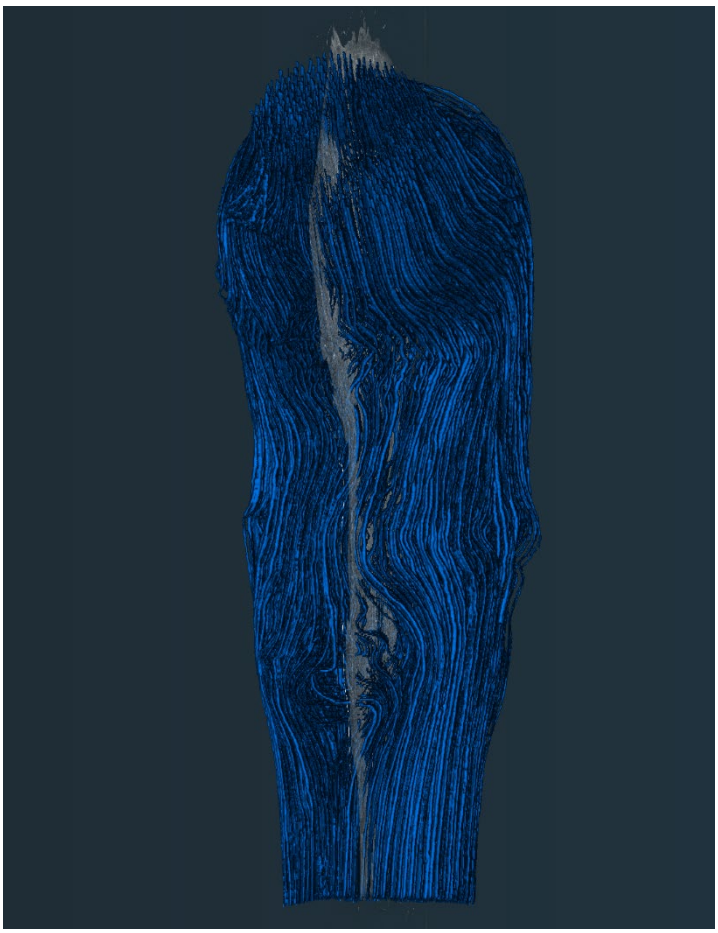
Australian Government

Wine Australia

CSIRO

Physiology of root:shoot interactions



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Physiology of root:shoot interactions

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Abstract

Rootstocks influence scion vigour and physiology, but the mechanisms remain unclear. This project tested the roles of root-derived hormones and hydraulics using controlled and field experiments. Results showed no significant differences in hormone production or scion hydraulics after accounting for growth variations. Microscopic analysis also found no structural changes in scion xylem due to rootstock genotype. However, rootstocks consistently affected scion growth and some links with root traits were observed. It seems unlikely that there simple genetic markers for rootstock selection exist, emphasizing the need for long-term trials focussed on the root system itself to better understand and optimize rootstock effects on scions.

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Executive summary

Rootstock conferred effects on scion vigour and, to a lesser extent perhaps, physiology, has long been observed and reported in the literature. However, the mechanisms behind these effects have proved elusive to pin down. Previous Wine Australia supported work on rootstock:scion interactions suggested, indirectly, that root derived hormones may play a role. In addition, whole plant hydraulics are impacted by rootstock conferred effects across a number of commonly grafted woody horticultural species.

The project described in this report exhaustively tested these hypotheses, utilising highly controlled environment studies, controlled greenhouse studies, a study with field grown young vines to match the greenhouse work, and a mature field planting. Overall, there was little evidence to support a physiologically significant difference in root derived hormone production in response to environmental changes, nor a rootstock derived difference in scion hydraulics once differences in scion growth were accounted for. Microscopic examination of new wood structure also didn't indicate any rootstock derived changes in the scions.

Despite these results, consistent rootstock conferred effects on the scion were observed in all of our experiments. Notably, although there was some evidence for scion effects on rootstock growth, wherever root growth and production were examined, specific rootstocks had consistent root growth habits. Consequently, it is likely that rootstock conferred effects on the scion are largely driven by inherent differences in root system growth form, which provides specific limitations on water and nutrient uptake, dependent on soil type and conditions.

The implication of this is that there is unlikely to be simple genetic markers or easy to measure rootstock phenotypes, such as xylem vessel density, that can be used for breeding, planting or management decisions. This does not mean that it is impossible to improve on using current experience with rootstocks for those decisions, but that improving our knowledge of rootstock conferred effects on the scion are likely to depend on an extensive network of long-term rootstock trials, where rootstock genotypes are not in competition belowground with other rootstocks that differ in their root system growth.

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Chapter 1

1.1 Project Background

Rootstocks are taking on an increasingly important role within Australian viticulture, with some nurseries, for example, reporting the overwhelming majority of new planting material sold being grafted vines (Glenavon Nursery pers. comm.). Far from simply providing protection against nematodes and phylloxera, rootstocks are being chosen for a range of other traits, including vigour and tolerance to abiotic stresses such as drought and salinity. Furthermore, Wine Australia funded project CSP0901 demonstrated a direct role of rootstock selection in determining vine water use efficiency as well as demonstrating clear rootstock effects on wine composition and sensory parameters; the latter conclusion also being supported by more recent CSIRO research in this area.

These rootstock effects on scion vigour and fruit composition are driven by a variety of root-to-shoot signals, which further vary depending on the rootstock:scion combination. For example, scion response to soil water deficit is governed by root hormone signals, while rootstock conferred vigour is governed by a mechanism yet to be fully elucidated, but likely to involve hydraulics. This latter observation has also been the subject of related work by the group at INRA Bordeaux and has resulted in a collaborative paper with CSIRO (Rossdeutsch et al 2016).

As the parent material of commonly utilised rootstocks originates in varied natural environments from wetlands to arid environments it is also likely that root signals of different genotypes vary in response to a given soil environment or stress. Indeed previous work at CSIRO has hinted that differences in the production of the root-to-shoot signal ABA may vary between genotypes, driving different shoot responses in a given scion genotype. Better utilisation of these differences, based on an improved understanding of the processes and their regulation, provides an opportunity to not only improve rootstock choice for specific environments, but to develop management strategies in synchrony with rootstocks for specific viticultural aims. An example of such an integrated growing system in other horticultural industries, is the ‘Smarttree’ growing system in the Almond industry, based on the Rootpac rootstocks.

At present rootstock choice by growers is, ideally, guided by best available advice assisted by rootstock selector tools that attempt to bring together the past literature, much of which describes studies from very different environments or growing systems overseas and usually empirical in nature, rarely providing mechanistic understanding of differences between rootstock traits. More typically growers have relied on anecdotal reports from neighbours or, at worst, are persuaded to simply use whatever rootstocks are available.

1.2 Project Aims and Performance targets

The aim of the project was to build on the past work to further develop understanding of the role of rootstock choice and grafting in the regulation of water uptake, nutrient uptake and abiotic stress response. Specifically, the project objectives were:

- Improve rootstock selector tools with information for specific viticultural situations based on a mechanistic understanding of root to shoot communication (e.g. vigour control, hormone signalling). For example: selecting between rootstocks that lead to rapid stomatal closure and rootstocks that maintain open stomata (for rain-fed vineyards or high-production irrigated vineyards respectively).
- Improved management options for existing rootstock/scion combinations (based on the same mechanistic understanding as above).
- Identify key traits for future breeding targets (e.g. high/low vigour, specific root to shoot signalling).
- Provide improved knowledge to growers covering the use of current deficit irrigation strategies for vigour control and WUE.
- An assessment of the potential to develop 'precision planting' utilising multiple rootstocks within a single vineyard block based on soil type etc.

The Annual Operating Plans are given in Appendix 5. The strategic plan (revised in 2019) for the project included the following outputs.

Three-year outputs:

- Rootstocks that differ in production of root-derived signals (e.g. ABA, CK) under identical soil stress conditions identified.
- Effect of differences in graft structure on whole vine hydraulic conductance determined.
- Interactions between scions that differ in water deficit response (an/isohydric genotypes e.g. Shiraz/Grenache) and rootstocks that differ in drought signalling determined.

Five-year outputs:

- Crop management strategies matched to specific rootstock scion combinations demonstrated to improve resource use efficiency in the field.
- Relative roles of vine hydraulics and graft union structure in rootstock conferred vigour determined.
- Rootstocks that differ in production of root-derived signals (e.g. ABA, CK) under identical soil stress conditions identified and impact on scion function determined

Chapter 2: Materials & methods

The primary aim of the project was to objectively determine mechanisms whereby rootstock genotypes influence the growth and physiology of the scion, explaining rootstock conferred traits. Achieving this would open up improved crop management, specific to rootstock choice and also improve the information available in making rootstock choice.

The approach taken by the work was to combine short-term experiments under highly controlled conditions with longer-term (multi-season) experiments under field conditions, using a common set of rootstock:scion combinations and concentrating on rootstock conferred vigour and water stress response as traits of interest. The latter because the plant physiology is well understood, the former because it is perhaps the most obvious and commonly observed rootstock conferred trait, and, furthermore, because there ws a potential for the two to be linked. This series of experiments is summarised in Table 1.

2.1 Experiment 1: root ABA production of Merbein 5512 and 1103 Paulsen under soil water deficit

This experiment was completed in the planning phase of the project, prior to project initiation, and followed an observation in CSP0901 that suggested the commercially available CSIRO bred rootstock Merbein 5512 (M5512) may produce less ABA under stress than 1103 Paulsen (1103P).

2.1.1 Plant material

Cuttings of M5512 (obtained from Genavon Nursery [Langhorne Creek, SA]) and 1103P rootstocks were grafted to Cabernet Sauvignon (CS) using 'Omega' bench grafting and propagated by Yalumba Nursery (Nuriootpa, SA). Once both roots and shoots had emerged, the plants were potted in UC mix and placed in a glasshouse at CSIRO Waite Campus under natural light, with temperature controlled to 27°C maximum during the day and 18°C minimum at night. All pots were watered to field capacity daily at 9 AM using an automated irrigation system.

After approximately four weeks of growth the plants were randomly assigned to five blocks, each located on a different glasshouse bench. Within each block half of the plants from each rootstock:CS combination were randomly assigned to either well-watered (WW) or water deficit (WD) treatments. Within each block eight pots (two of each irrigation*rootstock combination) were instrumented with 15 cm capacitance soil moisture sensors (ICT International, Armidale, NSW), logging at 30 minute intervals, providing ten instrumented pots per irrigation*rootstock combination.

Table 1 - summary of experiments undertaken through the project.

Experiment #	Primary aim	Genotype combinations (rootstock:scion)
1	Confirm/deny possible difference in root derived hormone between Merbein 5512 and 1103 Paulsen. **Greenhouse**.	Merbein 5512 : Cabernet Sauvignon 1103 Paulsen : Cabernet Sauvignon
2	Compare short-term root response to controlled water deficit in contrasting rootstocks. **DroughtSpotter platform**.	Merbein 5512 : Cabernet Sauvignon 1103 Paulsen : Cabernet Sauvignon Ramsey : Cabernet Sauvignon
3	Determine medium-term rootstock derived differences in plant physiology without water stress. **Greenhouse**.	Merbein 6262 : Cabernet Sauvignon Merbein 5489 : Cabernet Sauvignon Merbein 5512 : Cabernet Sauvignon 140 Ruggeri : Cabernet Sauvignon 1103 Paulsen : Cabernet Sauvignon Mi 0907 : Cabernet Sauvignon Ramsey : Cabernet Sauvignon
4[1]	Link root phenotypes to conferred vigour over longer-term; interaction with scion. **Greenhouse**.	Merbein 6262 : Cabernet Sauvignon Merbein 5489 : Cabernet Sauvignon 140 Ruggeri : Cabernet Sauvignon 1103 Paulsen : Cabernet Sauvignon Ramsey : Cabernet Sauvignon RGM[2] : Cabernet Sauvignon Cabernet Sauvignon : Cabernet Sauvignon Merbein 6262 : Shiraz Merbein 5489 : Shiraz 140 Ruggeri : Shiraz 1103 Paulsen : Shiraz Ramsey : Shiraz RGM : Shiraz Shiraz : Shiraz
5	Link root phenotypes to conferred vigour and water deficit response over longer-term; interaction with scion. **Field**.	Merbein 6262 : Cabernet Sauvignon Merbein 5489 : Cabernet Sauvignon 1103 Paulsen : Cabernet Sauvignon Ramsey : Cabernet Sauvignon RGM : Cabernet Sauvignon Cabernet Sauvignon : Cabernet Sauvignon Merbein 6262 : Shiraz Merbein 5489 : Shiraz 1103 Paulsen : Shiraz Ramsey : Shiraz RGM : Shiraz Shiraz : Shiraz

[1] Experiment was curtailed through impact of COVID-19 pandemic.

[2] Riparia Gloire de Montpellier

2.1.2 Experimental procedure

Five plants (one per block) per treatment combination were sampled immediately prior to the WD treatment being imposed and then 24 hours, 31 hours, 2 days, 3 days and 5 days after the start of the WD treatment. After sampling on the 5th day the WD treatment was ended and the plants returned to watering to field capacity. A further set of samples, to assess recovery, was then taken 23 days after the start of the WD treatment. The same plant was never sampled twice.

The water deficit was applied by removing irrigation to the WD assigned pots until the soil moisture (averaged across the instrumented pots) reached 10% soil moisture by volume. Water was then provided daily in 50 ml increments to maintain soil moisture above 10% and below 15%.

The sample procedure consisted of taking stomatal conductance measurements on two leaves per plant, using a Delta-T AP4 Porometer (Delta-T, UK), followed by a leaf water potential and stem water potential (using a leaf equilibrated, whilst on the vine, in a silvered ziplock plastic bag for a minimum of 30 minutes) measurement using the balance pressure method (Boyer 1967) with a Plant Water Potential Console (Soil Moisture Equipment, USA). Following the leaf water potential measurement 0.2 MPa of overpressure was applied and the exuded sap collected and frozen in liquid nitrogen. At the same time, a leaf was taken and immediately frozen in liquid nitrogen and a root sample was extracted by hand, roughly cleaned and frozen in liquid nitrogen.

2.1.3 Hormone and gene transcription analysis

The frozen tissue samples were subsequently ground under liquid nitrogen into a fine powder. An aliquot of that power was used for LC/MS/MS analysis of abscisic acid (ABA) and its metabolites phaseic acid (PA) and di-hydro-phaseic acid (DPA) according to the method of Spiers et al (2013). The xylem sap samples were also analysed for ABA and its metabolite using the same method.

A second aliquot of the tissue samples was used to determine the transcription level of the key ABA synthesis genes NCED1 and NCED2 and the key ABA catabolic genes HYD1 and HYD2 using RT-qPCR (Spiers et al 2013).

2.2 Experiment 2: root hormone production of three contrasting rootstocks under matched soil water deficit

This experiment followed up on experiment 1, but utilised the DroughtSpotter platform at the University of Adelaide's Australian Plant Phenomics Facility node. The platform allows precise, minute by minute, control of soil moisture, thereby eliminating potential effects of plant vigour on soil moisture between watering events, ensuring all root systems were subjected to exactly the same soil conditions.

2.2.1 Plant material

Cuttings of M5512, Ramsey and 1103P rootstocks were grafted to Cabernet Sauvignon (CS) using 'Omega' bench grafting and propagated by Yalumba Nursery (Nuriootpa, SA). On being received at CSIRO they were managed as per 2.1.1 above.

On 7th Jan 2019 16 vines per genotype were transplanted into pots at the Plant Accelerator and made up to matching total weights by adjusting the soil amount. The following day the pots were flushed with water to saturate the soil and thinned to a single shoot. The pots were then set up on the DroughtSpotter platform in six randomised blocks (12 vines per genotype) and set to water to maintain 3400 g. The LED supplemental lighting was set up and turned on for 16 hour days.

2.2.2 experimental procedure

Stomatal conductance was used to assess the status of the vines as they grew. On 11th Feb half the vines of each genotype were set to reduced irrigation by lowering the target weight by 100 g. Stomatal conductance was monitored daily for the next four days and the target weight reduced by 50 g for each day.

The first whole vine harvest was undertaken on 15th Feb. Stomatal conductance was again measured daily and the target weight reduced by a further 50 g on 18th Feb due to reduced signs of water stress. The final harvest was undertaken on 19th Fe.

The harvest days began with stomatal conductance, stem water potential and leaf water potential all being measured as described in 2.1.2 above. Sap was collected whilst taken the leaf water potential measurement. Two further leaves were harvested and immediately placed into liquid nitrogen, before being stored at -80°C for further use. At the second harvest, the stem cut just above and below the graft union either frozen immediately liquid nitrogen or kept in 80% ethanol for further use.

Root samples were obtained by gently shaking soil from the roots, a sample taken and briefly washed, then frozen in liquid nitrogen.

Plant hormones and gene expression were determined using the frozen samples as described in 2.1.3.

2.3 Experiment 3: physiology of rootstock conferred vigour

Seven rootstock genotypes grafted to one scion genotype were grown over a nine months in semi-controlled conditions (greenhouse), with roots and shoot, plant physiology, hydraulic and xylem structure traits all examined in order to link any phenotypic differences with conferred vigour.

2.3.1 Plant material

Cuttings of M5512, M5480, M626, 1103 Paulsen, 140 Ruggeri, Ramsey and Mi0907 (a CSRIO breeding line) rootstocks were grafted to Cabernet Sauvignon (CS) using 'Omega' bench

grafting and propagated by Yalumba Nursery as previously. On receival by CSIRO (3rd Jan 2019) they were maintained as per 2.1.1 above. On 22nd Jan they were repotted into 3L pots for the experiment. On 24th Jan they were thinned to a single shoot. On 28th Feb the vines were pruned back to three nodes and the shoot material weighed then oven dried for dry mass determination.

2.3.2 experimental procedure

Three vines of each genotype were randomly assigned into each of eight blocks and maintained with full watering and slow release fertiliser. The first whole vine harvest was completed on 13-14th March 2019.

Pre-dawn leaf water potential was measured before sunrise as per leaf water potential in 2.1.2. Gas exchange measurements were made with a LiCOR 6400XT mid-late morning. Leaf and stem water potential were then measured as per 2.1.2 and xylem sap collected. Two leaves were collected and stored in liquid nitrogen for further use. Scion and rootstock stem tissue either side of the graft union was collected for future microscopy analysis. The roots were subsampled with a soil corer for root length assessment and the rest of the root system cleaned and dried for total root weight. Leaves were cut from the stem and measured for leaf area using a LiCOR 3000x leaf area meter. All plant parts were then oven dried for dry mass determination.

A second harvest was undertaken in the same manner in December 2019, following the shoots being cut back in May and August, with the biomass being measured as previously.

Plant hormones and associated gene expression were assessed as previously described.

2.4 Experiment 4: root phenotypes associated with rootstock conferred vigour

Following on from experiment 3, this was intended to be a longer term (<12 month) study, concentrating on root phenotypes that might be linked to conferred vigour and addressing potential rootstock:scion interactions. Unfortunately, this study was significantly impacted by the COVID-19 pandemic, limiting the work undertaken.

2.4.1 Plant material

Cuttings of M6262, M5489, 1103 Paulsen, Ramsey and Riparia Gloire de Montpellier were obtain and grafted to Cabernet Sauvignon and Shiraz as previously by Yalumba Nursery. In addition, Cabernet Sauvignon and Shiraz canes were self grafted in the same way toi obtain grafted plants on own roots. All vines were managed as previously until use.

2.4.2 experimental procedure

Replicates of each genotype were assigned to eight blocks and maintained as per experiment 3. On 30th Sept. To 2nd Oct 2020 a harvest of all rootstock : scion combinations was undertaken

as per experiment 3. Plant hormone and associated gene expression was analysed as per 2.1.3. Root sample were analysed using WinRhizo (Regent Instruments, Canada).

2.5 Experiment 5: the role of the field environment in determining rootstock-scion interactions

It is necessary to control conditions as much as possible when studying fundamental differences in rootstock traits and rootstock conferred traits, as those traits are strongly influenced by the environment and, in the case of water deficit or nutrient uptake, it is the root system response to the environment that may differ between genotypes. Consequently, a dedicated field study was set up at CSIRO Irymple field station using a selection of rootstock : scion combinations from the greenhouse experiments, to determine the extent to which the rootstock derived traits were impacted by natural conditions vs semi-controlled or controlled conditions. Unfortunately, this study was significantly impacted by the COVID-19 pandemic, limiting the work undertaken.

2.5.1 Plant material

Plant material matching experiment 4 was obtained at the same time, but shipped to the CSIRO Irymple site. It was maintained there in a shade house for approximately three months until being planted out in a cleared block with new, low, trellis (Figure 1). After being fully irrigated the vines were split into fully irrigated, or reduced irrigation by using a second dripper line with low output drippers for the reduced irrigation treatment.

2.5.2 experimental procedure

When travel was available, stomatal conductance and soil moisture were measured to assess the treatment and plant status (12th Jan 2021). In late March 2021 further stomatal conductance measurements were made, together with gas exchange using a LiCOR 6400XT.

A 50 mm diameter 1 m soil corer was then used to collect a soil core to 60 cm depths 25 cm from the vine trunk. Following this the shoots were cut from the vine and the leaves removed. A sub-sample of the leaves were used for leaf area measurement as in previous experiments. The subsample was dried and weighed separately to the remainder of the leaves in order to estimate total leaf area per vine by with scaling the dry weights. The stem was separated into recent growth and woody stem and dried and weighed separately.

A local contractor was employed to scoop out approximately 0.5 m^3, to a depth of 60 cm centred on the cut off vine stump. The soil was sifted on site to collect the root systems. Some rootstock genotypes, particularly Ramsey had woody roots extending beyond this region already, but visually, the majority of the root system was within the soil sampled.

The remaining vines were spur pruned to a cordon in June 2021, with fresh pruning weight recorded. The vines fruited and harvest yield was determined in March 2022.

		Main-plot 1			Main-plot 2			
		Rows						
Plant	Panel	1	2	3	4	5	6	Block/Rep
1	1	RS4	RS2	RS8	RS11	RS9	RS7	1
2		RS4	RS2	RS8	RS11	RS9	RS7	
3		RS4	RS2	RS8	RS11	RS9	RS7	
4		RS4	RS2	RS8	RS11	RS9	RS7	
5	2	RS1	RS3	RS12	RS2	RS5	RS3	
6		RS1	RS3	RS12	RS2	RS5	RS3	
7		RS1	RS3	RS12	RS2	RS5	RS3	
8		RS1	RS3	RS12	RS2	RS5	RS3	
9	3	RS10	RS7	RS6	RS8	RS4	RS6	
10		RS10	RS7	RS6	RS8	RS4	RS6	
11		RS10	RS7	RS6	RS8	RS4	RS6	
12		RS10	RS7	RS6	RS8	RS4	RS6	
13	4	RS5	RS9	RS11	RS1	RS10	RS12	
14		RS5	RS9	RS11	RS1	RS10	RS12	
15		RS5	RS9	RS11	RS1	RS10	RS12	
16		RS5	RS9	RS11	RS1	RS10	RS12	
17	5	RS7	RS10	RS6	RS8	RS7	RS5	2
18		RS7	RS10	RS6	RS8	RS7	RS5	
19		RS7	RS10	RS6	RS8	RS7	RS5	
20		RS7	RS10	RS6	RS8	RS7	RS5	
21	6	RS3	RS1	RS12	RS1	RS4	RS10	
22		RS3	RS1	RS12	RS1	RS4	RS10	
23		RS3	RS1	RS12	RS1	RS4	RS10	
24		RS3	RS1	RS12	RS1	RS4	RS10	
25	7	RS8	RS11	RS4	RS12	RS2	RS11	
26		RS8	RS11	RS4	RS12	RS2	RS11	
27		RS8	RS11	RS4	RS12	RS2	RS11	
28		RS8	RS11	RS4	RS12	RS2	RS11	
29	8	RS2	RS5	RS9	RS6	RS3	RS9	
30		RS2	RS5	RS9	RS6	RS3	RS9	
31		RS2	RS5	RS9	RS6	RS3	RS9	
32		RS2	RS5	RS9	RS6	RS3	RS9	
33	9	RS6	RS8	RS10	RS11	RS5	RS4	3
34		RS6	RS8	RS10	RS11	RS5	RS4	
35		RS6	RS8	RS10	RS11	RS5	RS4	
36		RS6	RS8	RS10	RS11	RS5	RS4	
37	10	RS12	RS2	RS4	RS3	RS9	RS2	
38		RS12	RS2	RS4	RS3	RS9	RS2	
39		RS12	RS2	RS4	RS3	RS9	RS2	
40		RS12	RS2	RS4	RS3	RS9	RS2	
41	11	RS9	RS7	RS1	RS10	RS12	RS8	
42		RS9	RS7	RS1	RS10	RS12	RS8	
43		RS9	RS7	RS1	RS10	RS12	RS8	
44		RS9	RS7	RS1	RS10	RS12	RS8	
45	12	RS11	RS3	RS5	RS7	RS6	RS1	
46		RS11	RS3	RS5	RS7	RS6	RS1	
47		RS11	RS3	RS5	RS7	RS6	RS1	
48		RS11	RS3	RS5	RS7	RS6	RS1	
49	13	RS2	RS6	RS7	RS2	RS10	RS9	4
50		RS2	RS6	RS7	RS2	RS10	RS9	
51		RS2	RS6	RS7	RS2	RS10	RS9	
52		RS2	RS6	RS7	RS2	RS10	RS9	
53	14	RS8	RS10	RS12	RS7	RS5	RS6	
54		RS8	RS10	RS12	RS7	RS5	RS6	
55		RS8	RS10	RS12	RS7	RS5	RS6	
56		RS8	RS10	RS12	RS7	RS5	RS6	
57	15	RS3	RS4	RS11	RS4	RS11	RS8	
58		RS3	RS4	RS11	RS4	RS11	RS8	
59		RS3	RS4	RS11	RS4	RS11	RS8	
60		RS3	RS4	RS11	RS4	RS11	RS8	
61	16	RS1	RS5	RS9	RS12	RS1	RS3	
62		RS1	RS5	RS9	RS12	RS1	RS3	
63		RS1	RS5	RS9	RS12	RS1	RS3	
64		RS1	RS5	RS9	RS12	RS1	RS3	
65	17	RS7	RS2	RS4	RS6	RS9	RS11	5
66		RS7	RS2	RS4	RS6	RS9	RS11	
67		RS7	RS2	RS4	RS6	RS9	RS11	
68		RS7	RS2	RS4	RS6	RS9	RS11	
69	18	RS12	RS5	RS10	RS8	RS5	RS4	
70		RS12	RS5	RS10	RS8	RS5	RS4	
71		RS12	RS5	RS10	RS8	RS5	RS4	
72		RS12	RS5	RS10	RS8	RS5	RS4	
73	19	RS6	RS1	RS8	RS12	RS3	RS10	
74		RS6	RS1	RS8	RS12	RS3	RS10	
75		RS6	RS1	RS8	RS12	RS3	RS10	
76		RS6	RS1	RS8	RS12	RS3	RS10	
77	20	RS9	RS11	RS3	RS1	RS7	RS2	
78		RS9	RS11	RS3	RS1	RS7	RS2	
79		RS9	RS11	RS3	RS1	RS7	RS2	
80		RS9	RS11	RS3	RS1	RS7	RS2	

Figure 1 - field plan for experiment 5.

Chapter 3: Experimental results and discussion

3.1 Experiment 1: root ABA production of Merbein 5512 and 1103 Paulsen under soil water deficit

Although growth and biomass were not assessed as part of this experiments, there was a difference in vine size, with the vines grafted to 1103 Paulsen larger than those grafted to Merbein 5512. Despite daily watering this led to the 1103 Paulsen vines having lower soil moisture than the Merbein 5512 vines, meaning that the imposition of treatments combined drying down the deficit irrigated treatments, with increasing the soil moisture in the 1103 Paulsen control vines by watering twice per day (Figure 2). However, the control and deficit treatments were well separated for the experimental period and differences ion soil moisture between rootstock genotypes were limited. Due to the timing of the experiment, over the Christmas holiday period, the plants could only be watered daily during the recovery period, resulting in the differences in soil moisture between rootstocks reappearing, although differences between treatments were immediately removed.

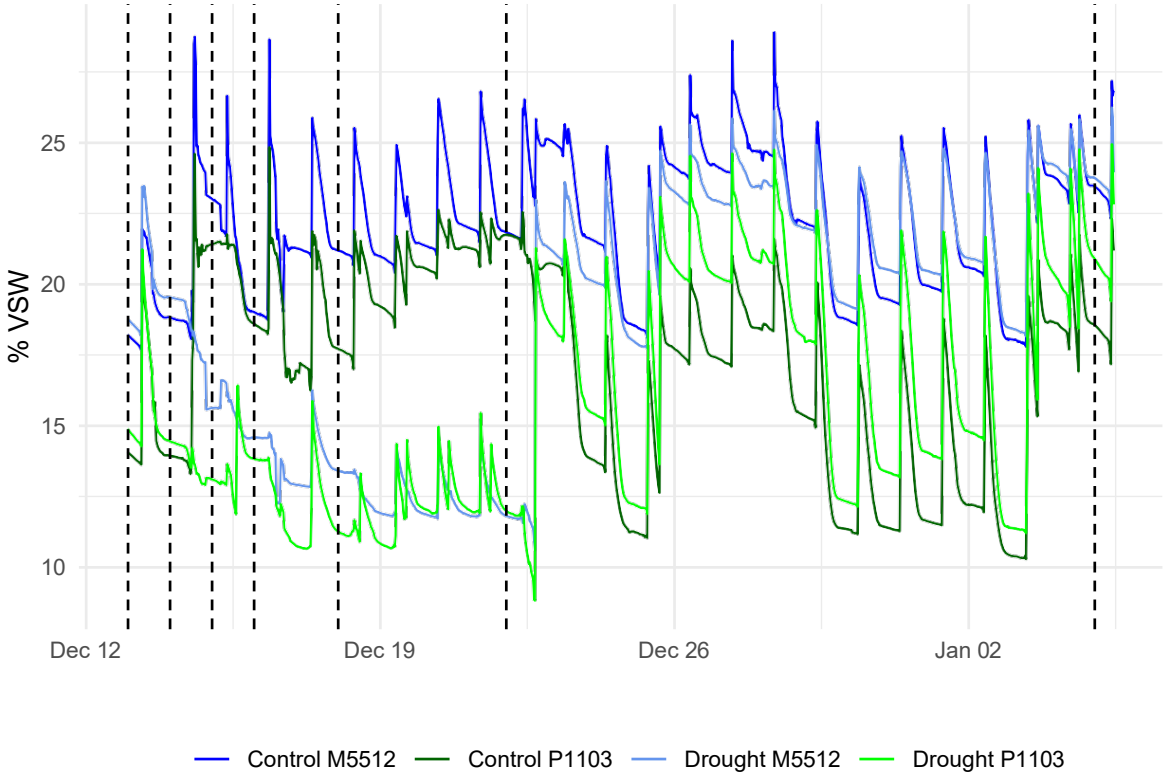


Figure 2 – mean volumetric soil moisture during the experimental period, dashed lines indicate measurement/sampling times.

The impact of the water deficit treatment on the physiology of the vines was confirmed by regular measurements of stomatal conductance, midday leaf water potential and stem water potential.

Stomatal conductance (g_s) of scion leaves was not significantly affected by rootstock genotype prior to the water deficit treatment, or during the water deficit period, but was significantly lower after recovery from water deficit, despite having slightly higher soil moisture during the latter period (Figure 3).

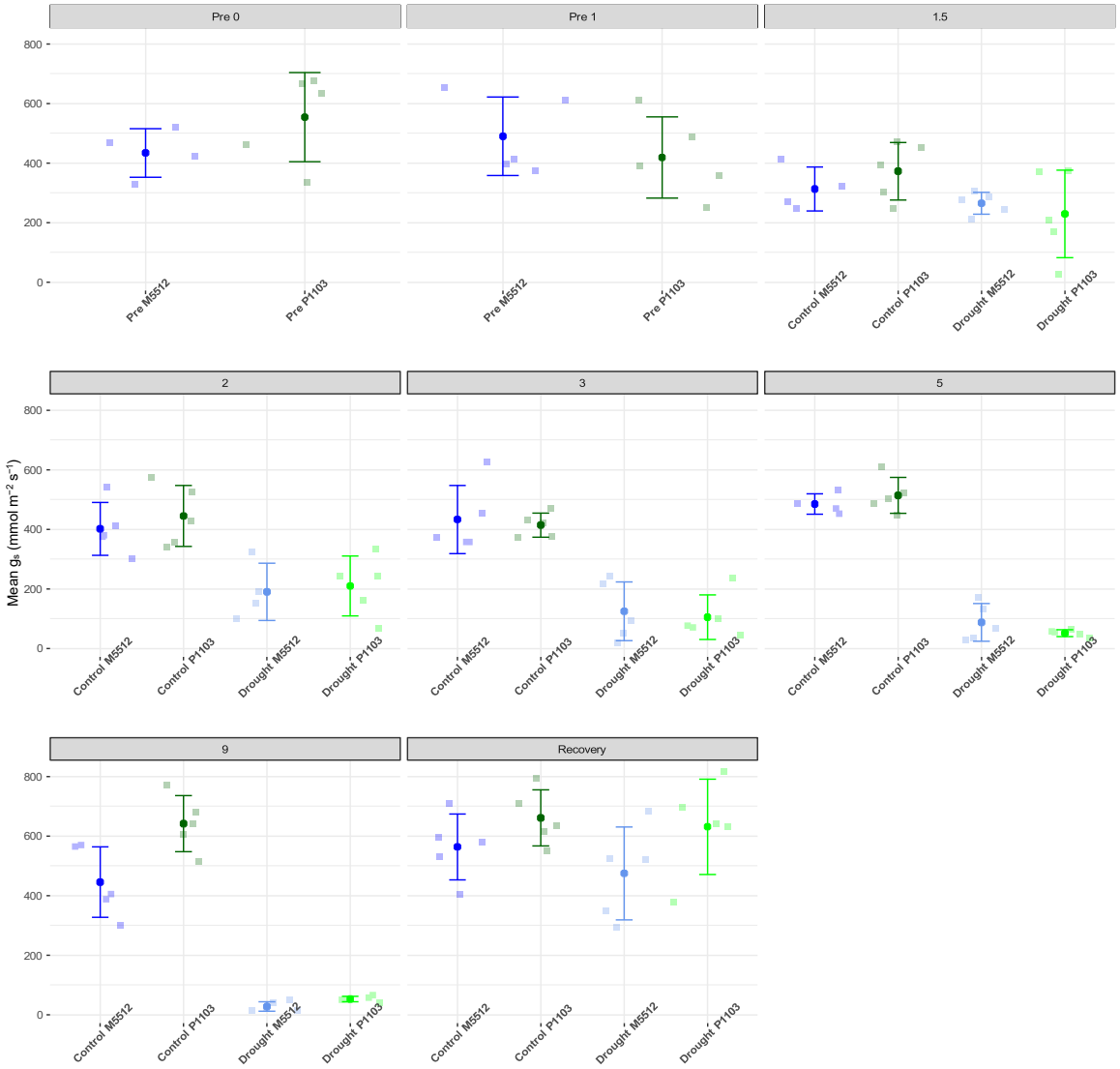


Figure 3 - stomatal conductance (g_s) of Cabernet Sauvignon vine grafted to M5512 or 1103P rootstocks and well-watered or subject to a soil water deficit. Data are means +/- SD

The water deficit significantly reduced g_s, with the impact increasing from to day five of treatment as soil moisture reduced. There was no significant difference between the rootstocks in rate of g_s decrease (p=0.063).

Neither midday leaf water potential (Ψ_{leaf}), nor stem water potential (Ψ_{stem}) were affected by rootstock prior to the start of the water deficit treatment (Figure 4 and Figure 5 respectively). The impact of the water deficit on Ψ_{leaf} was less pronounced than on g_s, and took longer to reach its maximum effect, but was still indicative of moderate water stress (Carbonneau 1998). There was no significant effect of rootstock on the Ψ_{leaf} response.

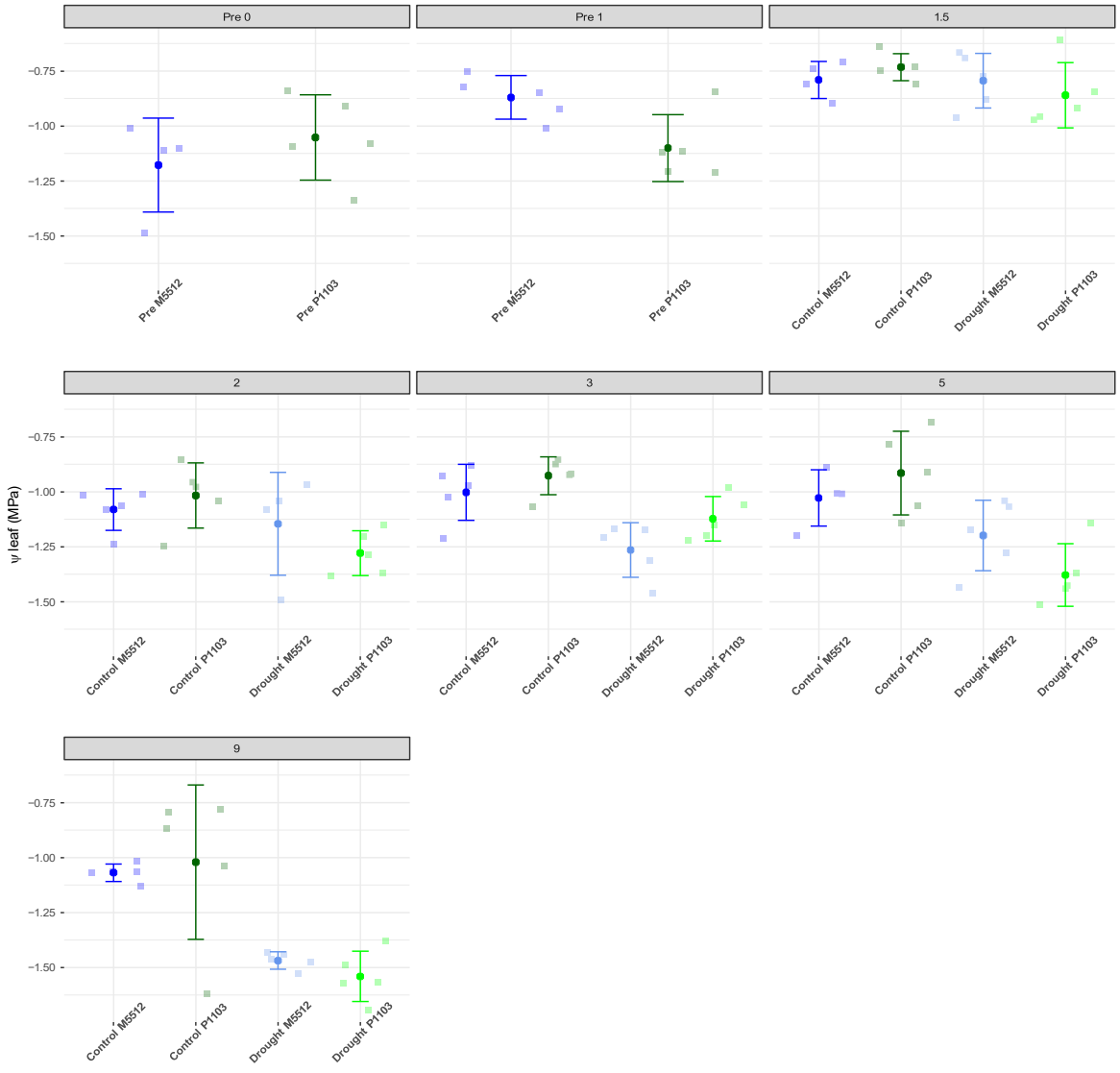


Figure 4 – midday leaf water potential (Ψ_{leaf})of Cabernet Sauvignon vine grafted to M5512 or 1103P rootstocks and well-watered or subject to a soil water deficit. Data are means +/- SD

The treatment effect on Ψ_{stem} was more apparent than on Ψ_{leaf} on the first day of treatment (Figure 5 vs Figure 4, day 1.5) on the relative decrease in response to water deficit larger. This was likely because of the high evaporative demand on the leaves due to the summer growing conditions, as the greenhouse used had limited cooling capacity. Again, there was no significant effect of rootstock on the response.

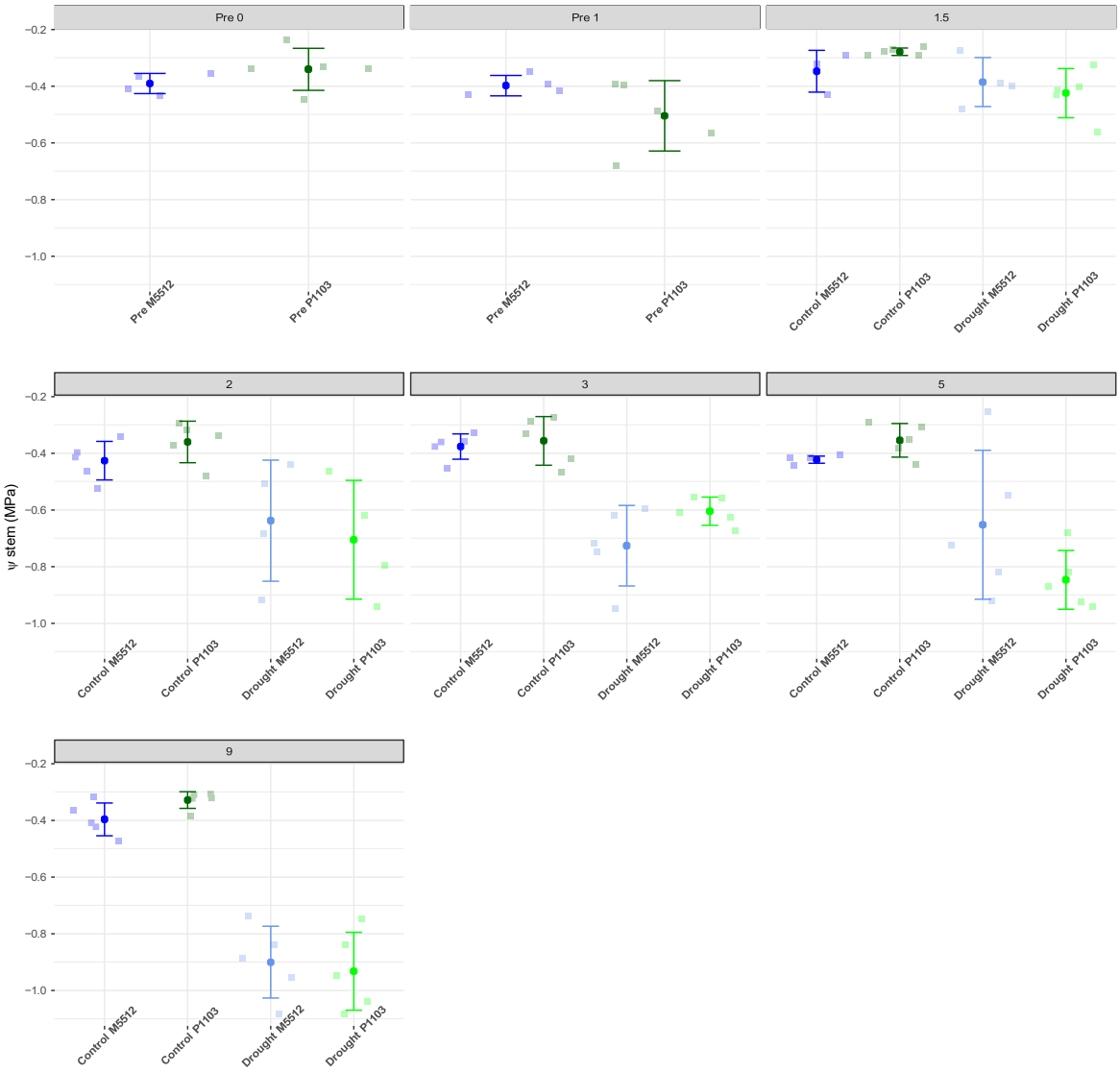


Figure 5 - stem water potential (Ψ_{stem}) of Cabernet Sauvignon vine grafted to M5512 or 1103P rootstocks and well-watered or subject to a soil water deficit. Data are means +/- SD.

Plotting the relationship between Ψ_{stem} and g_s also indicated no effect of rootstock genotype on the physiological response of the vines to the water deficit treatment (Figure 6). Taken as whole, the vine physiology results reported here demonstrated that the water deficit

treatment was successful in imposing a moderate to severe water stress on the vines, with no difference between the rootstocks.

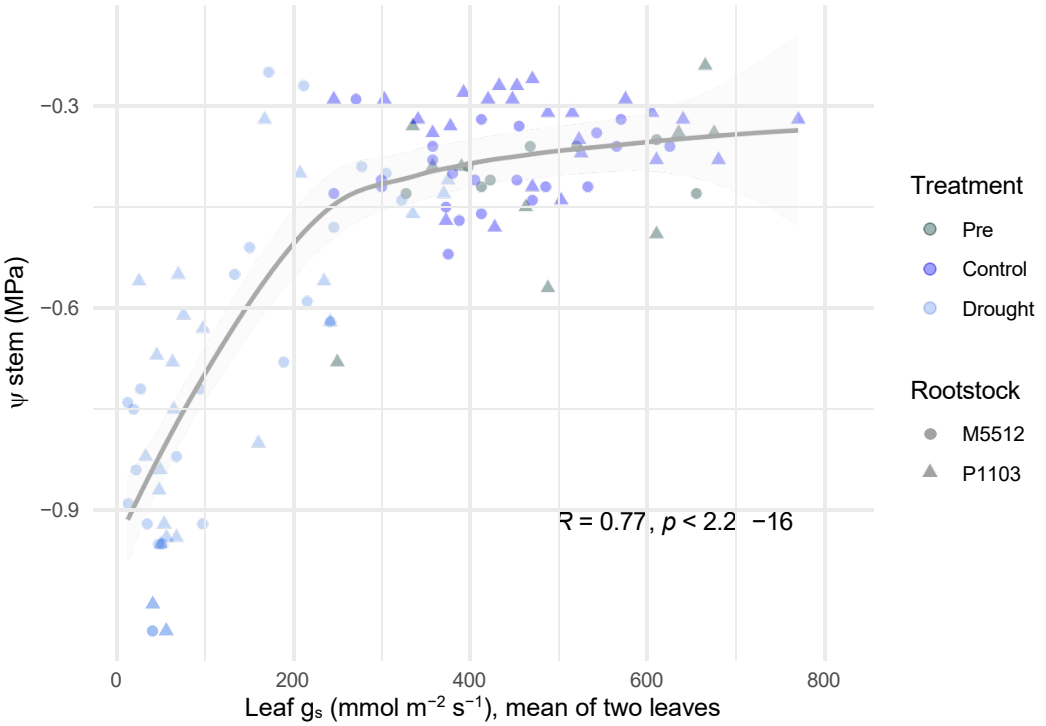


Figure 6 - relationship between stem water potential (Ψ_{stem}) and stomatal conductance (g_s) of Cabernet Sauvignon vine grafted to M5512 or 1103P rootstocks under increasing water deficit. Data are individual leaves, with measurements taken throughout the nine-day experimental period.

Abscisic acid (ABA) is a key signal of water stress within the vine and mediator plant responses to this stress, such as changes in g_s. ABA concentrations in xylem sap, leaf tissue and root tissue were measured to look for differences as a result of rootstock, although it is not possible to determine directly whether the ABA measured originates from the root system or the vine canopy.

Xylem sap and root tissue ABA concentrations were slightly, but significantly, higher in the 1103 Paulsen vines, compared with the Merbein 5512 vines. This was consistent with the difference observed in soil moisture between the two rootstock genotypes prior to the deficit treatment. There was no significant difference in the leaf tissue, however.

On the first day of water deficit there was already a small response in xylem sap ABA in both genotypes, and this was higher in 1103 Paulsen for the first two days than Merbein 5512 (Figure 7), leading to a significant rootstock effect (p=0.023). There was no difference between the rootstocks on the subsequent days of the water deficit, however, and the differences at the start of the treatment were consistent with the soil moisture differences (Figure 2) that

were assumed to have resulted from canopy size differences. Consequently, there was no evidence of a difference in xylem sap ABA between the rootstocks in response to water deficit.

Sap ABA in the control vines ranged from 70 to 150 ng ml^{-1} whereas the vines under water deficit, once established at day 3, had a sap ABA concentration of 500-700 ng ml^{-1}.

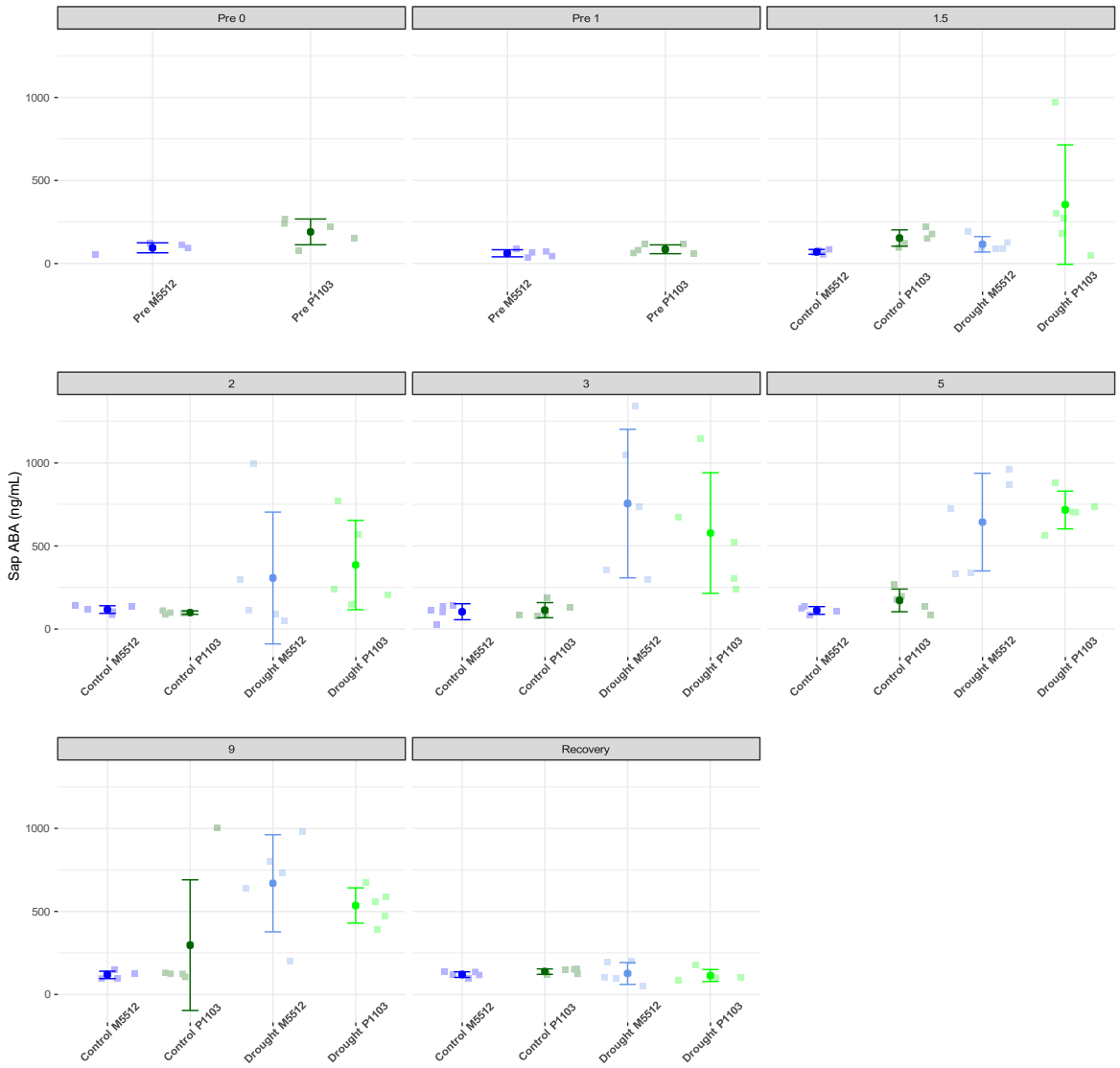


Figure 7 - xylem sap ABA concentration of Cabernet Sauvignon vines grafted to M5512 or 1103P rootstocks and well-watered or subject to a soil water deficit. Data are means +/- SD.

Sap ABA concentrations over 200 ng ml^{-1} were clearly associated with changes in g_s (Figure 8), with g_s dropping from around 300 mmol m^{-2} s^{-1} to under 50 mmol m^{-2} s^{-1}. Variation in g_s above 300 mmol m^{-2} s^{-1}, was likely driven by other environmental factors, particularly the light environment (not assessed). The g_s vs Ψ_{stem} relationship (Figure 6) had an inflexion point at

the same g_s value, supporting this conclusion. Xylem sap ABA under 200 ng ml^{-1} was associated with soil moisture vales over 18% volumetric soil water and concentrations over 250 ng ml^{-1}, with soil moisture of 15% or under. The bulk density of the UC mix used was not measured, so these values cannot be associated with a specific soil water potential.

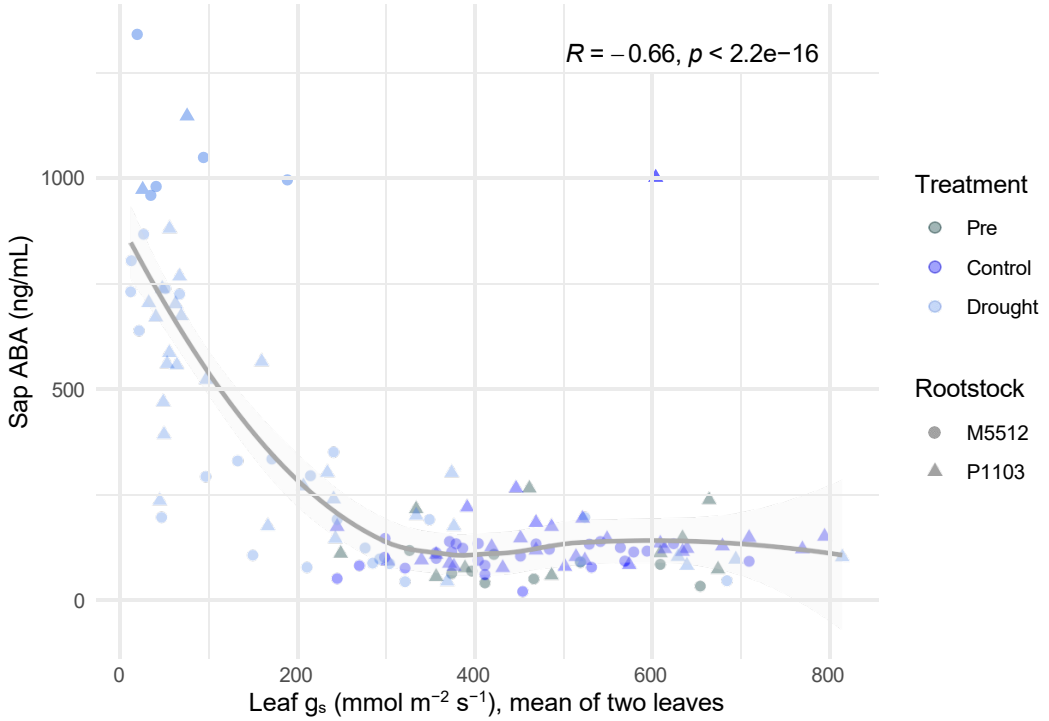


Figure 8 - relationship between xylem sap ABA concentration and stomatal conductance (g_s) of Cabernet Sauvignon vine grafted to M5512 or 1103P rootstocks under increasing water deficit. Data are individual vines, with measurements taken throughout the nine-day experimental period.

The ABA metabolites, phaseic acid (PA), di-hydrophaseic acid (DPA) and ABA-glucose ester (ABA-GE) were also analysed. PA concentration also increased in xylem sap, with the increase apparent on the first day without water, but the proportional increase was less than seen with ABA (data not shown). The increase in DPA was smaller again and delayed further following the imposition of water deficit. No significant concentrations of ABA-GE were seen in xylem sap, consistent with the prior suggestions that ABA-GE is stored in the cell vacuole once glucosylated (Bray & Zeevaart 1985).

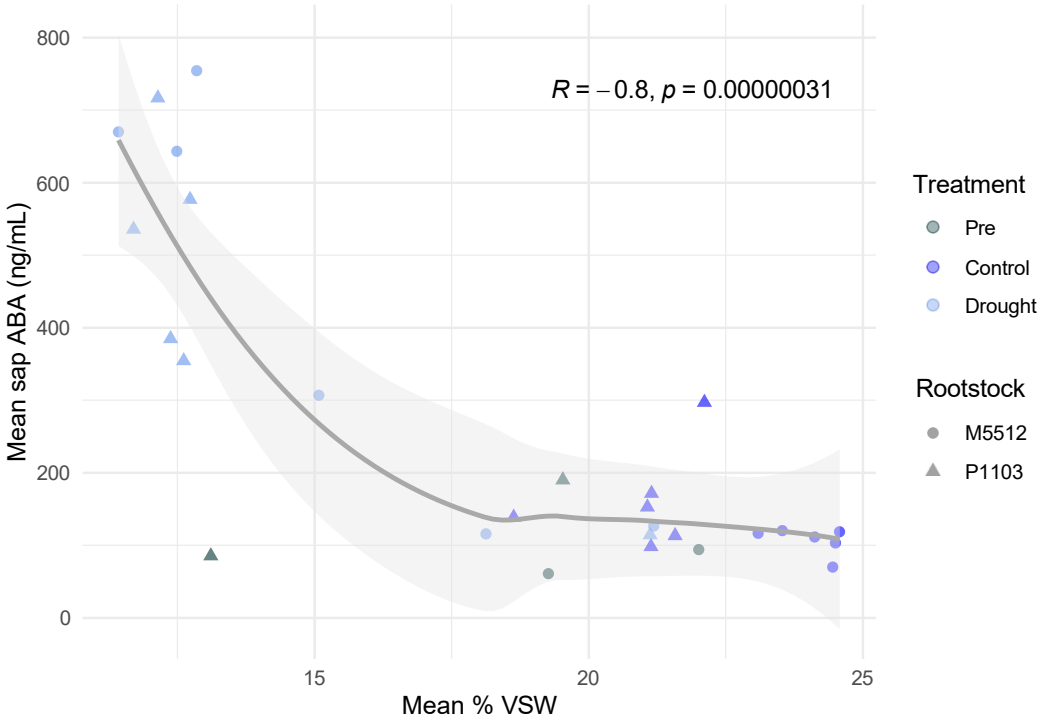


Figure 9 - relationship between soil moisture and xylem sap ABA concentration of Cabernet Sauvignon vine grafted to M5512 or 1103P rootstocks under increasing water deficit. Data are individual vines, with measurements taken throughout the nine-day experimental period.

Whether synthesised in the root system or the leaf, extra-cellular ABA is likely to accumulate in the leaf until metabolised due to the leaf being major the site of water loss from the vine. As a result, leaf tissue ABA concentrations are often higher than sap or root. Prior to the water deficit treatment the leaves had 550-750 ng g^{-1} of ABA, with no difference between rootstocks (Figure 10) and these values were similar in the control vines throughout the experiment.

Leaf ABA concentration was significantly higher in vines under water deficit and increased to day 3, at which point the soil moisture levels were maintained, with no difference between rootstock genotypes. At day nine of the deficit, leaf ABA concentrations were approximately three times controls (1,800 ng g^{-1} vs 450-600 ng g^{-1}), a similar relative increase to the sap ABA concentrations.

The increase in PA concentration in the leaf was delayed, relative to ABA, only being clear at day three of water deficit (data not shown). A change in leaf DPA concentrations with water deficit was similarly delayed and concentrations were about a third of PA (data not shown).

Root tissue ABA concentrations were already raised, compared with controls, on the first day of water deficit, although the difference was much larger in the 1103 Paulsen vines (Figure 11). Overall though, there was no difference in root ABA concentration between rootstocks.

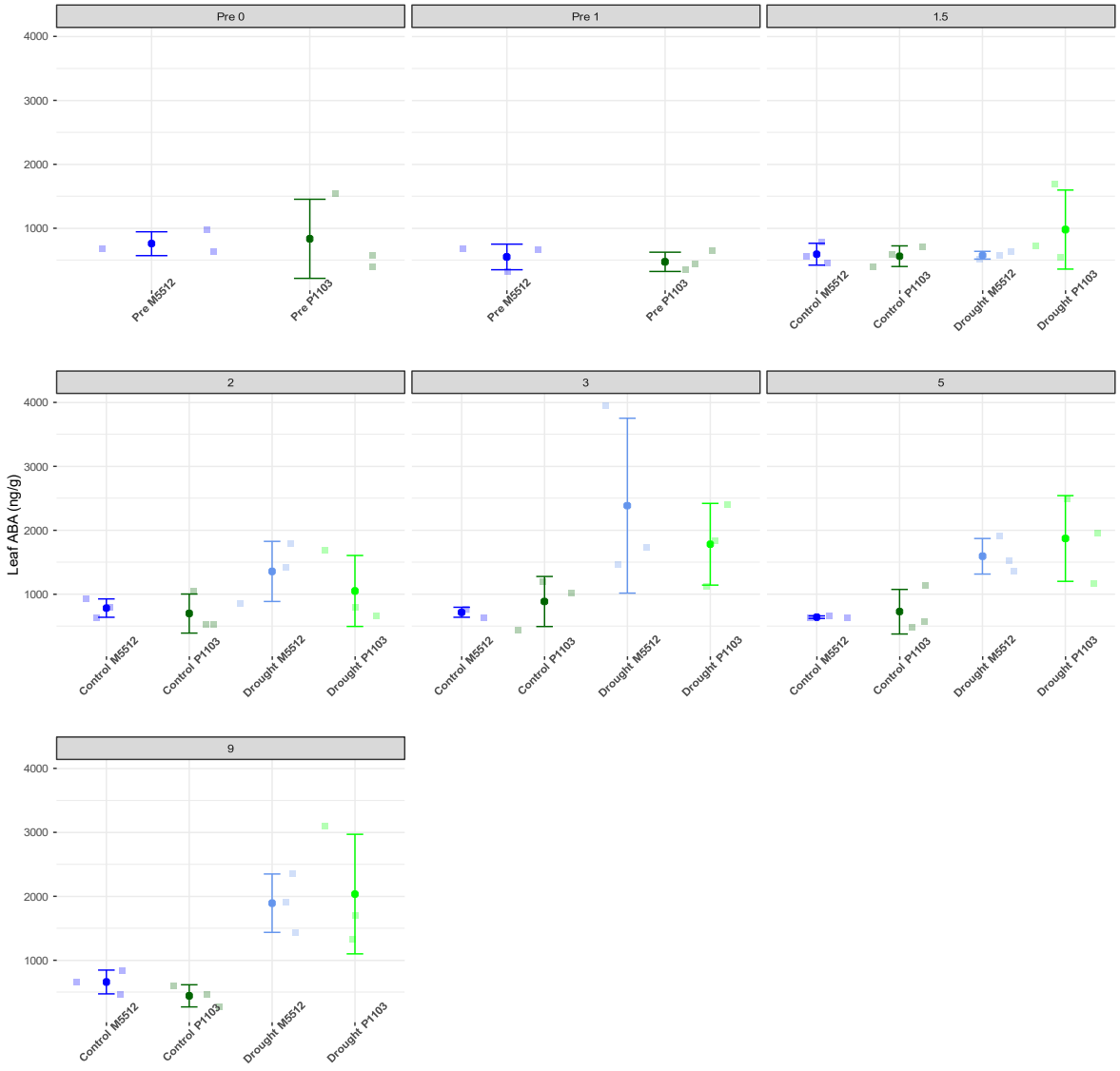


Figure 10 - leaf ABA concentrations of Cabernet Sauvignon vines grafted to M5512 or 1103P rootstocks and well-watered or subject to a soil water deficit. Data are means +/- SD.

Mean root ABA concentrations were always below 3 ng g^{-1} in the control vines and always above this in the deficit irrigated vines, reaching 200 ng g^{-1} on day three and 300 ng g^{-1} on day nine of treatment, a hundred-fold increase.

A change in PA concentration in root tissues of deficit irrigated vines was not observed until day three of the treatment and was significantly higher in the 1103 Paulsen vines ($p<0.001$; data not shown). However, in in the 1103 Paulsen vines, root PA only reached 74 ng g^{-1}. Root DPA concentrations were much higher, 550-1200 ng g^{-1} from days 3-9, and there was no significant effect of rootstock (data not shown). The difference in patterns of PA and DPA accumulation under water deficit between roots and leaves suggests that PA is converted to DPA more quickly than ABA to PA in roots, with either the reverse true in leaves.

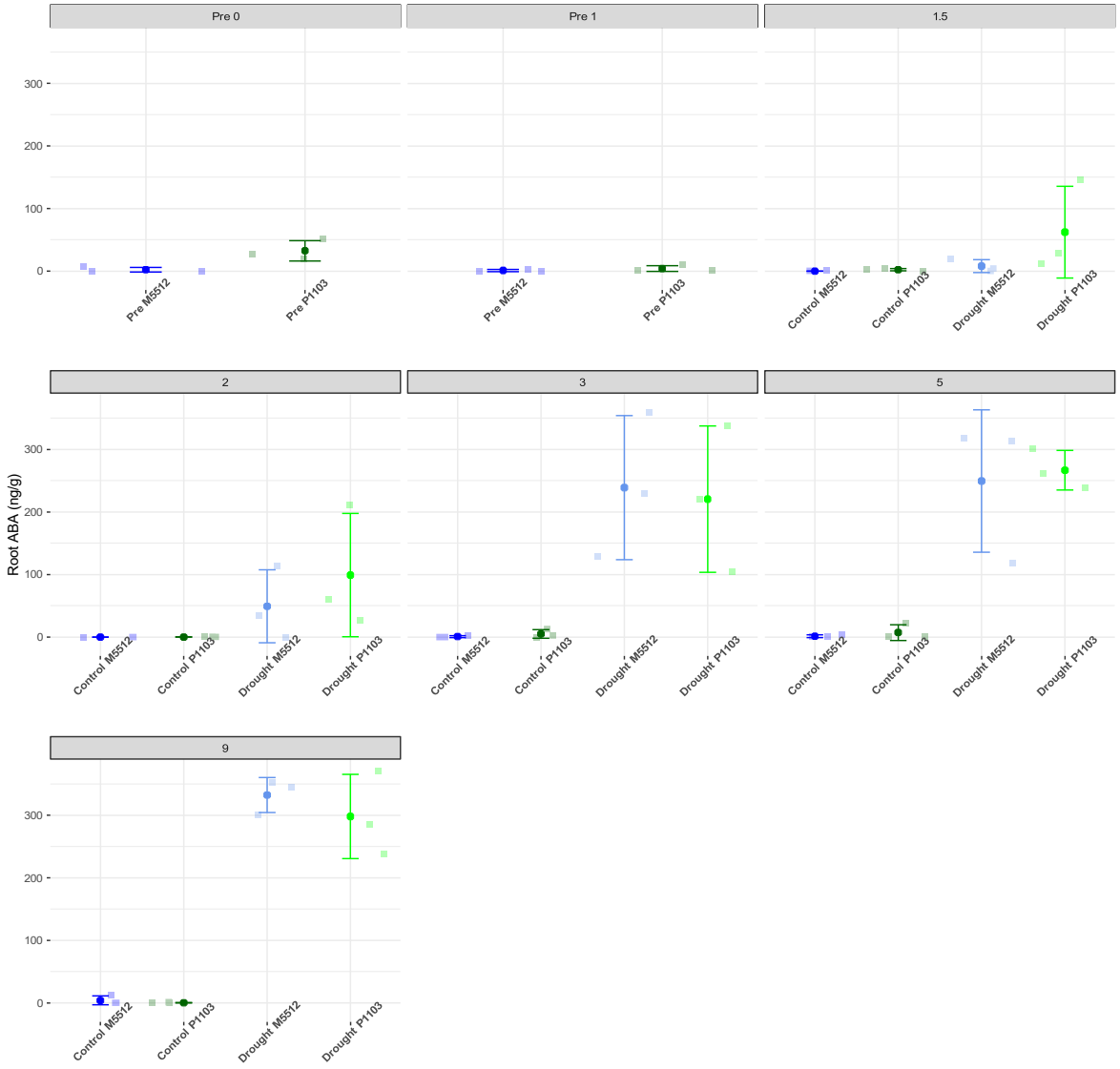


Figure 11 - root ABA concentrations of Cabernet Sauvignon vines grafted to M5512 or 1103P rootstocks and well-watered or subject to a soil water deficit. Data are means +/- SD.

Although it was not possible to identify the source of ABA in any of the experiments undertaken in this project, transcription of the key genes involved in ABA synthesis (NCED1; Zeevaart & Creelman 1998) and metabolism (HYD1; Speirs et al 2013) was examined in both root and leaf tissue. Transcription levels are reported relative to actin, a key 'housekeeping' gene least affected by the water deficit treatments.

Prior to the water deficit treatments being applied, there was no significant difference between rootstocks in leaf NCED1 expression (Figure 12), but a significant rootstock effect in root NCED1 expression ($p=0.006$), being higher in 1103 Paulsen (Figure 13), likely again as a

consequence of the lower available soil moisture for these vines and consistent with the results above.

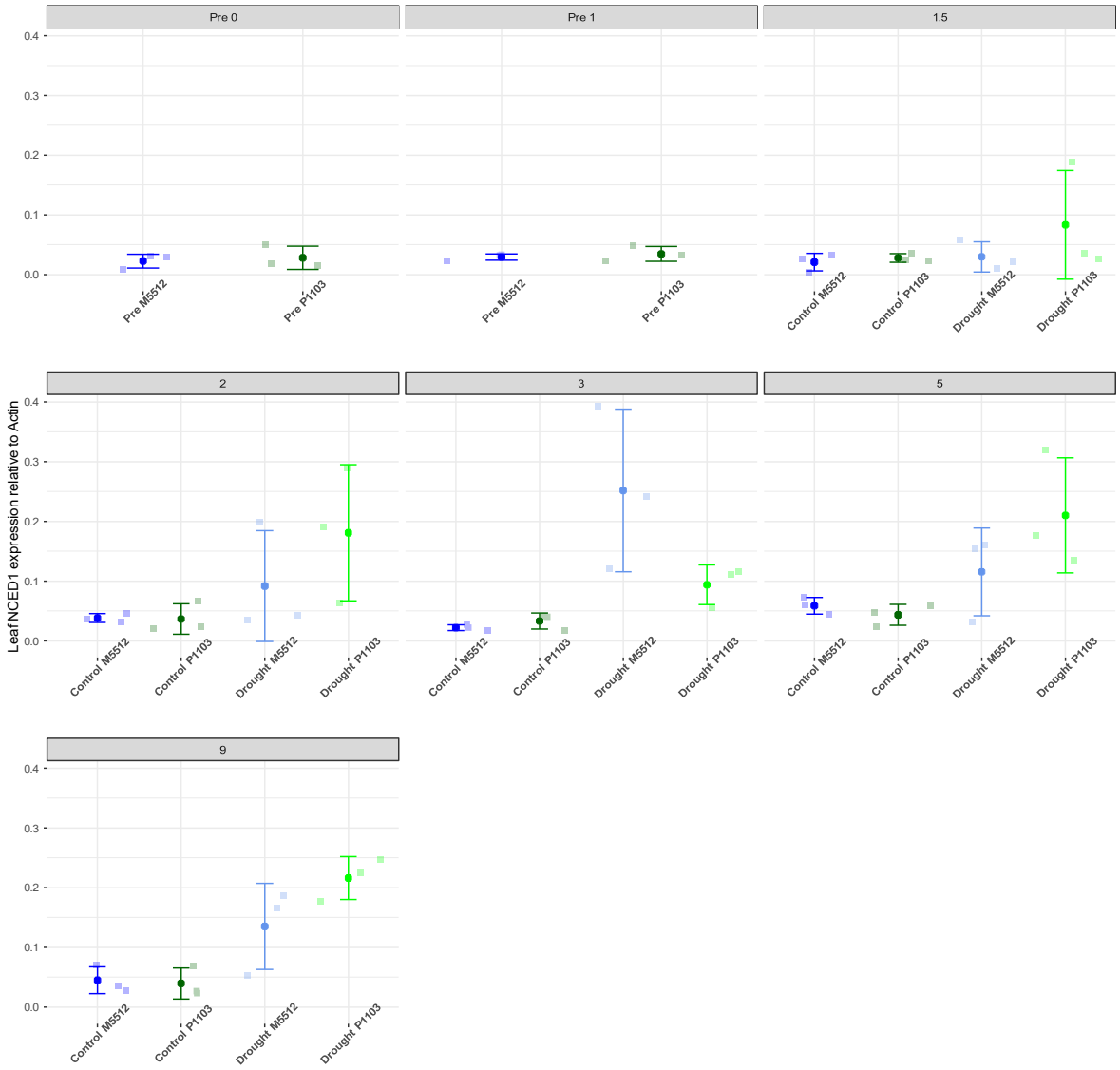


Figure 12 - NCED1 expression relative to actin in leaf tissue of Cabernet Sauvignon vines grafted to M5512 or 1103P rootstocks and well-watered or subject to a soil water deficit. Data are means ± SD.

During the water deficit treatment period, NCED1 expression was significantly higher in both leaf and root tissue of the deficit irrigated vines, with no effect of rootstock, and increased to day 3 in both tissues. On the first day of treatment, relative NCED1 expression in root tissues exposed to deficit was 0.025±0.011 and 0.065±0.029 for Merbein 5512 and 1103 Paulsen respectively, vs 0.004±0.002 and 0.003±0.001 for control vines. In the leaf tissues the results were 0.023±0.009 and 0.056±0.021 vs 0.015±0.006 and 0.027±0.010 respectively. Whilst

having limited statistical power, these results suggest a rapid initiation of NCED1 transcription in response to decreasing soil moisture, but also perhaps that the larger relative increase in NCED1 expression in the roots may indicate a faster response in those tissues. Furthermore, the NCED1 expression levels, relative to actin, ranged from 0.34 to 1.08 in the root tissues (days 3-9), but only 0.09 to 0.23 in the leaf tissues, again potentially indicating a stronger transcription response to water deficit in the root system.

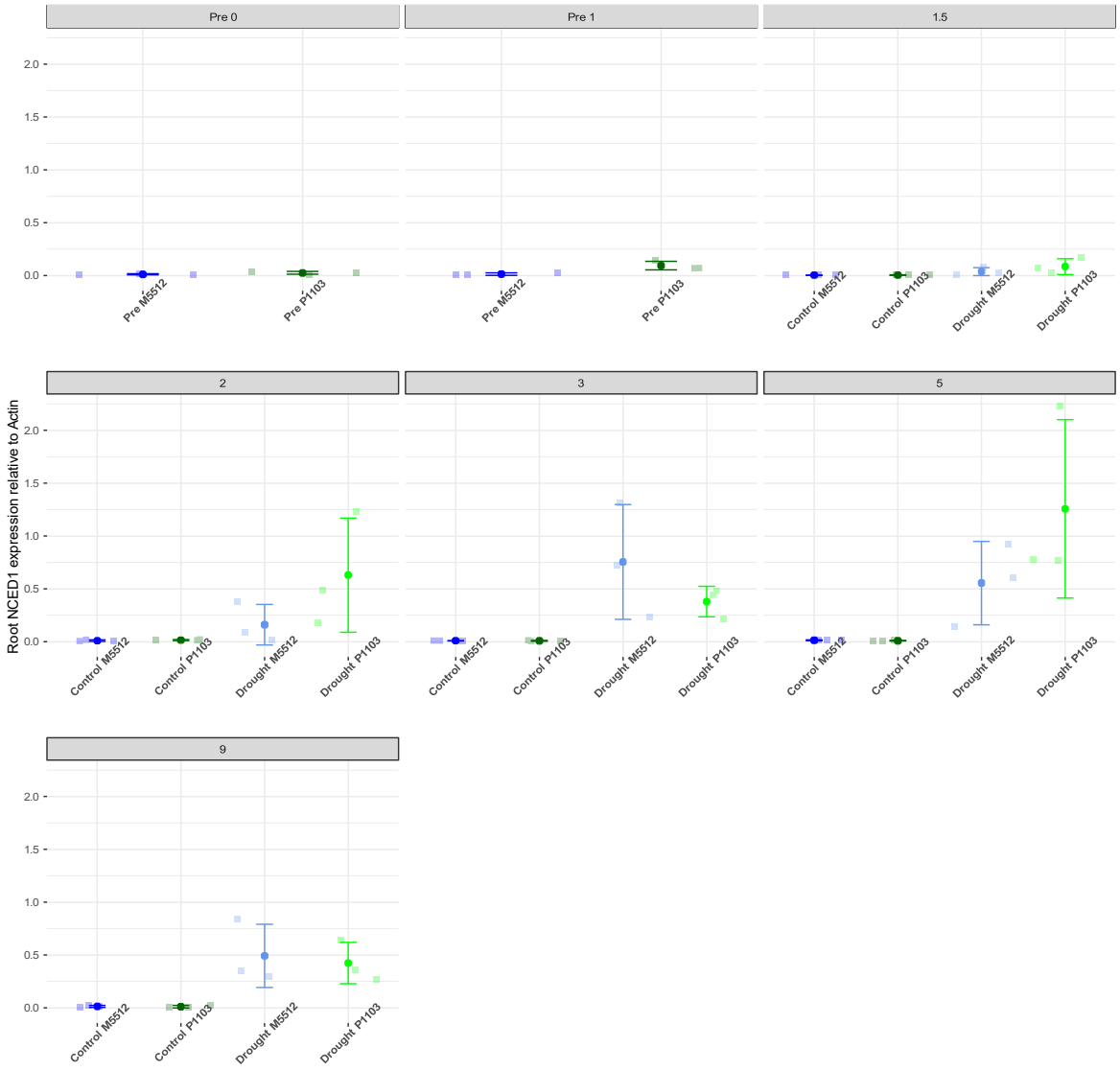


Figure 13 - NCED1 expression relative to actin in root tissue of Cabernet Sauvignon vines grafted to M5512 or 1103P rootstocks and well-watered or subject to a soil water deficit. Data are means +/- SD.

Relative NCED2 expression in the leaves was very low (<0.02) and not significantly affected by water deficit (data not shown). In contrast, NCED2 expression in the roots was already higher in deficit irrigated vines than control vines on the first day of deficit (Figure 14) and reached

expression levels close to NCED1 in leaves on day 3 (0.132±0.040 and 0.167±0.051 for Merbein 5512 and 1103 Paulsen respective).

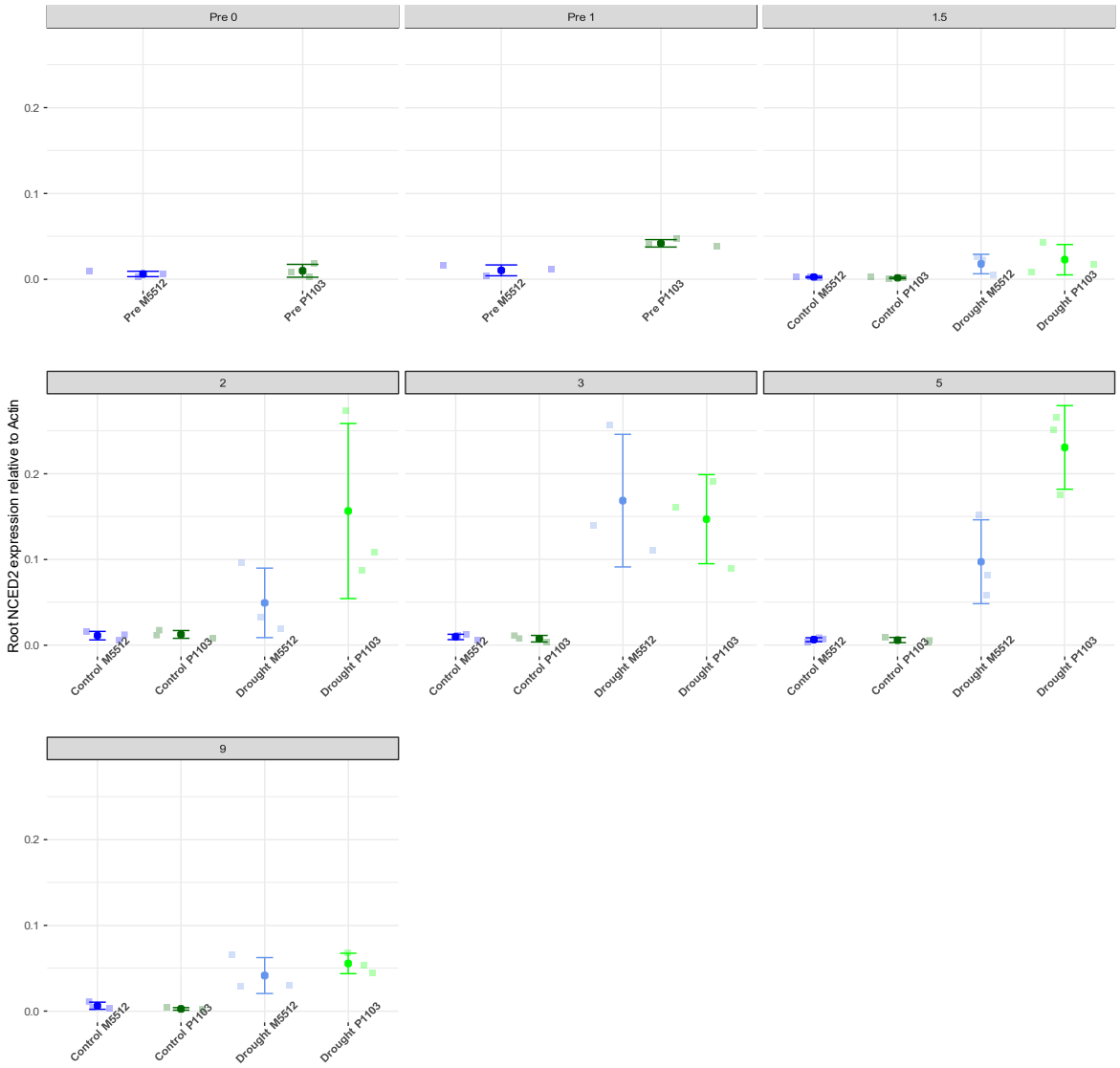


Figure 14 – NCED2 expression relative to actin in root tissue of Cabernet Sauvignon vines grafted to M5512 or 1103P rootstocks and well-watered or subject to a soil water deficit. Data are means +/- SD.

Relative expression of the HYD1 gene, which encodes for a protein which catalyses the rate limiting step in ABA metabolism, in root tissues was low (<0.02) and although there was a significant treatment effect it was small and inconsistent, with a larger day-to-day variation (data not shown). There was also a significant variation between days in leaf tissue expression of HYD1, but this was overlaid with a reduction in HYD1 expression in the deficit irrigated vines () from day 3 onwards ($p<0.001$). The mean relative expression of HYD1 in control vines ranged

from 1.2-1.8, cf. 0.5-1.0 in the deficit vines. The higher expression of HYD1 than NCED in the leaves was likely indicative of the relatively high ABA concentrations in those tissues and the decrease with water deficit a mechanism to ensure g_s remains depressed to conserve water, through reduced ABA metabolism.

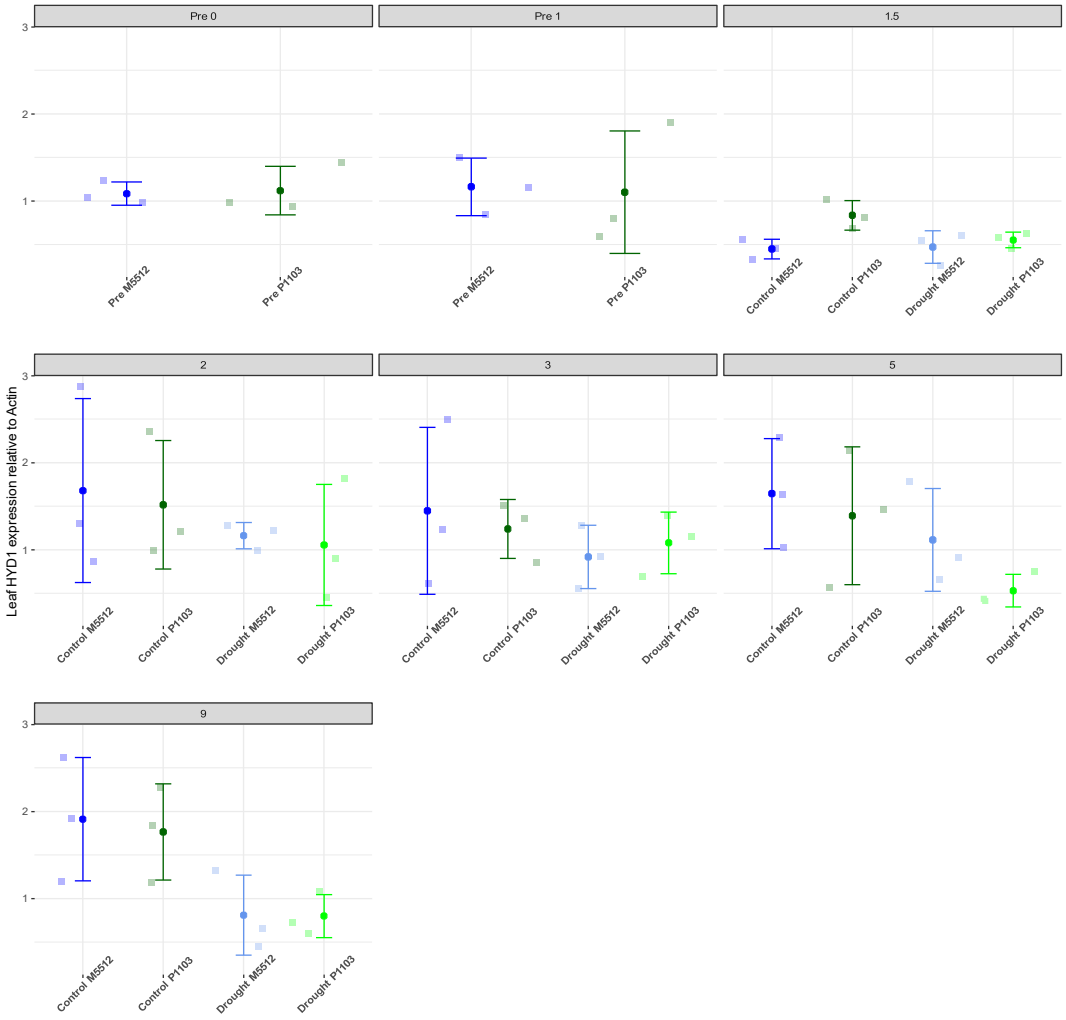


Figure 15 – HYD1 expression relative to actin in root tissue of Cabernet Sauvignon vines grafted to M5512 or 1103P rootstocks and well-watered or subject to a soil water deficit. Data are means +/- SD.

3.2 Experiment 2: water deficit response of three contrasting rootstocks under fine-grained control of soil moisture

The previous experiment did not find evidence of differences between two rootstocks in their response to soil water deficit, but control of soil moisture was limited by the requirement to hand water to weight and there were rootstock driven differences in starting conditions as a result. The APPF DroughtSpotter platform offered an opportunity to precisely control soil moisture by continuously weighing the pots and watering up to every 15 minutes as required to maintain a set weight. The automated nature of the platform also had the capacity to add in a third rootstock and Ramsey was chosen, due to its known high conferred vigour (ref).

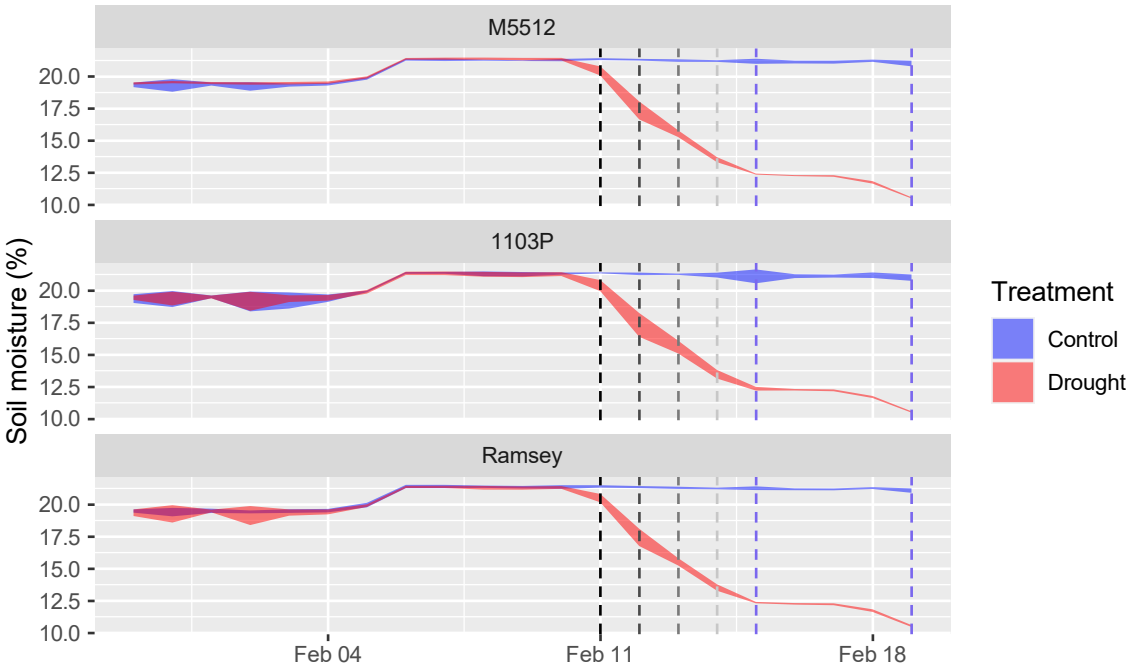


Figure 16 - volumetric soil moisture of Cabernet Sauvignon vines grafted to three rootstock genotypes. The ribbons show mean daily soil moisture ±SD (n=6). Black/grey dashed lines indicate changes in set points for soil moisture, blue dashed lines indicate sampling dates (15 and 19 Feb).

The platform was successfully used to step down the soil moisture, ensuring there was no effect of rootstock conferred vigour on the rate of dry down and all three rootstocks reached soil moisture targets at the same time (Figure 16). The initial soil moisture target was chosen based on experience, but g_s was tracked to ensure that an adequate impact on leaf physiology occurred. The intention was to reach a g_s of approximately 75-100 mmol m^{-2} s^{-1} , take an initial tissue sample from the vines and then maintain that soil moisture level before taking a second sample set. However, it was observed that the g_s of the water deficit treated vines began to rise again almost immediately and after three days was approaching the g_s of the control vines, despite a much lower available soil moisture (Figure 17). In addition, the data from the

DroughtSpotter indicated that the water use by the deficit treated vines was rising (data not shown). The platform doesn't limit the amount of water provided to an individual pot, but simply replaces what has been used until it reaches a set value. Thus, if a vine is able to adjust its physiology to the lower soil moisture level, perhaps by osmotic adjustment or a similar mechanism but still take up water it is able to use as much water as a vine at a much higher soil moisture level. It appears that this is exactly what was happening. Although an interesting observation that is worth following up, it was a potential issue for our experiment. Consequently, a further reduction in soil moisture was established and the second tissue sampling undertaken before further adjustment could be made.

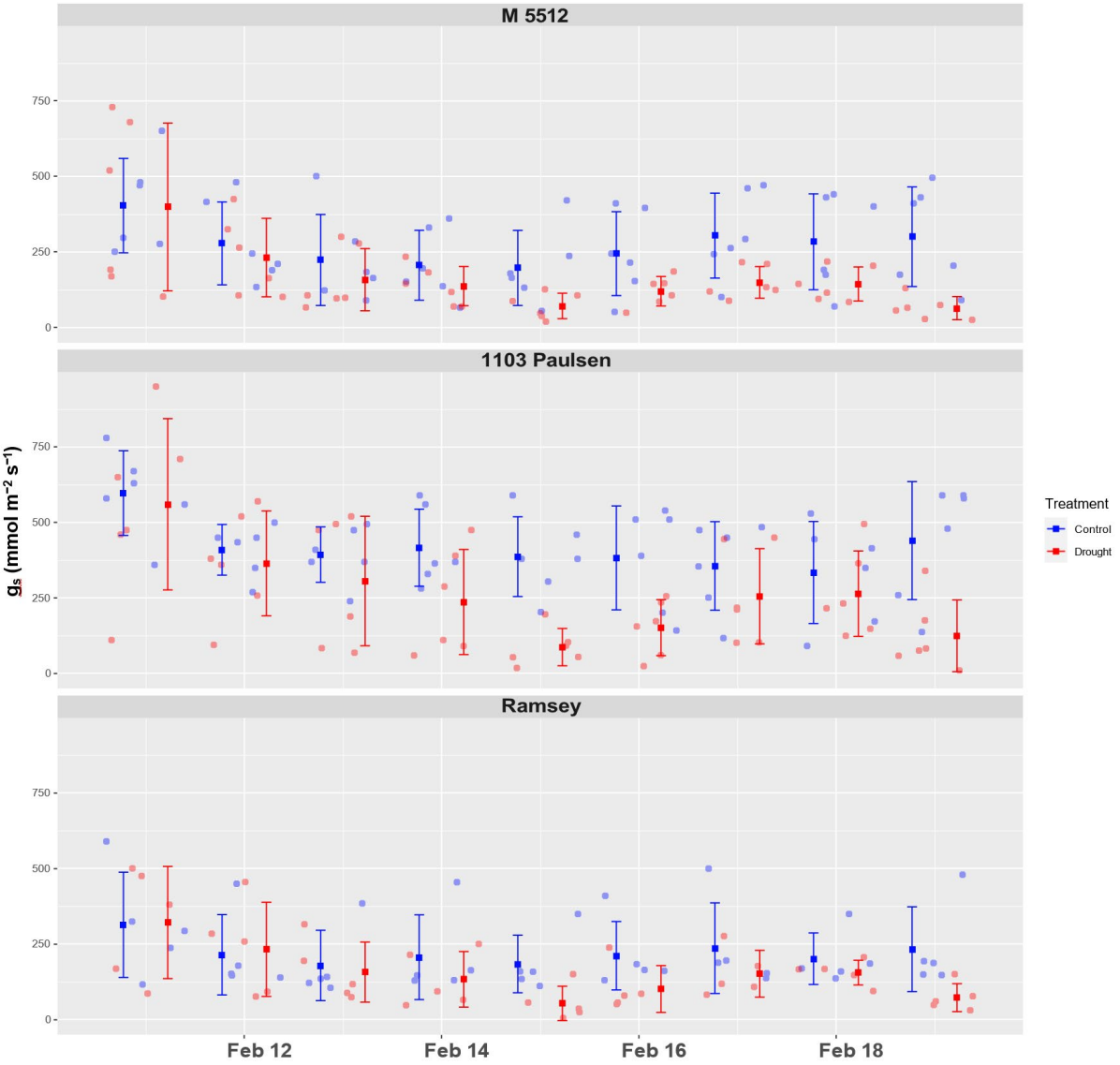


Figure 17 - stomatal conductance (g_s) of Cabernet Sauvignon vines grafted to M5512, 1103P or Ramsey rootstocks and well-watered or subject to a soil water deficit. Data are means ±SD and displayed as pairs (control/drought) around measurement date.

A second issue that can be seen in the g_s results was a decline in the g_s of the control vines, despite adequate soil moisture being maintained (Figure 17). The DroughtSpotter platform had an LED system set up as supplemental lighting to ensure the vines didn't begin to go into dormancy with the declining daylength, but either because of this, or despite this, some senescence of the leaves occurred, which was particularly noticeable in the Ramsey vines (visual observation). These differences in g_s of the control vines resulted in a significant rootstock effect ($p<0.001$), despite similar values between rootstocks under water deficit.

At the two tissue sampling dates, the end of the initial dry down and following a second dry down, Ψ_{stem} measurements were made as an additional estimate of the physiological impact of the water deficit. Despite the issues noted above, the water deficit treatment resulted in significantly lower Ψ_{stem} values, indicative of moderate water stress, with no significant effect of rootstock (Figure 18). Consequently, with the lack of any difference in soil moisture between rootstocks at any time in the experiment and the physiological evidence of water stress as a result of the water deficit treatment, the experiment was considered as a suitable basis to assess possible differences in grafted rootstocks to a given soil stress.

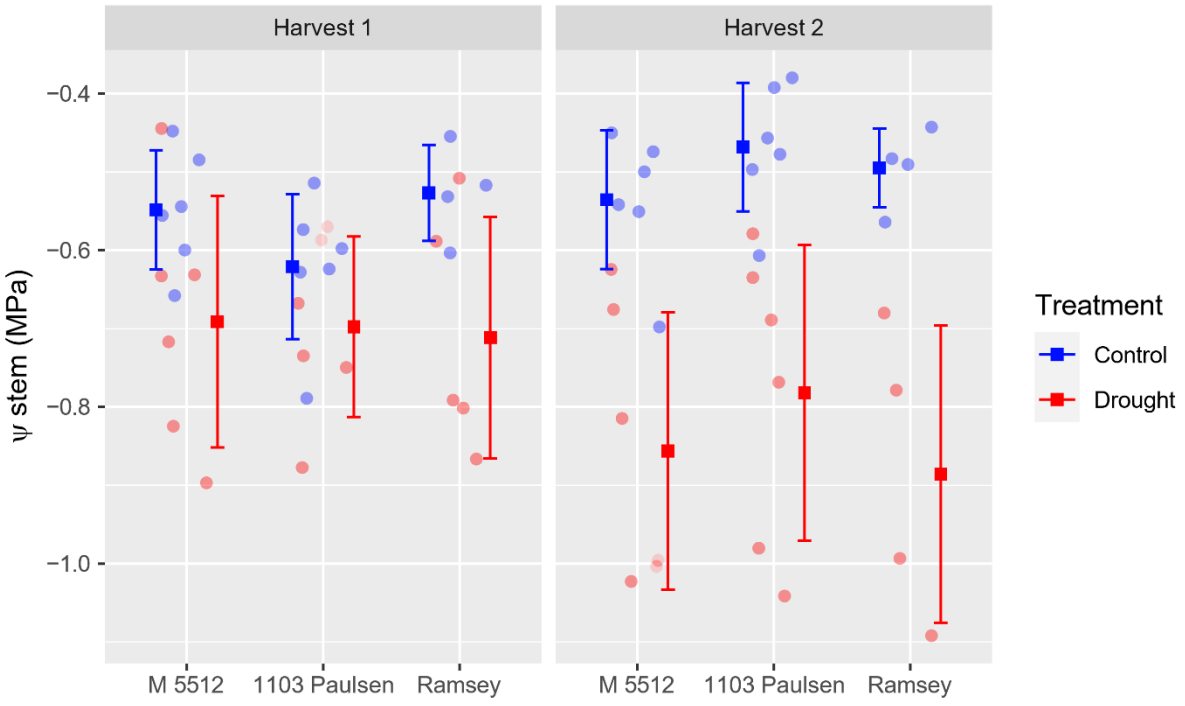


Figure 18 - stem water potential (Ψ_{stem}) of Cabernet Sauvignon vines grafted to M5512, 1103P or Ramsey rootstocks and well-watered or subject to a soil water deficit. Data are means ±SD.

Potential differences in rootstock responses to identical soil moisture conditions were examined through changes in plant hormone concentrations and changes in key genes involved in ABA synthesis and metabolism, similarly to experiment 1.

Xylem sap hormone concentrations were assessed using the same method as for experiment 1. ABA concentrations were not dissimilar from the steady state water deficit period during experiment 1, indicating a similar degree of soil water stress. The increase in sap ABA with water deficit treatment was highly significant and there was no effect of rootstock, or of sample time, despite the differences in soil moisture (Figure 19). Of the ABA metabolites, ABA-GE was only present in trace amounts and not impacted by rootstock or treatment, DPA was also present only in small amounts, albeit slightly, but significantly higher at the second sampling date. PA was the major metabolite present in the sap samples, as with experiment 1, but the results were very variable and although the water deficit significantly increased the concentrations present, the increase was not large (Figure 19).

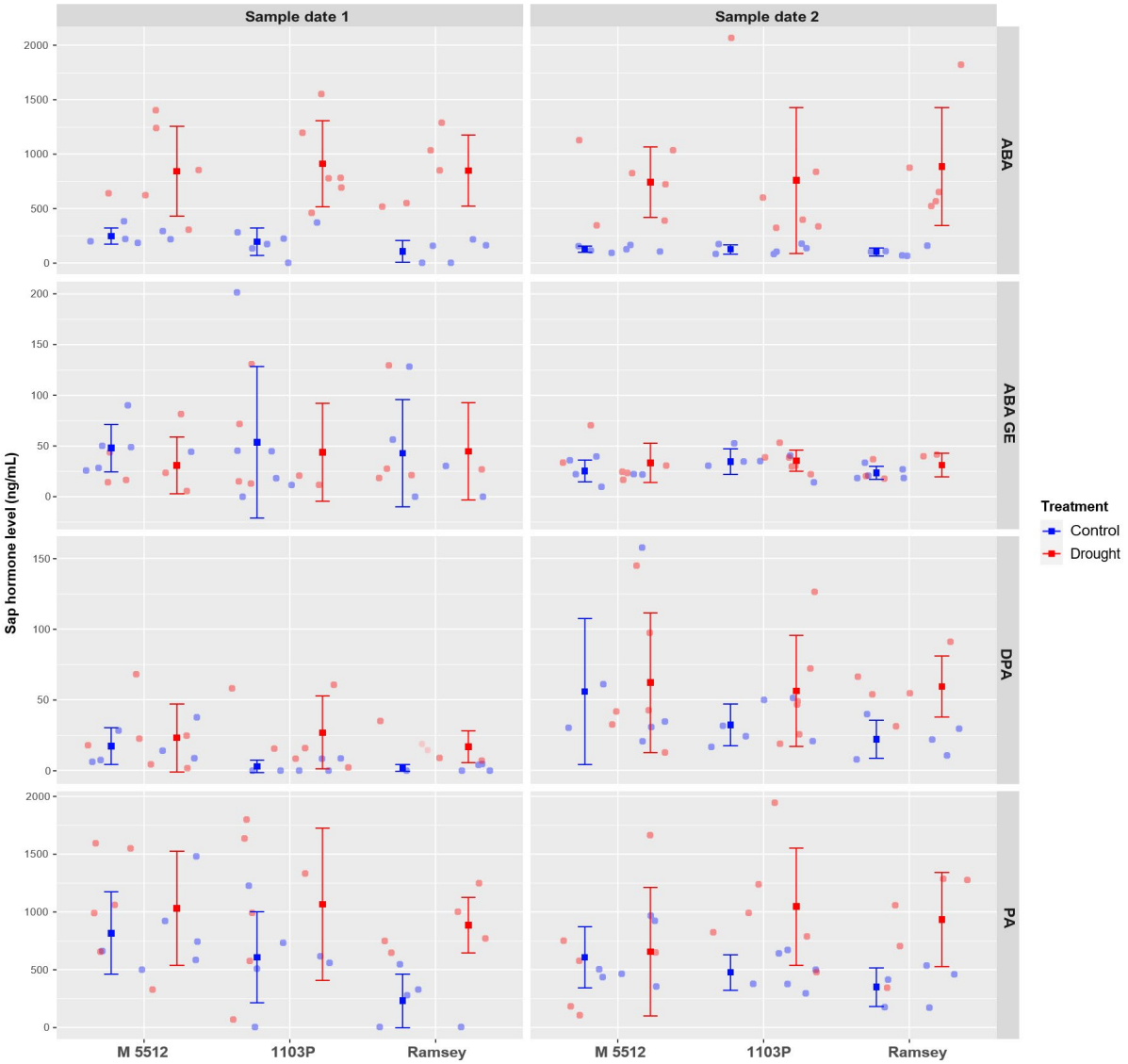


Figure 19 – xylem sap ABA and its metabolites, PA, DPA and ABA-GE of Cabernet Sauvignon vines grafted to M5512, 1103P or Ramsey rootstocks and well-watered or subject to a soil water deficit. Data are means ±SD.

The leaf and root tissue samples were analysed with an expanded assay that also provided the other major plant hormones, auxin (IAA), jasmonic acid (JA), salicylic acid (SA) and an additional ABA metabolite, neo-phaseic acid (neo-PA).

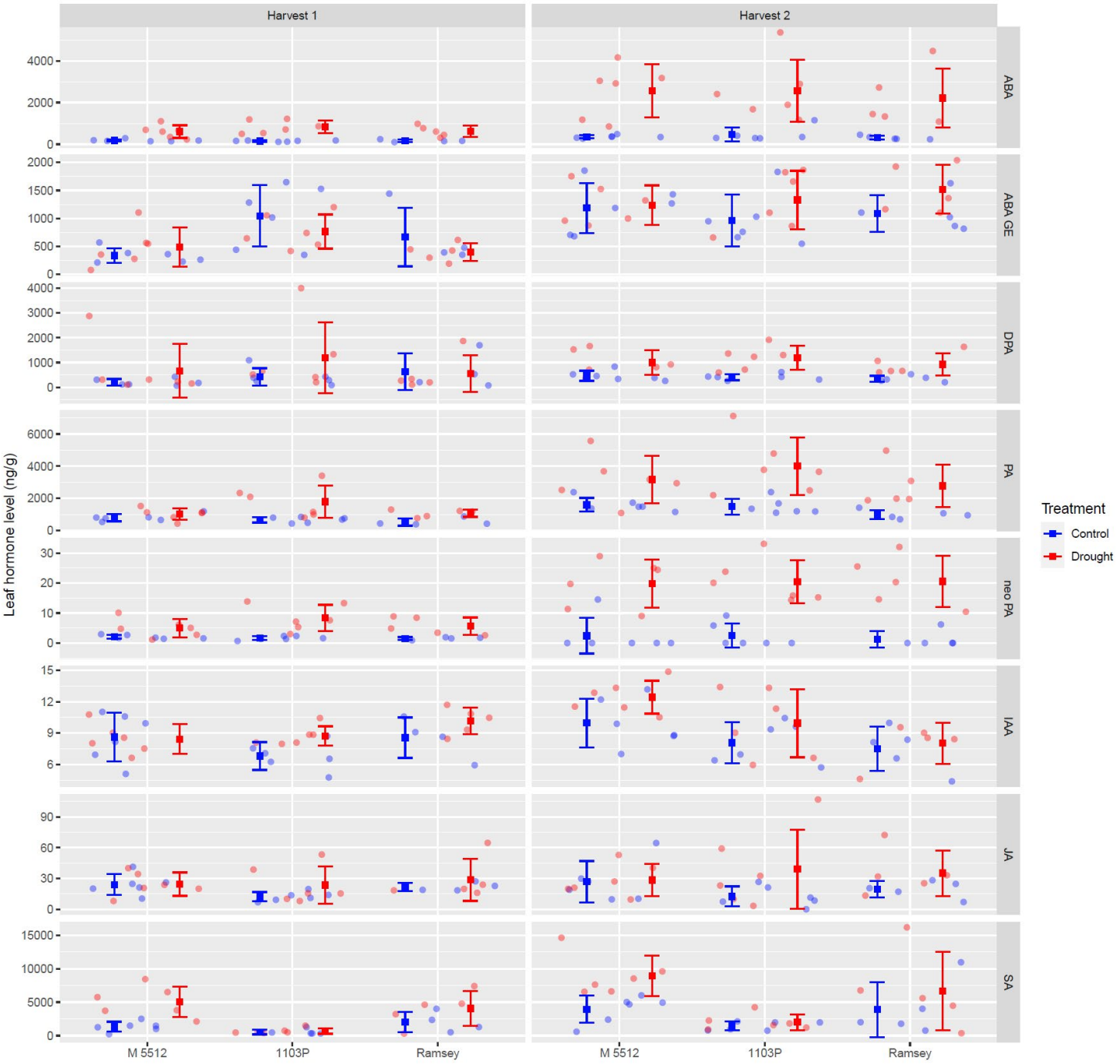


Figure 20 – concentration of key plant hormones at each sampling date in leaves of Cabernet Sauvignon vines grafted to M5512, 1103P or Ramsey rootstocks and well-watered or subject to a soil water deficit. Data are means ±SD.

Leaf ABA concentrations in the water deficit treatment increased between the two sampling dates (i.e. with duration and severity of water stress), having similar values to the steady state water deficit period of experiment 1 at the second sample date (Figure 20). Leaf ABA also increased in the control treatment, albeit to a much lesser extent, possibly due to the partial leaf senescence observed. However, at the second sample date the control values were no higher than those of control leaves in experiment 1. There was no rootstock effect.

The ABA metabolite PA was present in the leaves at higher concentrations than ABA in all the samples, with PA at the second sample date also significantly higher than the first and significantly higher under water deficit. There was also a significant rootstock effect, 1103 Paulsen accumulated more leaf PA than the other rootstocks ($p=0.005$). Leaf DPA followed a similar pattern, but the rootstock effect was not significant and, overall, DPA concentrations were lower than PA. Neo-PA was significantly higher under water stress and this effect was increased at the second sampling date. However, the amounts were extremely small (<20 ng g^{-1}) and not physiologically meaningful. It is likely that the neo-PA production is a side reaction (Bai et al 2022) and simply reflects increase amounts of ABA in the leaf tissue.

There were significant effects of water deficit on the other three hormones measured, IAA, JA and SA, but they were not large enough to be meaningful and not very consistent (Figure 20).

Root samples were only collected at the final sampling date. ABA concentrations were 10-20 times higher under the water deficit treatment, and there was a significant rootstock effect, with Merbein 5512 highest and Ramsey lowest (Figure 21). Given the high concentrations compared to experiment 1 (500-785 ng g^{-1} vs 300 ng g^{-1}), the high xylem sap ABA concentrations and the low g_s values, it is difficult to be sure whether this limited rootstock effect is meaningful, particularly as recirculation can occur with ABA moving to the root system from the shoot via the phloem. Furthermore, this difference was not reflected in the ABA metabolites, PA and DPA. DPA was again being the primary metabolite accumulating in the root, with much higher concentrations than PA (Figure 21). There were rootstock effects ($p=0.03$ for DPA, $p=0.02$ for PA), but they were not large effects or consistent between metabolites or with ABA.

As with the leaf hormones, there were small inconsistent effects of treatment or rootstock on the other hormones, IAA, JA, SA, but they were unlikely to be physiologically meaningful.

Expression of the ABA synthesis gene NCED1 was examined in leaf and root tissue (Figure 22). In both tissues NCED1 expression was significantly upregulated in response to water deficit, relative to actin expression. There were weakly significant effects of rootstock in leaf ($p=0.050$) and root ($p=0.063$), but neither were clear and there was no rootstock:water deficit interaction, so no evidence of a difference of rootstock NCED1 expression in response to drought, matching the lack of any observed interaction in ABA concentration. As for experiment 1, NCED2 was expressed in roots to a similar degree as NCED1, but there was no change in leaf tissues (Figure 22). Again, there was no interaction between rootstock and water stress.

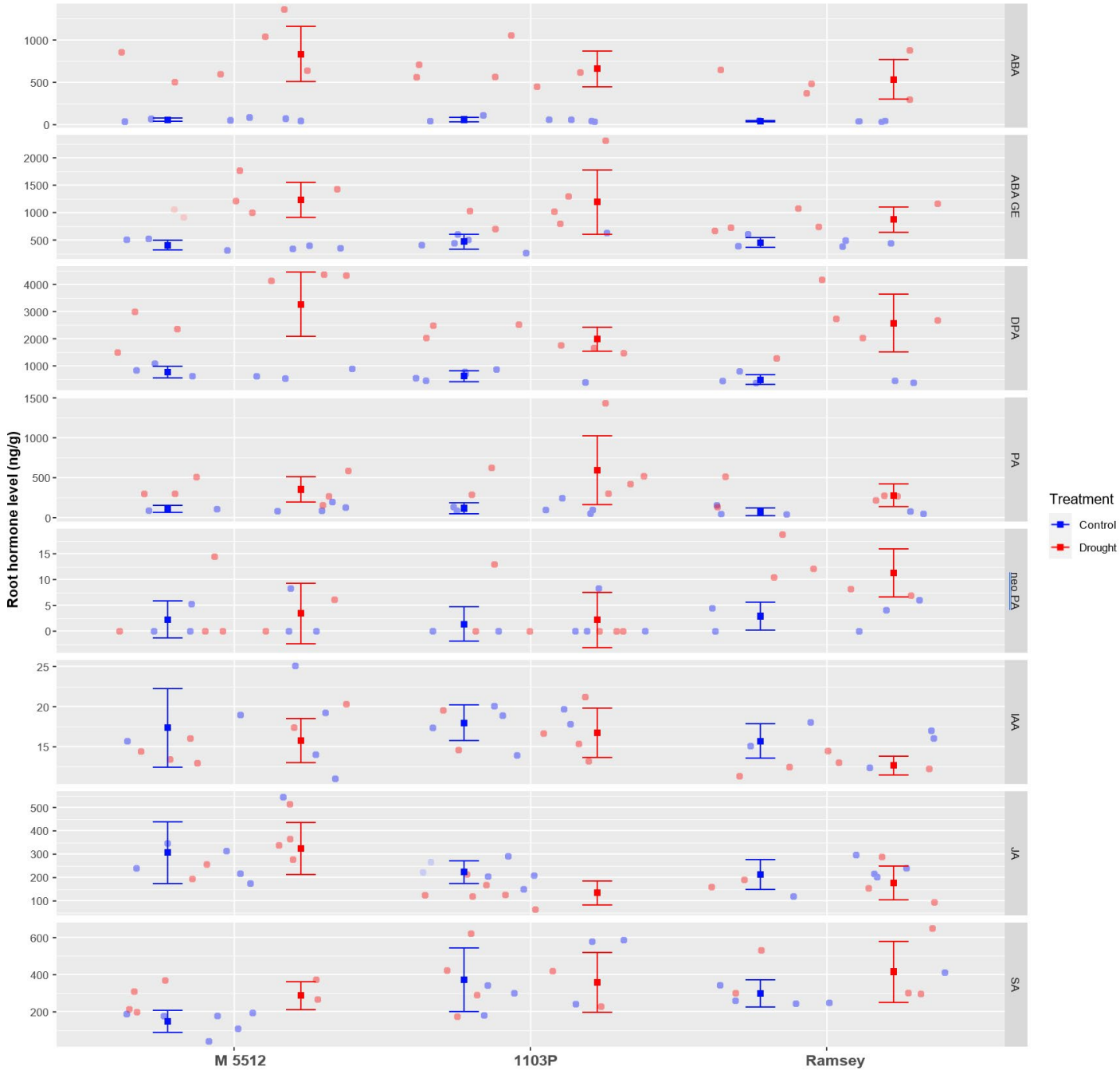


Figure 21 - concentration of key plant hormones at the final sampling date in roots of Cabernet Sauvignon vines grafted to M5512, 1103P or Ramsey rootstocks and well-watered or subject to a soil water deficit. Data are means ±SD.

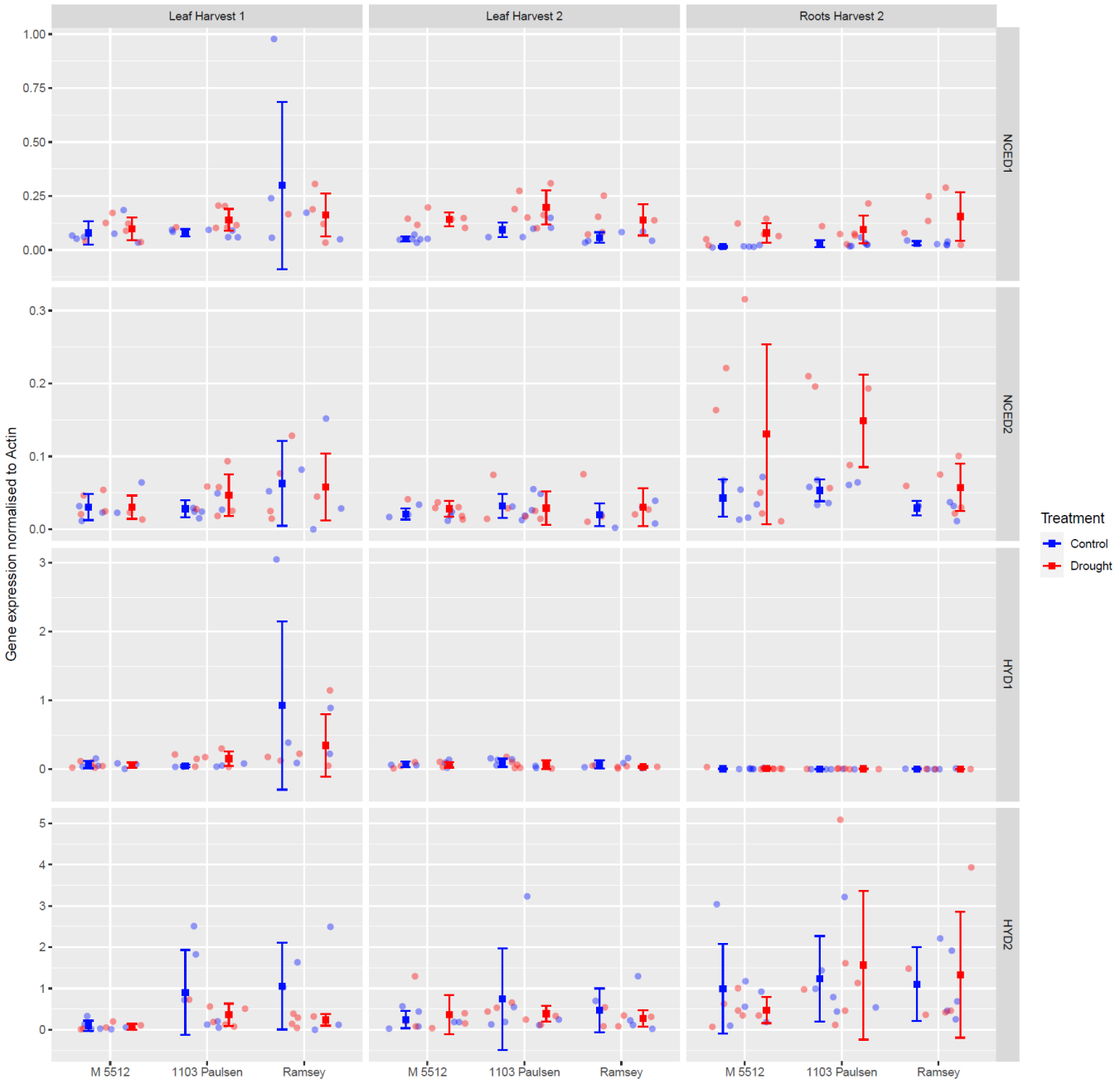


Figure 22 – expression of key genes related to ABA synthesis and metabolism in leaves (sample date 1 & 2) and roots (sample date 2) of Cabernet Sauvignon vines grafted to M5512, 1103P or Ramsey rootstocks and well-watered or subject to a soil water deficit. Data are means ±SD.

Transcription of the ABA metabolism genes HYD1 and HYD2 was also examined, but neither were affected by water stress in either leaf or root tissue (Figure 22). Overall expression levels of HYD1 were low, whereas expression levels of HYD2 were similar to those of HYD1 in experiment 1.

3.3 Experiment 3: physiology of rootstock conferred vigour

Shoot vigour is perhaps the most prominent and commonly and consistently reported rootstock conferred trait, but the mechanism behind it is still unconfirmed. Experiments 1 and 2 did not support a role for differences in root responses to the soil environment between rootstock genotypes, which could result in cumulative growth differences over time in the field. We previously reported differences in maximum sap flow under high evaporative demand between scions grafted to different rootstocks (Edwards et al 2022), with a hypothesis that differences in hydraulics could be a potential explanation. The first of our experiments to address this used seven different rootstocks grafted to a single scion, grown under well-watered conditions to exclude possible cumulative effects of differences in soil stress responses. The vines were grown in pots under semi-controlled conditions, with growth and physiology differences assessed over two whole plant harvests, nine months apart.

Conferred vigour was assessed through the total shoot length produced, the canopy size and aboveground dry matter production. Harvest 1 was undertaken at the end of the first growing season and represented the total growth since grafting. Harvest 2 was undertaken in early summer of the following season and represented the initial growth flush as a result of increasing daylength. Supplemental light was provided over winter to ensure the vines didn't enter dormancy, but this wasn't, and was not intended to be, adequate to maintain significant growth.

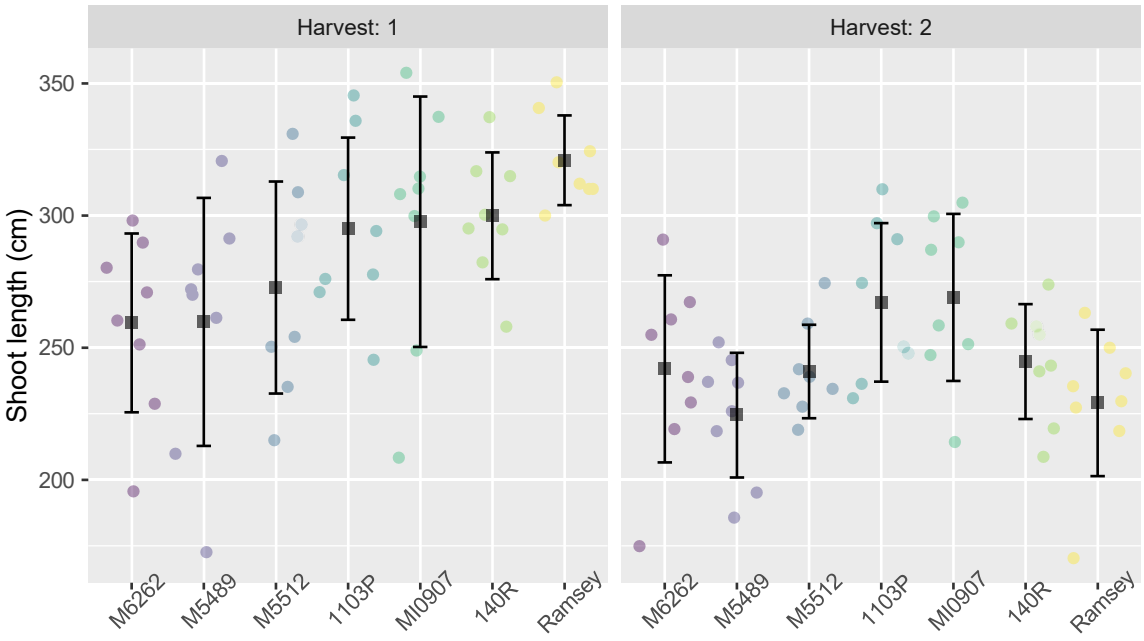


Figure 23 – total shoot length produced up to plant harvest of Cabernet Sauvignon vines grafted to seven different rootstocks. Data are means ±SD.

Shoot length produced (p<0.001; Figure 23), canopy area (p<0.001; Figure 24) and shoot dry mass (p=0.001; Figure 25) all varied significantly according to rootstock genotype grafted to the scion. There was a significant difference between the plant harvest dates in each case, expected given the natural difference in light environment between the two growing periods (season). However, there was also a significant interaction between rootstock and harvest.

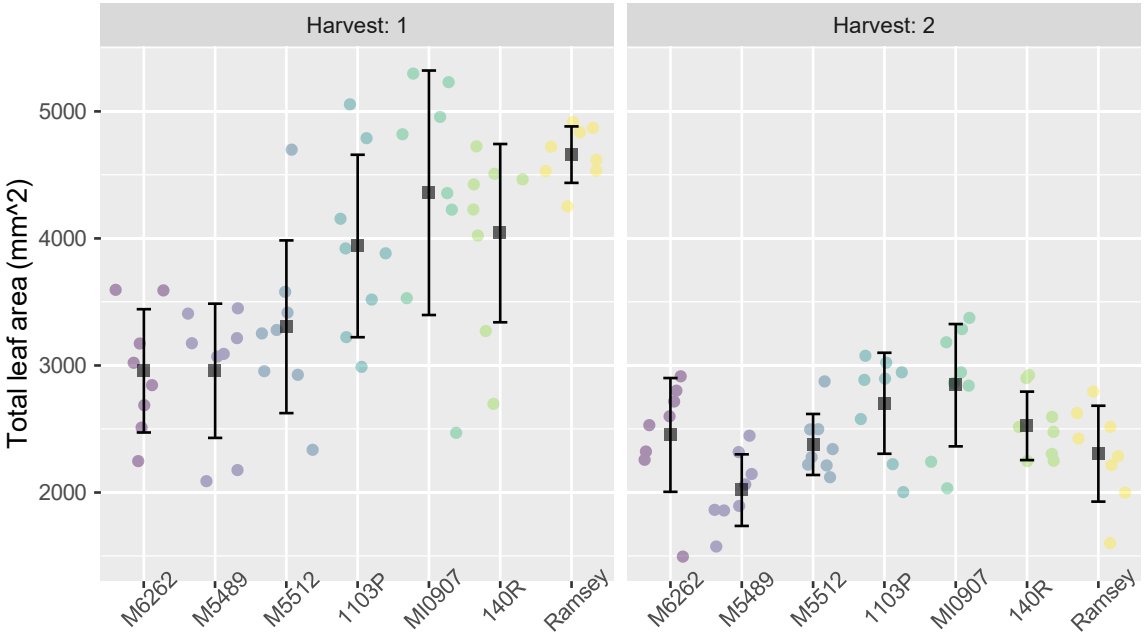


Figure 24 - total canopy area at plant harvest of Cabernet Sauvignon vines grafted to seven different rootstocks. Data are means ±SD.

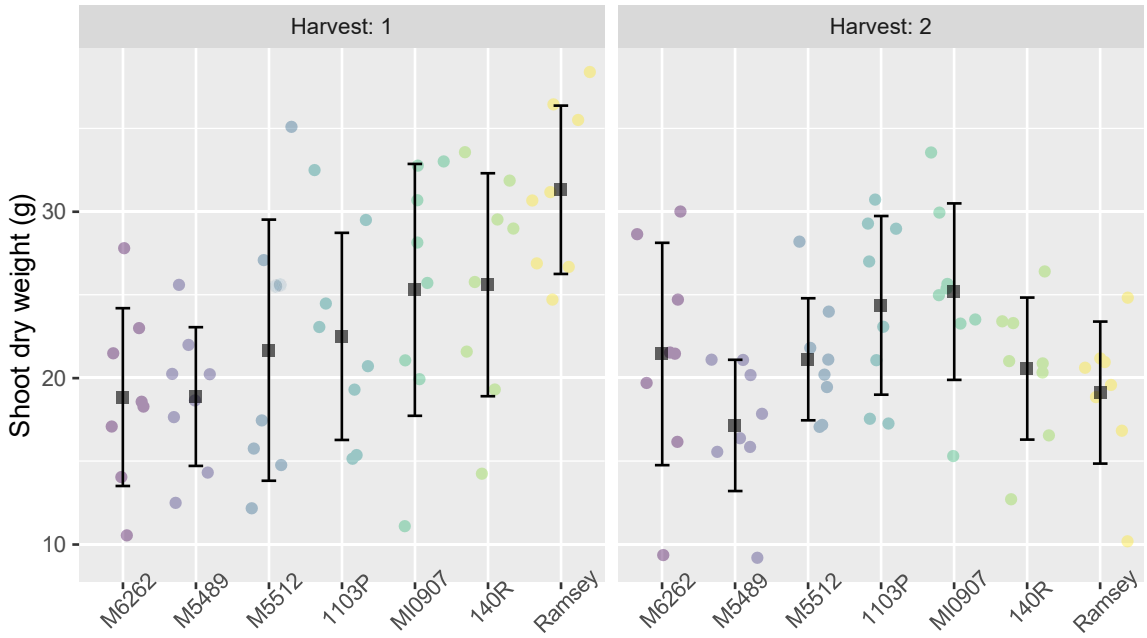


Figure 25- - total shoot dry matter produced up to plant harvest of Cabernet Sauvignon vines grafted to seven different rootstocks. Data are means ±SD.

The main driver of the rootstock by harvest interaction was Ramsey, which had the highest stem length, shoot dry mass and canopy size at harvest 1, but the second lowest, to Merbein 5489, at harvest 2. The remaining rootstocks had some variation in vigour order, but were strongly correlated between the harvests (e.g. r^2=0.689 for mean shoot dry mass, n=6). Across both harvests canopy dry mass and shoot dry mass were strongly correlated (data not shown), with the canopy dry mass forming between 50-60% of shoot dry mass.

Despite the reduction in Ramsey biomass production at harvest 2, Ramsey and Mi0907 exhibited the highest total vigour across the course of the experiment (Figure 26) and Merbein 5489 the lowest. With the exception of Merbein 5489 having lower shoot dry matter production than Merbein 6262, rootstock conferred vigour of the seven rootstocks matched previously reported effects on mature field grown vines.

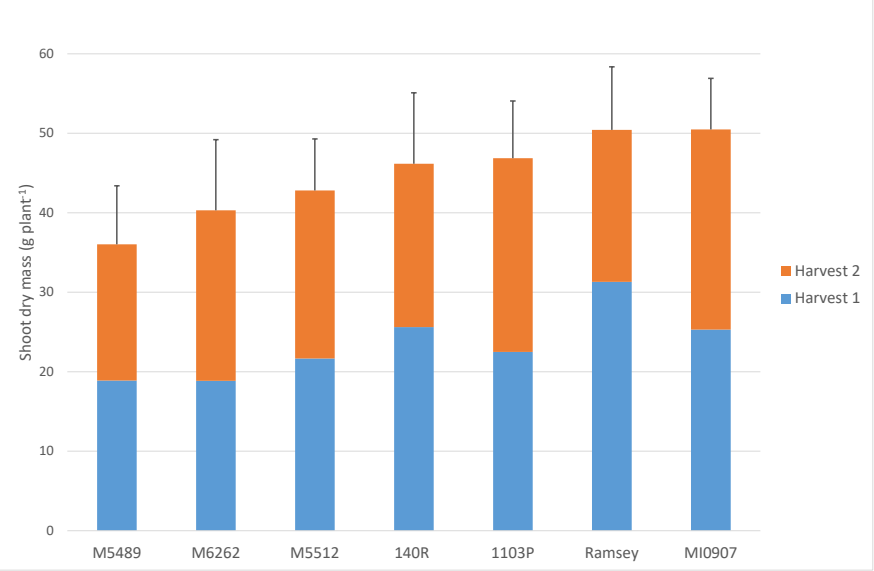


Figure 26 - total shoot dry matter produced over 16 months by Cabernet Sauvignon vines grafted to seven different rootstocks. Data are means ±SD.

Intriguingly, there was a strong correlation between the mean single leaf size and the total canopy area (Figure 27), suggesting that the major driver of canopy vigour was node/leaf number. Stem length and canopy size were also highly correlated (data not shown r^2=0.944), but the stem length and shoot dry matter correlation was offset by harvest (data not shown, r^2>0.9 for each harvest separately), likely due to the different time of season the plants were harvested for growth data (Dec vs March) and vine age.

Trunk diameter of the scion was also measured at the second harvest (data not shown) and correlated with total shoot dry matter production (r^2=0.428, n=7), however Merbein 5489 was an outlier (lower than expected shoot dry matter) and excluding this rootstock improved the correlation to r^2= 0.781 (n=6).

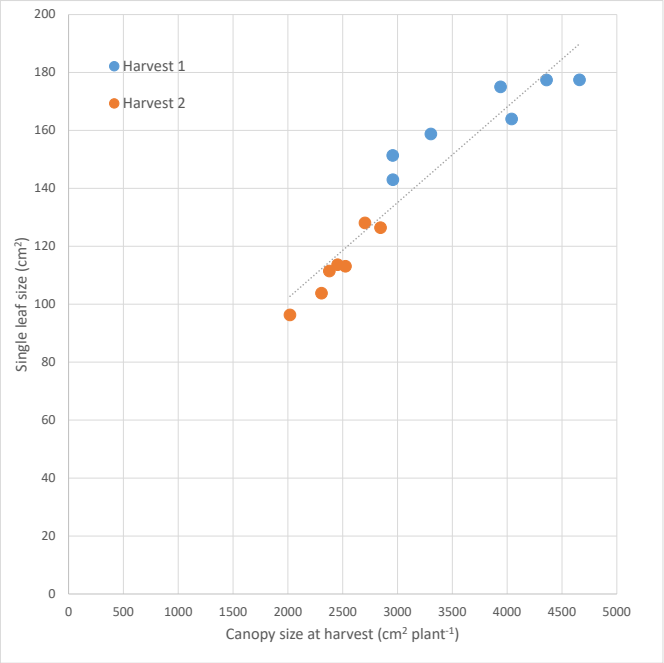


Figure 27 - correlation between single leaf size and total canopy area at harvest (r^2=0.903). Data are means of eight replicates.

Although this was a pot experiment, which will typically affect root growth in different ways to natural environments (Passioura 2006), the root systems were examined in order to make comparisons with the observed rootstock conferred shoot vigour. Root dry mass differences between rootstocks were small at the first plant harvest (Figure 28, left panel), but there was a significant effect of rootstock at the second plant harvest (Figure 28, right panel). Unlike the shoots, there was no pruning of roots occurring, so the total root dry mass was greater at the second harvest, except for Merbein 5489.

Although the vines with the lowest shoot biomass production, Merbein 5489, had the lowest root biomass at harvest 2 and the vines with the highest shoot biomass production, Mi0907, had the highest root biomass harvest 2, there was no overall correlation between root biomass production and shoot biomass production at harvest 2 (r^2=0.203), or harvest 1 or when comparing the harvest 2 root biomass with total shoot biomass production across the entire experiment. Furthermore, there was no correlation between root length or canopy size at either harvest or across harvests.

The ratio of root and shoot biomass at harvest 1 was lowest in Ramsey grafted vines and highest in Merbein 6262 grafted vines (data not shown). When calculated across the whole experiment (root dry mass at harvest 2 / total shoot production up to harvest 2) Ramsey still had the lowest root:shoot ratio, but Mi0907 had the highest, with Merbein 6262 second (Figure 29).

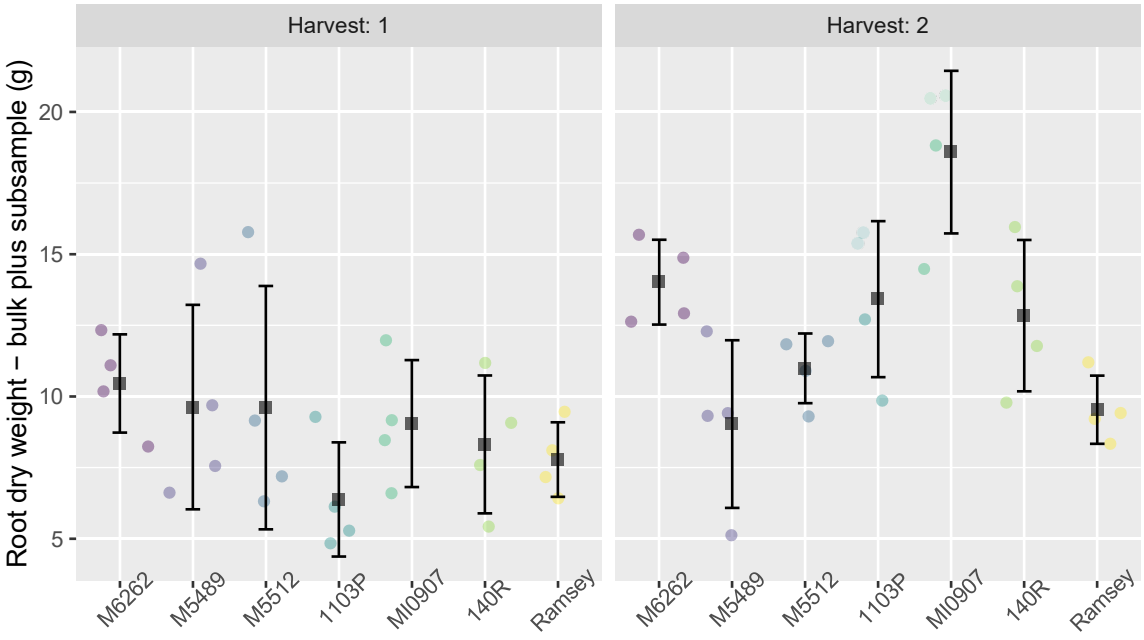


Figure 28 - total root dry matter produced up to plant harvest of Cabernet Sauvignon vines grafted to seven different rootstocks. Data are means ±SD.

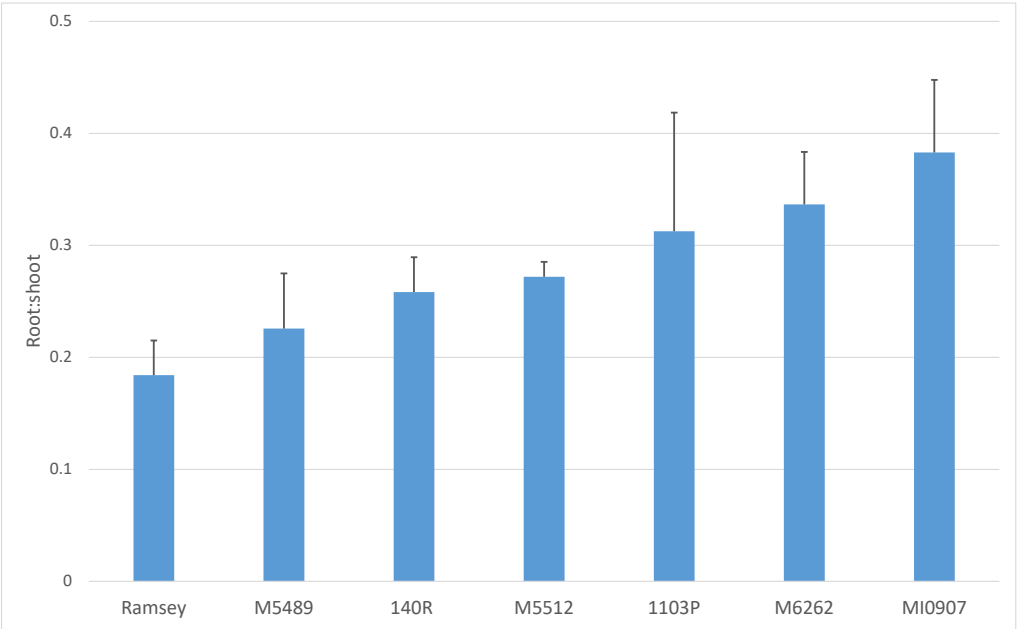


Figure 29 - ratio of root production to shoot production of Cabernet Sauvignon vines grafted to one of seven rootstocks over 16 months. Data are means ±SD.

Leaf physiology measurements were undertaken immediately prior to each plant harvest to determine whether there were any rootstock effects and any link with rootstock conferred

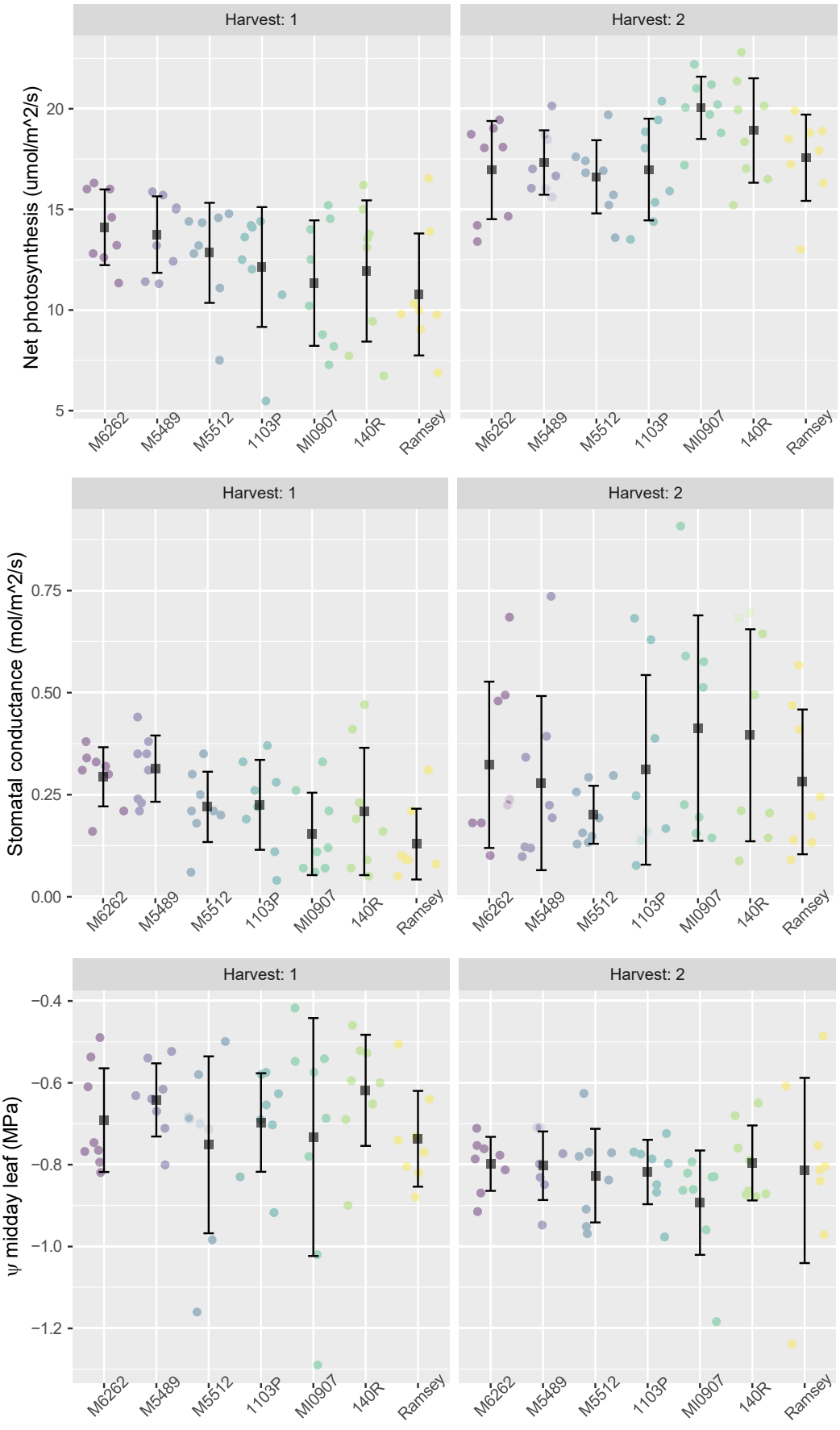


Figure 30 – photosynthesis under saturating light (top), stomatal conductance (middle) and midday leaf water potential (bottom) of Cabernet Sauvignon vines grafted to seven different rootstocks at the two plant harvest dates. Data are means ±SD.

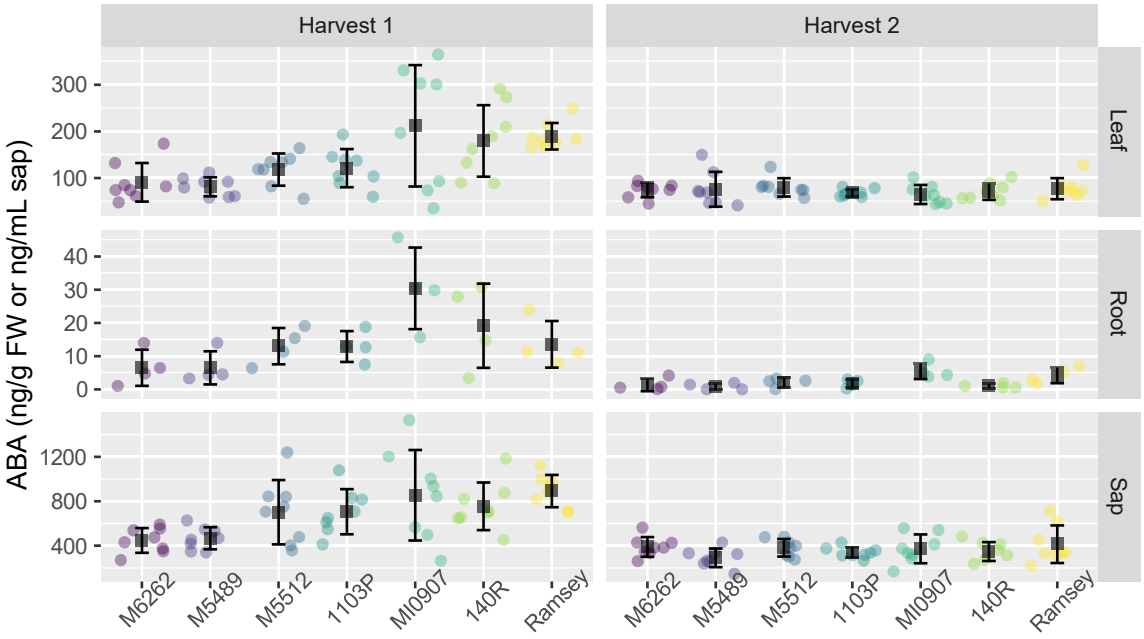


Figure 31 – ABA concentration in leaf tissue (top), root tissue (middle) and xylem sap (bottom) of Cabernet Sauvignon vines grafted to seven different rootstocks at the two plant harvest dates. Data are means ±SD.

vigour. Although there were some differences between the two measurements dates, no impact of rootstock genotype was observed on photosynthetic rate, stomatal conductance, transpiration (data not shown) or midday leaf water potential (Figure 30). A small difference in pre-dawn leaf water potential between the higher conferred vigour rootstocks (Mi0907, Ramsay and 140 Ruggeri) and the rest at harvest 1 (data not shown), likely due to greater water use during the preceding day, as the measurements were made prior to daily watering.

The interpretation of the pre-dawn leaf water potential results at harvest 1 was supported by an analysis of tissue and sap ABA concentrations (Figure 31). ABA of leaf tissue, root tissue and sap were all correlated with pre-dawn leaf water potential, but leaf and root concentrations were relatively low, particularly compared with those observed in experiments 1 and 2, suggesting that any water deficit was mild and short-term. Furthermore, the highest ABA concentrations were observed in the sap samples, which had the strongest correlation with pre-dawn water potential (r^2=0.893). No significant effect was observed at the second plant harvest, suggesting adequate soil moisture was present, even for the higher conferred vigour rootstocks. Overall, there was no indication of a direct link between rootstock conferred vigour and leaf physiology of the scion.

The primary aim of the experiment was to examine the potential role of plant hydraulics in rootstock conferred vigour. Three methods for assessing hydraulics were used, a high-pressure flow meter (HPFM) to determine the hydraulic conductivity of the root system, the evaporative flux method (EFM) to determine hydraulic conductivity based on physiology measures, and a microscopy method that uses xylem vessel diameter.

The EFM was used to determine hydraulic conductivity of the leaf, based on the difference between stem and midday leaf water potential (K_{leaf}; mmol m^{-2} s^{-1} MPa^{-1}) and of the whole vine water path, based on the difference between pre-dawn water potential and midday leaf water potential (K_{plant}; mmol m^{-2} s^{-1} MPa^{-1}). Neither demonstrated statistically significant differences between rootstock genotypes, nor any relationship with rootstock conferred vigour (determined as total shoot dry mass production over the experiment) but both had a weak correlation between harvest dates if the lowest vigour rootstock, Merbein 5489 was excluded (r^2=0.547, p=0.093, n=6 and r^2=0.585, p=0.076, n=6 respectively; Figure 32).

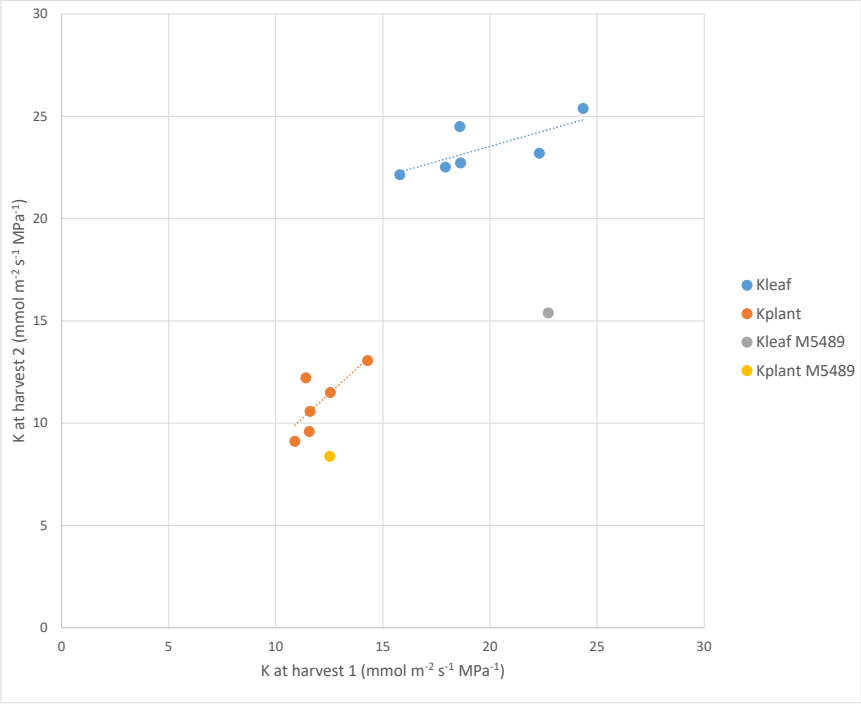


Figure 32 - relationship between harvests of K_{leaf} and K_{plant} of Cabernet Sauvignon vines grafted to seven different rootstocks at the two plant harvest dates. Data are means of eight replicates.

The HPFM method was used to determine the whole root system hydraulic conductivity (Lp_r; m^3 s^{-1} MPa^{-1}) and also scaled this to root dry mass ($LP_{r(dm)}$; m^3 g^{-1} s^{-1} MPa^{-1}). As with the EFM method at the leaf level, there was no significant effect of rootstock for either measure of root hydraulic conductivity. There was also no significant correlation between harvests for mean Lp_r, but this was not surprising as the root system size did not change consistently between the two harvests. However, there was a weak correlation between the harvests when root system hydraulic conductivity was adjusted for root biomass and 1103 Paulsen, which had lower root biomass measured at harvest 1 than the other rootstocks, was excluded ($LP_{r(dm)}$; r^2=0.791, p=0.061; Figure 33 top).

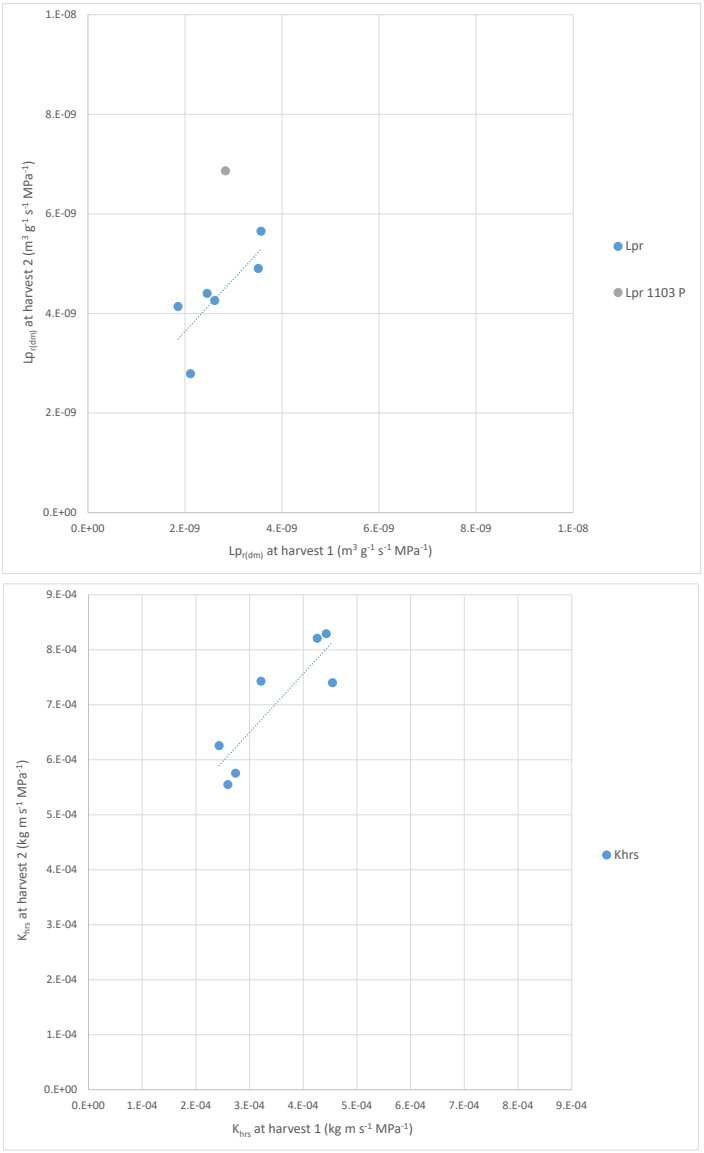


Figure 33 - relationship between harvests of $Lp_{r(dm)}$ (top) and K_{hrs} (bottom) of Cabernet Sauvignon vines grafted to seven different rootstocks at the two plant harvest dates. Data are means of eight replicates.

The microscopy method calculated hydraulic conductivity of the stem, for both the new scion growth (K_{hss}, kg m s^{-1} MPa^{-1}; post-pruning at each plant harvest) and for the rootstock (K_{hrs}, kg m s^{-1} MPa^{-1}; below the graft union). For the scion stem section there was no significant effect of rootstock, but K_{hss} was significantly lower at the time of the second harvest than the first (Figure 34 right), potentially due to the lower canopy size (Figure 24).

K_{hrs} was the only hydraulic measurement conducted that exhibited a statistically significant effect of rootstock genotype ($p<0.001$; Figure 34 left). There was also a strong correlation between harvest dates, with no outliers ($r^2=0.756$, $p=0.011$, $n=7$; Figure 33 bottom). However, there was no correlation between K_{hrs} and rootstock conferred vigour. There was also no correlation between K_{hss} and K_{hrs}, suggesting that at this early growth stage after grafting (6-

16 months) the xylem structure of the two parts of the vine stem are somewhat independent, retaining their genotypic differences.

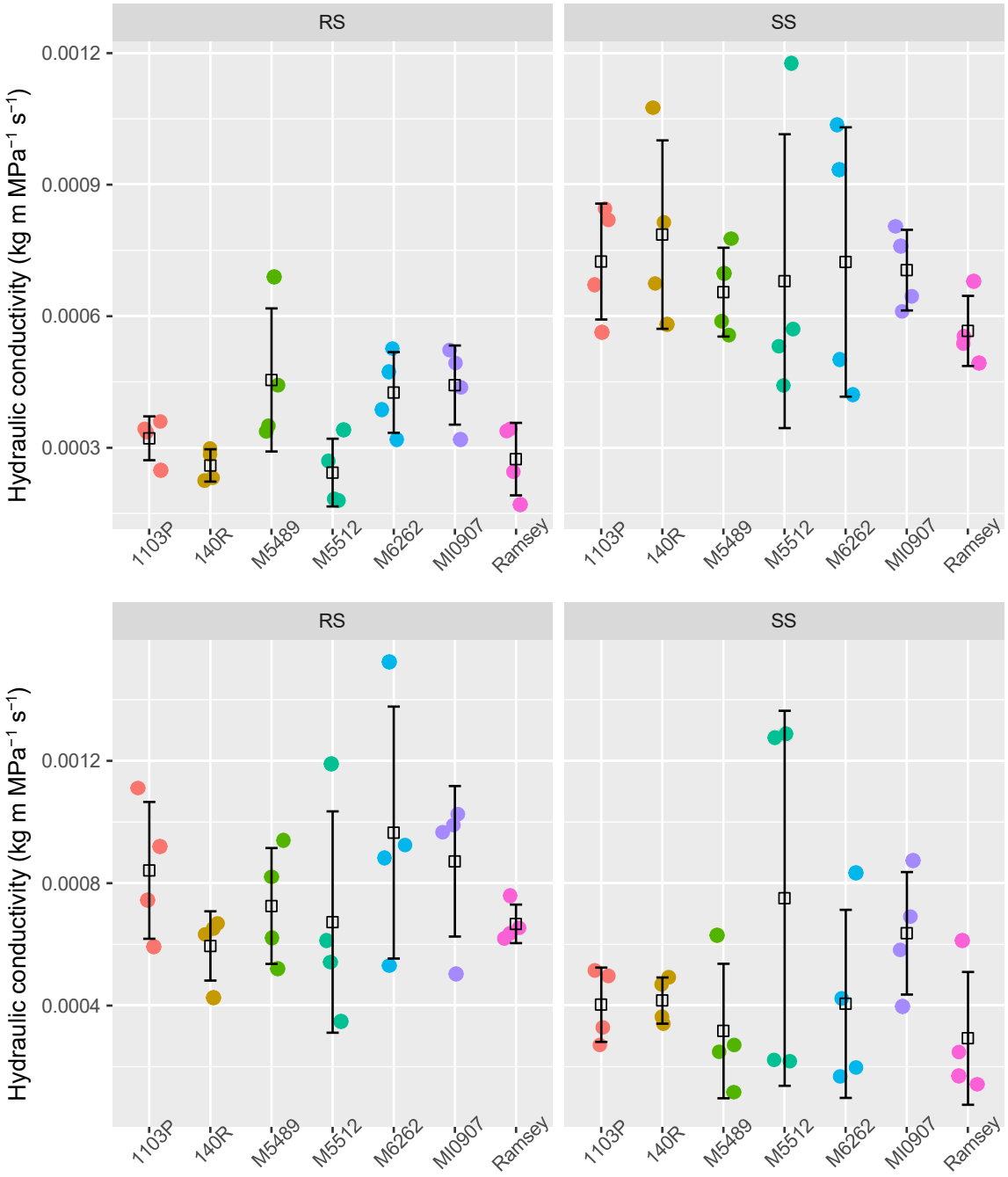


Figure 34 - stem hydraulic conductivity (Kh) of the rootstock wood (left) and new scion stem growth (right) of Cabernet Sauvignon vines grafted to seven different rootstocks at plant harvest 1 (top) and plant harvest 2 (bottom). Data are means ±SD.

In summary, although the experiment was successful in regards to establishing a platform that simulated rootstock conferred vigour in a semi-controlled greenhouse environment, despite the necessity to use potted plants, there was no evidence of rootstock driven effects on plant

hydraulics and, therefore, no evidence that plant hydraulics might explain rootstock conferred vigour. As noted above, past work indicated rootstock limitations on leaf physiology under high evaporative demand conditions, which were not present here, but these results suggest that any such limitations are not likely to be due to physical differences in plant hydraulics (xylem structure). This doesn't rule out differences in short term adjustment of hydraulics, e.g. via changes in aquaporins.

3.4 Experiment 4: rootstock scion interactions and root system traits

The prior experiments found differences in root system traits between different rootstocks grafted to the same scion, albeit no clear links to rootstock conferred shoot traits. This experiment as intended to use the same experimental system as experiment 3, but with a focus on rootstock:scion interactions, particularly on the root system. Unfortunately, it was significantly affected by the COVID-19 pandemic and limitations on access to conduct the planned measurements.

The design was to use five rootstock genotypes, grafted to two scions and include self-grafts of the scions. Reciprocal grafts of the two scions varieties were not included. The vines were to be grown for at least 18 months, with three whole vine harvests, to ensure more than a full growing season was completed, but was cut short with a single whole vine harvest and set of physiology measurements.

As with the previous experiment, total aboveground production was estimated using the dry mass of all new growth (from the scion bud after grafting), including leaves & stem, with multiple pruning times combined (Figure 35). Note that although 140 Ruggeri:Shiraz is included in the figures, the rootstock was not included in the statistical analysis due to the 140 Ruggeri:Cabernet vines failing to grow.

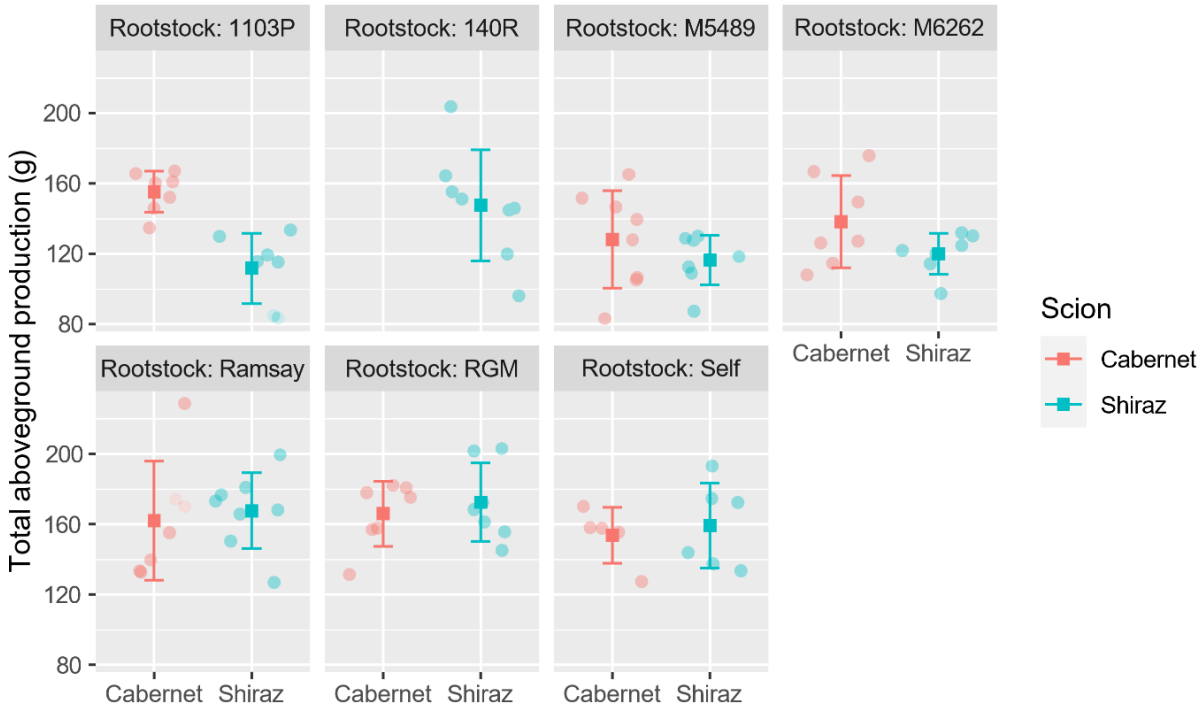


Figure 35 - aboveground biomass production during nine months after budburst of Cabernet Sauvignon and Shiraz scions grafted to six rootstocks or self-grafted. Data are means ±SD.

Rootstock had a highly significant effect on scion vigour ($p<0.001$), with self-grafts, Ramsey and RGM having high vigour with both scions and the two Merbein stocks having low vigour with both scions. There was also a significant rootstock by scion interaction ($p=0.011$), most evident with 1103 Paulsen, where aboveground production of the Cabernet Sauvignon scion was significantly higher than aboveground production of Shiraz as scion (Figure 35 top left). Furthermore, Shiraz aboveground production with 1103 Paulsen was the lowest with any included rootstock. Overall, the impact of rootstock on Shiraz aboveground production (whether high or low vigour) was greater than on Cabernet.

Belowground biomass was also significantly affected by rootstock ($p=0.003$, Figure 36) and, as with the earlier experiments, the ranking was not the same as for scion production. For example, Ramsey had lower belowground biomass than either Merbein 6262 or self-grafts. There was no rootstock by scion interaction, but there was a significant scion effect ($p=0.034$). In general, belowground biomass was lower with a Shiraz scion, except with Ramsey.

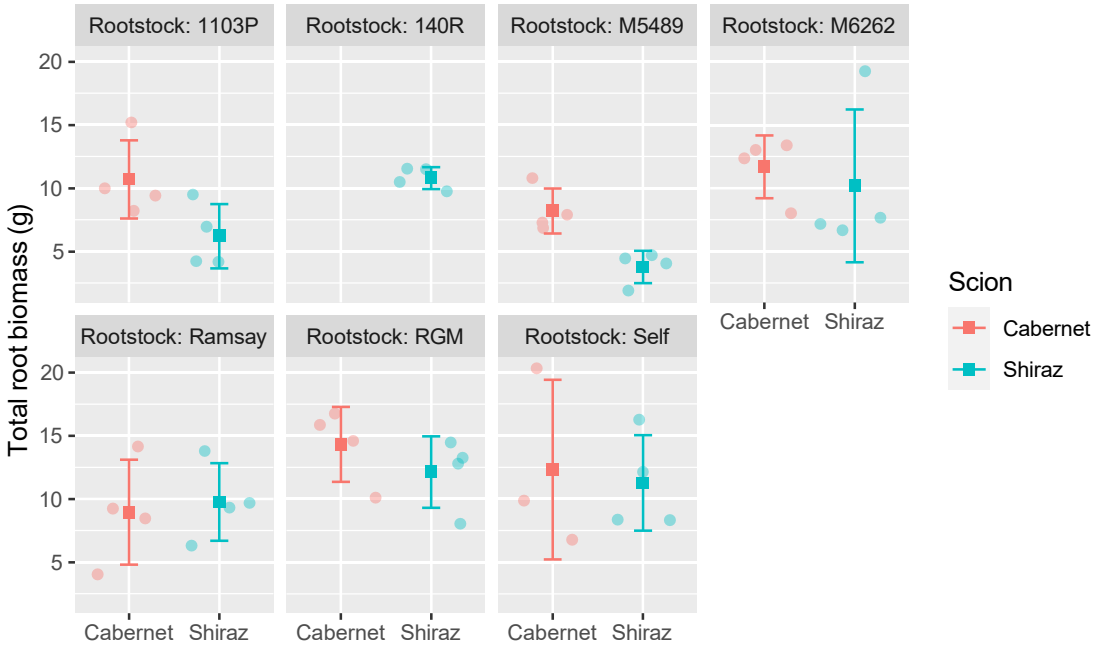


Figure 36 - belowground biomass nine months after budburst of Cabernet Sauvignon and Shiraz scions grafted to six rootstocks or self-grafted. Data are means ±SD.

Root fraction is the proportion of whole vine biomass in the root system, it is a more robust measure than root to shoot ratio when root to shoot ratio is either very small or very high. There was a significant effect of rootstock on root fraction of the grafted vine ($p<0.001$; Figure 37), but no rootstock by scion interaction or scion effect. RGM, Merbein 6262 and self-grafts had the highest root fraction, with Merbein 5489 and Ramsey the lowest. The difference in ranking between root fraction, root biomass and aboveground production demonstrates the

complexity of the interplay between rootstock and scion with no correlation between root production and shoot production, whether positive or negative.

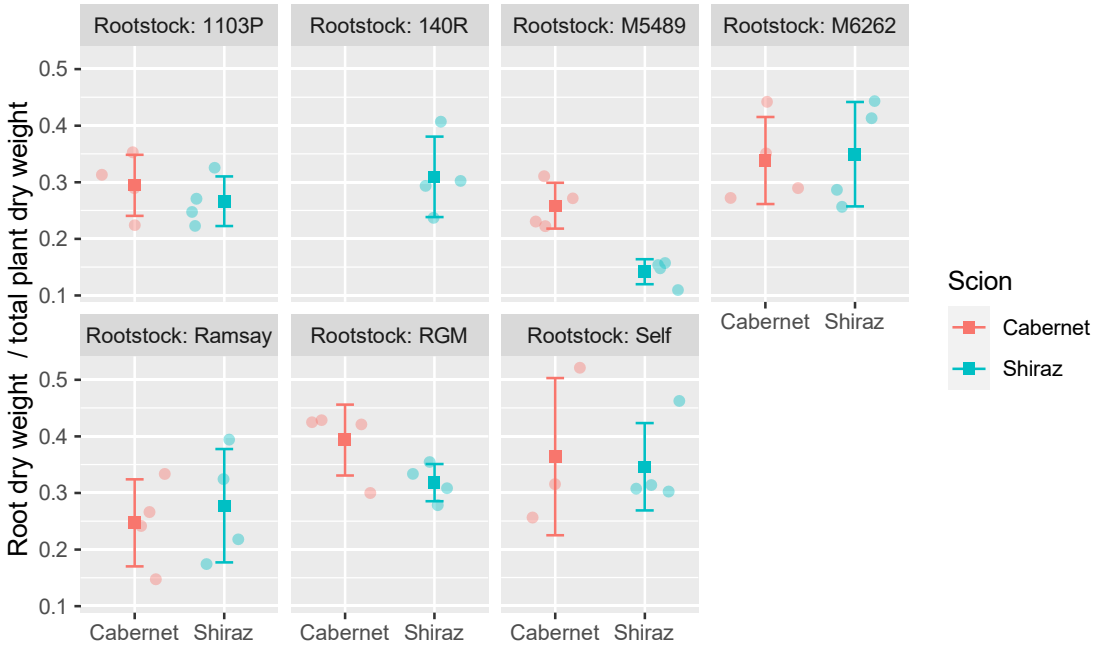


Figure 37 - root fraction nine months after budburst of Cabernet Sauvignon and Shiraz scions grafted to six rootstocks or self-grafted. Data are means ±SD.

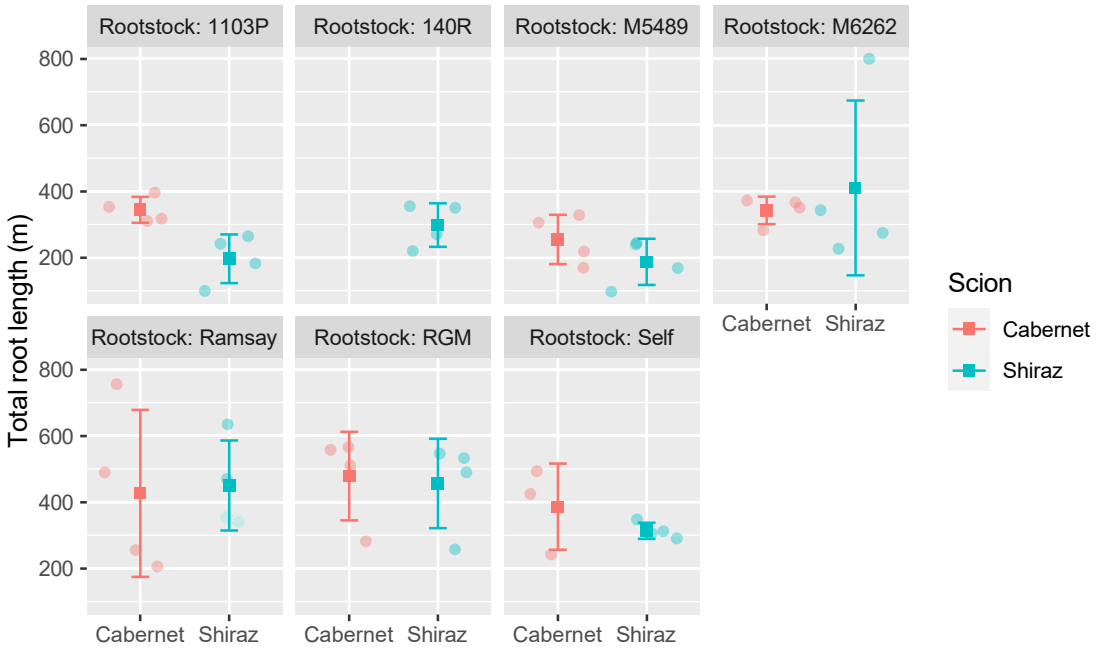


Figure 38 - root length nine months after budburst of Cabernet Sauvignon and Shiraz scions grafted to six rootstocks or self-grafted. Data are means ±SD.

Total root length per vine was also significantly affected by rootstock ($p<0.001$; Figure 38), but although root length was correlated with root biomass, it was non-linear and there were outliers, both scions grafted with Ramsey in particular (data not shown). There was no rootstock by scion interaction and, although root length was numerically lower with Shiraz as scion in some rootstocks, but not others, there was no significant scion effect either ($p=0.100$). Root length per vine was highly correlated with aboveground production ($r2=0.831$, $p<0.001$; Figure 39), demonstrating how finely the absorptive area of the root system was associated with the productivity of the shoot system.

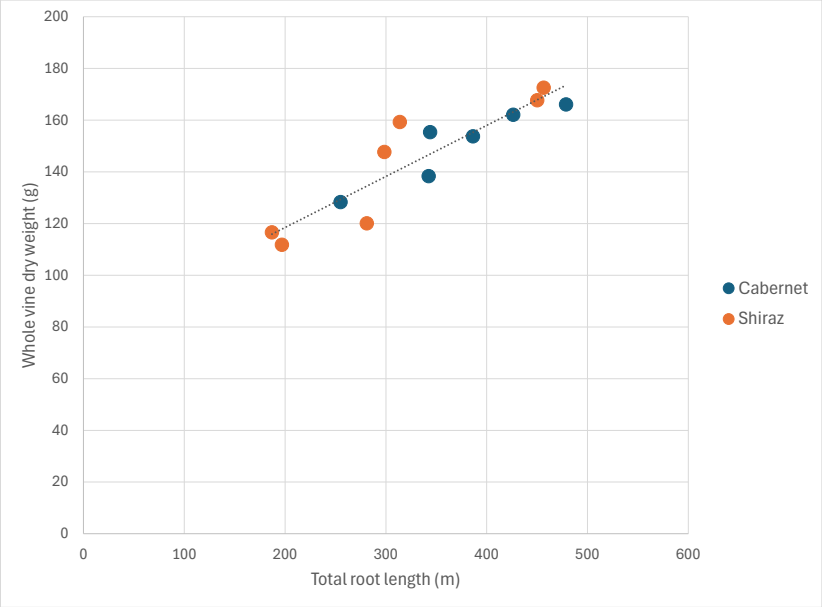


Figure 39 - relationship between aboveground production and vine root length of Cabernet Sauvignon and Shiraz scions grafted to six rootstocks or self-grafted. Data are means of four replicates.

The correlation of root length to other traits where root biomass was not suggested a change in specific root length (SRL: length per unit dry mass). Indeed, this was the case, with rootstock significantly affecting SRL ($p=0.005$; Figure 40), most notably Ramsey, and a weak interaction with the scion ($p=0.068$). The latter due to Shiraz grafted vines having higher SRL with the two Merbein rootstocks, but not the other rootstock genotypes.

Differences in SRL can be caused differences in root diameter, to examine this the roots were split into three diameter classes (<0.5 mm, 0.5-1.5 mm and >1.5 mm). There was a significant effect of rootstock in each case ($p=0.026$, $p=0.025$ & $p=0.003$ respectively), with Ramsey and Merbein 6262 having more root length in the finest class and the self-grafted vines (i.e. *V. vinifera* as a rootstock) having more root length in the largest diameter class. However, scion also had a highly significant effect on the two finer classes, with Shiraz having less root length below 0.5 mm ($p<0.001$) and more in the 0.5-1.5 mm class ($p<0.001$). There was no scion effect on the roots >1.5 mm in diameter.

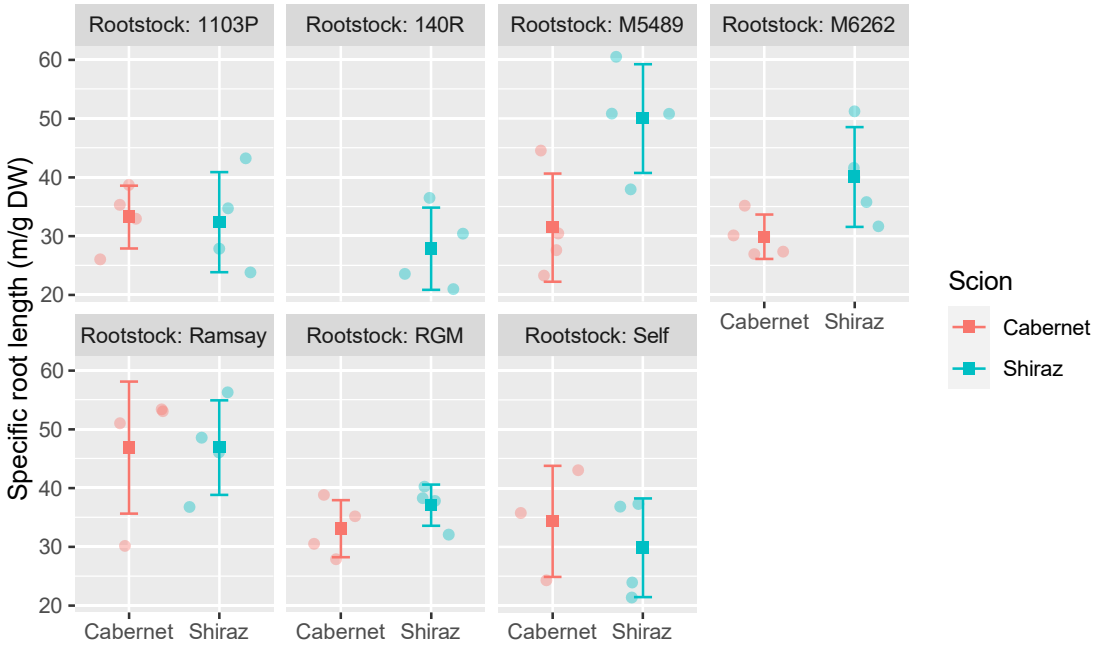


Figure 40 - specific root length nine months after budburst of Cabernet Sauvignon and Shiraz scions grafted to six rootstocks or self-grafted. Data are means ±SD.

The interplay between rootstock and scion in determining the root traits and distribution of roots between specific traits that resulted in the relationship between total root length and aboveground production suggested a possible link between the root absorptive area (root surface) and the shoot absorptive area (leaf surface). However, there was no correlation between total root surface area and total leaf surface area (data not shown). When that relationship was expressed as a ratio (root system surface area : vine canopy area) there were significant effects of rootstock (p=0.002) and scion (p-0.025), with Shiraz vines generally having a higher canopy area per unit root surface area and some rootstocks, e.g. 1103 Paulsen & Merbein 5489 having a higher canopy area per unit of root surface area (data not shown).

Although this experiment was limited in the scope of measurements and curtailed by the pandemic, it nonetheless demonstrated how there is an interplay between rootstock and scion in conferred traits (shoot vigour, root morphology). This experiment was carried out in pots for practicality and the limitations in available soil volume as a result, not to mention the limited exposure to environmental variation that a greenhouse imposes, means that experimental artefact in the results cannot be ruled out. Furthermore, these are traits that are not straightforward to measure in the field, but rootstock effects on scion vigour were still observed and if that interplay in root and scion traits is retained in field plantings, those traits offer a means by which rootstock by scion interactions may be more clearly quantified and rootstocks selected.

3.5 Experiment 5: linking field and greenhouse observations

While the greenhouse experiments were successful in replicating the rootstock conferred vigour observed in field plantings and allowing detailed vine traits and physiology to be examined, as well as being pot experiments they used young vines. To bring together greenhouse and field observations a field trial was established using young vines in a section of the CSIRO Irymple field station, where the irrigation could be managed separately to the rest of the site and the vines could be dug up to allow the root systems to be recovered.

The experiment included 12 rootstock : scion combinations and Cabernet Sauvignon on own roots for comparison with self-grafted Cabernet Sauvignon (Table 2). The rootstocks and scions were chosen to match previous experiments, particularly experiment 4. In addition to the rootstock : scion combinations an irrigation treatment was applied, with the intention of producing mild-moderate water stress.

Unfortunately this experiment was also impacted by the COVID-19 pandemic, meaning travel to the site was difficult, impacting the management and some intended measurements. To partially rectify this, the experiment was extended, allowing additional measurements.

Table 2 - rootstock : scion combinations used in field nurery experiment.

Scion	Rootstock	Graft	Combination code
Cabernet Sauvignon	Cabernet Sauvignon	Self-graft	RS1
Cabernet Sauvignon	Merbein 6262	Yes	RS2
Cabernet Sauvignon	Merbein 5489	Yes	RS3
Cabernet Sauvignon	1103 Paulsen	Yes	RS4
Cabernet Sauvignon	Ramsey	Yes	RS5
Cabernet Sauvignon	RGM	Yes	RS6
Shiraz	Shiraz	Self-graft	RS7
Shiraz	Merbein 6262	Yes	RS8
Shiraz	Merbein 5489	Yes	RS9
Shiraz	1103 Paulsen	Yes	RS10
Shiraz	Ramsey	Yes	RS11
Shiraz	RGM	Yes	RS12
Cabernet Sauvignon	Cabernet Sauvignon	No, own roots	RS13

The vines were planted in the 2019/20 season, with some replants for failed vines in September 2020. The planned physiology measurements for the 2020/21 season were limited and this resulted in the reduced irrigation treatment not being well matched to the vine size, meaning that despite receiving half-irrigation the impact on soil moisture availability was small, averaging 16.8±4.4 % VSW over the top 30 cm in the low irrigation treatment vs 18.4±3.8 % in the full irrigation treatment in the first measurements that were able to be taken (December 2020). Stomatal conductance measurements made at the same time indicated limited effect on vines (p=0.009), being 132±45 mmol m^{-2} s^{-1} in the low irrigation treatment vs 166±47 mmol m^{-2} s^{-1} in the full irrigation treatment. A second set of measurements in January 2021 found no difference in stomatal conductance (356±83 and 336±99 mmol m^{-2} s^{-1} for low vs full irrigation respectively). Vine size meant that the whole vine harvests needed to go ahead at the end of the 2020/21 season as planned (Figure 41), to be practical, but the irrigation system was adjusted and the remaining vines maintained for the 2021/22 season.



Figure 41 - shoot and root harvest of whole vines.

Due to the difficulties in applying the irrigation treatment there were no significant impacts of irrigation on any of the whole vine harvest measures and the irrigation treatment has been ignored in the results below. This was despite a small (2%), but significant (p=0.046) difference in weight of the soil cores, indicating that there was less moisture in the cores from the low irrigation treatment at the time of vine harvest.

Unlike experiment 4, there was no significant interaction between rootstock and scion on aboveground growth, defined as total new dry matter. Both rootstock (p<0.001) and scion (p=0.011) had significant impacts (Figure 42). The ranking of rootstock conferred vigour was very similar as experiment 4, with RGN and Ramsey having the highest aboveground production, followed by self grafts, then the two Merbein rootstocks and 1103 Paulsen. However, the scion effect did not match experiment 4, with Shiraz scions having greater aboveground production than Cabernet Sauvignon. The consistent rootstock effect and lack of interaction with the scion was further demonstrated by a strong correlation between the scions for mean aboveground biomass per rootstock (r^2=0.743, n=6).

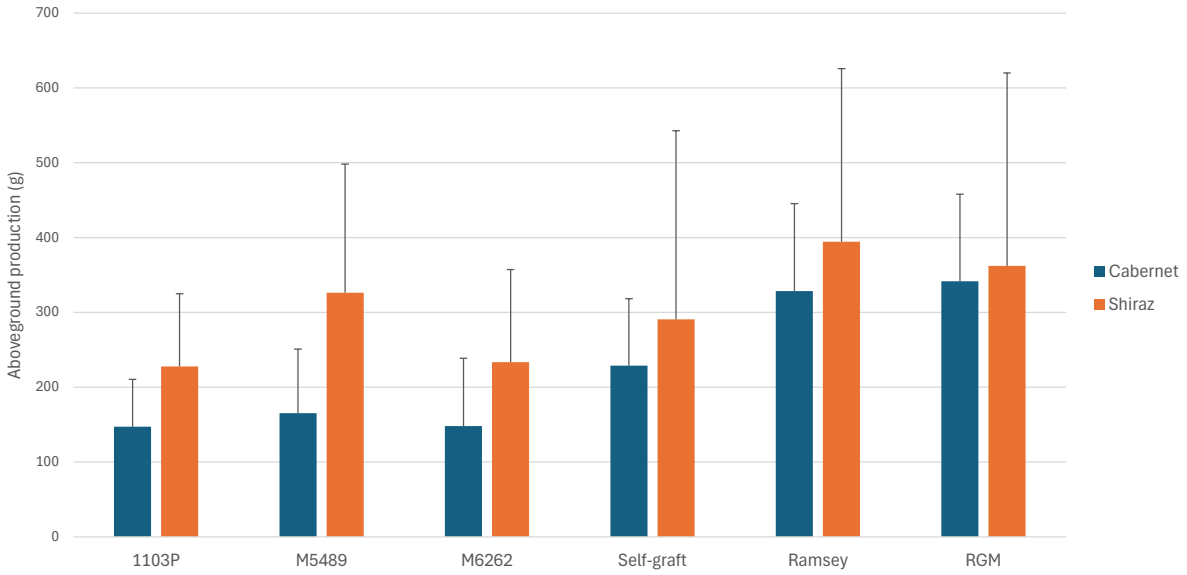


Figure 42 - aboveground dry matter of Cabernet Sauvignon and Shiraz vines grafted to five rootstocks or self-grafted 16 months after budburst. Data are means ±SD.

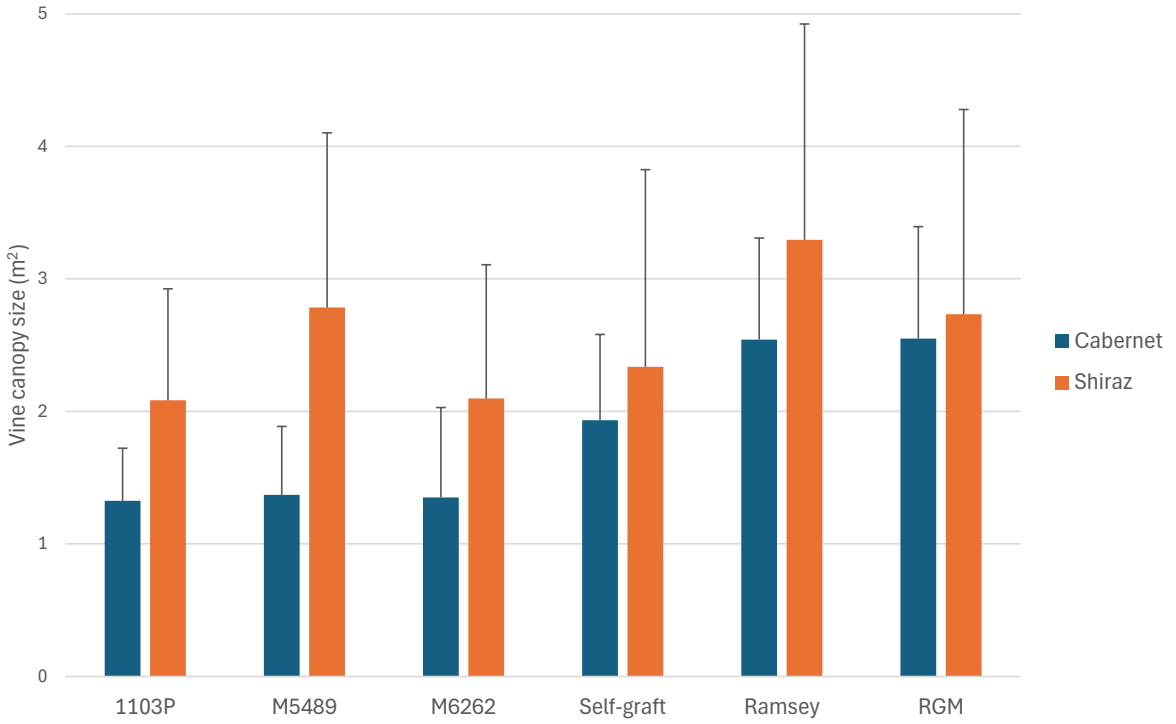


Figure 43 - canopy size of Cabernet Sauvignon and Shiraz vines grafted to five rootstocks or self-grafted 16 months after budburst. Data are means ±SD.

Vine canopy area followed the same pattern as aboveground production, with rootstock ($p=0.002$) and scion ($p<0.001$) both having a significant effect with no rootstock by scion

interaction (Figure 43). The Shiraz vines had the larger canopy, which matched experiment 4, where total leaf area was generally higher with Shiraz (data not shown).

The pot grown vines in experiment 4 were protected from dormancy by the greenhouse conditions and pruned to prevent excessive size, managing a total aboveground production of around 110 to 170 g per plant, whereas the field grown plants did go through dormancy, when they were pruned if required (excessive shoot above the wire), but had more time to grow and were in natural conditions, reaching around 190-360 g per plant – approximately double the greenhouse experiment. However, the difference in management meant that the field grown vines had approximately 10x the canopy size of the greenhouse grown vines. Belowground, the field grown vines had no absolute limits on root growth (although soil depth was around 60 cm) and the bulk root recovery was undertaken on about 0.5 m^3 of soil, 50x the pot volume used.

Air-dried root biomass recovered was around 10x higher than from the pots, although some roots had grown outside of the soil volume excavated. As with the pot experiment, the ranking of rootstocks by belowground biomass varied from the ranking with aboveground biomass, but unlike with aboveground biomass, the order didn't match the pot experiment. The self-grafted vines had the highest belowground biomass, followed by Ramsey and Merbein 6262, and then the remaining rootstocks (Figure 44). The rootstock effect was statistically significant ($p<0.001$) and there was a significant rootstock by scion interaction ($p=0.050$), due to the higher belowground biomass in self-grafted Cabernet Sauvignon vines compared with self-grafted Shiraz vines, but the Shiraz vines having higher belowground biomass with the other rootstocks. This meant that the correlation between the scions by rootstocks was less strong than for aboveground biomass ($r^2=0.534$, n=6).

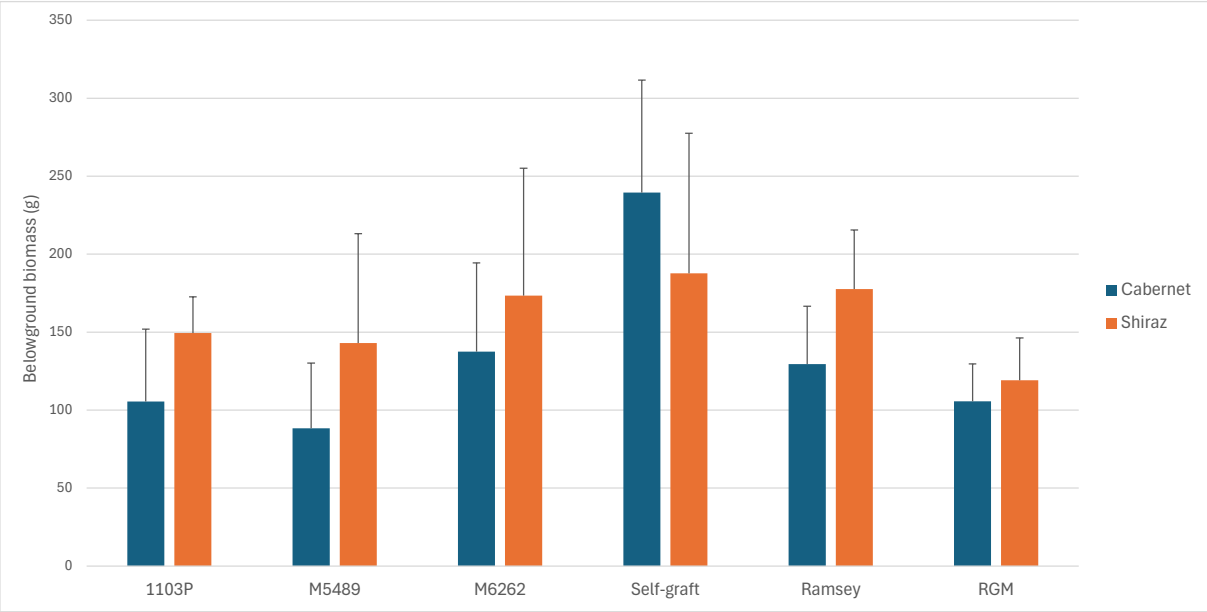


Figure 44 – air-dried belowground biomass of Cabernet Sauvignon and Shiraz vines grafted to five rootstocks or self-grafted 16 months after budburst. Data are means ±SD.

Prior to the bulk root excavation, soil cores were taken for estimation of fine root length, expressed as root length density (m per core). Matching visual observations whilst extracting the bulk roots, RGM had the greatest root length density and Merbein 6262 the least (Figure 45). There was a significant rootstock by scion interaction (p=0.006) due to Shiraz having a higher root length density with some rootstocks, compared with Cabernet, but lower with others. The major differences with the results from the pot experiment, was the rootstock scion interactions, which were often the reverse of the pot experiment, and Merbein 6262 having the lowest root length density. There was no relationship between root length density and aboveground production, but unlike the pot experiment, the unrestricted root volume meant that the fine root density estimate was unlikely to be representative of the whole root system root length.

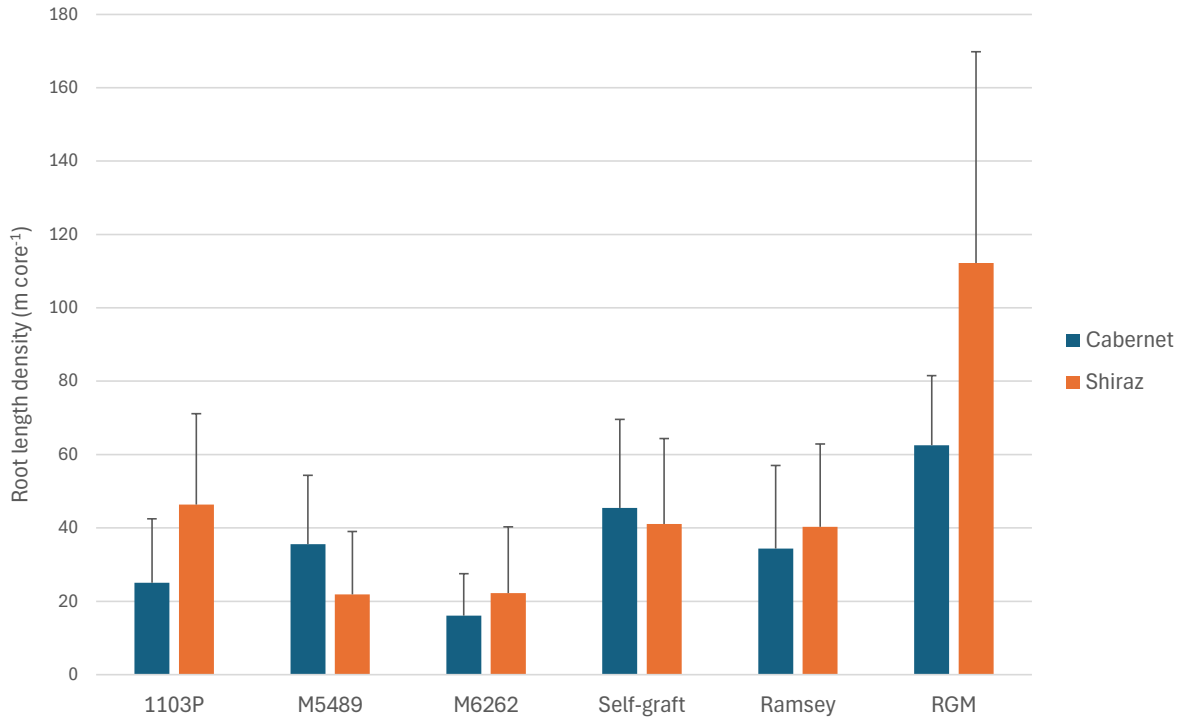


Figure 45 - root length density of Cabernet Sauvignon and Shiraz vines grafted to five rootstocks or self-grafted 16 months after budburst. Data are means ±SD.

Despite the impacts of the pandemic on field trial, overall the whole vine harvest results supported the conclusions from the pot experiment around the complexity of rootstock by scion interactions, but also demonstrated the difference of a field environment (greater growth of Shiraz cf. Cabernet Sauvignon, where the reverse was true in the greenhouse) and of changes in the rooting volume available.

Retaining the vines that weren't harvested allowed the irrigation treatments to be attempted in the subsequent growing season (2021/22). Soil moisture monitoring demonstrated that

around a 30% reduction in soil water to 60 cm depth, although the difference at 30 cm depth was more difficult to maintain and generally smaller (Figure 46). However, this was enough to have a physiological effect, unlike the previous season.

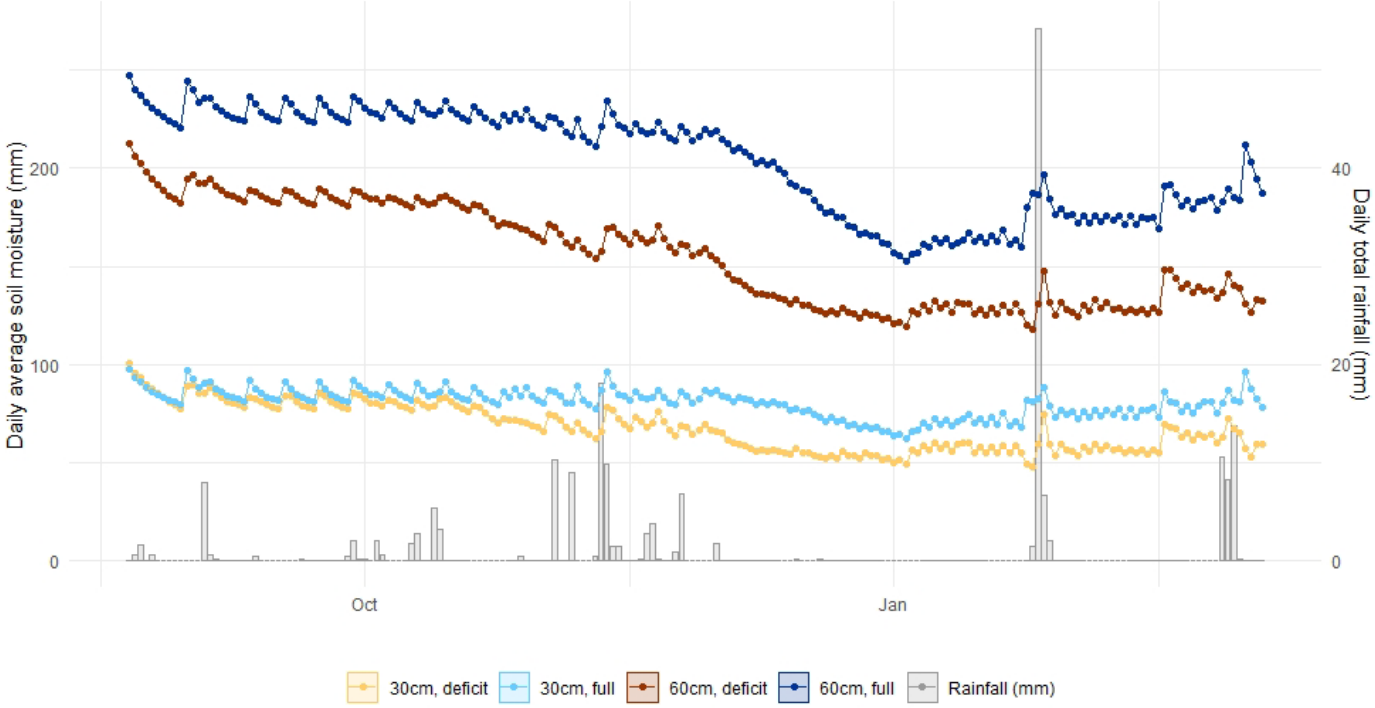


Figure 46 - soil moisture to 30 cm and 60 cm (mm) under full and deficit irrigation and recorded rainfall events, during the 2021/22 growing season (from August to April).

After leaf-fall in the 2020/21 season, all vines were spur pruned back to a cordon on the wire. Pruning weights were recorded and compared back to vine aboveground production from the whole vine harvest for each rootstock : scion combination (Figure 47). Despite the lack of leaves and difference in selection of material to prune a strong relationship remained between the two (r^2=0.700), indicating that the remaining vines going in to the 2021/22 growing season were representative of the vines harvested during prior season.

Despite the lack of an irrigation effect on the whole vine harvest components and the short part of the growing season remaining after the whole vine harvest in which the changes to the irrigation management could have an effect, there was a significant irrigation effect on the 2021 pruning weights (p=0.038), with lower fresh weight in all rootstocks (Figure 48). In contrast, there was no difference in pruning weight between scions, suggesting that the scion difference at the whole vine harvest was a result of the increased canopy size and primary stem/cordon.

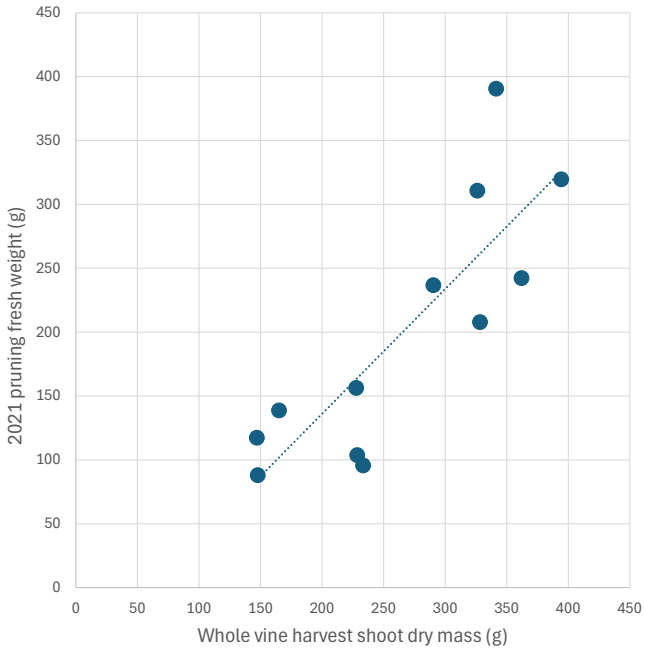


Figure 47 - comparison of 2020/21 season pruning weights and whole vine harvest aboveground production. Data are means.

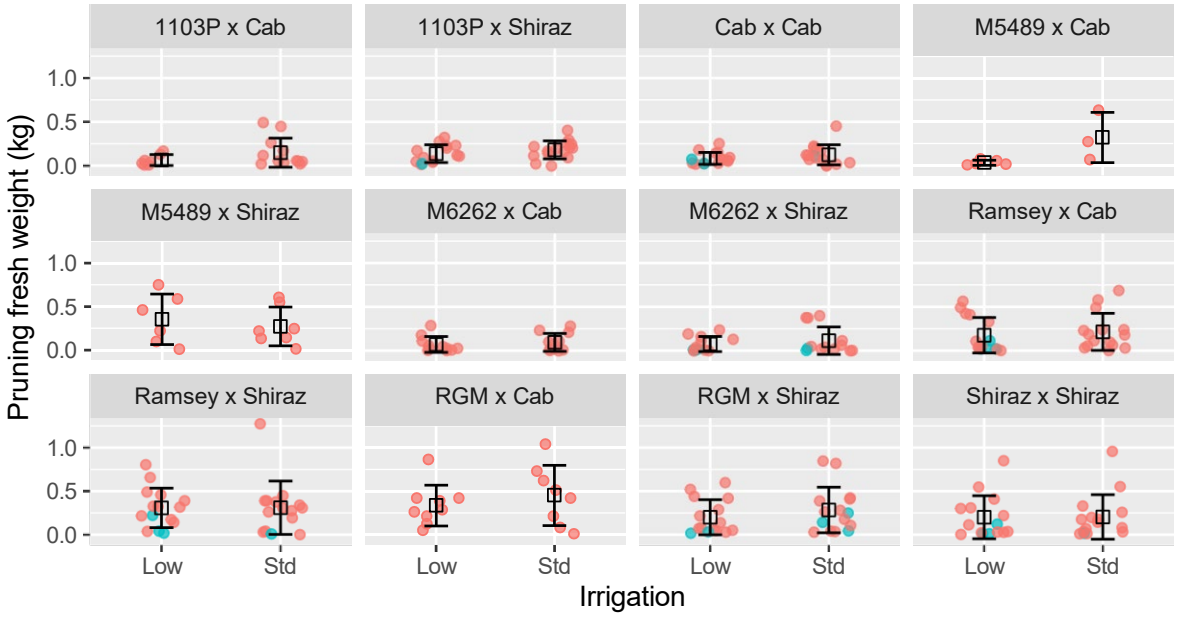


Figure 48 - pruning weight of Cabernet Sauvignon and Shiraz vines grafted to five rootstocks or self-grafted after two growing seasons. Replant vines are marked with turquoise. Data are means ±SD.

With the irrigation treatment established, vine physiology was assessed across the remaining vines and, being in their third growing season, the fruit were harvested at the end of the season to determine any rootstock by scion interactions. The physiology measurements

demonstrated a mild-moderate water stress, as intended, but no significant rootstock or scion effects (e.g. stomatal conductance, irrigation effect p<0.001; Figure 49).

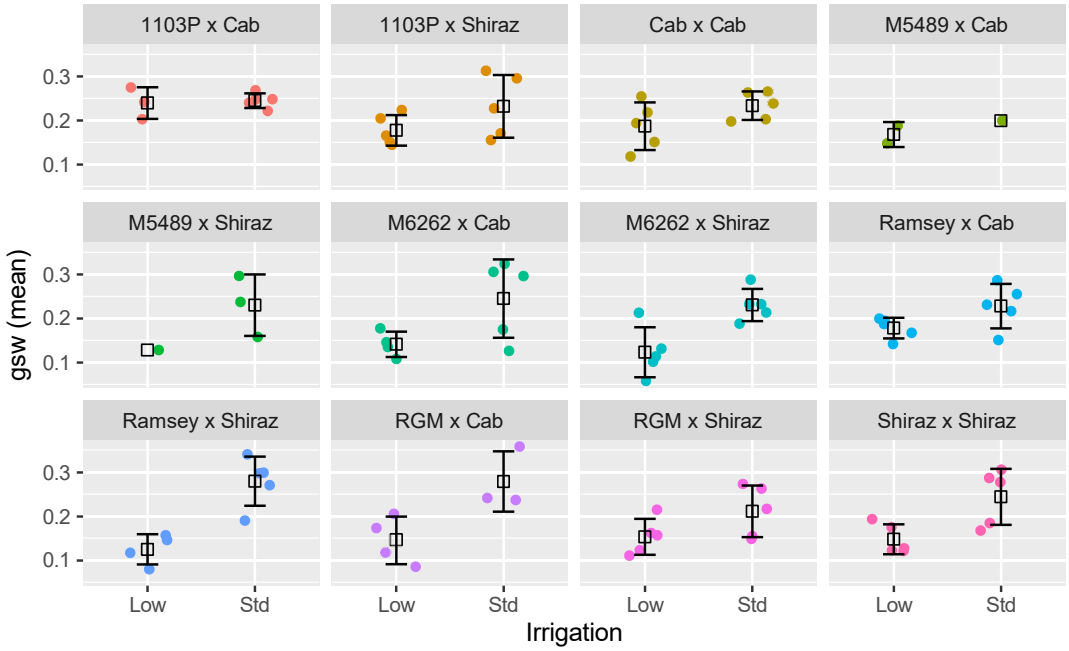


Figure 49 - stomatal conductance (g_{sw}) of two-year old Cabernet Sauvignon and Shiraz vines grafted to five rootstocks or self-grafted. Data are means ±SD.

Harvest yield at the end of the 2021/22 season was assessed as bunch number and fruit fresh weight per vine. There was no effect of irrigation treatment on bunch number and only a weak effect on yield (p=0.083). Rootstock had a significant influence on both parameters, with Ramsey producing the highest in both cases, followed by RGN, 1103 Paulsen and the self-grafted vines, then Merbein 5489, with Merbein 6262 having the lowest yield.

In summary, whilst the pandemic meant the field trial was not able to accomplish what was planned, it did provide support for the greenhouse experiments, thereby validating that approach to study aspects that are difficult to measure in the field. Furthermore, it once again demonstrated the consistent impact on the scion that rootstock choice can have.

Chapter 4: Conclusions

The experiments described here used increasingly tight control of the soil environment to determine whether root system signals to the scion differ in response to changes in the soil environment, namely water deficit. No evidence was found for such differences in plant hormone production and in an additional study, no evidence was found for gene transcription products in the xylem stream. Where apparent differences were observed, they were due to rootstock conferred vigour impacting the water use and transport by the vine.

The project had a hypothesis that rootstock genotype may affect the hydraulic capacity of the grafted vine, with low conferred vigour rootstock likely to have a lower hydraulic capacity than a high conferred vigour rootstock. Real world differences in water transport under periods of high demand would accumulate over time to impact growth. However, despite taking multiple approaches to examining hydraulic conductivity, no evidence for differences induced by rootstock genotype were found. This doesn't completely rule out short-term differences in hydraulic conductivity (i.e. not due to xylem structure) in response to specific environmental cues, but no evidence for such were seen either.

Despite these hypotheses not being confirmed, rootstock conferred differences in vine vigour were invariably observed, even in very young vines and these were generally consistent between different temporal scales, field and greenhouse, or scion choice, although only two scions were used in our studies. Where differences between experiments, or scales, were seen, it is likely that they were due to changes in root growth traits imposed by limited soil volumes.

The aim of this project was to find measurable, objective, quantifiable traits, either in the rootstock itself, or conferred to the scion, which were validated through precise experimental control of the root environment. Even if difficult to measure in a field environment, such a trait could be used to predict genotype response and improve management of grafted vines. Although some aspects of the project were impacted by the COVID-19 pandemic, limiting the planned experimental work, such a trait was not found. In conjunction with related work from other agencies, such as ISVV, this suggests that the consistently observed rootstock conferred traits are driven over time by subtle impacts on whole vine physiology. This also means that these traits are likely to be complex multi-genic traits making marker discovery for breeding more difficult also.

Future work is likely to benefit from combining molecular physiology studies in carefully controlled environments with long-term field trials that maintain contrasting rootstock genetics without belowground competition from other genotypes with a very different growth habit.

Appendix 1: Intellectual property

No patents were taken out during the term of this project.

The information generated by this project will enter the public domain and will be accessible for future reference.

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Appendix 2: References

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Appendix 3

Project staff

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Appendix 4: Planned Project Outputs

Outputs and activities from the annual operating plans.

FY1

Output	Target	Activities
Project staff appointed	1/10/2017	PDF position approved, advertised and appointed. Other staff allocated to project (EJE 30%, MW 10%, CSOF3 technical officer 100%).
Validated LCMS/MS method for analysis of key cytokinins available	1/1/2018	Brief literature review of cytokinin (CK) analysis undertaken. Appropriate standards identified and obtained. Method developed for existing CSIRO Waite Campus LCMS/MS facility, tested and validated.
Differences in ABA production of M5512 and 1103 Paulsen rootstocks characterised.	30/6/2018	Rootstock material obtained from commercial nursery (1103 Paulsen) and CSIRO Irymple (M5512), grafted with a single scion clone and propagated to provide up to 100 plants per genotype for characterisation experiments (by 1/1/2018). Experimental conditions (degree of soil water stress and method of application) finalised and plant responses (e.g. leaf physiology, growth) measured. Samples taken for analysis of ABA content and expression of key genes for ABA synthesis and catabolism. Data analysed and available in summary form.
Screen for ABA and CK production developed. At least five commercial rootstocks screened.	30/6/2018	Simple screen (e.g. specific growth conditions, timing and type of sampling, etc. determined) to test grafted and/or ungrafted rootstock material for difference in root derived plant hormone production (ABA and CK). At least five commercially available rootstocks (chosen based on ecological background of parent material) obtained from commercial nursery and trialled with screen.
Literature review on graft structure and development in woody perennials available	30/6/2018	Literature review undertaken to establish the current knowledge on development of graft unions and their structure, including regulatory control and influence of genetic differences between rootstock and scion. Suitability of review for publication in scientific or industry journal determined.

FY2

Output	Target	Activities

Experimental plan for collaborative work with INRA Bordeaux developed	31/10/2019	EJE + PDF visit INRA Bordeaux for collaboration discussions with Greg Gambetta and other staff. Site visits to examine current INRA rootstock research plantings. Agreement on collaborative experiment plan (matched experiments and/or genotypes) towards improved understanding of rootstock conferred vigour, graft union impact on vine hydraulics and hormonal control of the shoot by root. Agreement on data sharing determined (collaboration is informal aligning of experimental work and not governed by a sub-contract or sharing of funds).
Experimental plan for collaborative work with University of Adelaide developed	31/10/2019	Schedule of work agreed with Vinay Paguy at UA for 2018/19. Contract signed by CSIRO and UA for provision of service (i.e. CT sample scanning, contribution towards hydraulic conductivity experimental work).
Plant material available for 2018/19 season glasshouse experiments	30/11/2019	Rootstock material obtained, grafted with a single scion clone and propagated to provide 30+ plants per genotype (as required). Genotypes representing different growth rates/conferred vigour chosen (e.g. RGM, 1103 Paulsen & M5512) for characterisation of graft union structural properties at different ages (1-18 months). Further genotypes chosen for additional screening of rootstocks for differences in plant hormone production (ABA, CK) and investigations into additional root:scion communication mechanism (e.g. small RNAs).
2018/19 season glasshouse experiments complete	30/6/2019	Potted vine experimental work for 2018/19 season finalised and planned in discussion with INRA Bordeaux and UA, following commencement of PDF position (appointed June 2018). Graft union material available for UA CT scanning. Water deficit experiments using the 'DroughtSpotter' complete, physiological data collated and all samples collected. Additional glasshouse experiments (to be determined) complete, physiological data collated and all samples collected. Sample processing underway for plant hormone and other signal molecule (to be determined) analysis.
Basic growth and production data from new CSIRO Irymple rootstock trial (planted 2017)	30/6/2019	Pruning weights and yield measured in 10 cultivars x six replicates. Data utilised for comparison of conferred traits (e.g. vigour) with various glasshouse experiments undertaken and archived for future use.

FY3

Output	Target	Activities

Experimental plan for collaborative work with University of Adelaide developed.	31/10/2020	Schedule of work agreed with Vinay Paguy at UA for 2019/20. Contract signed by CSIRO and UA for provision of service (i.e. CT sample scanning, contribution towards hydraulic conductivity experimental work).
Plant material available for 2019/20 season glasshouse experiment and new nursery planting at Irymple.	30/11/2019	Rootstock material obtained, grafted with two scions and propagated to provide 20+ plants per genotype/rootstock interaction (as required). Genotypes representing different growth rates/conferred vigour chosen (e.g. RGM, Ramsay, 1103 Paulsen & M6262) and grafted with two scions for characterisation of rootstock traits and their association with scion physiology.
2019/2020 season glasshouse experiments complete.	30/06/2020	Remaining harvests completed for the long-term glasshouse experiment begun in the 2018/19 season. Physiological data collected and all samples collected for plant hormonal and molecular analysis. Graft union and other tissues collected for CT scanning and microscopy analysis of vascular system. New glasshouse experimental work with potted vines developed in conjunction with INRA Bordeaux. Allied experiments at Bordeaux to be undertaken in their 2020 season. The CSIRO work will focus on rootstock induced differences in growth and development of the grafted vine, whilst the Bordeaux work will focus on rootstock induced differences in scion physiology.
Protocols/method development for project experimental work successfully established.	30/06/2020	After the recent visit to Bordeaux (France), two methods identified for future experimental work at CSIRO; 1) Low pressure flow system (to measure hydraulic conductance), and 2) plants tissue microscopy (to efficiently measure anatomical characteristics of rootstocks). A low pressure flow system will be developed and validated at the Waite Campus and the microscopy workflow for xylem structure analysis established and utilised with new and existing tissue samples.
Basic growth and production data from new CSIRO Irymple rootstock trial (planted 2017).	30/6/2020	Pruning weights and yield measured in 10 cultivars x six replicates. Data utilised for comparison of conferred traits (e.g. vigour) with various glasshouse experiments undertaken and archived for future use. Data shared with INRA Bordeaux 'GraphAdapt' project for cross-hemisphere comparison of matching scion/rootstock combinations.

New nursery planting established at CSIRO Irymple farm.	31/12/2019	A new short-term (6-18 months) nursery trial will be used to evaluate specific rootstock traits (especially root traits) in the field, whilst allowing a whole plant harvest for hydraulic or other measurements. Moreover, two scions (Cabernet, Shiraz) will be utilised, to ensure that observed traits, such as conferred vigour and plasticity of response under field conditions, are cross-genotype and any mechanistic conclusions are broadly valid. Grafted vines will be allowed to establish for the first season (2019-20) with a final plant harvest in the 2020-21 season. Plant growth will be assessed and an initial assessment of root characteristics will be examined if rate of establishment allows.

FY4

Output	Target	Activities
Experimental work (CT scanning) with collaborator at University of Adelaide complete.	31/06/2021	After successfully signing the contract between UA and CSIRO, the CT scanning of graft unions (obtained from previous experiment) completed at UA, followed by quantitative data analysis pipeline established.
Plant material available for 2020/21 season glasshouse experiment.	15/12/2021	Rootstock material obtained, grafted with two scions and propagated to provide 40+ plants per genotype/rootstock interaction (as required). Genotypes representing different growth rates/conferred vigour chosen (e.g. RGM, Ramsay, 1103 Paulsen & M6262) and grafted with two scions (Shiraz and Cabernet) to explore interaction of rootstock induced vigour and plant water use efficiency under deficit irrigation.
New glasshouse experiment at Plant Accelerator (2020/21) complete.	31/06/2021	A new short-term (few weeks) trial completed at Plant Accelerator facility at University of Adelaide which provide tight control of drought treatment and accurate measure of plant water use. The exact amount of plant water use together with the scion physiological responses under drought stress and then recovery period would allow to understand rootstock (with varying scion vigour) induced effects on scion phenotypic plasticity for physiological traits and possible mechanisms associated with this response such as hormonal regulation. An important aim is to test if scion phenotypic plasticity is affected by rootstocks or independent of rootstock induced vigour effects. Rootstocks with different conferred vigour chosen (e.g. RGM, Ramsay, 1103 Paulsen, M5489 & M6262) and grafted with two scions (Shiraz and Cabernet) to ensure that observed traits and their plasticity are cross-genotype and any mechanistic conclusions are broadly valid. Grafted vines established for few weeks and then subjected to two levels of irrigation (full and deficit irrigation) followed by a recovery to full irrigation. Physiological data recorded and different tissue samples collected for plant hormonal and molecular analysis.

2019/2020 season glasshouse experiment complete.	30/12/2020	The glasshouse experiment was due to be harvested in March 2020; however, due to COVID-19 situation the harvest was delayed. Plants are continuingly growing in the glasshouse and the harvest to be completed in Oct-Nov 2020. Physiological data recorded and different tissue samples collected for plant hormonal and molecular analysis.
Nursery experiment at CSIRO Irymple farm complete.	30/06/2021	A short-term (6-18 months) nursery trial was established to evaluate specific rootstock traits (especially root traits) in the field, whilst allowing a whole plant harvest for hydraulic or other physiological measurements. Grafted vines, six rootstocks grafted with two scions, established for the first season (2019-20) and final plant harvest completed in the 2020-21 season. Plant growth (including root traits) assessed, scion physiological observations recorded while collecting tissues samples for hormonal and molecular analysis.
Low pressure flow system successfully established.	30/12/2020	After the visit to Bordeaux (France), two methods identified for future experimental work at CSIRO; 1) Low pressure flow system (to measure hydraulic conductance), and 2) plants tissue microscopy (to efficiently measure anatomical characteristics of rootstocks). The microscopy workflow for xylem structure analysis has been established and successfully utilised with new tissue samples. However, low pressure flow system validation has been delayed due to COVID-19 situation. The system is established and system test on grapevine stems to be completed.
Basic growth and production data from new CSIRO Irymple rootstock trial (planted 2017).	30/6/2021	Pruning weights and yield measured in 10 cultivars x six replicates. Data utilised for comparison of conferred traits (e.g. vigour) with various glasshouse experiments undertaken and archived for future use. Data shared with INRA Bordeaux 'GraphAdapt' project for cross-hemisphere comparison of matching scion/rootstock combinations.

FY5

Aim **(Year 3 / Year 5 deliverables)**	**Outputs**	**Activities**	**Activity target date**
Relative roles of vine hydraulics and graft union structure in rootstock conferred vigour determined. Rootstocks that differ in production of root-derived signals (e.g. ABA, CK) under identical soil stress conditions identified and impact on scion function determined	Plant material available for 2021/22 season experiments.	• Rootstock material contrasting in conferred traits (e.g. vigour) obtained, grafted with two scions contrasting in stress response available for glasshouse experiment. • CSIRO Irymple nursery trial maintained and remaining vines available for experimental work. • Nuriootpa field site (originally part of CSSA1701-1.5) maintained.	15/12/2021

Relative roles of vine hydraulics and graft union structure in rootstock conferred vigour determined. Crop management strategies matched to specific rootstock scion combinations demonstrated to improve resource use efficiency in the field.	Basic growth and production data from new CSIRO Irymple rootstock trial (planted 2017).	• Canopy size, pruning weight, yield, yield components and basic fruit composition measured in 10 cultivars * six replicates. • Data utilised for comparison of conferred traits (e.g. vigour) with various glasshouse experiments undertaken and archived for future use. • Data shared with INRA Bordeaux 'GraphAdapt' project for cross-hemisphere comparison of matching scion/rootstock combinations.	30/6/2022
Rootstocks that differ in production of root-derived signals (e.g. ABA, CK) under identical soil stress conditions identified and impact on scion function determined	Potential for rootstock:scion communication via sap RNA determined.	• Xylem sap in volume obtained from two contrasting rootstocks grafted to the same scion using root pressure chamber. • Xylem sap examined for presence of messenger and micro RNA and basic transcriptome described if viable. mRNA and miRNA candidates of interest identified. • Matching or same sap samples used for multi-hormone determination. • All identified signals correlated with vine growth and/or physiology parameters.	30/6/2022
Relative roles of vine hydraulics and graft union structure in rootstock conferred vigour determined.	Influence of graft union structure on sap flow confirmed.	• Live feeding of contrast agent to glasshouse grown plants with contrasting rootstock genotypes grafted to the same scion via cut stem and intact root system. • Transpiration assessed. • Micro-CT imaging to confirm active vessels and structure.	
Relative roles of vine hydraulics and graft union structure in rootstock conferred vigour determined. Crop management strategies matched to specific rootstock scion combinations demonstrated to improve resource use efficiency in the field.	Relative roles of vine hydraulics and graft union structure in rootstock conferred vigour determined.	• All relevant project data collated, including 21/22 growing season measurements of hydraulic conductivity in 2017 planting and nursery planting at CSIRO Irymple on at least two occasions and 21/22 growing season glasshouse experiment results. • Influence of graft union structure on vine hydraulic conductivity and actual vine transpiration assessed. • Influence of vine total hydraulic conductivity on scion transpiration and growth assessed.	30/06/2022
New Yr 5 output: Communication of results of project	Submission of final project report. Communication of results as applicable.	• Prepare final report • Prepare papers for publication • Disseminate project outputs to industry where practical (e.g. AWRI Roadshow, industry seminars).	30/06/2022

Appendix 6: Budget reconciliation

A budget reconciliation will be provided separately.