower arm of chromosome 18 for the individual informative progeny. Segments coloured gold, purple, or grey show markers scored as being from the resistant parent, susceptible parent, or unknown, respectively. Putative delineation of the *MJR2* is shown in red boxes for each population and assumes congruency in marker order between the mapping populations and the Pinot Noir reference genome. **(B)** Scaled physical position of SNPs (horizontal lines) linked to *MJR2* from each population that align to chromosome 18 in the PN40024 (12xV2) reference genome. The physical scale is depicted to the left in Mbp. The region in red shows the putative delineation of the RKN-resistance locus shown in (A) and is based on the caveat described previously.

TAIR10 accession	Gene description	Putative gene location on Ch18 (bp)	E value
AT3G02760.1	Class II aaRS and biotin synthetases superfamily protein	32,833,902 -> 32,839,885	5.11e-174
AT4G10100.1	Co-factor for nitrate, reductase and xanthine dehydrogenase 7	32,856,375 -> 32,851,337	2.49e-36
AT4G23160.1	cysteine-rich RLK (RECEPTOR-like protein kinase) 8	32,871,830 -> 32,874,409	2.88e-41
AT1G71410.1	ARM repeat superfamily protein	32,871,830 -> 32,874,409	2.6e-142
AT1G71410.1	ARM repeat superfamily protein	32,902,170 -> 32,905,120	1.38e-26
AT1G71410.1	ARM repeat superfamily protein	32,906,290 -> 32,910,244	4.76e-134
AT4G23160.1	cysteine-rich RLK (RECEPTOR-like protein kinase) 8	32,917,400 -> 32,921,490	2.61e-76
AT1G69550.1	disease resistance protein (TIR-NBS-LRR class) family	32,925,805 -> 32,922,131	5.63e-26
AT3G51560.1	disease resistance protein (TIR-NBS-LRR class) family	32,979,005 -> 32,971,250	5.24e-26
AT5G17680.1	disease resistance protein (TIR-NBS-LRR class) family	33,057,482 -> 33,053,140	3.36e-68
AT1G65850.1	disease resistance protein (TIR-NBS-LRR class) family	33,063,662 -> 33,062,021	2.43e-15
AT5G58120.1	disease resistance protein (TIR-NBS-LRR class) family	33,083,882 -> 33,079,286	1.09e-46
AT4G15020.1	hAT transposon superfamily	33,087,407 -> 33,090,419	6.97e-84
AT4G23160.1	cysteine-rich RLK (RECEPTOR-like protein kinase) 8	33,091,727 -> 33,095,540	8.62e-88
AT5G66460.1	Glycosyl hydrolase superfamily protein	33,102,582 -> 33,100,612	7.24e-25
AT3G10890.1	Glycosyl hydrolase superfamily protein	33,122,102 -> 33,120,238	1.85e-24

Table 6.5.4 Annotation of predicted genes in delineated *MJR2* locus. Predicted genes in the region between ~32.83Mbp and ~33.20Mbp on chromosome 18 were identified using the Augustus plugin in the Geneious Prime software. Gene annotation was performed by BlastX of the predicted gene sequences against the *Arabidopsis* TAIR10 protein database. The top hit of each search is shown with the associated *Arabidopsis* accession number, *Arabidopsis* gene description, PN40024 chromosome 18 position, and the BlastX Expect value (E).

6.5.4 Discussion

Grape phylloxera and root knot nematode are major pests to Australian viticulture. Developing next generation rootstocks with durable resistance to root knot nematode, as well as grape phylloxera, is a key goal of our research program. Durable resistance to grape phylloxera and root knot nematode require the ability to combine or stack two non-redundant resistance loci to each of these pests into an individual rootstock. In CSP 1304, we successfully determined the genomic location of *MJR1*, as well as *RDV2* from *V. cinerea* C2-50 (Smith et al., 2018). DNA-markers linked to these loci were developed and used to select first-generation rootstocks (see section 6.6). In this project, we determined that combining *MRJ1* with *MJR2* would be a viable pathway to generate durable resistance, as the resistance mechanisms exhibited by *V. cinerea* and *V. champinii* appear to be distinct (Boyden and Cousins, 2003). Moreover, the pattern of localised cell necrosis in response to root knot nematode infection differs between these *Vitis* species.

Using F_1 Ramsey x Riesling and F_1 Dogridge x Riesling mapping populations, as well as the F_1 K51-40 x 140 Ruggeri population, we confirmed that *V. champinii* contains a single root knot nematode locus, termed *MJR2*. To genetically map *MJR2*, we utilised *in-vitro* and glasshouse-based screening procedures. In response to root knot nematode infection, *MJR2* elicits a cell necrosis, which likely functions to prevent the migration and feeding site establishment required for reproduction. Moreover, we showed that *MJR2* provides resistance to three *Meloidogyne* species populations, *M. javanica* 'pt 1103P', *M. arenaria* 'Barossa' and *M. incognita* 'SARDI'. Thus, *MJR2* is a single locus that confers broad spectrum resistance to the three major root knot nematode species that infest vineyards in Australia.

To identify DNA-markers linked to *MJR2*, we increased the number of candidate markers linked to the root knot nematode resistance locus by identifying pseudo-test cross SNPs, as well as SNPs with hemizygous and nullizygous configurations from the DArTseq datasets. The increase in marker density enabled us to better resolve informative recombinants and further delineated the *MJR2* locus to an ~370kb region in chromosome 18. Further, the utilisation of these SNPs in genetic mapping suggests that these different classes of SNPs may also be utilised in marker-assisted selection to further improve our ability to identify individuals harbouring *MJR2* when we select for second-generation rootstocks.

6.6 Marker-assisted selection of first-generation rootstocks

The development of next generation rootstocks with durable resistance to grape phylloxera and root knot nematode was initiated in this project. A next generation breeding scheme was developed with aim of stacking two resistance loci for grape phylloxera and root knot nematode into new durable resistant rootstock selections (Figure 6.6.1). To achieve this objective, we crossed *V. cinerea* C2-50 with Börner (*V. riparia* Gm183 x *V. cinerea* Arnold) in order to combine *RDV2* and *MJR1* with *RDV1* from each parent, respectively. F_1 *V. cinerea* C2-50 x Börner individuals containing *RDV1*, *RDV2* and *MJR1* resistance loci are referred to as first-generation rootstocks. Note: we initially proposed to cross *V. cinerea* C2-50 with *V. aestivalis* bicolor, as this accession appeared to harbour a phylloxera resistance determinant(s). However, due to difficulties with propagating *V. aestivalis* bicolor, as well as F_1 individuals crossed with this *Vitis* accession, we substituted the *V. aestivalis* bicolor parent with Börner.

Figure 6.6.1. Initial breeding and selection scheme for developing next generation rootstocks with durable resistance to grape phylloxera and root knot nematode. DNA-markers linked to each of the resistance loci are used to select for first- and second-generation rootstocks using marker-assisted selection. Second-generation rootstocks will undergo further screening to select for individuals that maintain root knot nematode resistance at high soil temperatures and exclude chloride and sodium ions from the scion. After vine multiplication, nursery and field trials will be used to evaluate and select next generation rootstocks for their ability to initiate roots from cuttings, good graft compatibility, and favourable yield, berry and wine associated traits.

To develop second-generation rootstocks, Ramsey (*V. x champinii*) will be crossed with first-generation rootstocks, to combine *MJR2* with *RDV1*, *RDV2* and *MJR1* (Figure 6.6.1). As shown

in Section 6.5, Ramsey is one of the *V. champinii* rootstocks used to identify DNA-markers tightly linked to *MJR2* (Section 6.5). Marker-assisted breeding will be used to select for second-generation rootstocks using DNA-markers linked to the grape phylloxera and root knot nematode resistance loci segregating in the F_1 Ramsey x first-generation individuals. As shown in table 6.4.3, a number of Ramsey hybrids were evaluated for *M. javanica* 'pt 1103P' resistance and results showed that two Ramsey hybrids, C114 and MG 16-47 were resistant to this nematode pest. C114 is derived from Ramsey x Mazade (*V. berlandieri)* and this root knot nematode resistant rootstock displays a high level of chloride exclusion (CSP 1304, Clingeleffer and Smith 2018; section 6.1; Table 6.4.1). Therefore, F_1 C114 x first-generation rootstocks containing *RDV1*, *RDV2*, *MJR1* and *MJR2* will be evaluated for chloride exclusion in order to select durable resistant rootstocks with the ability to exclude Cl- ions from the scion. MG 16-47, is derived from Ramsey x MS 27-12, a *M. rotundifolia* hybrid that displays incomplete resistance to G4 grape phylloxera (Table 6.4.2). Moreover, by crossing Ramsey, C114 and MG 16-47 with first-generation rootstocks, the genetic diversity of second-generation rootstocks will be substantially increased for improved pest resistance.

According to the breeding scheme, second-generation rootstocks will be evaluated for their ability to provide a high level of immunity to root knot nematodes at high soil temperature, as well as chloride exclusion (Figure 6.6.1). In addition, we propose that these rootstock selections will need to be evaluated for resistance to the seven grape phylloxera genotypes maintained at Ag VIC via collaboration with Dr. Catherine Clark. During the phylloxera testing period, second-generation rootstocks will be planted at the CSIRO-Irymple Farm and the SARDI Research Station at Nuriootpa for vine multiplication. Thus, after completion of the phylloxera evaluation trials, second-generation rootstocks with heat-stable and broad-spectrum resistance to root knot nematode and grape phylloxera, respectively, as well as high chloride exclusion, will be propagated for nursery and field trial evaluations. In this phase of rootstock development, selections will be evaluated for graft compatibility, ability to produce roots from nodal cuttings, and vine performance, including vigour, yield, berry and wine associated traits.

To marker-assisted select first-generation rootstocks, two sets of DNA-markers were utilised. Simple sequence repeats (SSRs) tightly linked to *RDV1* derived from Börner were used to select for this grape phylloxera resistance locus (Zhang et al., 2009). Single nucleotide polymorphism (SNP) linked to *RDV2* and *MJR1* in *V. cinerea* C2-50 were used to select for these grape phylloxera and root knot nematode resistance loci (Smith et al., 2018) (CSP 1304, Clingeleffer and Smith 2018). Kompetitive allele specific PCR (KASP)-markers were developed for SNPs tightly linked to *RDV2* and *MRJ1* in order to select F_1 *V. cinerea* C2-50 x Börner individuals containing these resistance loci. KASP-markers were chosen, as this PCR-based approach is cost effective and results from the genotyping has shorter turn-around time (Semagn et al., 2014). In our first-generation marker-assisted selection, 300 F_1 *V. cinerea* C2-50 x Börner individuals were screened for the presence or absence of *RDV1*, *RDV2* and *MJR1*.

In total, 23 first-generation rootstocks were selected, which harboured *RDV1*, *RDV2* and *MJR1*. These experimental rootstocks were planted at the SARDI Research Station and future crosses will be performed to marker-assisted select second-generation rootstocks (Figure 6.6.2).

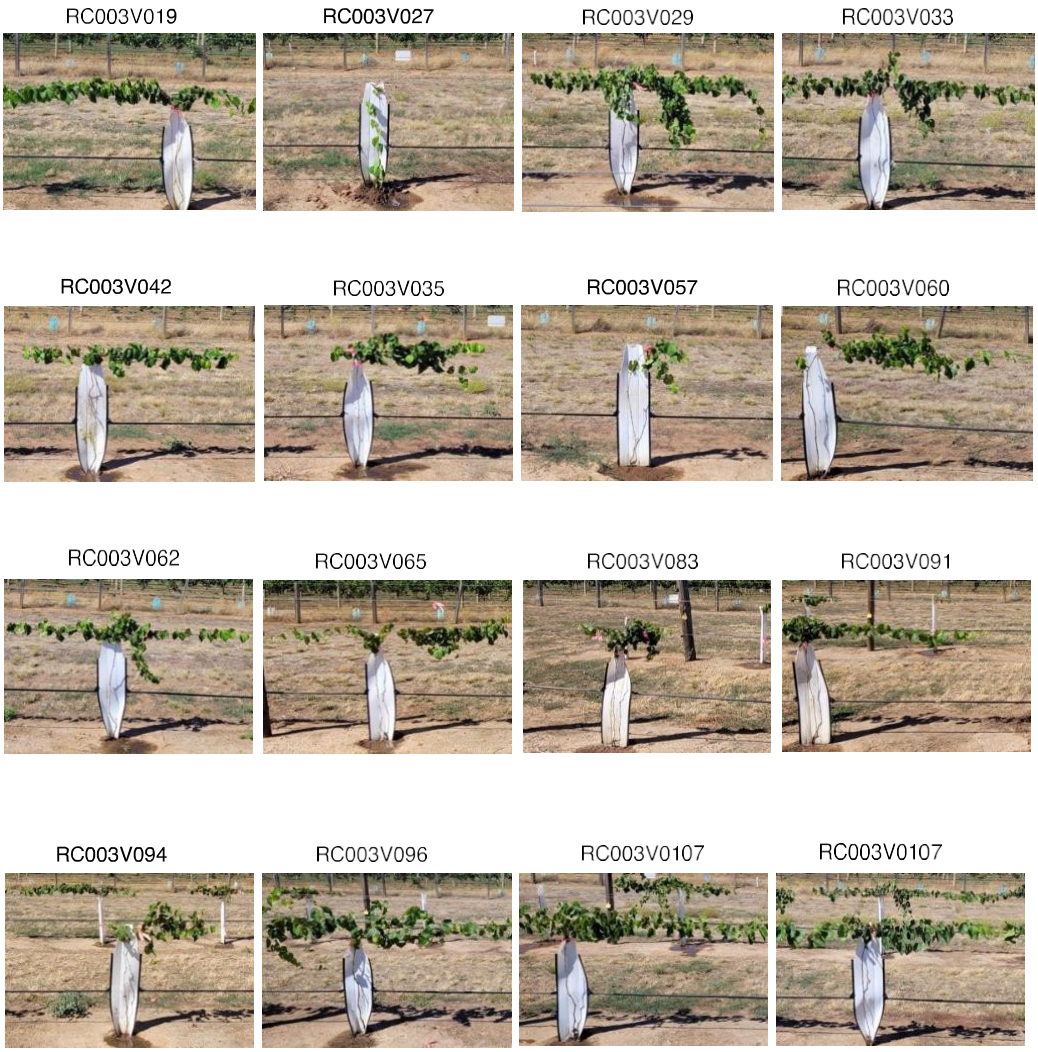


Figure 6.6.2. Establishment of first-generation rootstocks harbouring *RDV1*, *RDV2* and *MJR1*.
This figure displays 16 out of 23 first-generation rootstocks harbouring *RDV1*, *RDV2*, and *MJR1*. Based on a preliminary genetic mapping study, the first-generation rootstocks displayed in this figure likely contain *RDV38*.

To validate the first-generation rootstocks for root knot nematode resistance, a subset of F_1 individuals with and without *MJR1* were screened for *M. javanica* 'pt 1103' resistance. Results showed that all F_1 *V. cinerea* C2-50 x Börner individuals containing *MJR1* were resistant to *M. javanica* 'pt 1103P' (Table 6.6.1). Further, this subset of the F_1 *V. cinerea* C2-50 x Börner individuals were also screened for resistance to *M. arenaria* 'Barossa' and *M. incognita* 'SARDI'. All F_1 *V. cinerea* C2-50 x Börner individuals containing *MJR1* were resistant to *M.*

arenaria 'Barossa' and *M. incognita* 'SARDI' indicating that *MJR1* provides broad spectrum resistance to the three major root knot nematode species that infect grapevine (Table 6.6.1).

Table 6.6.1. *MJR1* is resistant to three *Meloidogyne* species populations

Rootstock ID	*M. javanica* 'pt 1103P'	*M. arenaria* 'Barossa'	*M. incognita* SARDI	Rating	*MJR1*
RC003V019	R	R	R	R	**+**
RC003V027	R	R	R	R	**+**
RC003V042	R	R	R	R	**+**
RC003V057	R	R	R	R	**+**
RC003V083	R	R	R	R	**+**
RC003V091	R	R	R	R	**+**
RC003V124	R	R	R	R	**+**
RC003V130	R	R	R	R	**+**
RC003V166	R	R	R	R	**+**
RC003V171	R	R	R	R	**+**
RC003V172	R	R	R	R	**+**
RC003V176	S	S	S	S	**-**
RC003V008	S	S	S	S	**-**
RC003V017	S	S	S	S	**-**
RC003V020	S	S	S	S	**-**
RC003V023	S	S	S	S	**-**
RC003V025	S	S	S	S	**-**
RC003V028	S	S	S	S	**-**
RC003V039	S	S	S	S	**-**
RC003V040	S	S	S	S	**-**
RC003V044	S	S	S	S	**-**
RC003V048	S	S	S	S	**-**
RC003V050	S	S	S	S	**-**

Phylloxera resistance was also evaluated in F_1 *V. cinerea* C2-50 x Börner individuals containing *RDV1*, *RDV2* and *RDV1* + *RDV2*, as well as progeny lacking these resistance loci. In the first evaluation trial, these sets of F_1 *V. cinerea* C2-50 x Börner individuals were screened for G4 grape phylloxera resistance. Results showed that all F_1 *V. cinerea* C2-50 x Börner individuals containing *RDV1*, *RDV2* and *RDV1* + *RDV2* were resistant to this biotype-A grape phylloxera genotype (Table 6.6.2). In addition, F_1 *V. cinerea* C2-50 x Börner individuals lacking *RDV1* and *RDV2* were resistant to G4 grape phylloxera, presumably because of the uncharacterised resistance determinants inherited from *V. riparia* Gm183.

Table 6.6.2. Summary of G4 grape phylloxera resistance in select F_1 *V. cinerea* C2-50 x Börner individuals

Grape Phylloxera Genotype	*V. cinerea* C2-50	Börner	*RDV1/rdv1 rdv2/rdv2*	*rdv1/rdv1 RDV2/rdv2*	*RDV1/rdv1 RDV2/rdv2*	*rdv1/rdv1 rdv2/rdv2*
G4	R	R	R	R	R	*R

*The G4 grape phylloxera resistance phenotype in individuals lacking *RDV1* and *RDV2* is likely derived from uncharacterised resistance determinants derived from *V. riparia* Gm183

With the recent emergence and characterisation of the aggressive G38 grape phylloxera (https://vinehealth.com.au/pests/pests-and-diseases/phylloxera/phylloxera-strains/), which parasitises 101-14 Mgt, Schwarzmann and 3309C, we tested a subset of F_1 *V. cinerea* C2-50 x Börner individuals containing *RDV1*, *RDV2*, and *RDV1* + *RDV2*, as well as Börner and *V. cinerea* C2-50, for G38 grape phylloxera resistance. In this trial, we also evaluated F_1 *V. cinerea* C2-50 x Börner individuals lacking *RDV1* and *RDV2* for G38 grape phylloxera resistance. Results from the screening assay showed that Börner was susceptible to G38, while *V. cinerea* C2-50 was resistant to this grape phylloxera genotype (Table 6.6.3). Interestingly, all F_1 *V. cinerea* C2-50 x Börner individuals harbouring *RDV1* were resistant to G38, which is surprising given that Börner is susceptible. Based on this result, we hypothesise that Börner is homozygous for a recessive gene that blocks *RDV1* resistance against G38 grape phylloxera. The presence of the recessive susceptibility factor in Börner that blocks *RDV1* function and possibly other *RDV* resistance determinants is of concern for breeding, as it is imperative that these alleles are not inherited in selected next generation rootstocks. Therefore, future studies should be aimed at understanding the molecular basis of the recessive *RDV1* resistant blocking factor.

Another unexpected result came from screening the F_1 *V. cinerea* C2-50 x Börner individuals with G38 grape phylloxera in which 56% of F_1 *V. cinerea* C2-50 x Börner individuals containing *RDV2* were resistant to this biotype-C genotype, while 44% were susceptible. In addition, 56% of the F_1 *V. cinerea* C2-50 x Börner individuals that did not inherit *RDV1*, *RDV2*, and/or G38 were resistant and 44% were susceptible to G38 grape phylloxera (Table 6.6.3). Together these results suggest that: (1) *RDV2* does not provide resistance to G38 grape phylloxera and (2) *V. cinerea* C2-50 contains a resistance locus that may be specific to the G38. We performed a preliminary genetic mapping study and results indicate that the G38 resistant determinant is potentially located on chromosomes 13 in *V. cinerea* C2-50 (data not shown). *RDV1* is also located on chromosome 13; however, the position of this putative G38 phylloxera resistance locus, termed *RDV38*, is located at the opposite end of the chromosome. Therefore, *RDV38* appears to be a distinct locus from *RDV1*. Taken together, *V. cinerea* C2-50 contains two grape phylloxera resistance loci, *RDV1* and *RDV38*, which provide resistance to G1/G4 (biotype-A) and G38 (biotype-C), respectively. Based on genotype data derived from F_1 *V. cinerea* C2-50 x Börner individuals, out of the 23 individuals harbouring *RDV1*, *RDV2* and *MJR1*, 16 appear to also contain *RDV38*. Therefore, these individuals will be used to develop second-generation rootstocks (Figure 6.6.3). However, it is imperative to determine the genetic location of *RDV38* in the *V. cinerea* C2-50 genome and identify DNA-markers linked to this locus in order to successfully select second-generation rootstocks with durable resistance to grape phylloxera, including the aggressive G38 strain.

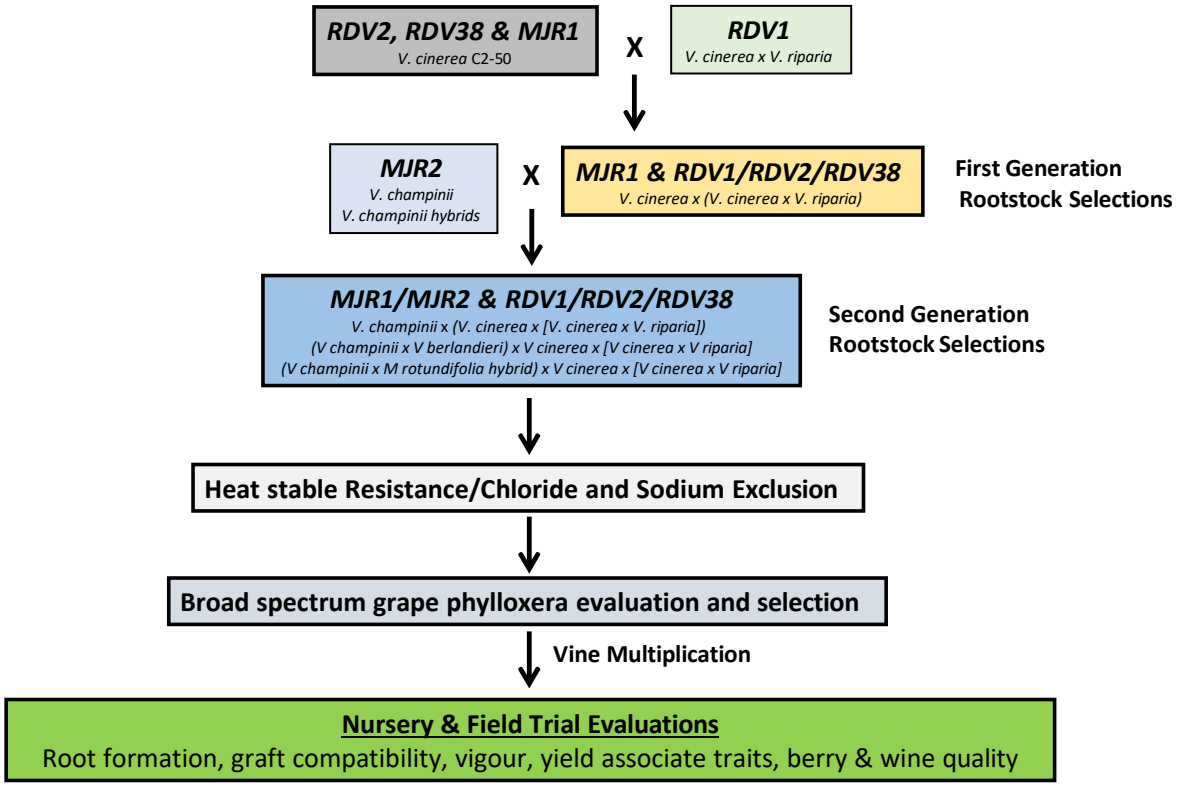


Figure 6.6.3. Revised breeding and selection scheme for developing next generation rootstocks with durable resistance to grape phylloxera and root knot nematode. With the recent identification of RDV38, we have incorporated this locus in the breeding scheme. To select for the *RDV38* locus, it will be essential to determine the position of this locus and tightly linked markers in the *V. cinerea* C2-50 genome. DNA-markers linked to each of the resistance loci are used to select second-generation rootstocks using marker-assisted selection. Second-generation rootstocks will undergo evaluation to select for individuals that maintain root knot nematode resistance at high soil temperatures and exclude chloride and sodium ions from the scion. In addition, individual *RDV* loci will be evaluated for their ability to provide resistance to the diverse panel of grape phylloxera genotypes maintained at AgVIC. After vine multiplication, nursery and field trials will be used to evaluate and select next generation rootstocks for their ability to initiate roots from cuttings, good graft compatibility, and favourable yield, berry and wine associated traits.

As grape phylloxera is the number one biosecurity threat to the Australian grape and wine industry, it is essential that future studies address the specificity of *RDV1*, *RDV2* and *RDV38* to the representative grape phylloxera genotypes maintained at AgVIC. These data will provide critical information regarding grape phylloxera resistance in second-generation rootstocks to assist growers on selecting the appropriate rootstocks for production in phylloxera infestation zones (Figure 6.6.3). In addition, it will also benefit growers who want to effectively safeguard vineyards from future outbreaks of grape phylloxera accidently introduced into phylloxera free zones.

Figure 6.6.4. Breeding and evaluation scheme for developing third-generation rootstocks.
To increase genetic diversity, first-generation rootstocks harbouring *RDV1*, *RDV2*, *RDV38* and *MJR1* will be crossed to *V. candicans*, which contains *MJR3*. Using DNA markers linked to the five resistance loci, marker-assisted selection will used to select third-generation rootstocks with durable resistance to grape phylloxera and root knot nematode. The marker-assisted rootstocks will be evaluated for their ability to: (1) provide root knot nematode resistance at high soil temperature and (2) exclude sodium and chloride ions. Third-generation rootstocks will also be evaluated for their ability to provide resistance to the major grape phylloxera genotypes maintained at AgVIC. After vine multiplication, nursery and field trail evaluations will be performed to evaluate the performance of the third-generation rootstocks prior to release to industry.

To further increase the genetic diversity of next generation rootstocks, we proposed to cross first-generation rootstocks with a *V. candicans* accession (Figure 6.6.4). Results from this project showed that *V. candicans* is resistant to *M. javanica* 'pt 1103P' in glasshouse and *in vitro* screening systems (Table 6.4.3 and 6.7.3). The resistance mechanism induced by *V. candicans* involves localised cell necrosis in the root tips (Figure 6.7.2M-O). Preliminary genetic mapping based on phenotype and genotype determination derived from 20 F_1 *V. candicans* x Chardonnay individuals indicates that the root knot nematode resistance locus, termed *MJR3*, is located on chromosome 18 (data not shown). Future research should be aimed at the defining the genetic position of *MJR3* and identifying DNA markers tightly linked to this root knot nematode resistance locus for breeding next generation durable resistant rootstocks (Figure 6.6.4). Results from our project suggest that grape phylloxera resistance loci may be biotype and/or genotype specific. While *V. candicans* is susceptible to the biotype-A G4 population, it would be beneficial to determine if *V. candicans* is resistant to G38 or other biotype-C genotypes maintained at AgVIC.

6.7 Impact of high temperature on root knot nematode resistance

In a changing climate, an increase in global temperature is expected to augment the frequency of heatwaves, as well as alter annual precipitation events (IPCC, 2022). As a result, global warming is expected to negatively affect plant development pathways and physiological processes that control crop productivity (Battisti and Naylor, 2009; Long and Ort, 2010). In addition, experimental modelling predicts that an increase in global temperature may also increase the performance of pests, which will further reduce yield (Deutsch et al., 2018). Further, an increase in temperature can negatively impact the ability of a plant to initiate a complete immune response in response to an invading pathogen (Alcazar and Parker, 2011; Velasquez et al., 2018). Therefore, an increase in temperature can breakdown resistance and allow the pathogen to reproduce and spread. In a changing climate, plant breeding programs must incorporate heat-stable resistant mechanisms into the development of new crops in order to reduce the impact of pests and pathogens on crop productivity.

Root knot nematode (*Meloidogyne* spp.) is a major economically damaging pest to agriculture and horticulture due to its wide host range (Sato et al., 2019; Rutter et al., 2022). Plants harbouring root knot nematode resistance are a key management solution to reduce the impact of these plant parasitic nematodes on crop production (Davies and Elling, 2015; Saucet et al., 2016). However, studies in crop plants show that resistance can break down in response to an increase in soil temperature (Dropkin, 1963; Holtzman.Ov, 1965; Dropkin, 1969; Griffin, 1969; Jatala and Russell, 1972; Carter, 1982; Mullin et al., 1991). In *Solanum lycopersicum* cultivars containing the *Mi-1* resistance gene, a breakdown in root knot nematode resistance in response to increased temperature occurs under in vitro conditions (Haroon et al., 1993), as well as in glasshouse and field production systems (Philis and Vakis, 1977; Tzortzakakis and Gowen, 1996; Noling, 2000). Further, a brief exposure of *S. lycopersicum* cultivars containing *Mi-1* to high temperature treatment is sufficient to temporality break root knot nematode resistance (de Carvalho et al., 2015). However, a subset of *S. peruvianum* accessions displayed heat-stable resistance when plants were infected with root knot nematodes at high temperature (Ammati et al., 1986; Veremis and Roberts, 1996, 1996). Genetic evidence supports the hypothesis that heat-stable resistance is mediated *R*-genes, including *Mi-9,* which is a homolog of *Mi-1* (Jablonska et al., 2007). As the genetic background influences the expressivity of *Mi-1* mediated root knot nematode resistance (Jacquet et al., 2005; Cortada et al., 2008), genetic modifiers may also play a role maintaining immunity at high soil temperatures (Verdejo-Lucas et al., 2013).

Root knot nematodes are a major pest to wine grape production, as damage caused by these pests reduces yield up to 20% (Nicol et al., 1999; Saucet et al., 2016). As domesticated wine grape cultivars derived from *V. vinifera* are highly susceptible to root knot nematode, rootstocks derived from North American *Vitis* species containing resistance determinants are used to manage production in soils infested with these pests (Walker and Stirling, 2008;

Saucet et al., 2016). As wine grapes are often grown in warm to hot Mediterranean environments (Walker and Stirling, 2008; Saucet et al., 2016), climate change is expected to increase soil temperatures, which may impact resistance mechanisms in rootstocks. The impact of increased soil temperatures on root knot nematode resistance was examined on UCD-GRN1-5 and Harmony rootstocks (Ferris et al., 2013). Results showed that a significant breakdown in root knot nematode resistance occurred in Harmony when soil temperatures were $\geq 30^0$C. In contrast to Harmony, little or no breakdown in root knot nematode resistance occurred in the GRN1-5 rootstocks at high temperature (Ferris et al., 2013).

To better understand the performance of commercial rootstocks in response to root knot nematode infection at high temperatures, we evaluated the impact of root knot nematode resistance at 32^0C in a subset of commercial rootstocks and accessions to identify material containing heat-stable determinants. Glasshouse and *in vitro* system screening systems were used to evaluate the stability or breakdown on root knot nematode resistance at 32^0C. Results from this study indicate that heat-liable resistance exists in current rootstocks and the screening systems developed in this project can be utilised to select for new rootstocks containing heat-stable determinants that maintain a high level of root knot nematode resistance in a changing climate.

6.7.1 Soil temperatures in a hot climate exceed 30^0C during the summer months

As reported by Ferris et al., 2013, soil temperatures at or greater than 30^0C, cause a significant breakdown in root knot nematode resistance in the Harmony rootstock. To extend this work, we asked whether soil temperatures are capable of reaching and/or exceeding 30^0C in a hot-climate viticulture region in Australia. In this analysis, daily soil temperature data at 20 cm were collected from the Loxton Weather Station at the Australian Government, Bureau of Meteorology and Agriculture Observations Bulletin for South Australia (http://www.bom.gov.au/products/IDS65176.shtml). At this soil depth, root knot nematodes are expected to live and infect the roots of susceptible domesticated grapevines and rootstocks (Stirling, 1976). Over a 37 day period from 30 December 2021 to 4 February 2022, the soil temperature exceeded 30^0C for 27 days (Figure 6.7.1). The highest temperature reached during this period was 33.1^0C on 2 February 2022. At the CSIRO Irymple Farm, the average soil temperature at 20 cm from 1 November 2021 to 1 February 2022 was 36.2^0C (=/- 1.2), which is well above 30^0C (Figure 6.7.2). During this period, the lowest and highest temperatures were 34.0^0C and 38.8^0C. Therefore, in hot-climate viticultural regions of Australia, summer time soil temperatures exceed 30^0C, which may impact root knot resistance in commercial rootstocks.

6.7.2 Breakdown of root knot nematode resistance at 32^0C in glasshouse conditions

In a glasshouse trial, a number of commercial rootstocks with *V. champinii* parentage, including Ramsey, Dogridge, Freedom, K51-40 and C114, displayed complete resistance, in which the roots of all three replicates were devoid of egg masses after infection with *M.*

javanica 'pt 1103P' at 24^{0}C (Table 6.7.1). In addition, Schwarzmann and *V. cinerea* C2-50 also showed complete resistance to *M. javanica* 'pt 1103P' due to absence of egg masses in all three replicates at 24^{0}C. A low level of susceptibility was shown in 101-14 Mgt at 24^{0}C after inoculation with *M. javanica* 'pt1103P', such that an average 1.3 egg masses per vine and 0.9 egg masses per total root weight (g) was detected on the roots of this rootstock (Table 6.7.1). At 24^{0}C, *M. javanica* 'pt 1103P' effectively reproduced on the roots of susceptible replicated vines derived from 140 Ruggeri, du Lot and *V. vinifera* 'Riesling' control (Table 6.7.1).

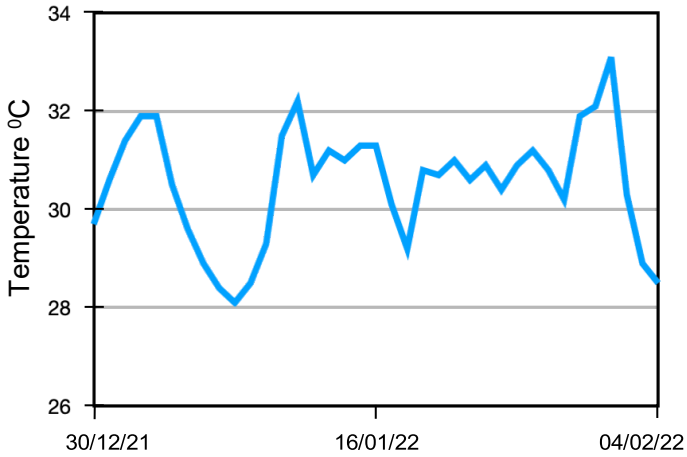


Figure 6.7.1. Soil temperature (^{0}C) at 20 cm at Loxton Weather Station, SA, from 30 December 2021 to 4 February 2022. Note: temperatures are measured beneath a maintained lawn.

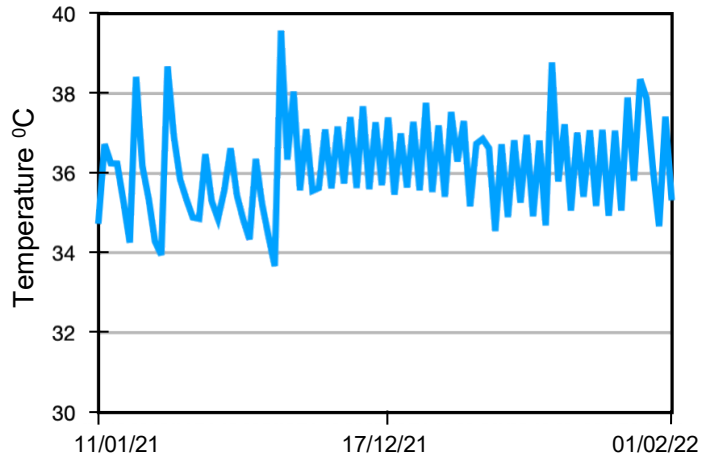


Figure 6.7.2. Soil temperature (^{0}C) at 20 cm at CSIRO Irymple Farm, VIC, from 01 November 2021 to 1 January 2022. Note: temperatures taken under drip irrigated vines

To evaluate the impact of increased temperatures on root knot nematode resistance, glasshouse screening was performed at 32^{0}C on material screened at 24^{0}C for comparison using *M. javanica* 'pt 1103P'. In this trial, all grapevine material was screened in quintuplicate. Under these conditions, only two rootstocks with *V. champinii* parentage, Dogridge and K51-

40, maintained complete resistance to *M. javanica* 'pt 1103P' in all five replicates at 32^{0}C (Table 6.7.2). A slight breakdown in *M. javanica* 'pt 1103P' resistance occurred at 32^{0}C in Ramsey, C114 and *V. cinerea* C2-50, in which an average 1.0, 0.4 and 3.0 egg masses per vine, respectively, were detected on the roots of these rootstocks (Table 6.7.2). In addition, an average 0.29, 0.04 and 1.1 egg masses per total root weight (g) was observed on the roots of Ramsey, C114 and *V. cinerea* C2-50, respectively. In contrast, a more substantial breakdown in *M. javanica* 'pt 1103P' resistance occurred at 32^{0}C for Schwarzmann and Freedom, in which an average 9.2 and 19.8 egg masses per vine, respectively, was identified on the root of these rootstocks (Table 6.7.2). Also, an average 2.08 and 3.89 number egg masses per total root weight (g) was detected on the roots of Schwarzmann and Freedom, respectively. The level of susceptibility in 101-14 Mgt increased from 24^{0}C to 32^{0}C, in which the average number of egg masses per vine increased from 1.3 to 31.0, respectively. At 32^{0}C, the average number of egg masses in Riesling, 140 Ruggeri and du Lot was >65, and egg masses per total root weight (g) ranged from 12 to 32.2 (Table 6.7.2). There was no apparent difference in susceptibility in Riesling and du Lot at 24^{0}C and 32^{0}C. Lastly, a slight increase in susceptibility was observed when the temperatures were increased from 24^{0}C to 32^{0}C for 140 Ruggeri (Table 6.7.2).

Table 6.7.1. Glasshouse evaluation of *M. javanica* 'pt 1103P' resistanct at 24^{0}C

Cultivar/accession	Mean Egg Mass/Vine	SD	Mean Egg Mass/g root	SD	*n*
Ramsey	0.0	0.0	0.0	0.0	3
Dogridge	0.0	0.0	0.0	0.0	3
Freedom	0.0	0.0	0.0	0.0	3
K51-40	0.0	0.0	0.0	0.0	3
C114	0.0	0.0	0.0	0.0	3
Vitis cinerea C2-50	0.0	0.0	0.0	0.0	3
Schwarzmann	0.0	0.0	0.0	0.0	3
101-14	1.3	1.5	0.7	0.8	3
140 Ruggeri	51.3	14.6	20.6	16.8	3
du Lot	79.0	29.3	3.0	1.4	3
Riesling	95.0	23.5	23.1	7.7	3

*1613-59 is derived from a cross between Couderc 1613 x Couderc 3306 (Garris et al., 2009).

6.7.3 Cell necrosis is associated with *M. javanica* 'pt 1103P' resistance

An in vitro screening system developed in grapevine showed that inoculation of *V. cinerea* C2-50 roots with *M. javanica* 'pt 1103P' induced cell necrosis in the root meristem and vascular initials, which appeared to trap and prevent feeding site establishment required for reproduction (Smith et al., 2018). If a similar localised pattern of cell/tissue necrosis occurred in the roots of commercial rootstocks infected with *M. javanica* 'pt 1103P' than the *in vitro* screening system could be utilised to evaluate how an increase in temperature alters the resistance response.

As a first step, we characterised the resistance response to *M. javanica* 'pt 1103P' infection at 27^{0}C, in the roots of the commercially available rootstocks. In addition, the resistance response to *M. javanica* 'pt 1103P' was also evaluated for *V. candicans* at the same temperature. Uninfected roots for *V. vinifera* 'Chardonnay' and commercial rootstocks including Ramsey, Dogridge, Freedom and Schwarzmann, as well as *V. candicans* were devoid of gall development, as well as cell/tissue necrosis. (Figure 6.7.3A, D, G, J, M and P). However, after inoculation of Chardonnay with *M. javanica* 'pt 1103P', galls developed on the roots without any visible signs of cell necrosis (Figure 6.7.3B). As galls continue to grow, they split open producing a mass of callus-like cells approximately 18 days after inoculation (Figure 6.7.3C). Thirty to forty days after inoculation, juvenile nematodes appeared in the media and infected roots causing new galls to develop. As the proliferative callus-like protrusions from galls produced in Chardonnay served as an indicator of susceptibility, we scored these structures during the course of the trial (see below). Inoculation of *V. champinii* parentage rootstocks, Ramsey, Dogridge and Freedom, with *M. javanica* 'pt 1103P' induced cell necrosis in the root meristem and vasculature initials, as well as cortex cells (Figure 6.7.3E, F, H, I, K, and L). After inoculating the roots of *V. candicans* and Schwarzmann with *M. javanica* 'pt 1103P', cell necrosis was also induced in the root meristem and vascular initials, as well as the cortex cells (Figure 6.7.3N, O, Q and R).

Table 6.7.2. Glasshouse evaluation of *M. javanica* 'pt 1103P' resistance at 32^{0}C

Cultivar/accession	Mean Egg Mass/Vine	SD	Mean Egg Mass/g root	SD	*n*
Ramsey	1.0	2.2	0.6	1.4	5
Dogridge	0.0	0.0	0.0	0.0	5
Freedom	19.8	10.8	4.4	1.9	5
K51-40	0.0	0.0	0.0	0.0	5
C114	0.4	0.9	0.0	0.1	5
Vitis cinerea C2-50	3.0	2.9	1.4	1.9	5
Schwarzmann	9.2	9.2	1.9	1.9	5
101-14	31.0	25.8	37.9	72.2	5
140 Ruggeri	82.6	27.1	41.7	32.2	5
du Lot	65.8	10.5	24.6	17.4	5
Riesling	85.6	13.2	25.2	12.0	5

*1613-59 is derived from a cross between Couderc 1613 x Couderc 3306 (Garris et al., 2009).

The response of rootstocks and accessions to *M. javanica* 'pt 1103P' was quantified to better characterise the resistant and susceptible phenotypes using the *in vitro* screening system. In this analysis, we scored the number of cell/tissue necrosis responses associated with *M. javanica* 'pt 1103P' resistance, as well as susceptibility determinants, galls and callus-like protrusions, in three replicates for each commercial rootstock and *Vitis* accession at 27^{0}C. Results showed complete resistance involving the induction of cell/tissue necrosis with no gall development occurred in Ramsey, Dogridge, K51-40, *V cinerea* C2-50 and *V. candicans* (Table

6.7.3). While Freedom, C114 and Schwarzmann produced a relatively high level of cell/tissue necrosis in response to *M. javanica* 'pt 1103P' infection, a limited level of gall development occurred on the roots of these rootstocks (Table 6.7.3). However, these galls did not progress to produce callus-like protrusions. The roots of 101-14 Mgt and 1613 Couderc displayed a low-level of gall development and callus-like protrusions (Table 6.7.3). In Riparia Gloire and Chardonnay, a substantial number of galls and callus-like protrusions developed on the roots of these cultivars in response to *M. javanica* 'pt 1103P' infection, with no visible signs of cell/tissue necrosis (Table 6.7.3).

6.7.4 Impact of resistance and susceptibility response to *M. javanica* 'pt 1103P' infection at 32^0C

As *M. javanica* 'pt 1103P' resistance is associated with the induction of localised cell necrosis, the next step in our analysis was to evaluate how temperature impacts this resistance response in Ramsey, Dogridge, Freedom, *V. candicans* and Schwarzmann. In addition, we also included 101-14 Mgt and Couderc 1613 in this analysis to determine how temperature affects susceptibility. In response to an increase in temperature from 27^0C to 32^0C, the induction of cell/tissue necrosis was accompanied with gall formation followed by the development of callus-like protrusions, albeit at relatively low levels in Ramsey, Dogridge, K51-40, C114, *V. cinerea* C2-50 and *V. candicans* (Table 6.7.4). In response to *M. javanica* '1103P' infection at 32^0C, the average number of callus-like protrusions per vine and per total root weight (g) did not exceed 10 and 50, in these rootstocks and *Vitis* accessions. In contrast, a higher level of resistance breakdown occurred in Freedom and Schwarzman, in which the average number of callus-like protrusions per vine increased to from 22.5 and 23.5, respectively, at 32^0C (Table 6.7.4). In addition, the average number of callus-like protrusions per root weight (g) was 102.3 and 117.5 for Freedom and Schwarzmann, respectively. An increase in susceptibility occurred in 101-14 Mgt and Couderc 1613 at 32^0C compared to 27^0C in response to *M. javanica* 'pt 1103P', such that the average number of callus-like protrusions per vine increased to 24.5 and 26.0, respectively (Table 6.7.4). In addition, the average number of callus-like protrusions per total root weight (g) also increased to 111.4 and 165.7 in 101-14 Mgt and Couderc 1613, respectively. In both Riparia Gloire and Chardonnay, the average number of callus-like protrusions per vine and total per root weight (g) increased when infections were performed at 32^0C compared to 27^0C (Table 6.7.4).

In response to *M. javanica* 'pt 1103P' infection, the apparent breakdown in resistance at 32^0C was further evaluated in Freedom and Schwarzmann. At 32^0C, the morphology of Freedom

and Schwarzmann roots were similar when these rootstocks were grown at 27^{0}C (Figure 6.7.4A and E). Further, in the absence of the *M. javanica* 'pt 1103P', the roots of Freedom and Schwarzmann lacked galls, callus-like protrusions and localised cell necrosis at 32^{0}C. After *M. javanica* 'pt 1103P' infection, a subset of roots from Freedom and Schwarzmann initiated cell/tissue necrosis in the root meristem and vascular initials (Figure 6.7.4B and F). In the other set of infected roots, gall development was initiated in the roots of Freedom and Schwarzmann, often with little or no cell necrosis (Figure 6.7.4C and G). In Freedom and Schwarzmann, as galls grew in size, proliferative callus-like protrusions formed (Figure 6.7.4D and H). Thirty to forty days post-infection, free-living juvenile *M. javanica* 'pt 1103P' nematodes appeared in the media and a new cycle of infection occurred.

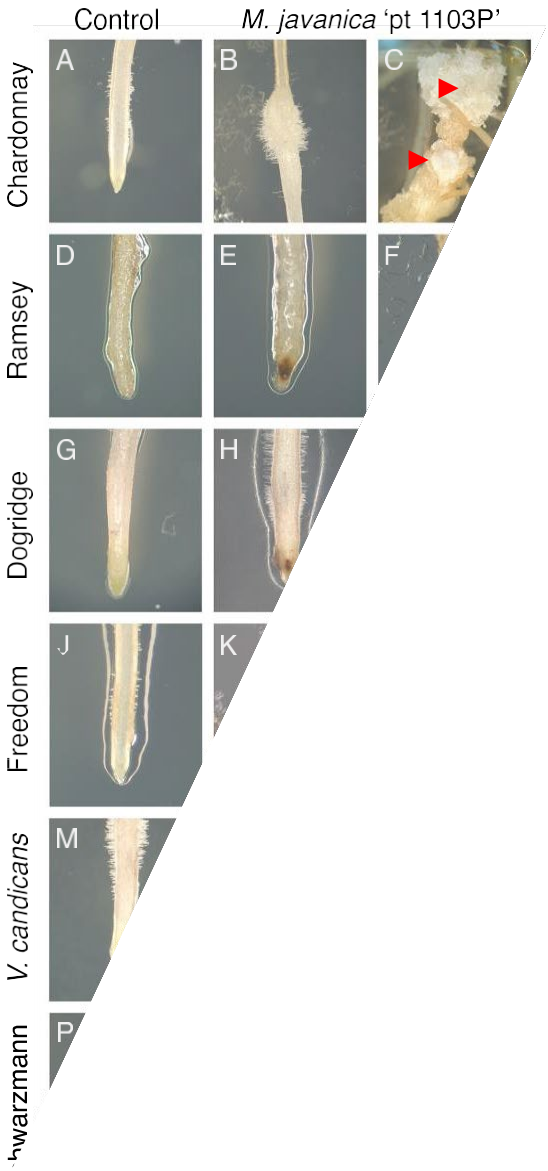


Figure 6.7.3 Resistance responses in root knot nematode resistant rootstocks.

(A, D, G, J, M and P). Morphology and appearance of roots in the absence of *M. javanica* 'pt 1103'. Infection of Chardonnay roots results in the formation of (B) galls, which eventually split open producing (C) callus-like protrusions. (E, f, H, I, K, L, N, O, Q and R) In response to *M. javanica* 'pt 1103P' infection, localised cell necrosis occurs in tissues of the roots.

6.7.5 Discussion

In a changing climate, the intensity and duration of heatwaves are predicted to increase (IPCC, 2022). Understanding how temperature affects root knot nematode resistance will provide a basis for breeding new rootstocks in a changing climate. Daily soil temperatures obtained from Loxton in the Riverland likely reflect the hot, dry conditions experienced in the Murray-Darling basin, the major grape production region Australia (Walker and Stirling, 2008). Here, we demonstrate that soil temperatures reach and exceed 30^0C for the majority of days during the peak of summer in the Riverland, SA in the 2021/22 season. If climate change increases the duration of days above 30^0C throughout the summer in the Riverland and Murray-Darling basin, this could have a significant impact on resistance mechanisms that function to prevent root knot nematode infection in existing rootstocks, particularly with Freedom and Schwarzmann.

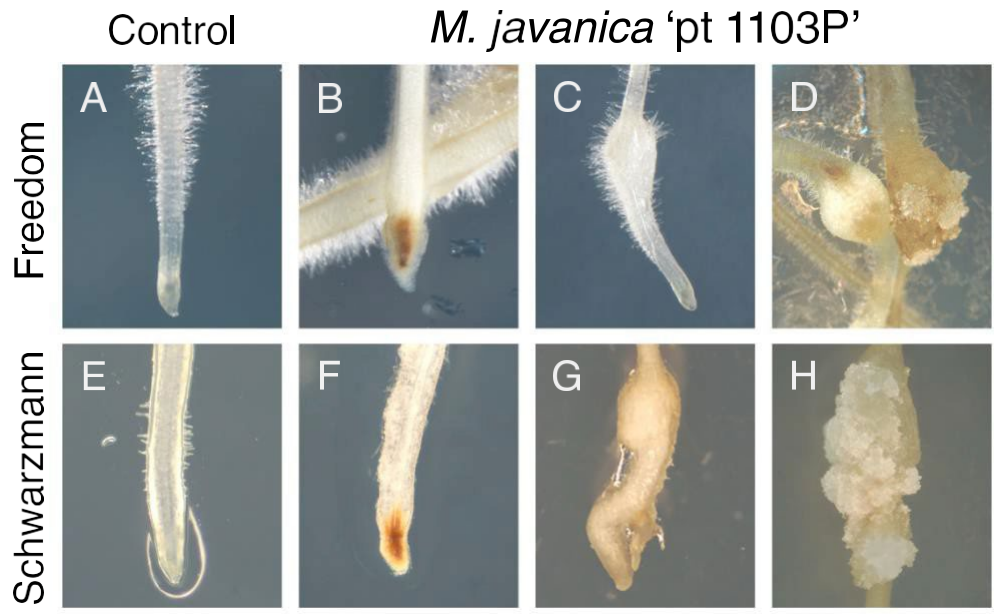


Figure 6.7.4. Breakdown of root knot nematode resistance in Freedom and Schwarzmann at 32^0C. (A and E) Uninfected *M. javanica* 'pt 1103P' roots of Freedom and Schwarzmann at 32^0C. (B and F) Localised cell necrosis in response to *M. javanica* 'pt 1103P' infection in the root tips of Freedom and Schwarzmann at 32^0C. (C and D) in response to *M. javanica* 'pt 1103P' infection, (C and G) galls and (D and H) callus-like protrusions developed on the roots of Freedom and Schwarzmann at 32^0C.

In this study, we utilised a glasshouse trial and an *in vitro* system to evaluate the impact of *M. javanica* 'pt 1103P' resistance in commercial rootstocks and accessions targeted for breeding. Data derived from glasshouse and in vitro screening are highly comparable indicating that the *in vitro* system can be used as a tool to select new rootstocks with heat-stable *M. javanica* 'pt 1103P' resistance. This system could also be adapted to better understand the impact of high temperature and resistance to other pests, including grape phylloxera.

Based on the results from our study, *M. javanica* 'pt 1103P' resistance displayed in commercial rootstocks involves the induction of cell necrosis and programmed cell death in different tissues of the roots. In *V. cinerea* C2-50, cell necrosis was induced primarily in the root meristem and vascular initials of infected roots (Smith et al., 2018). In response to *M. javanica* 'pt 1103P' infection, Dogridge and Ramsey, two *V. x champinii* rootstocks, initiated cell necrosis in the cortex and root meristem of the roots. The difference in cell necrotic patterns induced in the cortex of *V. cinerea* C2-50 and *V. x champinii* rootstocks suggest that the mechanism of resistance may not be conserved. Therefore, combining resistance from *V. cinerea* C2-50 with *V. x champinii* will provide a pathway to breed for durable root knot nematode resistance.

Dogridge is the parent of K51-40 (Smith et al., 2016), while Ramsey is a parent of C114 (P. Clingeleffer, personal communication). In addition, Dogridge is one of the grandparents of Freedom (Garris et al., 2009). If *M. javanica* 'pt 1103P' resistance is inherited from the *V. x champinii* rootstocks, it is interesting to point out that resistance breakdown in Freedom is more severe than in Ramsey, Dogridge, K51-40 and C114. According to the hypothesis above together with the resistance ratings, suggests that the genetic background may influence the stability of resistance at high temperature.

Sunraysia and hot-climate

In January 2022, the soil temperature at 20 cm in Loxton was $\geq 30^0$C for 24 days and the highest temperature recorded during this period was 33.1^0C.

Heatwaves and stabilisation of temperatures

Under these conditions and based on our research, a temporary breakdown in resistance is expected to occur in Freedom and Schwarzmann. In addition, the low susceptibility displayed in 101-14 Mgt and Couderc 1613 is expected to increase. In a changing climate, a major selection criterion for selecting root knot nematode resistance rootstocks should include whether resistance can be maintained at higher temperatures.

In our study, we examined how an increase in temperature impacts resistance in five rootstocks with *V. champinii* parentage including Ramsey, Dogridge, K51-40, MG 60-114 and Freedom. In K51-40, MG 60-114 and Freedom, the resistance displayed in these rootstocks is likely derived from the *MJR2* locus in *V. champinii* (Cousins and Walker, 2002) (Henderson, Dunlevy and Smith, unpublished). While Ramsey, Dogridge and K51-40 display a slight breakdown in *M. javanica* 'pt 1103P' resistance, Freedom displays a considerable breakdown

in resistance.

If *M. javanica* ‘pt 1103P’ resistance in these rootstocks is mediated by *MJR2,* then the stability of the immune response to this nematode pest may be controlled by the genetic background of the rootstock. Therefore, understanding the genetic modifiers that alter the function of root knot nematode resistance genes to maintain an effective immune response at a higher temperature would be of considerable value for the development of new durable resistant rootstocks for a changing climate.

Table 6.7.3: Resistance and susceptibility responses to *M. javanica* 'pt 1103P' infection at 27^{0}C

Cultivar/accession	Mean NR (MNRs)	SD	Mean Galls (MG)	SD	Mean CLP (MCLP)	SD	Mean RW (MRW)	SD	MNR/MRW	SD	MG/MRW	SD	MCLP/MRW	SD
Ramsey	23.0	5.6	0.0	0.0	0.0	0.0	0.30	0.10	83.8	32.8	0.0	0.0	0.0	0.0
Dogridge	29.0	16.2	0.0	0.0	0.0	0.0	0.23	0.03	124.0	59.1	0.0	0.0	0.0	0.0
Freedom	53.5	15.5	2.8	2.5	0.0	0.0	0.47	0.08	112.3	19.7	6.6	6.9	0.0	0.0
K51-40	18.8	6.9	0.0	0.0	0.0	0.0	0.19	0.02	96.9	23.4	0.0	0.0	0.0	0.0
C114	36.5	9.0	1.0	1.2	0.0	0.0	0.38	0.23	116.7	50.7	3.2	3.8	0.0	0.0
Vitis cinerea C2-50	16.5	5.1	0.0	0.0	0.0	0.0	0.30	0.09	57.4	16.2	0.0	0.0	0.0	0.0
V candicans	30.8	13.5	0.0	0.0	0.0	0.0	0.41	0.20	88.7	45.3	0.0	0.0	0.0	0.0
Schwarzman	20.3	3.7	2.5	2.1	0.0	0.0	0.19	0.05	112.9	25.1	12.3	8.6	0.0	0.0
101-14 Mgt	10.5	4.0	20.3	4.9	8.3	3.9	0.22	0.09	48.9	3.4	99.0	19.6	39.0	10.6
Couderc 1613	9.5	1.3	13.3	4.3	10.3	1.7	0.13	0.02	72.4	9.3	105.6	50.6	80.5	25.5
Gloire de Montpellier	0.0	0.0	16.3	9.1	15.5	7.1	0.19	0.09	0.0	0.0	81.1	14.2	86.8	30.5
Chardonnay	0.0	0.0	22.3	5.4	38.5	11.7	0.22	0.05	0.0	0.0	102.0	13.8	175.2	34.1

*1613-59 is derived from a cross between Couderc 1613 x Couderc 3306 (Garris et al., 2009).

NR, necrotic responses; CLP, callus-like protrusions; RW, root weight (g); *n* =4

Table 6.7.4: Resistance and susceptibility responses to *M. javanica* 'pt 1103P' infection at 32^{0}C

Cultivar/accession	Mean NR (MNRs)	SD	Mean Galls (MG)	SD	Mean CLP (MCLP)	SD	Mean RW (MRW)	SD	MNR/MRW	SD	MG/MRW	S
Ramsey	25.0	7.0	2.0	1.8	2.8	1.8	0.25	0.12	106.6	23.7	7	
Dogridge	37.8	7.9	5.3	3.7	9.8	2.5	0.23	0.03	161.5	14		
Freedom	13.0	5.0	12.0	2.2	22.5	4.4	0.22	0.09	59.			
K51-40	32.0	4.7	3.0	2.3	5.5	5.1	0.42	0.33				
C114	34.5	3.5	5.3	4.7	7.0	5.0	0.36					
Vitis cinerea C2-50	22.5	2.1	3.0	2.9	4.3	3.0						
V candicans	23.0	7.9	8.3	7.1	4.5							
Schwarzman	18.3	3.0	14.8	4.3								
101-14 Mgt	20.8	3.9	18.8									
Couderc 1613	0.0	0.0										
Gloire de Montpellier	0.0											
Chardonnay	0											

*1613-59 is derived fr

NR, necrot

7. Outcomes & Conclusions

The project addressed objectives involving the evaluation of new rootstocks and advanced breeding material, which included genotype x environment and scion x rootstock interactions, all of which will likely provide significant benefits to both grapegrowers, winemakers and the environment. In addition, the project made a significant step toward the development of new durable grape phylloxera and root knot nematode resistance rootstocks for a changing climate in Australia.

Submission of Plant Breeders Rights applications for four CSIRO rootstocks

Plant Breeders Rights (PBR) provides legal protection for the release of new varieties. Four new rootstocks, C113, C114, C20 and C7 were prepared for PBR, with the near completion of Stage 1. For Stage 2, the Distinctness, Uniformity and Stability (DUS) trials have been established and all ampelographic data have been collected and analysed. Upon approval of the Stage 1 application, which is expected to occur in December 2022, Stage 2 will be submitted for rootstock release.

C113 and C114 are two high vigour rootstocks with the potential to replace 1103 Paulsen and Ramsey, respectively. With the ability of these rootstocks to exclude chloride from leaves and berries, their ease of propagation and graft compatibility, C113 and C114 have the potential to also replace 140 Ruggeri, a chloride excluder that is difficult to propagate. C113 and C114 also provide resistance to root knot nematode, have strong performance with major wine cultivars and are able to promote fruit maturity. In addition, C114 maintains productivity under sustained deficit irrigation compared to other high vigour rootstocks and is drought tolerant.

C20 is a rootstock with reduced vigour potential, high water use efficiency, and produces yields similar 1103 Paulsen or 140 Ruggeri. Fruit composition was enhanced in C20 due in part to lower juice pH and malic acid, as well as higher accumulation of berry anthocyanins, compared to 1103 Paulsen and 140 Ruggeri. It provides resistance to one species population of root knot nematode and G4 grape phylloxera. In contrast to 101-14 Mgt, a rootstock with reduced vigour potential, C20 is drought and salinity tolerant.

C7 is a rootstock with a relatively high vigour potential with production and fruit quality traits similar to 1103 Paulsen and 140 Ruggeri. C7 was selected based on its capacity to maintain acceptable productivity when supplied with saline irrigation water and under deficit irrigation in long-term trials. In addition, C7 provides resistance to one species population of root knot nematode and G4 grape phylloxera.

Addressing the concept of a universal rootstock ideotype

In the rootstock evaluation component of the project, rootstock x scion interactions were evaluated using seven rootstocks grafted with Shiraz, Chardonnay and Cabernet Sauvignon. Of the seven rootstocks, 1103 Paulsen and CSIRO rootstocks, C113, C114 and C20, were used in this study. The significance and conclusion of this activity shows that scion performance

and fruit composition are influenced by the rootstock cultivar. In addition, results indicate that rootstock performance is influenced by the environment. Therefore, developing a universal rootstock ideotype is an unrealistic goal and growers must consider rootstock x scion x environmental interactions when selecting a rootstock. In addition to the trials performed at Irymple and Padthaway, regional trials will further identify rootstock ideotypes for specific scions and growing regions.

Impact of rootstocks on growth, yield, and quality traits under different irrigation and salinity conditions

Results from deficit irrigation showed that the impact of rootstocks on vigour and yield-associated traits were stable over time, despite significant effects of season on these traits. The influence of rootstocks on vigour is linked to yield, bunch weight, berry weight and berries per bunch, as well as fruit composition traits. Further, yield is a function of both bunch number and bunch weight. While deficit irrigation had a minimal effect on yield, a small but significant increase in bunch number and a decrease in bunch weight, berry weight, berries per bunch and pruning weight, which were associated with an increased TSS, pH, tartaric acid, anthocyanins and phenolics and a reduced TA and malic acid. No direct links were observed between pruning weight, as well as wine must, composition or spectral properties following addition of tartaric acid for pH adjustment. Across the rootstock genotypes, bunch and berry weight were more important drivers of juice and wine composition than yield and bunch number. Further, the study confirmed that juice pH across the rootstocks was positively correlated with malic acid, YAN and K and that malic acid was positively correlated with YAN and K in both irrigation treatments. The addition of tartaric acid for pH adjustment during winemaking processes had a significant impact on wine composition and spectral properties. The requirement for pH adjustment was a very useful criterion in the final selection of new rootstocks.

In the Padthaway trial, rootstocks were selected based on a limited supply of moderately saline water. Results from this trial confirmed that rootstock-conferred vigour is linked to yield, as well as bunch and berry weight of the rootstocks. Under this water management practice, berry weight was a major driver of fruit composition across the rootstocks being negatively correlated with tartaric acid, anthocyanin and phenolics concentration. Further, juice pH across the rootstocks was positively correlated with malic acid, K and Mg concentration. The use of small-scale winemaking techniques in the evaluation of rootstock genotypes was compromised by differences in fruit maturity across the rootstocks and between seasons, as must TSS was linked to must pH, tartaric acid addition for pH adjustment, final wine pH (negative), % alcohol and wine spectral properties.

The above field trials were performed with F_1 individuals derived from a *V. cinerea* C2-50 x Schwarzmann cross and illustrate that the C2-50 parent likely contains key traits for managing vines under reduced irrigation and/or in saline soils, which is expected to occur in a changing climate. Thus, the selection of water use efficient and salinity-tolerant next generation rootstocks will be a priority, given the potential for reduced water availability in the future.

New Sources of pest resistance

New rootstock germplasm was evaluated for grape phylloxera and root knot nematode resistance. Results from the G4 grape phylloxera screening suggest that material with *M. rotundifolia* parentage (MS 27-12 & MG 17-10), as well as a *V. aestivalis* accession and *V. riparia x V. monticola* hybrid, are new potential sources of resistance. As the *V. aestivalis* accession is difficult to propagate, crosses should be performed with rootstock germplasm that readily produces roots from dormant cuttings. New germplasm with root knot nematode resistance was also identified, including *V. candicans* and Ramsey (*V. x champinii*) and Ramsey x MS 27-12. This material has been targeted for future development of second-generation rootstocks. Moreover, crosses between new *Vitis* sources of grape phylloxera and root knot nematode resistance would further increase the genetic diversity and value of the rootstock germplasm for future breeding.

Identification of DNA markers linked to *MJR2* from *V. champinii*

To develop second-generation rootstocks with durable resistance to root knot nematode, we identified DNA-markers linked to *MJR2* from *V. champinii*, which will be combined with *MJR1* to develop second-generation durable resistant rootstocks. We showed that the *MJR2* locus mapped to the distal end of chromosome 18, using three F_1 mapping populations: (1) Ramsey x Riesling, (2) Dogridge x Riesling, and (3) K51-40 x 140 Ruggeri. Based on results from the *in vitro* screening system, *MJR2* elicits an immune response in the cortex and root meristem. In contrast, *MJR1* elicits an immune response in the root meristem and vascular initials (Smith et al., 2018; CSP1304, Clingeleffer and Smith 2018), indicating that the resistance mechanism(s) mediated by *MJR1* and *MJR2* is likely different. We showed that *MJR2* is able to detect and mount resistance to the three *Meloidogyne* species populations: (1) *M. javanica* 'pt-1103P', (2) *M. arenaria* 'Barossa' and (3) *M. incognita* 'SARDI'. Therefore, these results suggest that *MJR2* provides broad spectrum resistance to the three major *Meloidogyne* species that parasitise grapevines. As *MJR1* and *MJR2* localise to a similar region at the distal end of chromosome 18, it indicates that this region is a 'hot-spot' for root knot nematode resistance, which is similar to the chromosomal localisation of *Mi*-root knot nematode resistance genes in *Solanum lycopersicum* (El-Sappah et al., 2019).

Development of next generation durable pest resistant rootstocks

A breeding scheme was developed to lay the groundwork for developing next generation rootstocks with durable resistance to grape phylloxera and root knot nematode. To achieve this breeding objective, *V. cinerea* C2-50 was crossed with Börner to stack *RDV2*, *RDV38* and *MJR1* with *RDV1*, respectively. After marker-assisted screening of 300 seedlings, 23 first-generation rootstocks containing *RDV1, RDV2*, and *MJR1*. Preliminary results suggest that 16

of the 23 first-generation rootstocks contain *RDV1, RDV2*, *RDV38* and *MJR1*. This will need to be validated through the genetic mapping and identification of molecular markers linked to *RDV38* (see below). In the next phase of this project, *V. champinii* material will be crossed with first-generation rootstocks to combine *RDV1*, *RDV2*, *RDV38* and *MJR1* with *MJR2*. Molecular markers linked to each of these loci will be used to select for second-generation rootstocks, which will be further screened for heat-stable root knot nematode resistance and chloride exclusion. In addition, it is essential to determine the specificity of *RDV1*, *RDV2* and *RDV38* to the representative grape phylloxera genotypes maintained at AgVIC, as this will help growers to identify appropriate rootstocks to safeguard vineyards from the diverse populations of grape phylloxera found in Australia. After the screening is completed and a final set of rootstock selections is identified, nursery and field trial evaluations will be performed to identify high performing rootstocks adapted to Australian conditions with potential for release.

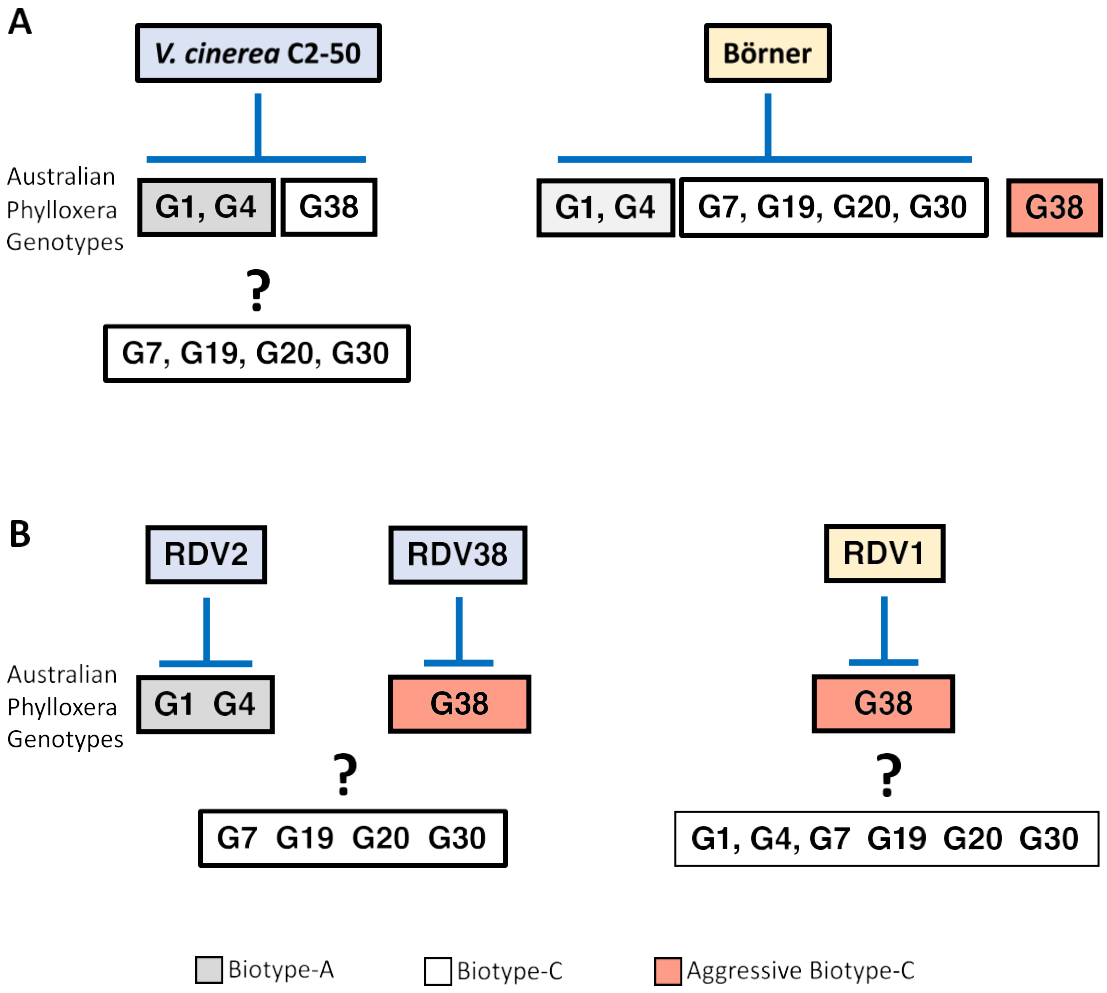


Figure 7.1.1: Complexities of grape phylloxera resistance in grapevine.
(A) *V. cinerea* C2-50 provides resistance to G1, G4 and G38, while Börner is resistant to G1, G4, G7, G19, G20, G30. However, Börner is susceptible to G38. At this time, it is uncertain whether *V.*

cinerea C2-50 is resistant to G7, G19, G20 and G30. (B) While *RDV2* mediates resistance to G1 and G4, *RDV38* provides immunity to the aggressive G38 genotype. While *RDV38* is specific for G38, future studies must address whether RDV2 and/or RDV38 provide resistant to other biotype-C genotypes, including G7, G19, G20 and G30. Research derived from this project showed that *RDV1* mediates resistance to G38. Whether *RDV1* and/or other *RDV* genes provide resistance to other grape phylloxera genotypes has yet to be determined. Lastly, preliminary results from this project indicate that Börner contains a susceptibility determinant that blocks *RDV1* mediated resistance to G38.

A G38 grape phylloxera specific resistance determinant identified in *V. cinerea* C2-50

A subset of F_1 *V. cinerea* C2-50 x Börner individuals were screened for resistance to the aggressive G38 grape phylloxera. Results showed that *RDV1* provides resistance to G38, while *RDV2* does not (Figure 7.1.1). In addition, preliminary results indicate that *V. cinerea* C2-50 contains a G38 specific resistance locus, *RDV38* (Figure 7.1.1). Using genotype data, preliminary results suggest that *RDV38* maps to chromosome 13. Additional genetic mapping studies will help resolve the chromosomal location of the *RDV38* locus and identify molecular-markers for marker-assisted selection of second-generation durable pest resistant rootstocks.

A G38 grape phylloxera-specific susceptibility factor identified in Börner

Börner contains the first grape phylloxera resistance locus identified, referred to as *RDV1*. Our results showed that Börner is susceptible to G38 grape phylloxera (Figure 7.1.1). However, F_1 *V. cinerea* C2-50 x Börner individuals harbouring *RDV1* were resistant to G38 grape phylloxera (Figure 7.1.1). Based on these results, we propose that Börner is homozygous for a recessive allele that acts to block *RDV1* mediated resistance to G38 grape phylloxera. Understanding the genetic nature of this 'susceptibility factor' will help to design further durable grape phylloxera resistance rootstocks for the future.

Heat-stable resistance is an essential selection criterion for next generation rootstocks

In a changing climate, heat-stable resistance to root knot nematode is a critical selection criterion for developing durable resistant rootstocks. Results from this project showed that a high level of resistance is maintained at 32^0C in Ramsey, Dogridge, K51-40, *V. cinerea* C2-50 and *V. candicans*. In contrast, a substantial breakdown in root knot nematode resistance occurred in Freedom and Schwarzmann at 32^0C. We propose that genetic background is an important factor for determining heat-stable resistance. Therefore, implementing the *in vitro* screening system in the selection of second-generation rootstocks will be an important step for selecting heat-stable root knot nematode resistance for a changing climate.

8. Recommendations

1. Ensure that nurseries have access to the four newly released rootstocks, C113, C114, C20 and C7, in order to provide the wine grape industry with access for uptake and adoption.

2. Continue breeding next generation rootstocks with durable grape phylloxera and root knot nematode resistance

5-year recommendation

A. Identify molecular markers linked to the G38 grape phylloxera specific resistance locus, *RDV38*, in *V. cinerea* C2-50.
B. Cross first-generation rootstocks to *V. champinii* material containing *MJR2*, including Ramsey and C114 (note: as C114 is a salinity tolerant rootstock with high chloride exclusion, the cross will enable the team to also select for chloride exclusion).
C. Select for second-generation rootstocks containing *RDV1*, *RDV2*, *RDV38*, *MJR1* and *MJR2*.
D. Address the genetics of the Börner G38 grape phylloxera susceptibility factor
E. Identify molecular markers linked to the third root knot nematode resistance locus, *MJR3*, in *V. candicans*.
F. Cross first-generation rootstocks to *V. candicans* and select for third-generation rootstocks containing *RDV1*, *RDV2*, *RDV38*, *MJR1* and *MJR3.*

10-year recommendation for second- and third-generation rootstocks

A. Establish rootstock plantings at the CSIRO Irymple Farm and SARDI Research Station in Nuriootpa for vine multiplication.
B. Select for heat-stable root knot nematode resistance and chloride exclusion.
C. Perform phylloxera testing in collaboration with AgVIC to determine the specificity of grape phylloxera resistance for *RDV1*, *RDV2* and *RDV38*.
D. Establish nursery trials to evaluate rooting ability and graft compatibility with at least two major wine cultivars.
E. Establish field trials with at least two major winegrape cultivars to identify high performing rootstocks with the following traits:
    a. A desirable range of vigour potential
    b. Good vine balance
    c. Optimal yield-associated traits
    d. Favourable berry and wine quality
F. Build off of the deficit irrigation trials in this project, to identify second- and third-generation rootstocks that perform well under reduced irrigation.
G. Identify the genetic determinant(s) that block *RDV1*-mediated resistance to G38 grape phylloxera in Börner.

9. Communication

The project actively engaged with the national and international grapevine research community and industry to disseminate scientific and practical knowledge on the progress, research findings and benefits of pest resistant rootstock in viticulture.

International Conferences/Meetings

1. International Grapevine Phylloxera and Nematode Conference at UC Davis, California in August 2017. Developing molecular markers for phylloxera and root knot nematode resistance for breeding new rootstocks for Australia conditions.

2. GiESCO presentation, Argentina, November 2017.Giesco Clingeleffer, PR., Morales, NB and Smith, H. (2017). Simple trait measurements across rootstock genotypes indicates performance as field grown, grafted vines. In proceedings 20th GiESCO International Meeting, Mendoza, Argentina, 5 -17 November 2017, 240-245.

3. GiESCO presentation, France, July 2019. Clingeleffer, P., Morales, N., Davis, H. and Smith, H. (2019). The significance of scion x rootstock interactions. Oeno One, 2019, 2. 335-346.

National Conferences/Meetings

1. CRUSH at Waite Campus, September 2018. Breeding new rootstocks with resistance to root knot nematode and phylloxera for Australia conditions.

2. Roots and Rhizosphere Symposium at University of Adelaide, April 2018. Breeding new rootstocks for Australia with resistance to root knot nematode and grape phylloxera.

3. 17th Australian Wine Industry Technical Conference, July 2019. Developing next generation rootstocks with long-term resistance to root knot nematode and phylloxera.

Industry Forum Presentations

1. Rootstock Development for the Australian Wine industry. Yarra Valley, 4 August 2017. (Yarra Valley Rootstock and Clone Workshop, with tasting)

2. AWRI Road Show 2018. Rootstock Development for the Australian Wine Industry. Mornington 28 November.

3. AWRI Road Show 2019. Rootstock Development for the Australian Wine Industry. Yarra Valley 29 January 2019 and Mornington 30th January

4. AWRI Road Show 2019. Pre-season Pest & Disease Workshop. Piangil 1 August and Cardross 2 August. Developing next generation rootstocks with long-term resistance to root knot nematode and phylloxera.

5. Phylloxera Immersion Tour in Yarra Valley, 26-27 November, 2019. Developing next generation rootstocks with long-term resistance to root knot nematode and phylloxera.

6. AWRI Road Show 22 October, 2020. Langhorne Creek & Adelaide Hills Seminar. Breeding next generation rootstocks with durable resistance to phylloxera and root knot nematode.

7. VineHealth Board Meeting, 7 December, 2021. CSA 1701-Rootstock genetics and improvement – New improved rootstocks with durable pest resistance.

8. Australian Grape & Wine Committee, 2 February 2022. Grape phylloxera – rootstock interactions and development of new durable resistant rootstocks for Australian conditions.

9. AWITC Rootstock workshop 28 June, 2022. Update: Development and release of new Australian Rootstocks.

Technical Publications:

1. Pat Iocco-Corena, Jamila Chaíb, Laurent Torregrosa, Don Mackenzie, Mark R. Thomas, Harley M. Smith (2021). *VviPLATZ1* is a major factor that controls female flower morphology determination in grapevine. *Nature Communications* 12: 6995. Note: this was a collaborative project with the former scion breeding team leader, Mark Thomas, aimed at developing a gene editing tool for scion and rootstock breeding.

2. Ian B. Dry, Christopher Davies, Jake Dunlevy, Harley Smith, Mark R. Thomas, Amanda Walker, Rob Walker and Peter Clingeleffer (2022). Development of new wine-, dried- and table-grape scions and rootstocks for Australian viticulture: past, present and future. *Australian Journal of Grape and Wine Research* 28, 177-195.

3. Jake Dunlevy, Peter Clingeleffer, Harley Smith (2019). Breeding next generation rootstocks with durable pest resistance using DNA marker-assisted selection. *Wine & Viticulture Journal* 34, 40-45.

4. Peter Clingeleffer, Norma Morales, Hilary Davis, Harley Smith (2019). The significance of scion x rootstock interactions. *OENO One* 53, 2.

5. Harley M. Smith, Catherine W. Clarke, Brady P. Smith, Bernadette M. Carmody, Mark T. Thomas, Peter R. Clingeleffer, Kevin S. Powell (2018). Genetic identification of SNP markers linked to a new grape phylloxera resistant locus in *Vitis cinerea* for marker-assisted selection. *BMC Plant Biology* 18, 360.

6. Harley M. Smith, Brady P. Smith, Norma B. Morales, Sam Moskwa, Peter R. Clingeleffer, Mark R. Thomas (2018). SNP markers tightly linked to root knot nematode resistance in grapevine (*Vitis cinerea*) identified by a genotyping-by-sequencing approach followed by Sequenom MassARRAY validation. *PLoS ONE* 13(2): e0193121.

Conference Proceedings

1. Peter Clingeleffer, Norma Morales, Hilary Davis, Harley Smith (2019) The myth of the universal rootstock revisited: Assessment of the importance of interactions between scion and rootstock. In: Proceedings of 21st GiESCO Conference.

2. Harley M. Smith, Jake Dunlevy, Norma B. Morales, Amy A. Smith, Peter Clingeleffer (2020) Developing next generation rootstocks with long-term resistance to phylloxera and root knot nematode. In: Proceedings of the 17th Australian Wine Industry Technical Conference.

10. Intellectual Property

The intellectual property (IP) arising from this project includes the four rootstocks undergoing Plant Breeders Rights assessment, namely, C113, C114, C20 and C7. Background IP (BIP) associated with this project includes family 86 selections used in the water deficit trial, *V. cinerea* C2-50, first-generation rootstocks, and new *Vitis* germplasm screened for root knot nematode and G4 grape phylloxera resistance. These were provided for use in the project, but they (and any derived new rootstocks) will not be licensed to the Commonwealth (DAFF) beyond the project on a perpetual, royalty-free basis for further use and adaptation or for any commercialisation. Any further use, adaptation or commercialisation of such IP will be determined by CSIRO and Wine Australia. Formal contracts between Wine Australia and CSIRO were tailored to manage the BIP.

Project IP arising from this project includes new knowledge on the molecular markers linked to *MJR2* in *V. champinii*, the presence of *RDV38* in *V. cinerea* C2-50, and heat-stable root knot nematode resistance in commercial rootstocks.

Outcomes generated from the project were:
- Released rootstocks derived from background intellectual property
- Data sets, genetic maps and DNA markers for breeding
- Journal manuscripts
- Reports and other communication material

Beneficial information disclosing breeding markers will be disseminated to Australian industry via scientific publications or other means as appropriate (after assessment for potential protection). Any new plant variety rights or other IP including the grapevine varieties derived from background intellectual property will remain under the management and control of CSIRO and Wine Australia. The PBR will be assigned to CSIRO. Only copyright material such as reports will be licensed to the Commonwealth on a royalty-free basis for Commonwealth use.

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