Hort Innovation

## Final Report

# Better tree performance and water use efficiency through root system resilience

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**Delivery partner:**

CSIRO Agriculture & Food

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Better tree performance and water use efficiency through root system resilience (AL13009)

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## Summary

Plant root systems are the organ that acquires both water and nutrients, key to maximising production in any horticultural industry. Roots are also the organ directly affected by soil stresses, be they physical or chemical, and they signal this stress to the shoot. Much of our knowledge of root systems is derived from annual plants, with far less information available on perennial plants. In grafted trees, such as almond, rootstock choice has the ability to impart particular traits on the scion of choice, including resistance to specific soil stresses.

The project reported on here has combined a variety of studies on almond root systems, from commercial orchards to research greenhouses, in order to generate information to assist growers maximise resource use efficiency and production.

In collaboration with Hort Innovation project AL14005, work was undertaken over four seasons at a 2.2 ha field trial at Lindsay Point in NE Victoria, examining root system dynamics and tree water use in response to a 30% reduction in irrigation, a 40% reduction in nitrogen application and a combination of the two. Minimal impacts of the reduced inputs on the root system were observed and the reduction in tree water use was less than the reduction in applied irrigation, in line with the lack of any significant effect on yield reported by AL14005. This suggests that almond root systems are able to maintain adequate nutrition to the shoot in a broad range of input scenarios and improvements in resource use efficiency over current standard practice are possible.

This field study was complimented with detailed greenhouse studies using the stable isotope $^{15}N$ as a label, allowing nitrogen uptake to be quantified and tracked over in as little time as a few hours. This work used six rootstocks and three scions in eight combinations, providing two key learnings. First, that the fine root system was able to swiftly reach maximum nitrogen uptake rate, with little difference between rootstocks, meaning that that shoot demand was likely the major driver of whole plant nitrogen uptake. Secondly, that despite having little effect on nitrogen uptake rate, rootstock genotype did appear to influence whole tree nitrogen allocation. If this effect is also present in mature trees, it has implications for rootstock choice and tree management.

Finally, the project adapted a plant growth analysis method to use as a rapid pre-screen for rootstock resilience to soil abiotic stress, taking just a few weeks. This was tested and simplified to determine its limitations and ensure it was able to be used robustly by tree nurseries and importers of new genetic material. The screen is intended to be used to test and categorise genotypes prior to selection for long-term field trials or similar. Results of screening for resilience to water deficit and high soil conductivity are reported for eleven rootstock genotypes.



## Keywords

Abiotic stress, nitrogen uptake, reduced inputs, root dynamics, rootstocks, rootstock:scion interactions, screening, tree water use.

# Introduction

The recent history of the almond industry has been extremely positive, with plantings rising from 5,600 ha in 2001, to almost 29,000 ha at the start of this project in 2014 (ABA 2015) and currently stands at over 45,000 ha (ABA 2019). During this period, almond kernel production has risen from 9,100 t (2.2% of global) to approximately 80,000 t (over 6% of global), surpassing Spain in 2012 to become the world's second largest producer (ABA 2015, 2019). Despite this, the industry was concerned that absolute production was not being matched by an improvement in production efficiency (averaging <2 t $ha^{-1}$ in both 2001 and 2018, below that of the USA) and seasonal yield variation meant that industry expectations of production increases were not being met. Furthermore, the cash costs of almond production in Australia were estimated as AU$9,800 $ha^{-1}$ compared with US$6,840 in 2013, at a time when the US$ and AU$ were at parity (ABA 2013). The major differences being labour, fertiliser and irrigation costs. Consequently, the Almond Board of Australia arranged a number of workshops with research providers in 2013, which culminated in a group of research projects being established as part of a productivity programme, including the project reported on here.

Improving production efficiency and reducing costs presents a number of challenges for industry, including maximising radiation use efficiency (conversion of sunlight into yield), irrigation use efficiency and fertiliser uptake. Two of these factors (water and nutrient uptake) are mediated by the root system of the tree and the third (light interception) can be affected by rootstock conferred vigour traits as almonds are uniformly grown as a grafted tree. The root system also acts as a barrier to adverse soil characteristics, such as soil compaction, water deficit or high soil conductivity (whether through nutrient application or salinity). Despite this, to date the almond root system has received limited attention from the research sector, particularly in Australia, where over 90% of plantings are on a single rootstock, Nemaguard (Brown et al, 2013).

Plant root systems contain a complex range of sensing and signalling mechanisms that interact with the shoot, facilitating both active and passive transport of water and mineral elements. The use of grafted plants adds further complexity due to the different genetic backgrounds of the rootstock and scion and the potential influence of the graft union on these mechanisms. The potential of rootstock:scion interactions to provide plant performance benefits are amply demonstrated in the existing literature, with a wide range of studies describing the impact of rootstock use on scion vigour, water use efficiency, nutrient use efficiency, root-shoot signalling and salt tolerance in almonds and other tree crops.

This project was established to improve almond production efficiency through a better understanding of *Prunus* root systems, including fine root phenology and lifespan and the role of root factors such as class, age and morphology in nutrient uptake. In order to achieve this, the project combined controlled experiments on potted trees with extensive fieldwork at the same field trial used by Hort Innovation project AL14005, run by Agriculture Victoria at a commercial orchard (Treeby 2019). This approach allowed the information on root phenology and its response to reduced water and nitrogen application to be combined with the in-depth study of spur productivity and canopy structure undertaken by that project. Finally, this project has also assessed the efficacy of rootstock screening against abiotic stress, following on from the rootstock nematode resistance screening previously undertaken by CSIRO (Clingeleffer et al 2014).

# Methodology

## Project reach

Project work was undertaken at two commercial orchards in north-west Victoria and at the CSIRO Waite Campus site in South Australia. The commercial orchards were typical of Australian almond orchards. The experimental work carried out on young potted trees at the Waite Campus included the most common rootstock and scion genotypes used in the Australian almond industry and all genotypes were obtained from commercial nurseries. All of the project work undertaken was, therefore, intended to be relevant to the entire Australian almond industry.

## Target audience

The work at the commercial orchards was aimed at almond producers, industry consultants, service providers and other researchers. The work on rootstock pre-screens was primarily aimed nurseries and importers of rootstock genotypes, but was intended to be of value to the broader industry through the data generated. The work on root function was aimed at other researchers, industry consultants and advanced growers.

## Methods used

A detailed description of the methods used is provided in Appendix A, with a summary provided here. The project consisted of three component parts, designed to meet the two project outputs. The first was stand-alone work to develop an early screening assay to assess rootstock resilience to abiotic stresses (output 1). The second was field and greenhouse work to better understand the root systems of almond rootstocks in respect of resource uptake and use (knowledge required to develop output 2). The final part was the application of part 2 to trial fertiliser and water application schedules that would improve almond resource use efficiency (output 2).

The rapid growth of the industry during the early part of the project resulted in significant difficulty in sourcing the plant material required for the first component, extending the work into subsequent growing seasons. This subsequently impacted the capacity and timing of the greenhouse work to be undertaken as part of the second component. Whilst the rootstock screening work was augmented to contribute towards achieving output 2, the mid-term review of the project by Hort Innovation recognised that the large amount of data collected would be of value to the industry and that the timing of the experimental work meant that the final project component, field trials, was unlikely to be achieved as originally envisaged. The review suggested a shift in focus to ensure that the ongoing work was completed effectively rather than move resources into significant field trials.

### Component 1: rootstock abiotic stress screening

The role of rootstocks in providing tree resilience to soil abiotic stress is most effectively assessed by multi-location, long-term field trials. Such an assessment is extremely costly to run, requires a full orchard lifecycle (15-30 years) and needs to incorporate multiple management practices to provide a comprehensive result. As a precursor to such trials our aim was to develop a rapid pre-screen that could be used with ungrafted potted rootstocks and to process as many of the rootstocks available to the Australian almond industry as possible through the pre-screen.

The use of young ungrafted rootstock saplings to minimise the time, cost and space required for the screen meant that only stresses that directly impact the root system could be tested, such as soil compaction, toxicity, water availability, etc. Soil conductivity (assessed as salinity) and water deficit were chosen. The former was tested by irrigating to run off with 50 mM mixed chloride solution daily, equivalent to irrigating with 3.3 dS $m^{-1}$ water, seen as the maximum likely to be used in a commercial setting (Pitt et al, 2018). Irrigating to run-off in freely draining pots was used to ensure soil conductivity did not build up excessively during the screening trials. Water deficit was applied by irrigating to a fixed fraction of maximum transpiration, with daily transpiration of fully irrigated saplings assessed through pot weighing prior to irrigation each day. A tree root system can have two broad mechanisms to avoiding water stress, i) maintain water extraction from soil at lower soil water potential, or ii) find alternate sources of water through additional soil exploration. Our method could only test the former of these two mechanisms, but this was considered to be more representative of current industry irrigation practice.

Resilience to a specific stress can be assessed by a suitable marker, but whole plant productivity is the ultimate measure and tree yield is well correlated with vigour in the absence of factors such as excessive shading of

flowering buds. In our case we could only measure plant productivity (i.e. biomass) rather than production of kernels. The difficulty with measuring whole plant productivity, particularly in perennial plants, is that the non-productive parts of the plant increase as the plant grows (Jarvis & Jarvis, 1964)). To minimise this effect, we used *relative growth rate* (RGR) as the measure of plant productivity. Further, we did not use RGR directly, but the proportional change in RGR between trees subject to abiotic stress and trees not subject to stress.

### Component 2: root function and phenology

The phenology of fine root production, lifespan, turnover and their response to changes in irrigation and nitrogen fertiliser were studied using Nonpareil trees (on Nemaguard rootstock) at the CMV Farms Lindsay Point trial site, managed by Agriculture Victoria and described in the HortInnovation final report for AL14005 (Treeby 2019). The trial was a 2x2 factorial design, with six replicates. The treatments were standard or reduced irrigation (-30%) and standard or reduced nitrogen application (-40%). Roots were studied *in-situ* using 1.5 m long minirhizotrons (transparent plastic tubes, 35 mm in diameter) buried in the rooting/wetting zone of the trees in the trial. As the tubes were angled, they provided access to roots up to 1.3 m deep. A videoscope was used to image the upper surface of the minrihizotrons at approximately three-week intervals from June 2015 to June 2019. The images were analysed using the open source RootFly software to track individual roots over time.

This field study was complimented with two major pot experiments at the Waite Campus. Five-year old trees (Nonpareil on Nemaguard) were grown in the experimental orchard in 200 L containers and fertilised with urea labelled with the stable isotope of nitrogen ($^{15}N$) at three timepoints during the growing season: canopy closure, pre-harvest and post-harvest. Leaf and root samples were taken to track labelled nitrogen uptake following each application, with root samples split into three root size classes based on diameter.

A second pot experiment using grafted trees was undertaken in a greenhouse environment. This examined $^{15}N$ labelled urea uptake across six rootstocks (Barrier 1, Brights Hybrid, Cornerstone, Garnem, GF677 and Nemaguard) all grafted with Nonpareil and three scions (Nonpareil, Carina and Carmel) all grafted to Nemaguard. As well as tracking uptake in leaves and roots, the entire tree was harvested after nine days and whole plant uptake of labelled N determined.

### Component 3: new management strategies

As noted above, following the mid-term review, this component was minimised. Two areas of work addressed this, firstly, the linkage between the work undertaken by this project at Lindsay Point and by project AL14005, allowed the effects of reduced irrigation and nitrogen on tree productivity to be combined with effects on the root system. In addition, sapflow sensors were installed in the final three seasons (2016/17, 2017/18 and 2018/19) from budburst until harvest to provide an additional link between root and shoot responses as well as determine the effect of reduced inputs on actual tree water use.

Secondly, work was undertaken with Select Harvests on a compost and fertiliser trial they implemented in 2018/19 and 2019/20, with soil and root samples taken to determine the interaction between the treatments applied (control, control+compost, compost+reduced P & K application), root responses and changes to the turnover of soil biota in regards to nitrogen cycling.

# Outputs

The major results from the project and their interpretation are provided in **Appendix A**. The project defined two main outputs, which are summarised here, together with ancillary outputs.

## Output 1: an assessment of the resilience of available *Prunus* rootstocks to abiotic stress

Discussion with industry representatives through the Almond Board of Australia settled on water deficit and soil conductivity as appropriate stresses to use for development of a pre-screen for abiotic stress tolerance. A total of 17 different rootstock genotypes were screened against soil water deficit and against irrigation with 3.3 dS $m^{-1}$ water (Figure 1). Five of these were screened multiple times, with three subjected to a further validation experiment. The work was completed in six tranches as planting material became available over several seasons, with the validation experiment completing three further tranches. Table 1 provides an index for each rootstock for tolerance of water deficit and tolerance of high soil conductivity from the screening development work (See also Figure 11, below). In addition the methodology has been shared with the tree crops industry through a publication in an industry journal (Edwards 2021b).

*Figure 1 - Images of greenhouse Prunus rootstock screening in progress.*

## Output 2: optimised management for root systems to improve resource use efficiency

The intention of the second output was to provide extension information to growers with advice on more efficient irrigation and/or fertilisation management strategies based on field trial results which would, in turn, be informed by improved knowledge of root activity and uptake capacity. As described below (M&E section), significant events affected the capacity to undertake new field trials and their timeliness. Consequently, it was suggested during the mid-term review with Hort Innovation that additional field trials would not be the most efficient way to use the remaining project resources.

Output 2 is therefore, the information and advice provided within this report, the information already provided to industry through the articles and presentations listed below and anticipated future presentations to industry based on the information within this report. Copies of the industry articles and a selection of conference posters is provided in **Appendix B**.

*Table 1 – stress indices for water deficit and high soil conductivity derived from greenhouse screening for a selection of Prunus rootstocks. Superscript letters indicate a statistically significant grouping.*

| Rootstock genotype | Water deficit index | Soil conductivity index |
|---|---|---|
| Barrier 1 | 1.0$^{A}$ | 1.0$^{A}$ |
| Bright's Hybrid | 0.4$^{B}$ | 0.8$^{A}$ |
| Controller 6 | 1.1$^{A}$ | 1.0$^{A}$ |
| Controller 7 | 0.4$^{B}$ | 0.9$^{A}$ |
| Controller 9.5 | 0.4$^{B}$ | 0.6$^{B}$ |
| Cornerstone | 0.5$^{B}$ | 0.8$^{A}$ |
| Garnem | 0.4$^{B}$ | 0.8$^{A}$ |
| GF677 | 0.5$^{B}$ | 1.0$^{A}$ |
| Krymsk 86 | 0.4$^{B}$ | 1.0$^{A}$ |
| Nemaguard | 0.5$^{B}$ | 0.6$^{A}$ |
| RootPac 20 | 0.4$^{B}$ | 0.8$^{A}$ |

### Industry publications:

Zhou, S.-X., Edwards, E.J., Walker, R.R., 2016. Better tree performance through root system resilience. *Australian Nutgrower*, December 2016, 32-34.

Zhou, S.-X., Edwards, E.J., Walker, R.R., 2016. Using minirhizotrons to monitor root production and turnover in an almond orchard. *Australian Nutgrower*, December 2016, 34-35.

Edwards, E.J., Zhou, S.-X., Lawlor, A., Semmler, A., Welti, N., MacDonald, L., Walker, R.R., 2019. Better tree performance through root system resilience – project update. *Australian Nutgrower*, Autumn 2019, 15-17.

Edwards, E.J., Zhou, S.-X., Lawlor, A., Semmler, A., Welti, N., MacDonald, L., Walker, R.R., 2020. Project update: better tree performance through root system resilience. *Australian Nutgrower*, Autumn 2020, 22-23.

Edwards, E.J., Lawlor, A., Semmler, A., Zhou, S.-X., Welti, N., Walker, R.R., 2020. Better tree performance through root system resilience. *In a Nutshell*, December 2020.

Edwards, E.J. 2021a. Understanding roots for greater efficiency. *Australian Tree Crop*, December/January 2021, 14-17.

Edwards, E.J. 2021b. Rapid screening of rootstocks for resilience to abiotic stress. *Australian Tree Crop*, February/March 2021, 36-37.

Edwards, E.J. 2021c. How do almond root systems respond to changed inputs? *Australian Nutgrower*, in press.

### Conference and industry presentations:

Edwards, E.J., Walker, R.R. Roots and rootstocks: better tree performance through root system resilience. Poster presentation at the 16th Australian Almond Conference, October 2014, Adelaide, SA.

Zhou, S.-X., Edwards, E.J., Walker, R.R. Assessing Prunus rootstocks for tolerance to water deficit and salinity. Poster presentation at the 9th International Symposium of Root Research, October 2015, Canberra, ACT.

Zhou, S.-X., Edwards, E.J., Lawlor, A. Better tree performance and water use efficiency through root system resilience. Oral presentation at the 2015 Activated Almonds Forum, October 2015, Renmark, SA.

Zhou, S.-X., Walker, R.R., Edwards, E.J. Differential sensitivity and acclimation of leaf and fine root traits among congeneric *Prunus* taxa under drought or salinity stress. Poster presentation at Combio 2016, October 2016, Brisbane, QLD.

Zhou, S.-X., Walker, R.R., Edwards, E.J. Better tree performance and water use efficiency through resilient root

systems. Poster presentation at the 17th Australian Almond Conference, November 2016, Melbourne, VIC.

Edwards, E.J., Walker, M.W. Resilient root systems for the Australian almond industry. Oral presentation at the 2017 Australian Almond R&D Forum, October 2017, Loxton, SA.

Zhou, S.-X., Edwards, E.J., Lawlor, A., Walker, R.R. Better tree performance and water use efficiency through resilient root systems - assessment of resilience of Prunus rootstocks to abiotic stresses. Oral presentation at the VIIth International Symposium on Almonds & Pistachios, November 2017, Adelaide, SA.

Zhou, S.-X., Lawlor, A., Walker, R.R., Edwards, E.J., Better tree performance and water use efficiency through resilient root systems. Poster presentation at the US Almond Conference, December 2017, Sacramento, CA, USA.

Zhou, S.-X., Walker, R.R., Edwards, E.J. Differential plasticity of leaf and root acquisitive traits among six *Prunus* taxa under short-term versus prolonged drought or salinity stress. Poster presentation at the 10th International Symposium of Root Research, July 2018, Tel Aviv, Israel.

Edwards, E.J. Better tree performance through root system resilience. Oral presentation to the attendees of the 2019 Almond Board of Australia Riverland field-walk, August 2019, Paringa, SA.

Edwards, E.J., Lawlor, A., Semmler, A., Zhou, S.-X., Walker, R.R. Better tree performance through root system resilience. Oral presentation to the 2019 Australian Almond R&D Forum and Field Day, October 2019, Loxton, SA.

# Outcomes

The intended long-term outcomes of this project are improved rootstock choice by the Australian almond industry and more efficient water and fertiliser use. As this project predates current Hort Innovation policy on M&E plans, monitoring data is not available to inform this section of the report. The project outcomes that support the intended long-term industry improvements are listed below.

## Simple screening method for *Prunus* rootstocks available for nurseries and growers

The project has adapted a plant growth analysis technique to use as a rapid pre-screen for almond rootstocks against soil imposed abiotic stresses that can be utilised by any nursery, importer, or even a grower The screen takes only a few weeks and can be undertaken in a greenhouse. The technique has been published in "Australian Tree Crop", with instructions and a clear description of its applicability and potential limitations (Edwards 2021b).

The screen has been tested using two stresses and the results are provided in this report. In addition, they will be provided to industry via the same mechanism. Some of this information has already been provided to industry via presentations and posters (see Appendix B).

This part of the project outputs will aid nurseries and others to rapidly assess genotypes against suitable factors in a robust way, minimising lengthy field trials.

## Location, timing and phenology of fine root growth determined under commercial orchard conditions to assist grower management decisions

A better understanding of the location in the soil of active fine roots, the timing of root growth flushes and the impact of phenology through the growing season has the potential to improve the accuracy, in both time and space, of fertiliser and irrigation inputs in almond orchards. The tools available to build this understanding are extremely labour intensive and this project is unique in applying them to almonds in Australia, in fact the author knows of only one published study anywhere in the world that has used minirhizotrons in an almond orchard (Franco et al. 1995), which looked at root development in a new almond orchard, and one unpublished study (Brown pers. com.).

The results from this work indicate that almond roots are relatively long-lived and seasonality of production and loss is not as pronounced as expected. Combined with the nitrogen uptake information, this suggests that, from a root system perspective, growers can be freer in the timing of fertiliser application during the season (noting the timing of shoot requirements and the role of the shoot in determining nitrogen uptake). A draft publication summarising this for growers has been prepared and submitted to Australian Nutgrower (Edwards 2021c), together with an article on the Agriculture Victoria work (AL14005).

The project has also worked with CSIRO to develop computer software that assists in preparing minirhizotron images for analysis and in aggregating the results, which will assist further studies in this area in the future. It is expected that CSIRO will release these as open source software in the future.

## Nutrient uptake capacity of fine root types determined together with effect of phenology to assist with grower management conditions

Unfortunately, one impact of COVID-19 on this project was to prevent the analysis of the samples collected to determine the effect of phenology on root nitrogen uptake in the natural environment being undertaken prior to the end of the project and full expenditure of funds. Other opportunities to complete this will be sought. It is anticipated that if these results were available they could be combined with the data on nitrogen uptake by roots in a greenhouse environment and the data on root production and turnover in the field to generate a simple model of potential nitrogen uptake by almond trees through a growing season, which would be of value to growers.

The greenhouse data collected indicates that fine roots of different size classes and ages are all able to take up available nitrogen, albeit uptake rate by older roots may be slower. Furthermore, the finding that shoot demand is the most significant driver of nitrogen uptake suggests that growers can concentrate on satisfying shoot requirements with less concern about the ability of roots to access and absorb nitrogen. This outcome will also be

communicated to growers in the near future via industry journal articles.

### Information on the potential impact of rootstock:scion combinations on nutrient uptake capability available

Whilst based on a greenhouse study and acknowledging the finding in this project that root nitrogen uptake is largely shoot determined, the observation that specific rootstock scion combinations (e.g. Garnem/Nonpareil) appear to strongly influence the allocation of nitrogen within the tree is an important finding. Understanding this has the potential to impact rootstock breeding, tree management and choice of genotypes by growers. This finding would need to be confirmed in the field environment through a future research project before being communicated broadly to industry, but is a potentially significant outcome of the project.

# Monitoring and evaluation

This project pre-dated the current Hort Innovation M&E planning policy and was established as a suite of projects developed with the Almond Board of Australia and funded through the Almond Levy to address perceived productivity shortfalls. Despite this, the individual projects, including this one, operated individually with limited interaction between the different research providers, even though most used a shared field trial at CMV Farms Lindsay Point. The effectiveness of this project would have been improved by more formal interaction between the various research teams, such as annual meetings. A more detailed knowledge of the progress of the other projects would have allowed measurements and data to be better aligned and more easily synthesised for grower communications. In particular, collating the various root and shoot data will likely provide improved outcomes for growers. This has been discussed by the project leads for this project and AL14005 and will occur in the future.

The project suffered three significant events which had ongoing ramifications for the life of the project. Firstly, the growth of the industry led to major difficulties in obtaining planting material for the rootstock screening studies. The original planning had the first year of the project split between greenhouse screening and fieldwork at Lindsay Point, but only one third of the genotypes were able to be obtained in that first season. The outcome of this was that the greenhouse and staffing capacities were still utilised, but with a reduced output (in terms of number of genotypes) that had to be completed in later seasons, affecting the amount of greenhouse work that could be undertaken to complete the root function studies planned. The reduced number of genotypes screened at any one time allowed more detailed data to be collected, such as leaf physiology measurements in the water deficit treatments and chloride uptake in the soil conductivity treatments, so the value of the staff capacity committed was maximised. However, the overall impact on project outputs may have been reduced if those outputs had been formally varied with Hort Innovation, rather than being completed.

The situation was discussed with Hort Innovation during the mid-term review, undertaken in mid-2017 with Byron de Kock (R&D Manager at Hort Innovation), with the advice that the project should minimise or cancel the new field trials planned for the final two seasons. As a result, the project team limited this final part of the work to collaborating with Select Harvests on a compost and nutrition trial established at their Kindalyn Park orchard.

The second significant event was the resignation of Post-doctoral Fellow (PDF), Shuang-Xi Zhou, in early 2018, after he was offered a permanent position in New Zealand. With the project originally due to end in 2019 there was little time to recruit and appoint a replacement, particularly with CSIRO limitations on short-duration PDF. Consequently, a no-cost variation was discussed with, and subsequently approved by, Hort Innovation, whereby the end date of the project would be extended for a year, allowing the remaining work to be spread over two years and a Technical Officer appointed to assist with that work. This allowed the project to complete it's agreed program of work and meet its revised outputs.

A third event, the COVID-19 pandemic, impacted the project six months prior to its completion. A final glasshouse trial, the third and final run of the rootstock screening validation experiment, was delayed, but completed in November 2020. Although staff were able to continue working prior to this date, subject to COVID-safe restrictions including limitation on staff numbers within critical infrastructure, the experiment could not be run earlier, due to the trees being in winter dormancy. The requirement to work from home for staff also slowed down some sample processing due to lack of lab access, meaning that a small amount of data was not available at the time of report submission ($^{15}N$ results from the outdoor 5-year old tree phenology experiment) and these will be made available to industry as they become available, early in 2021.

As described above, the project consisted of three components: i) rootstock screening, ii) root system function, production and phenology, and iii) nutrient and irrigation trials. Both i) and iii) are discussed earlier in this section. The field trial work at Lindsay Point crossed over ii) and iii) and accounted for a significant proportion of staff time, with over 70 field trips across more than four years, each trip representing a return journey of over six hours for 2-3 staff on top of the actual work at the site. The primary work undertaken at Lindsay Point, root imaging using minirhizotrons, also required a very significant amount of staff time to process, being a largely manual image analysis task. At no cost to the project, CSIRO Scientific Computing staff assisted this in building scripts to automate the initial image processing and to collate and summarise the results, saving hundreds of hours of staff time, but significant manual processing remained. The method used remains the only means by which root production and turnover can be studied under field conditions and the data set will provide further insights following additional analysis in the future, but industry investment would be reduced for future work of this nature if it is undertaken on a smaller scale at an orchard closer to the researchers home location.

The time required to complete the rootstock screening and the field studies at Lindsay Point resulted in less time

being available for component ii), the detailed root function studies, than originally planned. This resulted in simplifying the original planned experiments. Although the changes meant that the inference in terms of nitrogen uptake from different root types was more limited, it did offer an opportunity to directly examine a broad combination of rootstock:scion combinations, including multiple rootstocks grafted to the same scion and vice:versa, thereby providing additional information that may be valuable to assist growers in making planting choices.

# Recommendations

The rapid pre-screen for soil imposed abiotic stress resilience in rootstocks has the potential to be a useful tool for nurseries and importers of new genetic material. It also has the potential to be adopted for assessment of other abiotic stresses that can be controlled in a nursery environment, whether it be rootstock or scion. Whilst this project allocated more resources than originally envisaged to this tool and testing available genotypes, it would be valuable to work with nurseries in determining the areas of potential application and collating results from different sources to provide to industry more broadly.

The information on belowground root system activity and tree water use under different reduced input management regimes at the Lindsay Point field site corroborates the recommendations of AL14005, based on the same site, in that there is potential for inputs of both water and nitrogen to be reduced with minimal impact on yield. However, as with other perennial horticulture industries, the almond industry is strongly risk adverse in this respect, thus supporting grower communication with further, grower led, trials of reduced inputs with open collation of the results could provide confidence in adoption of lower input, more resource use efficient orchard management. Digital technologies for on-the-go assessment of tree stress and physiology may also assist.

Similarly, although the soil amendment trial at Robinvale was too limited (in duration and replication) to draw clear inferences on the impact of organic matter additions on resource use in almond orchards, the results did indicate the potential for value in terms of reduced fertiliser input and improved soil edaphic conditions. Further, scientifically designed trials, preferably combining the skills of researchers in soils, tree physiology and agronomy, are called for.

The results of this project indicate that, in general, shoot demand is more important than root factors in determining tree nitrogen uptake. It is therefore recommended, that industry focusses on the shoot in determining nitrogen management, with root systems likely to be able to provide supplied nitrogen when required. That said, the observation of specific rootstock:scion effects on nitrogen allocation in the tree should be followed up with further research.

Finally, this project invested significant resources into collecting a unique data set on root growth in a commercial orchard and the impacts of reduced inputs on that. The analysis to date has meet the project requirements and provided industry with some surety on root system resilience to these conditions, there is significantly more analysis and detailed information that could potentially be obtained from that data set. It is therefore recommended that the project team take any available opportunities to further utilise this dataset and provide a more nuanced recommendation to industry.

# Refereed scientific publications

Copies of scientific publications are provided in **Appendix C**.

Zhou, S.-X., Walker, R.R., Edwards, E.J., 2018. Better tree performance and water use efficiency through resilient root systems: rapid screening for drought and salinity stress tolerance using relative growth rate. *Acta Horticulturae*, **1219**, 151–156. https://doi.org/10.17660/ActaHortic.2018.1219.25

Zhou, S.-X., Walker, R.R., Edwards, E.J., 2020. Decoupled drought responses of fine-root versus leaf acquisitive traits among six Prunus hybrids. *Journal of Plant Ecology*, **13**, 304–312. https://doi.org/10.1093/jpe/rtaa015

# References

ABA, 2013. Almond orchard productivity: R&D Project Development Workshop background papers. Almond Board of Australia, Berri, SA, 55pp

ABA, 2015. Almond Insights 2014-15. Almond Board of Australia, Berri, SA, 20pp.

ABA, 2019. Almond Insights 2018-19. Almond Board of Australia, Loxton, SA, 20pp.

Beadle, C.L., Growth analysis. In: Photosynthesis and production in a changing environment. Eds. Hall, D.O., Sculock, J.M.O., Bolhar-Nordenkampf, H.R., Leegood, R.C. and Long, S.P. Chapman & Hall, London.

Brown, B., Clingeleffer, P., Morales, N., Smith, B., 2013. Tolerance of almond rootstocks to root-knot nematodes. All About Almonds Factsheet. Almond Board of Australia, Berri, SA, 20pp.

Clingeleffer, P., Morales, N., Smith, B., 2014. Tolerance of almond rootstocks to root-knot nematode. Final report to Horticulture Australia Ltd.

Edwards, E.J., Collins, M.J., Clingeleffer, P.R., Walker, R.R., 2013. Achieving water use efficiency and improved drought tolerance with rootstocks. Final report to the Grape and Wine Research and Development Corporation.

Franco, J. A., Abrisqueta, J. M., Hernansaez, A. 1995. Root development of almond rootstocks in a young almond orchard under trickle irrigation as affected by almond scion cultivar. *Journal of Horticultural Science*, **70**, 597-607.

Jarvis, P.G., Jarvis, M.S., 1964. Growth Rates of Woody Plants. Physiologia Plantarum, 17: 654-666.

Pitt, T., Stevens, R. and Cox, J.W., 2018. Assessing the salt sensitivity of mature almond trees by replacing the resident saline irrigation with fresh water at different growth stages. *Acta Hortic.* **1219**, 251-258.

Steppe, K. De Pauw, D.J.W., Doody, T.M. Teskey, T.O. 2010. A comparison of sap flux density using thermal dissipation, heat pulse velocity and heat field deformation methods. *Agricultural and Forest Meteorology*, **150**, 1046-1056.

Treeby, M., 2019. Identifying factors that influence spur productivity in almonds. Final report to Hort Innovation. 108pp.

# Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report

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# Appendix A: detailed report

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## Detailed Methods

### Rootstock screening

The rootstock screening was conducted on the principal that any stress that negatively affects the root system would necessarily have an impact on whole plant growth. Absolute growth, the change in plant mass over a period of time, is non-linear, particularly in perennial plants due to their woody tissues. Consequently, relative growth rate (RGR) was used to assess changes in plant production in response to stress. RGR was calculated according to Beadle (1993) as:

$$RGR = \frac{1}{W}\frac{dW}{dt} = \frac{d}{dt}(\ln W)$$

Where *W* is the whole plant dry mass and t is time. The fewest number of plant harvests able to provide a robust estimate of RGR is three, so to minimise the duration of the screening experiments three whole tree harvests were undertaken, approximately two weeks apart. As the saplings were grown in a naturally lit glasshouse, the duration between harvests was extended when the daylength was short to allow enough growth to be measurable.

The impact of applied stressed was then determined as the relative effect on RGR:

$$Stress\ Index = RGR(Stress)/RGR(control)$$

Two stresses were used, water deficit and high soil conductivity. In all cases, both stresses were run consecutively, to enable the same plants to act as controls. Water deficit was applied by weighing the control trees daily, prior to irrigation to determine water use and applying a set fraction of that water use to the deficit treatments. Water use was determined per genotype. A trial experiment was used to assess the deficit required to generate moderate stress, determined as stomatal conductance ($g_s$) below half of the control plants. This was estimated as approximately 1/3$^{rd}$ the water use of the controls (evapotranspirtion; ET). Following the first tranche of genotypes the deficit was adjusted to 50% of control ET.

High soil conductivity was simulated by irrigating using water with an electrical conductivity (EC) of 3.3 dS/m. This was achieved by using a mixed chloride solution consisting of 20 mM NaCl, 3.3 mM $MgCl_2$ and 3.3 mM $CaCl_2$, a total of 33. 2 mmol $Cl^-$ per litre.

RGR of seventeen rootstocks was determined in six tranches (Table 2) over a two-year period using greenhouse grown, ungrafted material in pots with UC mix potting medium for 3-10 months prior to being subjected to the two stress treatments. The first of the three harvests was performed only on untreated plants. At each harvest, the leaves were removed, leaf area determined using a LiCOR Li3000 (LiCOR Inc, Lincoln, Nevada, USA) leaf area meter, and the leaves dried in an oven at 60°C. The stem was removed and also dried. Finally, the soil was left to partially dry, following which the roots sifted from the soil and again oven dried. Splitting the plant into leaf, stem and root and determining leaf area, allowed RGR to be factored out specific leaf area (leaf area / leaf dry mass; SLA) and leaf

mass ratio (leaf dry mass / whole plant dry mass; LMR) to determine unit leaf rate (ULR; an estimate of whole plant assimilation rate) according to:

$$RGR = SLA.LMR.ULR$$

*Table 2 – Tranches of rootstock genotypes screened against abiotic stress*

| Tranche | Genotypes | Start date | Days growth | Water deficit | Irrigation conductivity (dS $m^{-1}$) | Received Radiation (MJ $m^{-2}$) |
|---|---|---|---|---|---|---|
| 1 | Bright's Hybrid<br>Cornerstone<br>Felinem<br>GF677<br>Monegro<br>Nemaguard<br>Viking | 1/6/2015 | 58 | 33% ET | 3.3 | 472 |
| 2 | Barrier 1 | 18/11/2015 | 28 | 50% ET | 3.3 | 738 |
| 3 | Cadaman<br>Garnem | 11/2016 | 29 | 50% ET | 3.3 | 710 |
| 4 | Atlas | 7/4/2016 | 28 | 50% ET | 3.3 | 352 |
| 5 | Krymsk 86 | 9/5/2016 | 29 | 50% ET | 3.3 | 235 |
| 6 | Bright's Hybrid<br>Controller 6<br>Controller 7<br>Controller 9.5<br>Cornerstone<br>Garnem<br>GF677<br>RootPac 20<br>RootPac 40 | 20/1/2017 | 34 | 50% ET | 3.3 | 770 |

The use of relative change in RGR was to provide some degree of robustness of the stress index to differences in plant size. As different genotypes are propagated using different techniques, it is not practical to ensure that all are the same mass at the start of a screen. Furthermore, as the screens were run at different times of the year, the light environment also varied. To test the robustness of the stress index a validation experiment was designed. Three genotypes, Nemaguard, Garnem and Cornerstone, were selected and planting material obtained. These were potted as described above and then the same three harvest and stress procedure performed at three different times, approximately one, three and thirteen months after planting.

### Root dynamics and tree water use under reduced nitrogen application and irrigation

The experimental site (Figure 2) was the same one used for Hort Innovation project AL14005 and described in Treeby (2019). Briefly, the site was a commercial orchard at Lindsay Point in NE Victoria, with irrigation (standard vs 70% of standard) and nitrogen (standard vs 60% of standard) treatments applied factorially in a randomised complete block design with six blocks, giving 24 individual plots. Treatments were applied from the 2015/16 season onwards for four seasons. Each plot consisted of eight trees and four rows, with two rows of Nonpareil trees alternating with Carmel and Monterey as pollinators.

In April 2014, minirhizotrons tubes were installed in each plot in the most central Nonpareil row (Figure 3). These were clear acrylic tubes with an internal diameter of 35 mm and 1500 mm long. They were installed at an angle of 70° to the horizontal, meaning they reached to soil depth of just over 1000 mm. The tubes were also angled from centre of the tree line, midway between two trees, at an angle of 45° with respect to the tree line towards a tree, meaning that they were placed through the wetting zone and anticipated rooting zone.

*Figure 2 – water and nitrogen treatments at the Lindsay Point field site, overlaid on a Google Earth image.*

Each tube was imaged down its entire length using an Olympus IPlex FX video scope on 69 occasions, at roughly three-week intervals from June 2015 until June 2019. The resulting images were corrected for variations in viewing angle and rotation, cropped and resized using bespoke software written by CSIRO Scientific Computing, based on software written for the South Australian Research and Development Institute. Roots in the images were then hand traced using the open source RootFly software (http://cecas.clemson.edu/~stb/rootfly/). The RootFly output was then aggregated and processed using a further software tool written by the CSIRO Scientific Computing to provide root number and length for each image, change from the previous image, root lifespan etc.

*Figure 3 – minirhizotrons installed at the Lindsay Point orchard (left) and root imaging in progress (right).*

The minirhizotron data was complimented with soil coring (Figure 4), to allow 3D root distribution to be determined. Soil coring was only practical during the winter, to avoid damage to the trees by the equipment. Cores were taken in each plot on five occasions, July 2015 (prior to the onset of the treatments), August 2016, July 2017, August 2018 and August 2019. Typically, a three core transect was taken, 1200, 2000 and 3500 mm from the tree line approximate 400 mm away from a tree trunk along the tree line. In 2016, a fourth core was taken 700 mm from the tree line and in 2015 only a single core was taken, close to the tree line. Each core was taken to 750 mm depth and split into three 250 mm sections. In 2015, the cores were taken to 1,000 mm deep and split into four sections, but this was not possible away from the treeline mound due to a hard calcareous layer approximately 800 mm below the soil surface.

The roots were extracted from each core section by washing and sieving, split into fine (<3 mm diameter) and other (>3 mm diameter) root components, the fine roots were scanned for root length using WinRhizo (Regent Instruments, Ontario, Canada) and then each component was oven dried to determine dry mass.

*Figure 4 – soil coring at the Lindsay Point field site for root density estimates.*

Tree water use of the same trees was determined using SFM1 sapflow sensors (ICT International, Armidale, NSW, Australia). These were trialled for part of the 2016/17 season and then used from budburst until harvest during the 2017/18 and 2018/19 seasons (Figure 5). One sensor was used per plot and these were installed midway between the soil surface and the first branching (approximately 300 mm from ground level). The sensors were wrapped in foil insulation to minimise rapid solar heating affecting the measurements. The sensors had to be removed prior to harvest to prevent damage during tree shaking and couldn't be reinstalled until after any second shake. Bark depth, sapwood depth and wood density were all determined on 5 mm diameter wood cores taken from trees adjacent to the experimental plots. Tree circumference was determined approximately once per season at the height where the sensors were installed.

### Nitrogen uptake capacity of *Prunus* roots

Root nitrogen uptake was examined using urea as a fertiliser, labelled using the stable isotope $^{15}N$ at 10 atom percent. This could be traced using sequential biomass samples over time and where full recovery was possible (complete harvest of plants and soil sampling), a mass balance could also be calculated, allowing the allocation of the newly assimilated nitrogen to be examined also.

Two experiments were implemented during the 2018/19 growing season. The first to examine the effect of

phenology on uptake, using five-year old trees growing in an orchard in 200 L pots (Fig 6). Labelled urea was applied to five trees and unlabelled urea applied to a further five on three occasions during the growing season: November, January and late February. Leaf samples were taken at application, then 1, 3, 6 and 10 days after application to be analysed for the quantity of labelled nitrogen present. Root cores were taken 1, 3 and 10 days after application. Roots were extracted without washing and also analysed for label content. In addition, leaf samples were taken from trees that had received label monthly after application until the end of the season and root cores were taken from all trees in June 2019.

*Figure 5 – sapflow sensors installed in Nonpareil trees at the Lindsay Point field site.*

*Figure 6 – Nina Welti (left) and Alex Lawlor (right) with the five-year old almond trees planted in 200 L pots at the Waite Campus, Adelaide for nitrogen uptake experiments.*

The second experiment was a glasshouse experiment using potted grafted trees with three scions and seven rootstocks (Table 3), designed so that each scion was grafted to a common rootstock (Nemaguard) and each rootstock grafted to a common scion (Nonpareil). Six trees from each combination were fertilised with labelled urea and six plants with non-labelled urea. Leaf samples were taken from the trees at the time of application, 16 hours after application, 21 hours after application, then 2, 3 and 6 days after application. Root cores were taken 18 hours after application and after three days. At day nine, the entire tree was harvested, with large samples taken of leaves, new stem growth, woody stem, and roots. Soil sample were also taken. Dry mass was determined for all samples and the remaining bulk tissue. All samples were then analysed for $^{15}N$ label content.

*Table 3 – rootstock:scion combinations used for labelled nitrogen experiment.*

| Scion | Rootstock | Comparison |
|---|---|---|
| Carina | Nemaguard | scion |
| Carmel | Nemaguard | scion |
| Nonpareil | Nemaguard | scion & rootstock |
| Nonpareil | Barrier 1 | rootstock |
| Nonpareil | Bright's Hybrid | rootstock |
| Nonpareil | Cornerstone | rootstock |
| Nonpareil | Garnem | rootstock |
| Nonpareil | GF677 | rootstock |

### Impact of soil organic matter amendment and reduced fertiliser application on roots and soil

A trial to examine the effect of adding a compost generated from almond hull & shell ash and orchard woody waste was established by Select Harvests at an orchard in NE Victoria. Compost application, applied as a band on the soil surface in the wetting zone at a rate of 4 t $ha^{-1}$, was combined with standard or reduced potassium and phosphorus fertiliser (Figure 7). Treatments were applied to entire blocks, meaning that the multiple sample sites used within a block only represented psesudo-replication.

Soil samples to 60 cm depth were taken immediately prior to compost application, August 2018, and approximately 4 and 16 weeks after compost application, in September and December 2018. Soil samples were analysed for C, N, P, & K as well as plant available nitrogen sources and 5:1 pH and electrical conductivity.

## Rootstock screening

The first tranche of seven rootstocks to be screened were grown under adverse lighting conditions due to the timing of staff appointments and the availability of greenhouse space and planting material. As a result, growth rates were extremely low (2-6 g $g^{-1}$ $d^{-1}$) and the results have been excluded from the calculation of stress indices provided below. Furthermore, physiological measurements made suggested the water deficit treatment was excessive and would only be representative of irrigation levels that would be unlikely to allow a commercially viable production in an orchard, resulting a less severe stress being applied to the following tranches. A significant effort was made to collect physiological and morphological data from this experiment, which was later published as a refereed paper: "Decoupled drought responses of fine-root versus leaf acquisitive traits among six Prunus hybrids" (a copy is provided in Appendix C). The primary conclusion was that under the experimental conditions, leaf and root morphology did not respond to stress in the co-ordinated manner as expected.

The following five tranches examined the response of thirteen genotypes to moderate water deficit and to moderate salinity. One of those thirteen, RootPac 40, did not perform well enough in the greenhouse to be considered representative (RGR = 8.7 g $g^{-1}$ $d^{-1}$) and has also been excluded from the data presented below.

Whole tree dry weight (DWt) at the start of the experiments varied from 5 to 33 g. No attempt was made to ensure that all saplings were the same size as the intention of the screening was to provide results that were independent of plant size to a moderate extent. Actual differences were due to propagation method employed by

the nursery (e.g. tissue culture vs seeding vs clonal) and the sapling age at the time of use. Experimental whole tree biomass data from the five genotypes with the most similar starting DWt are given in Figure 8, together with data for Nemaguard, taken from the second validation run. A range of stress responses can be seen in those results, but growth always occurred, allowing the growth rates to be compared as planned.

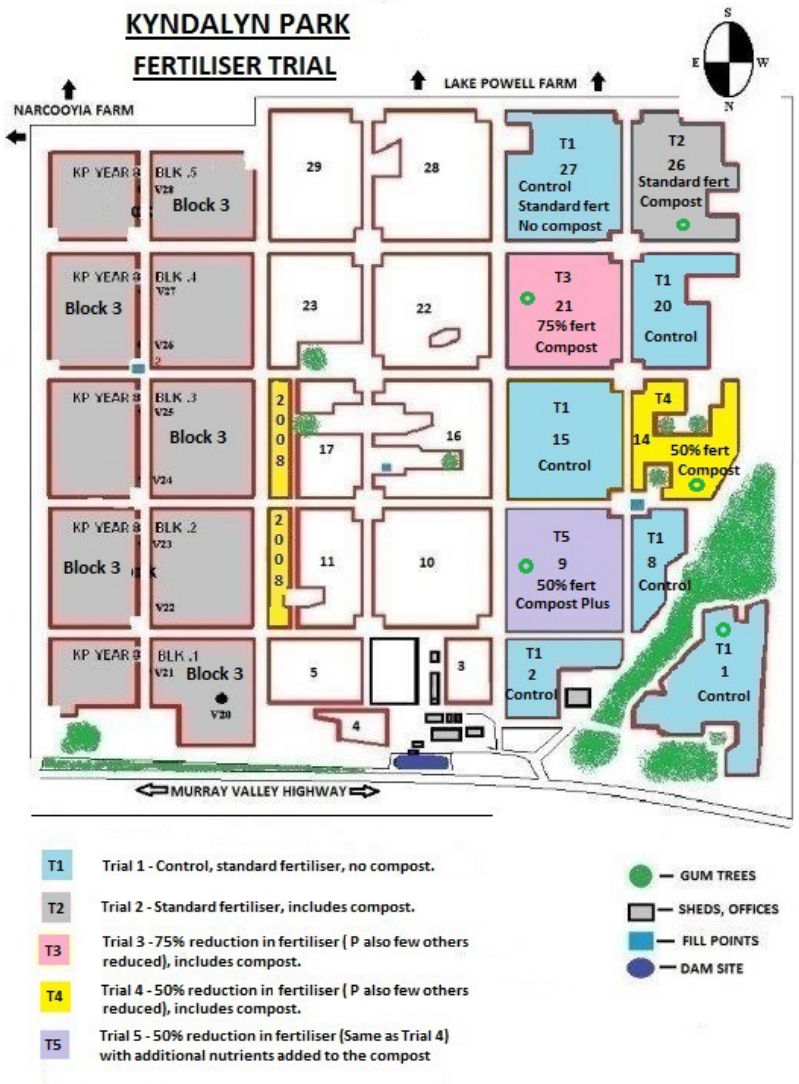


*Figure 7 – layout of compost and reduced fertiliser trial at Robinvale, NE Victoria. Image courtesy of Gunawardena.*

As expected, ontogenetic effects led to a significant relationship between measured RGR and plant size (Figure 9 top), despite RGR being a measure of growth per unit of existing biomass. This is typically due to an increased investment of biomass in supporting tissue and is more significant in perennial plants, such as *Prunus*. The relationship between the calculated stress indices and RGR of the control trees is provided as Figure 9 middle for twelve genotypes and the first two validation runs. For the water deficit screen it is clear that there is no relationship, as intended. However, there is a weak, but statistically significant, negative relationship between stress index and RGR for the salinity screen (adj $r^2 = 0.179$). This appears to not be a result of high RGR *per se*, but a susceptibility of very young saplings to salinity (see below) and the observed relationship between RGR and plant size.

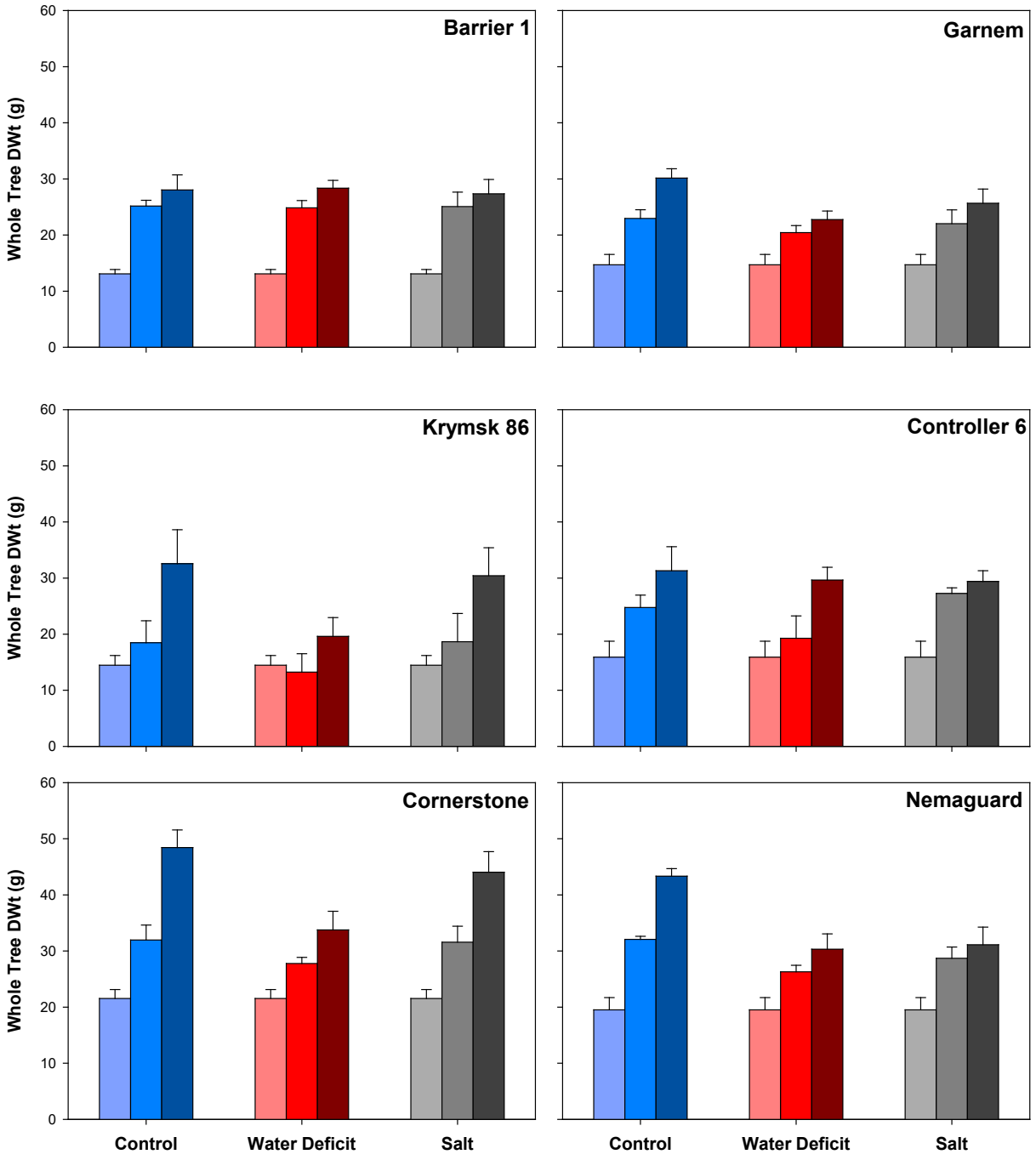


*Figure 8 – whole plant DWt at the three harvests used for RGR calculations under control, water deficit and salinity stress conditions for the six genotypes most closely matched in biomass. Each bar/shade represents a sequential harvest with approximately two weeks between harvests.*

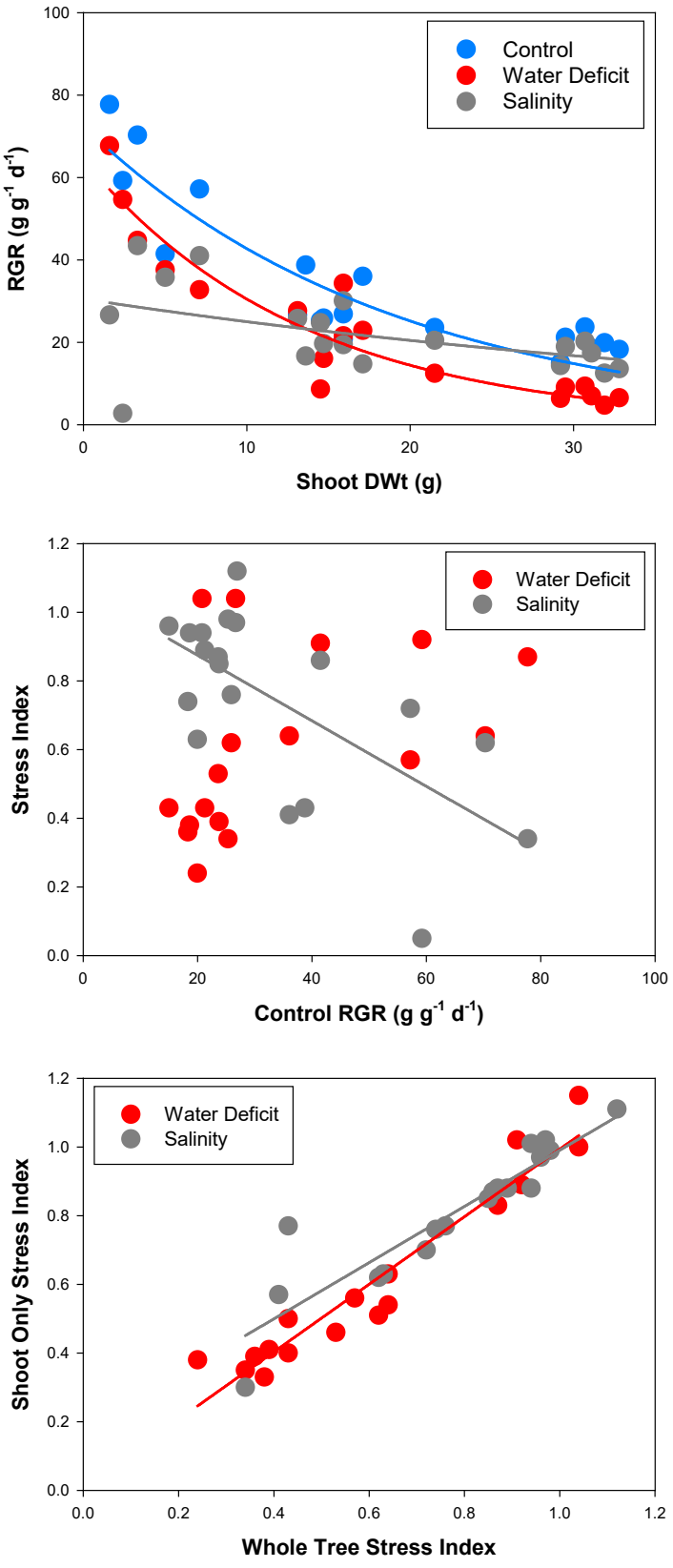


*Figure 9 – the relationship between RGR and shoot biomass (top); the relationship between calculated stress index and RGR of the control (middle) and the relationship between the stress index calculated using shoot biomass only and the stress index calculated using whole plant DWt (bottom) for the genotypes screened in tranches 2 to 6 and validation experimental runs 1 &2.*

The intention with the screening, was to provide a methodology that could be implemented by nurseries, breeders or importers of rootstocks for rapid pre-screening of large numbers of genotypes, prior to selection for longer-term rootstock trials. This was the purpose behind using simple measures of growth that require only a drying oven and a weighing scales, rather than detailed analytical equipment. The largest difficulty in assessing whole plant biomass is accurately extracting the root system, even in a research lab. This also typically prevents trees being grown in the field for these types of measurements, requiring growth in pots. However, because the stress indices are based on proportional differences in growth over a fairly limited timeframe (four weeks in good growing conditions), provided the stress being used does not lead to a large change in the fraction of the tree that is the root system, it may be possible to make the growth assessment on the aboveground parts of the tree. To test this, a 'shoot RGR' was calculated using the above-ground parts of the plant only. This was then compared to the whole tree RGR for the same datasets (Figure 9 bottom). The relationship was highly significant for stress indices, with an adj $r^2$ of 0.92 for the water deficit screen and 0.82 for the salinity screen. Slopes were close to 1:1 in both cases. This suggests that for short-duration screens such as this it is possible to assess stress resilience based on the shoot growth only.

The reproducibility of the screen was originally tested by repeating four of the genotypes screen in the first three tranches in the final set. However, three of these were in the first tranche that were subsequently excluded, leaving a single genotype, Garnem, that was repeated. Using the shoot based RGR stress indices, the result was 0.5 & 0.3 for water deficit and 0.8 & 0.9 for salinity in tranches 3 and 6 respectively. The differences between the two tranches would not be statistically significant, given the variations in tree size in the data used for the calculations and much greater numbers of trees would be required to accurately determine small differences in stress indices, beyond the scope of the current work. Instead a series of experiments was designed to examine reproducibility together with testing for an effect growth rate and received radiation, albeit not independently.

Screening of three genotypes, Nemaguard (current industry standard), Garnem (recently available high vigour rootstock with rapid uptake at present) and Cornerstone was repeated three times, at different plant growth stages and different times of the year, to ensure different light availability for growth. The average plant DWt was 2.4, 15.6 and 43.1 g for runs 1 to 3 respectively. This resulted in a shoot derived RGR of 68.1, 32.4 and 12.5 g $g^{-1}$ $d^{-1}$, again showing that RGR decreases with tree size.

Unfortunately, run 3, which was intended to begin in late March 2020, was delayed due to the COVID-19 pandemic. This had several implications, firstly, to be completed before the end of the project it had to be run early in the 20/21 growing season, whilst still emerging from dormancy. Secondly, run 3 occurred at a longer daylength (late Sept. to late Oct. vs late March to late April) and finally, the trees were larger than intended. The outcome was that for run 3, growth was primarily leaf material and likely still depleting tree reserves, meaning that tree to tree differences in biomass (woody stem) were larger than growth and the resulting data was highly variable and are unlikely to be accurate even though the water deficit results are broadly in line with run 2 (Figure 10).

Run 1 was, intentionally, run with very young plants at a time where received radiation was high due to long daylengths (Nov. to Dec.). Although this resulted in very high RGR, the young plants appeared to be particularly susceptible to salinity stress, recording a lower stress index than run 2 in all three varieties and in each case lower than they recorded in the original screening tranches. Consequently, it is suggested that to minimise possible excessive plant susceptibility to soil conditions at a very young age (equivalent to shortly after propagation), screening using this method does not occur until plants have a minimum dry weight of 10 g. During the screening tranches two genotypes, Atlas and Cadaman, were screened at weights below this and thus have been excluded from the screening results provided below.

In summary, although the validation experiment provided valuable results and demonstrated some additional requirements when screening using this method, it was unable to effectively validate, or otherwise, the screening method. Two of the three genotypes, Garnem and Cornerstone were run during the screening tranches and the results are compared in Table 4. These comparisons are not ideal, there are only two for Cornerstone and the reduced replicate number in the validation run 3 (n = 5) meant that they were more susceptible to plant to plant variation, which likely impacted the Garnem results through a greater proportion of smaller plants having been randomly assigned to the control group.

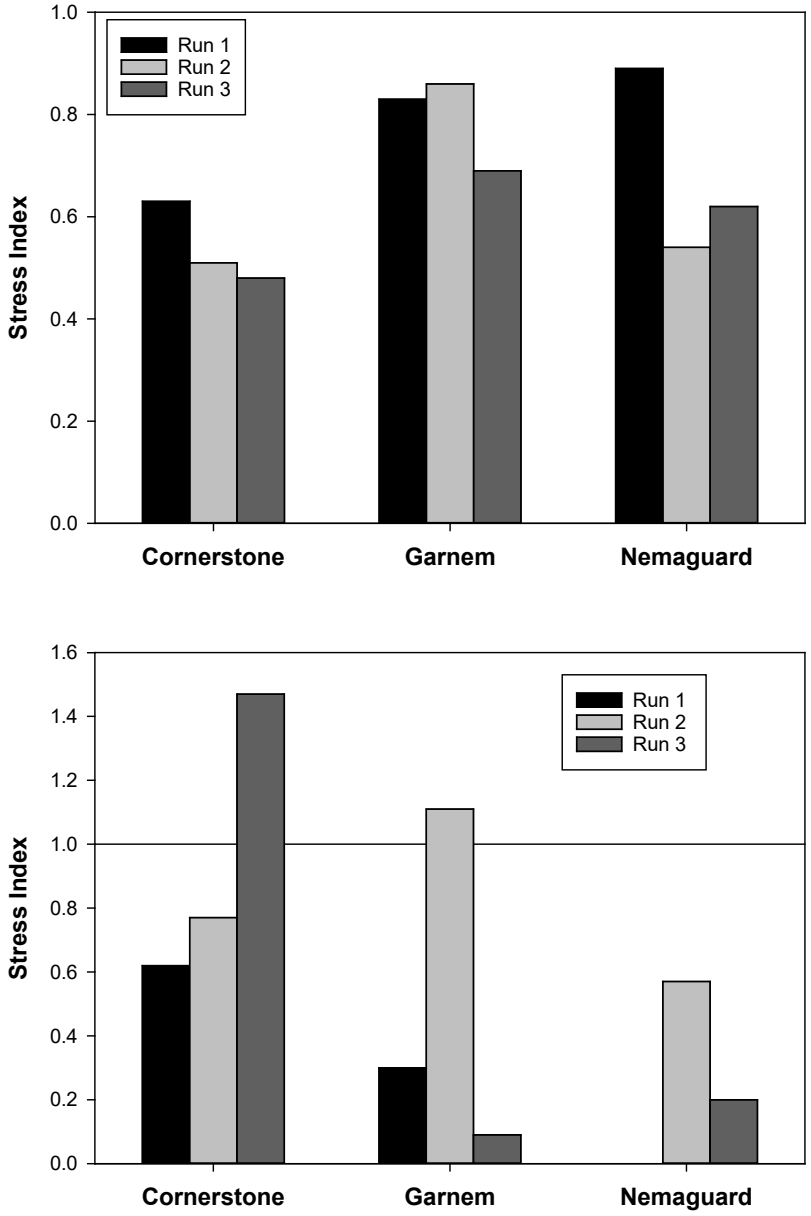


*Figure 10 – stress index calculated for each rootstock genotype for each of the three validation runs for water deficit (top) and salinity stress (bottom). Note that run 3 results should be considered unreliable.*

*Table4 – comparison of stress index from validation run 2 with original screening result.*

| Stress | Genotype | Tranche 3 | Tranche 6 | Validation Run 2 |
|---|---|---|---|---|
| Drought | Cornerstone | n/a | 0.5 | 0.5 |
| | Garnem | 0.5 | 0.3 | 0.9 |
| Salinity | Cornerstone | n/a | 0.9 | 0.8 |
| | Garnem | 0.8 | 0.9 | 1.1 |

Taking into account all of the learnings described above the proposed method for rapid pre-screening of ungrafted rootstock material to soil related abiotic stress is:

- Use planting material with at least 10 g dry mass and less than one year old;
- Include a minimum of 30 plants per genotype to maximise statistical power, size matched as much as possible;
- Grow during the October to March period, even in a greenhouse, unless adequate supplemental lighting is available;
- Undertake one harvest of 8+ plants prior to stress imposition, undertake at two subsequent harvests during stress application of 8+ plants per treatment (including control) at two-week intervals. Alternatively, more frequent harvests with fewer plants can be undertaken;
- Oven dry all harvested plant parts (either the entire aboveground part, or the whole plant) and weigh;
- Calculate the stress index using RGRstress / RGRcontrol, where RGR is calculated as the slope of a regression between ln(DWT) and days from start of stress;
- Determine groupings of similar stress indices, rather than using calculated indices to multiple decimal places to rank genotypes, unless very large numbers of plants used.

Using these criteria, stress indices for water deficit of 50% ET and for irrigation with 3.3 dS $m^{-1}$ water for eleven of the rootstocks screened by this project are provided in Figure 11 For the water deficit stress, Barrier 1 and Controller 6 performed better than the other genotypes, which should not be treated as different in this respect, without further testing. For salinity stress, most genotypes performed reasonably well, with only Nemaguard and Controller 9.5 significantly negatively affected.

## Root dynamics under commercial growing conditions and the influence of nitrogen and irrigation management

The dynamics of root growth are difficult to study, as they must be studied *in-situ*, and this is particularly so for perennial plants due to their root longevity. To assess root production, turnover and demographics there is currently only one method, minirhizotrons, which provide a view of roots growing in their environment. Unfortunately, obstacles in the path of roots have been shown to influence their growth and large root windows will cause proliferation, especially when the window offers a pathway for water movement along its surface. Consequently, minirhizotrons attempt to minimise the obstacle, allowing roots to grow around them as they would a stone in the soil. The downside of this approach is that it reduces the soil intersection area and therefore the amount of root that can be viewed. Nevertheless, it is only by studying the root system in this way that the allocation of plant production to the roots can be estimated and the periods of active root growth be determined.

Another problem with studying perennial root systems is that installation of minirhizotrons (or any other soil sensor) will cause damage to the roots, which in turn generates a flush of root growth. Accurate results can only be determined after that flush has died and been replaced, the duration of which is determined by root lifespan.

This effect can be seen in Figure 12, where the root birth rate has been calculated as new root number per day, averaged across all treatments. The tubes were installed in April and May 2015, shortly before the 2015/16 growing season and the start of data recording. The post-installation peak of root growth is clearly visible and is approximately five times the rate seen early in the 2018/19 growing season. Root death rate is also plotted and increases rapidly after the flush of root births and then steadily until autumn 2017. This suggest that a proportion of the roots born in the initial flush after installation are living for up to two years.

There was a surprising lack of seasonality in the root birth rate, with new roots appearing throughout the year, albeit at a slower rate than in spring. The difference between the root birth rate and the root death rate indicates the change in root numbers present, with numbers increasing where the birth rate is higher than the death rate and vice versa (Figure 13). Again, the seasonality is visible in in the root numbers, with increases typically occurring in early July, although delayed in the 2018/19 season.

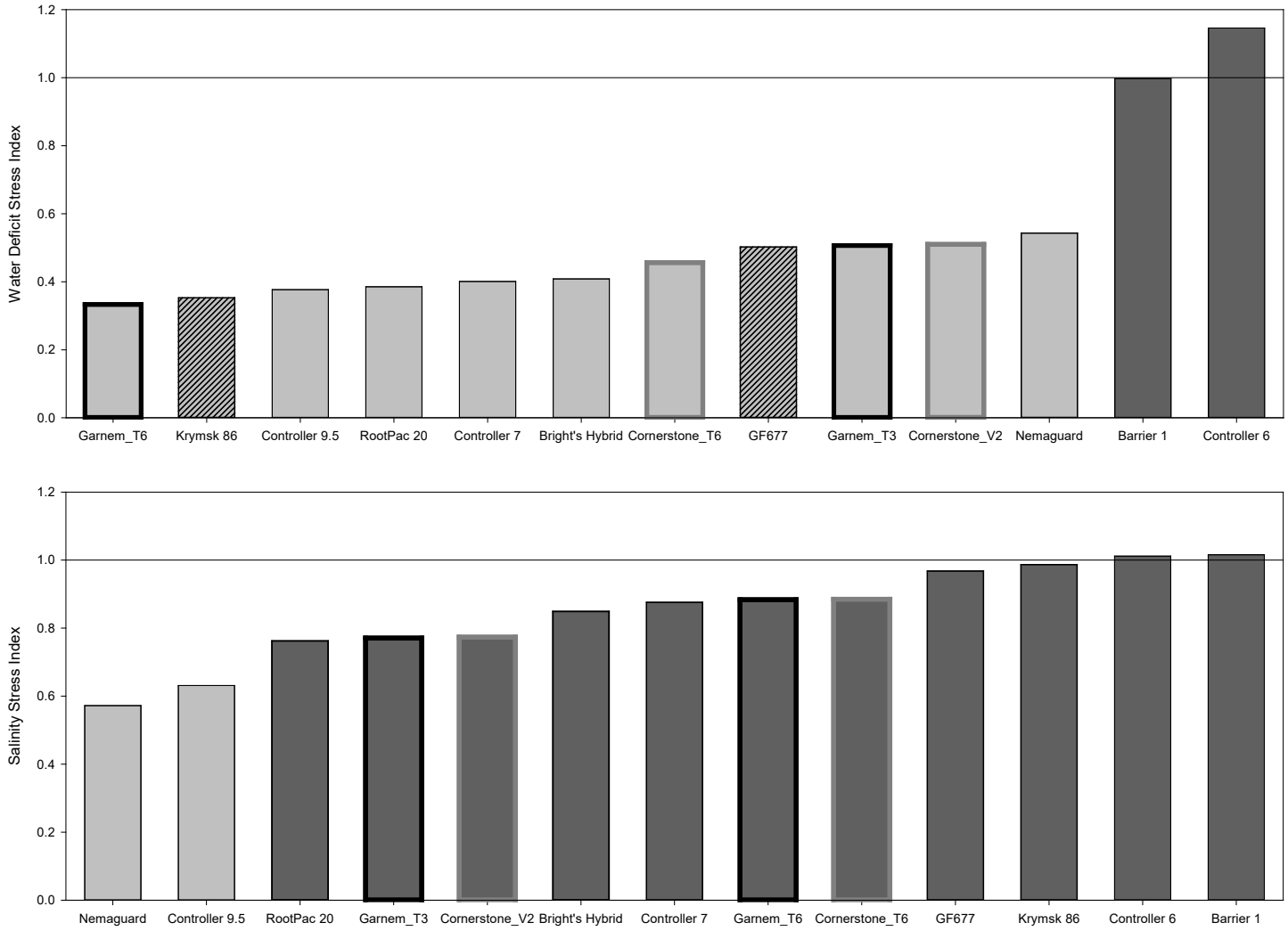


*Figure 11 – ranking of all genotypes with a calculated stress index considered reliable for water deficit (top) and salinity stress (bottom). Repeated measurements of the same genotype are indicated by the bar border and bars which are hatched were only moderately significant ($p < 0.100$).*

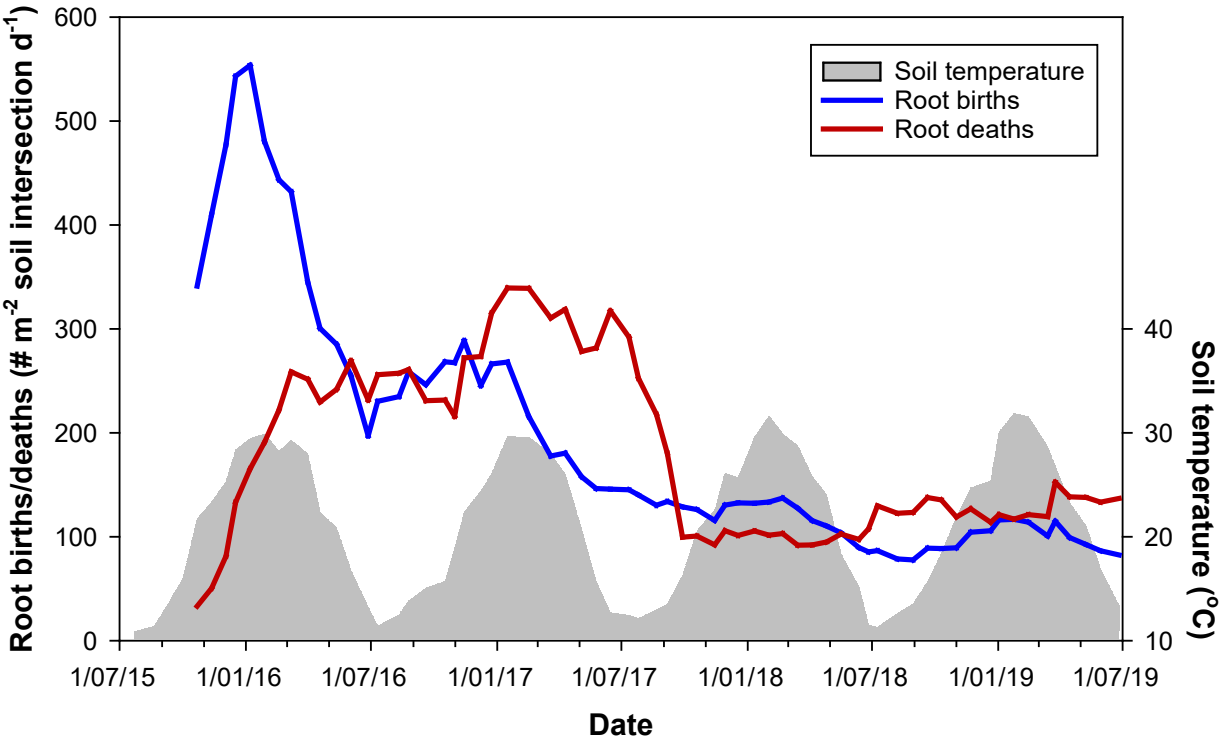


*Figure 12 – root birth (blue) and death (red) rates at the Lindsay Point field site, assessed at three-week intervals over four calendar years, left axis; soil temperature at 10 cm depth, right axis. Data are means of 12 positions across all treatments and represent the top 1 m of soil.*

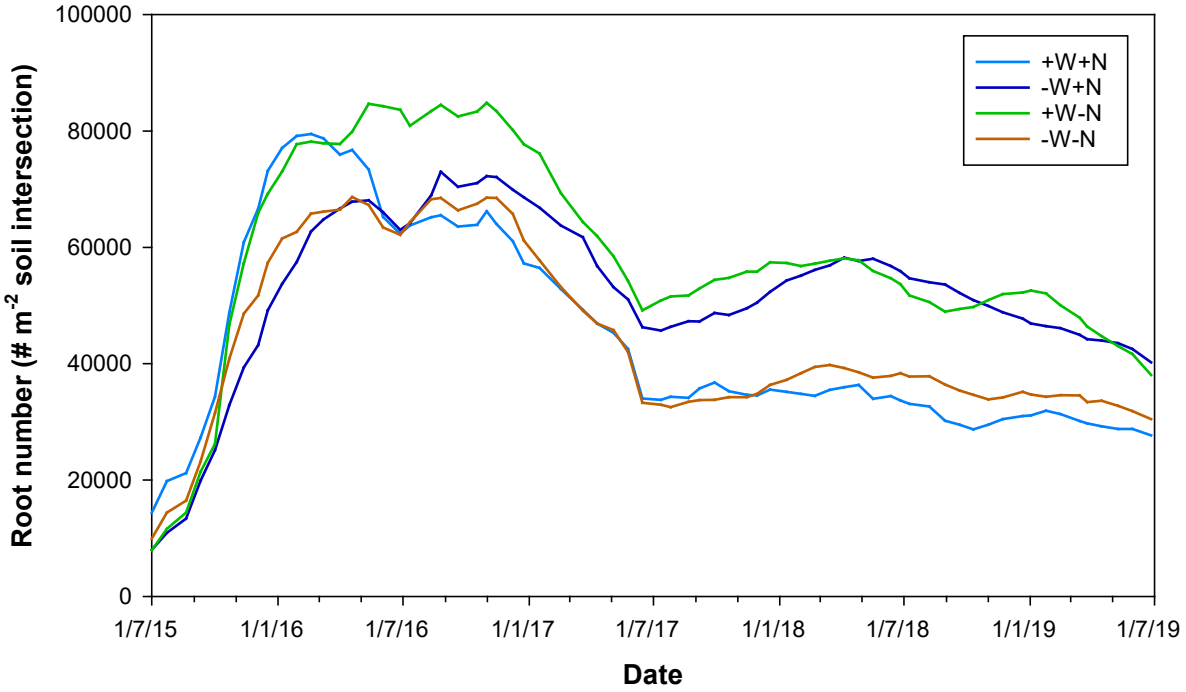


*Figure 13 – root numbers per area of soil intersection over the four years of treatments for standard irrigation and N fertigation (+W+N), 60% irrigation (-W+N), 70% N fertigation (+W-N) and 60% irrigation combined with 70% N fertigation (-W-N).*

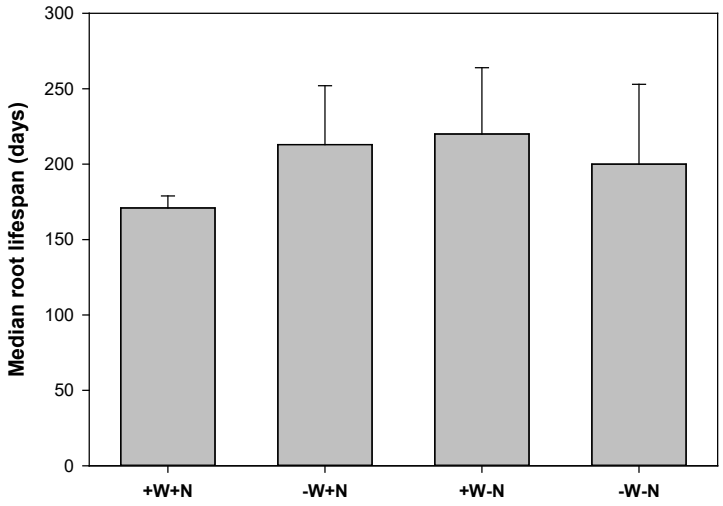


*Figure 14 – median root lifespan for all roots produced and lost with the four years of treatments for standard irrigation and N fertigation (+W+N), 60% irrigation (-W+N), 70% N fertigation (+W-N) and 60% irrigation combined with 70% N fertigation (-W-N). Data are means + SE.*

The irrigation treatments were imposed at the start of the 2015/16 season and the impact of this was a smaller post-installation root flush in the two reduced irrigation treatments. However, the die-back over winter 2016 was more pronounced in the +W+N treatment than the ones with reduced inputs and the two single input reduction treatments maintained a higher root number than the +W+N or -W-N treatment.

Median root lifespan was calculated for all of the roots that were not present at the start of the measurements and had died prior to the final measurement (Figure 14). This was approximately 200 days, meaning that half of all roots produced lived for longer than this. Furthermore, the majority of these roots were the ones produced in the initial flush after installation, so it is potentially an underestimate.

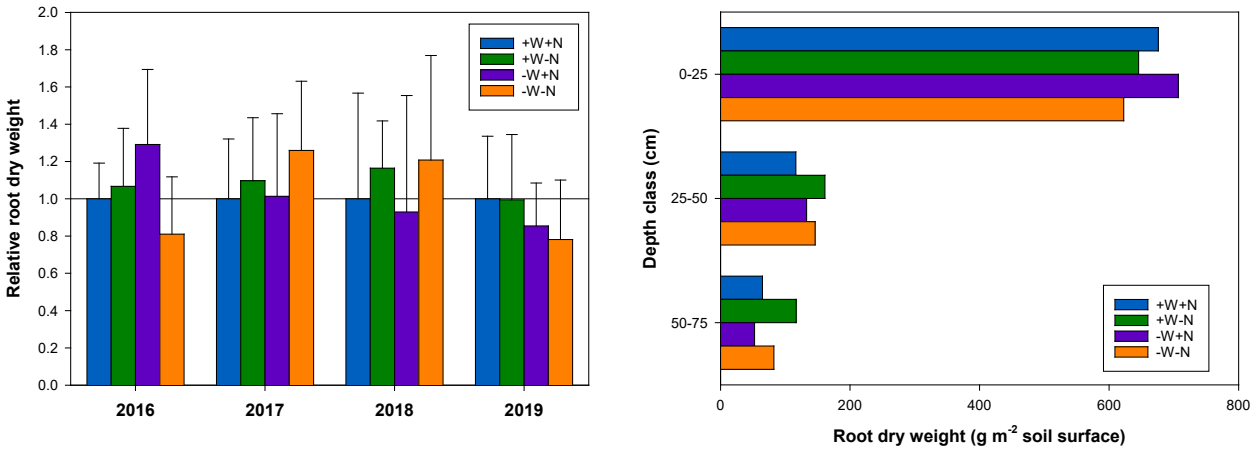


*Figure 15 – root dry mass to 0.75 m relative to control during dormancy (left) for each of four seasons of treatment by depth class (right) averaged over four seasons of treatment for standard irrigation and N fertigation (+W+N), 60% irrigation (-W+N), 70% N fertigation (+W-N) and 60% irrigation combined with 70% N fertigation (-W-N). Data are means, error bars are SE where present.*

The root coring during winter, corroborated the limited treatment effects seen in the minirhizotron data. With a small increase in root biomass in the reduced input treatments, the largest being 8% across the four years of treatments in the +W-N treatment (Figure 15). More consistently, there was a change in root distribution with depth. The reduced N treatments having less root mass in the top 25 cm of soil (where 75% of the recovered root biomass occurred over the entire dataset) and more root mass at the lower depths.

Overall there were small adjustments in root growth and location as a result of the reduced nitrogen and irrigation treatments at the Lindsay Point site, but no major changes were observed. This suggests that the trees were well able to adjust to the reduced inputs and that improvements in resource use efficiency could be made. Indeed, this is supported by the lack of overall yield impacts observed at the site over the four seasons by the AL14005 project.

## Effect of reduced nitrogen application and irrigation on tree water use

In conjunction with the belowground data collected at the Lindsay Point field site, sapflow sensors were used to monitor tree water use, with the intention of comparing this with the belowground data reported above and the canopy data reported by AL14005 (Treeby 2019). Data were typically collected from pre-budburst, but are reported from shortly after budburst (1st September) until shortly before harvest (1st February) here for consistency between trees and seasons. Very high flow rates were recorded, with sap velocity typically exceeding 25 cm $hr^{-1}$ and whole tree sap flow rates in excess of 2.5 L $hr^{-1}$ in the middle of the day once the canopy was established. These high flow rates resulted in some difficulties in maintaining reliable data during the middle of the day, particularly in 2018/19 leading to some underestimated data and therefore more variation in the results than was expected. Furthermore, the data presented have been calculated with average scaling values and do not account for possible small differences in properties between trees. Nonetheless, the data collected was generally well correlated with atmospheric conditions and canopy growth. Figure 16 top provides daily total water use for a typical tree for the bud-burst to harvest period. The responsiveness of the canopy to changes in the evaporative demand of the atmosphere, represented by reference evapotranspiration ($ET_o$), are apparent, as is the gradual increase in tree water use through September and October as the canopy grows. This period ties in with light interception data, reported by AL14005, which generally found peak light interception occurred in December.

The same daily tree water use forms a strongly curvilinear relationship with $ET_o$ (Figure 16 bottom), much more so than has been observed in grapevines (Edwards et al. 2013). This was seen with all of the sensors across all three seasons and was not due to a failure of the sensors to measure maximum flow (which can be determined from the minutely sensor data). Rather it suggests either leaf response to high evaporative demand, i.e. stomatal closure, which might be expected in a Mediterranean plant, or alternatively, an inability for the root system or tree hydraulics to transport enough water to meet the canopy demand.

Total seasonal water use ranged from 9 to 17 kL per tree for the post-budburst to harvest period (Figure 17), equating to 3.1 to 5.9 ML $ha^{-1}$. The irrigation applied to the site averaged 14.6 ML $ha^{-1}$ for the entire growing season, whereas the values for tree water use quoted here do not include the entire irrigation season, soil evaporation or deep drainage. Further, sapflow sensors, require accurate scaling for each installation and commonly provide an underestimate in comparison to weighing lysimeters (Steppe et al. 2010). Averaged over the 2016/17 and 2017/18 seasons, the reduced irrigation treatments resulted in a 20% decrease in growing season tree water use. If the 2018/19 season is included this becomes an 8% decrease, but the +W+N sapflow data for 2018/19 was particularly problematic and is certainly underestimated. $ET_o$ for 2018/19 was lower than the preceding seasons (Figure 17), canopy light interception the highest of the years reported in AL14005 (Treeby 2019), suggesting a larger canopy and the irrigation applied was the highest of the seasons the field trial was running, at 16.3 ML $ha^{-1}$. These factors combined to generate the highest tree water use of the three seasons in 2018/19.

If the tree water use per treatment is normalised to the +W+N treatment, the seasonal differences in water use can be minimised and treatment effects more clearly seen (Figure 18). Interestingly, the -N treatments had slightly higher water use than +N treatments irrespective of irrigation treatment. Ultimately, despite the variability in the sapflow data, they are consistent with the aboveground effects detailed by AL14005 and suggest that the reduction in tree water use under the -W treatments is less than the reduction in irrigation, suggesting an improvement in irrigation use efficiency by those trees and supporting the conclusion of AL14005 that the reduction in water applied do not drive a statistically significant reduction in yield over the four seasons studied.

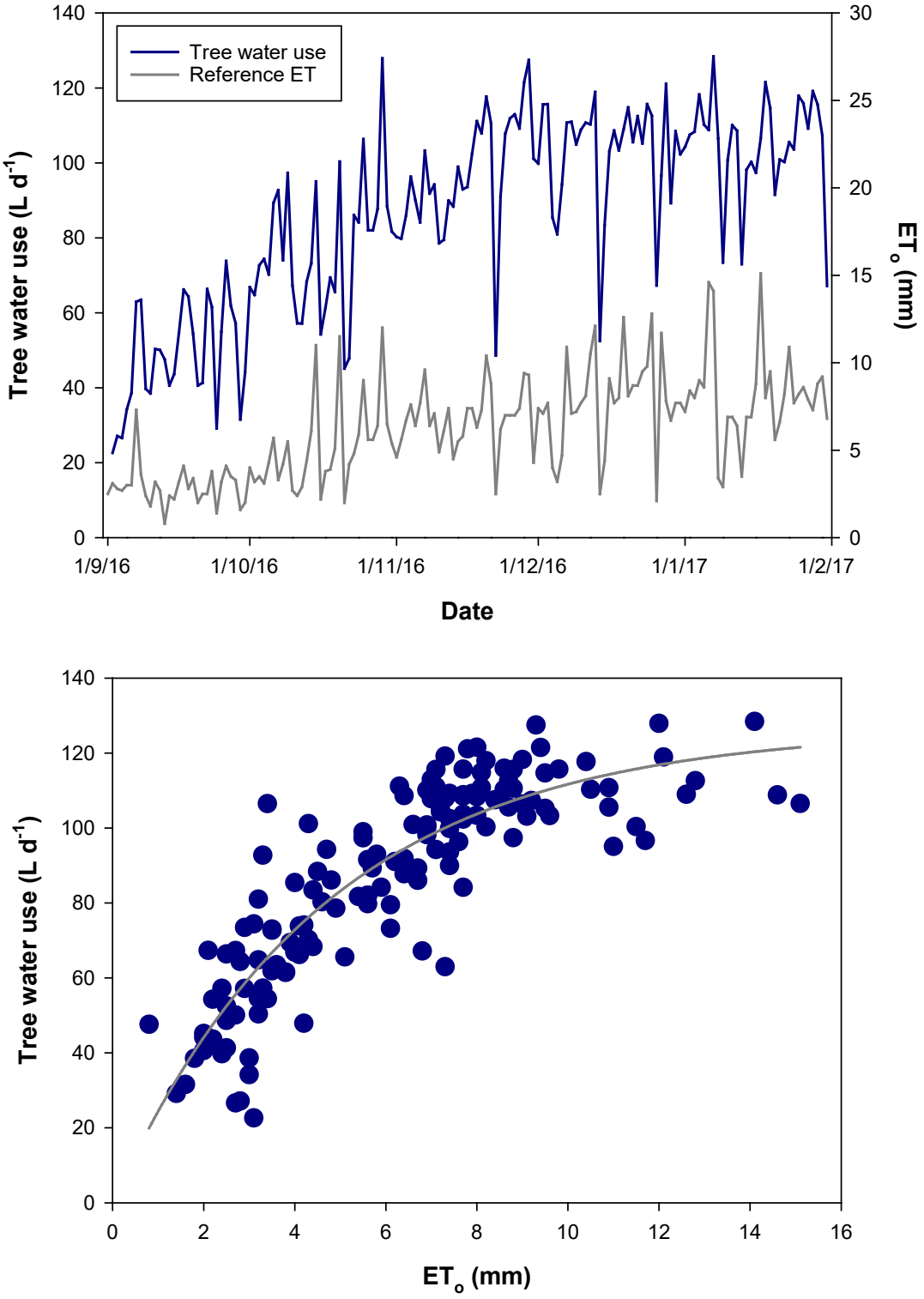


*Figure 16 – daily tree water use of a single tree under standard commercial management (control) plotted against time, together with $ET_o$ (top) or against $ET_o$ for budburst to harvest in the 2016/17 growing season.*

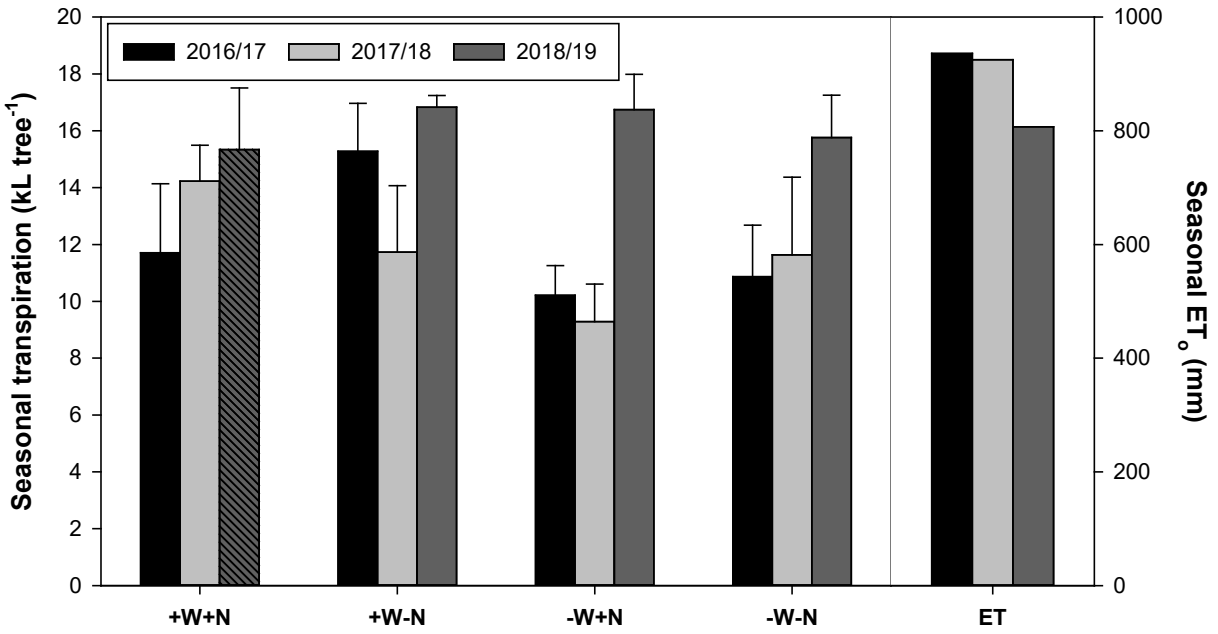


*Figure 17 – tree water use from budburst to harvest for trees under standard irrigation and N fertigation (+W+N), 60% irrigation (-W+N), 70% N fertigation (+W-N) and 60% irrigation combined with 70% N fertigation (-W-N) for three growing seasons. Data are means +SE and the hatched bar represents data that is under-estimated by an unknown amount due to sensor failure at periods of highest flow.*

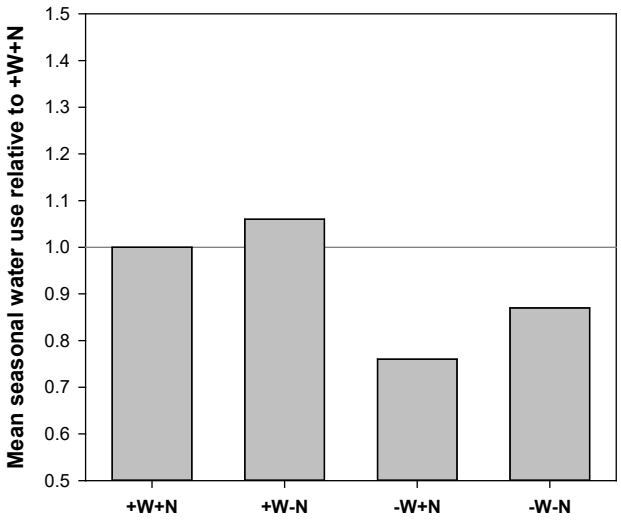


*Figure 18 – tree water use normalised to control (+W+N) and averaged over the 2016/17 and 2017/18 seasons for trees under standard irrigation and N fertigation (+W+N), 60% irrigation (-W+N), 70% N fertigation (+W-N) and 60% irrigation combined with 70% N fertigation (-W-N).*

## Effects of genotype, root age and class on nitrogen uptake capacity

Nitrogen uptake was estimated in greenhouse and outdoor pot experiments, using $^{15}N$ as a label, to examine the role of root class, age and genotype. The outdoor experiment followed trees through a growing season, with sequential applications of labelled urea and uptake monitored through leaf and root sampling, although only the leaf samples were able to be analysed.

Root age was also examined through a trial experiment with Nonpareil scions, grafted to Nemaguard rootstock, which included separating fine roots into young (white) and old (brown) fractions after application of $^{15}N$ labelled nitrogen fertiliser. Sampling at two time-points, two and five days after application, suggested difference in the rate of uptake, with a higher concentration of the label in the white roots than the brown roots after two days (Figure 19). In contrast, by day five, the brown roots had the same or higher amount of label. As with the following experiments, it was not possible to separate the nitrogen that had been incorporated in the root biomass from nitrogen stored in the root or nitrogen in the process of being transported elsewhere in the plant. However, at the end of the experiment, there was a greater amount of labelled nitrogen in the brown roots than white roots, the latter only making of 37% of the fine root biomass, and more labelled nitrogen in the root system than the leaves (data not shown).

The rate of uptake was examined in more detail in the main greenhouse experiment. Roots were sampled approximately 18 hours after labelled urea was applied and had already reached a concentration of 4-6 mg of newly taken up N per g of root dry mass (Figure 20 bottom). This only increased slightly during the remainder of the experiment, reaching 6-8 mg $g^{-1}$ nine days after application. There was little effect of rootstock genotype on the uptake of labelled N, although there was a small effect that was likely to be an artefact of root density in the pot (see below).

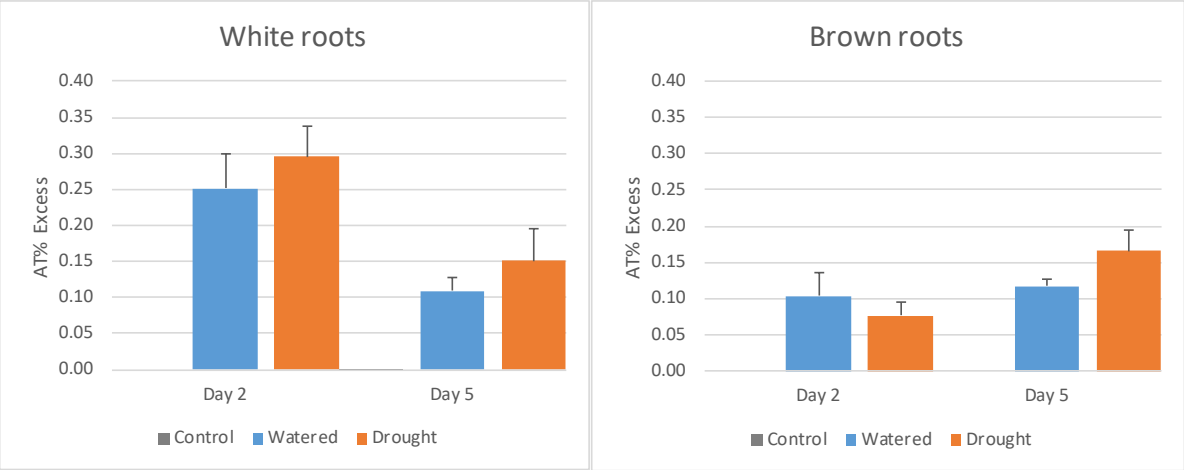


*Figure 19 – uptake of $^{15}N$ labelled nitrogen, expressed as increase in atom % over non-labelled plants, after 2 and 5 days of white (left) and brown (right) fine roots of a similar diameter.*

In contrast, the assimilation of labelled N in the leaves of the scion (all Nonpareil) was only 0.25 mg $g^{-1}$ at 16 hours and 1.0 mg $g^{-1}$ at 21 hours after application (Figure 20 top). Even after three days, labelled N in the leaves was at a lower concentration than the roots. There was also a rootstock genotype effect on N assimilation by the leaf, with a low concentration in trees grafted to Garnem (4.4. mg $g^{-1}$ after nine days) and a high concentration in trees grafted to Bright's Hybrid (10.7 mg $g^{-1}$), a much higher range than in the root tissues, which only varied between 7.1 and 8.1 mg $g^{-1}$ by rootstock genotype at harvest.

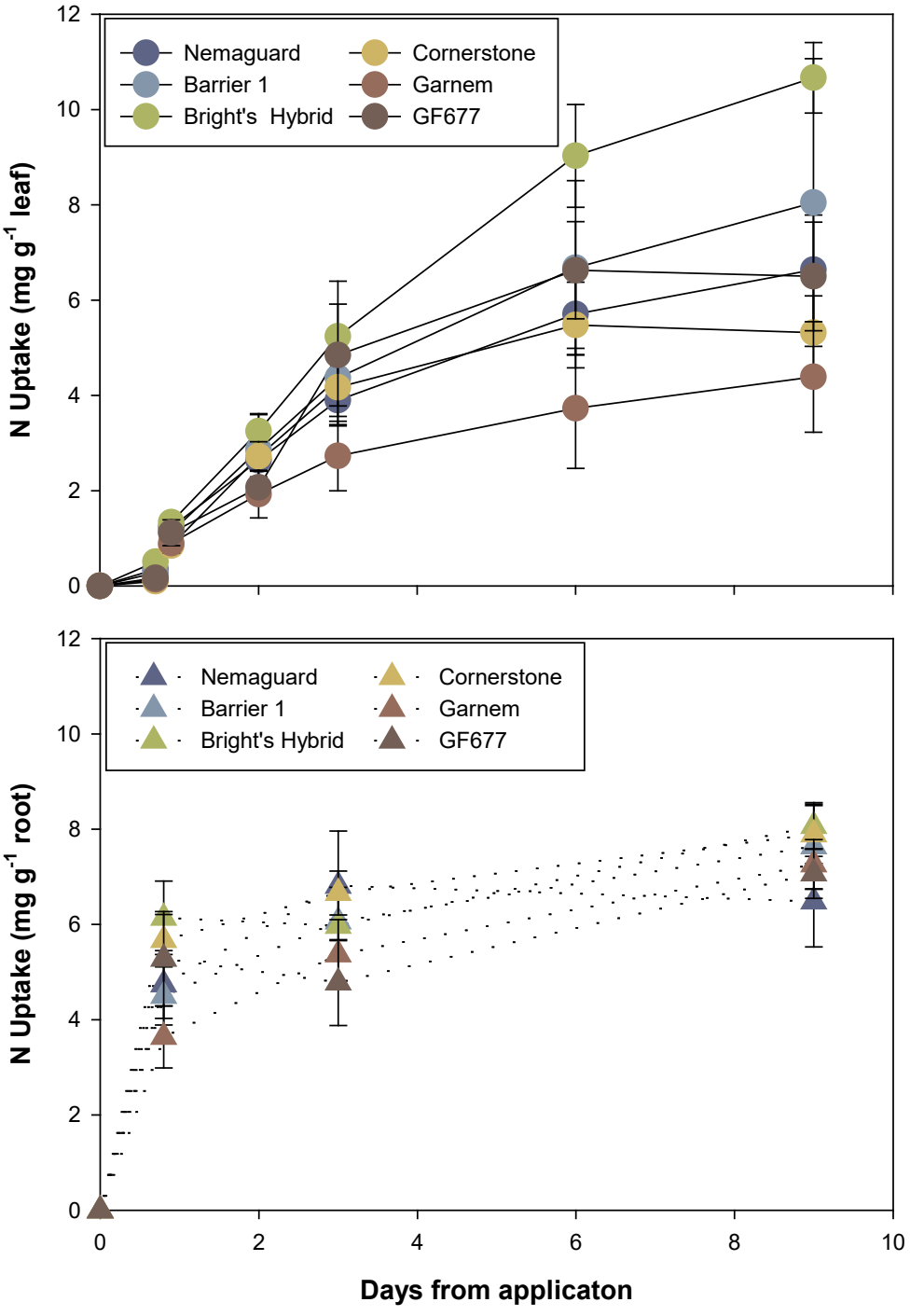


*Figure 20 – tissue concentration of newly assimilated N over nine days, estimated using $^{15}N$ labelled urea, of six rootstock genotypes grafted to Nonpareil in leaves (top) and roots (bottom).*

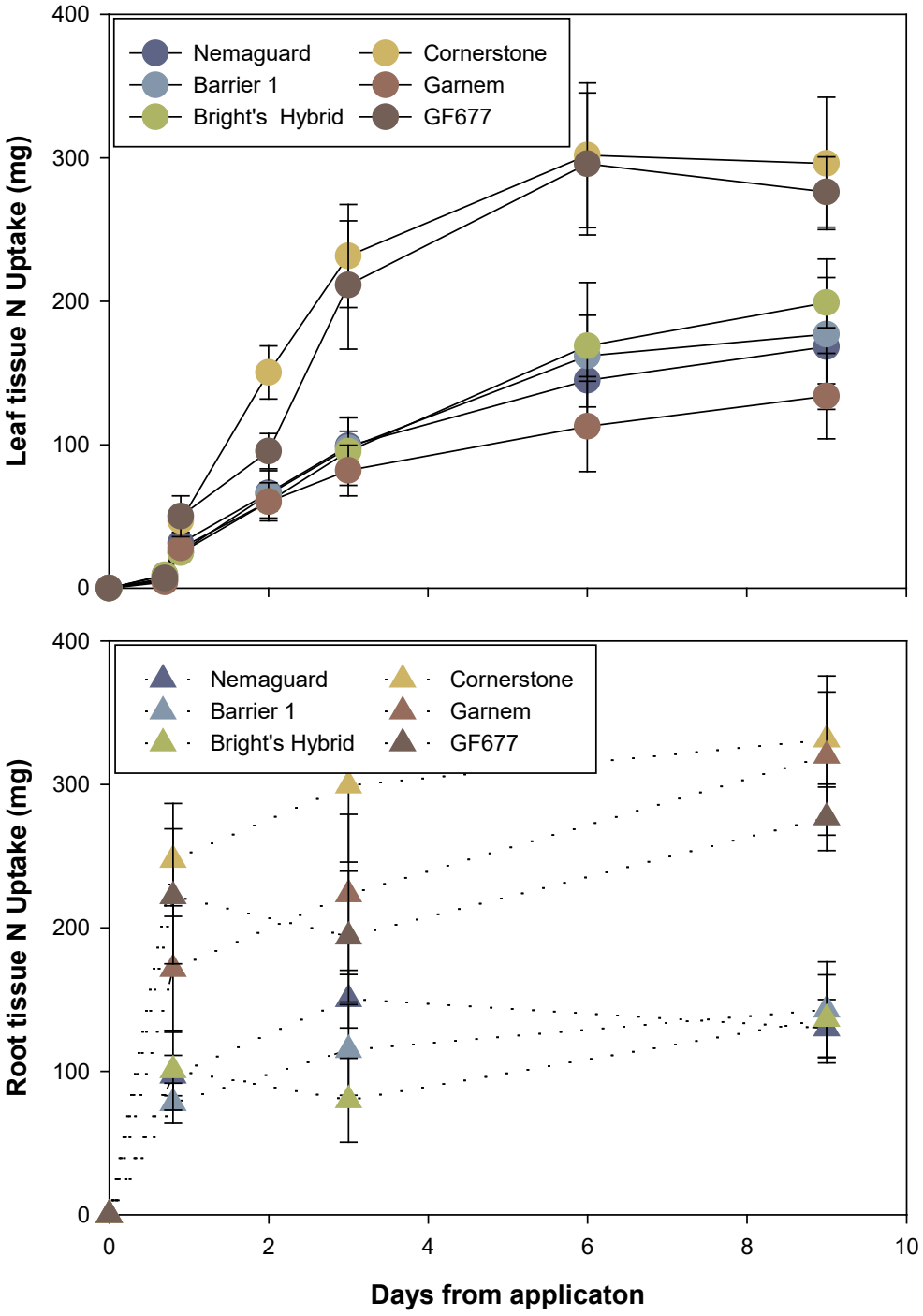


*Figure 21 – uptake per tissue of N over nine days, estimated using $^{15}N$ labelled urea and total tissue biomass from the harvest at day 9, of six rootstock genotypes grafted to Nonpareil, by leaves (top) and roots (bottom).*

Although growth was not measured, tissue dry mass at harvest was used to estimate total uptake of N into the leaf and root fractions for each sampling time by assuming that the increase in mass of those tissues was minimal during the experiment. What growth did occur would result in a small overestimate early in the experiment, decreasing to zero at day nine.

Whole of tissue N assimilation, calculated in this way, exhibited clear effects of rootstock genotype on both root and leaf (Figure 21). Cornerstone and GF677 grafted trees had much high leaf N uptake than the other genotypes, more than double at day 3, although by day six there was no further increase in either genotype, suggesting that the available labelled N in the pot had largely been exhausted. In contrast, the Garnem grafted trees had the lowest leaf tissue N uptake, despite having more leaf material than several of the other genotypes, due to the low rate of new N assimilation.

In the root system, Cornerstone and GF677 again had higher total N uptake than most of rootstock genotypes, due to larger biomass, but in contrast to the effect on leaf N assimilation, total N uptake by the Garnem root system was equal to Cornerstone.

The effect of scion genotype (all grafted to Nemaguard) on N uptake, was also modulated by the size of the tissue pool. With the exception of the data for Carina on day three, there was little effect of scion genotype on the concentration of labelled N in the root system (Figure 22 top). However, the concentration of newly assimilated N in the leaves of Nonpareil, was significantly higher than the other two scion genotypes (Figure 22 bottom). Even after adjusting for differences in the size of the leaf pool Nonpareil had more newly assimilated N than Carina, whereas the total N uptake in the rootsystem of the Nonpareil trees was lower than the other genotypes.

The sequential leaf and root samples suggested that N uptake was an interplay between plant genotype, plant size and within plant allocation. The whole tree harvests nine days after application of the label allowed this to be examined in more detail. Figure 23 provides the dry mass of each rootstock:scion combination and the allocation to leaf, new growth stem, old wood and root system. Neither the scion genotypes (on Nemaguard) or the rootstock genotypes (grafted to Nonpareil) were matched for biomass, due to the different sources, propagation methods and inherent vigour of these combinations. The data are also split according to labelled N application. Although there were some small absolute differences, there were no statistically significant differences in biomass between labelled and non-labelled trees for any genotype ($p > 0.1$, t-test) per genotype). The largest biomass allocation was to the woody stem of each tree, but this was assumed to primarily have a transport role in regards to N assimilation, and not sampled for $^{15}N$ analysis to reduce costs. Excluding belowground woody stem, the root fraction varied between 0.13 in Cornerstone and 0.16 in GFG677 and Bright's Hybrid. Tree N content and allocation to non-woody tissues is given in Figure 24. Genotype differences were not as large as for biomass due to the main N pools being leaf and root, with a lower allocation to the stem. Again, any differences between labelled and non-labelled trees were not statistically significant.

There was a very small amount of labelled N uptake by the non-labelled trees (Figure 25), this was assumed to have occurred due to volatilisation of the labelled urea in the soil, supported by a greater concentration in leaves than roots. Total N uptake of labelled trees during the nine days of the experiment was clearly influenced by tree size (also see Figure 26, adj $r^2 = 0.50$), but allocation was affected by tissue type and genotype. For example, 40-45% of all root N was absorbed during the experiment (Figure 27 right), whereas only 18% of leaf N was assimilated during the experiment. This was likely in part due to the lower N content of roots than leaves (Figure 27 left), but also reflected the different functions of the tissues. The stem had 33% newly acquired N, assuming this was a mix of N being transported and storage for growth, it suggests that the root system also had a significant storage role.

The new N pool in the roots was strongly influenced by genotype, and was not simply due to differences in vigour between rootstock:scion combinations. For example, root systems grafted to Nonpareil had a much lower proportion of total N acquired during the experiment in the roots systems than those grafted to Carmel or Carina (Figure 28), even though there was little difference in root fraction. Amongst the rootstocks, Garnem had the highest proportion of newly acquired N in the root system, despite a root fraction similar to the other rootstock:scion combinations. Interestingly, this contrasts with the shoot N for the Garnem plants, which had the lowest assimilation of new N of all the genotype combinations (Figure 25).

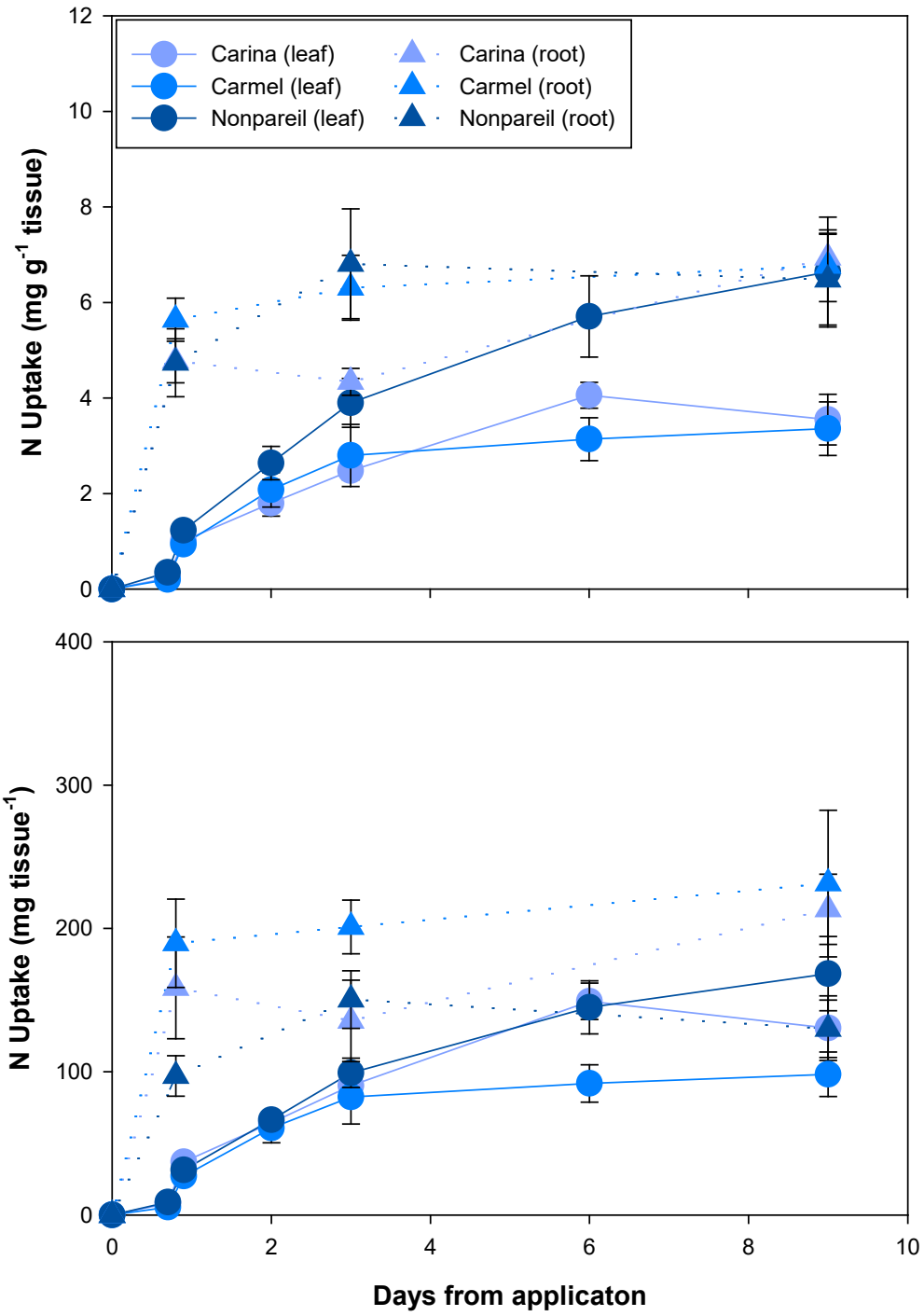


*Figure 22 - tissue concentration of newly assimilated N (top) and uptake per tissue (bottom) over nine days, estimated using $^{15}N$ labelled urea, of three scion genotypes grafted to Nemaguard.*

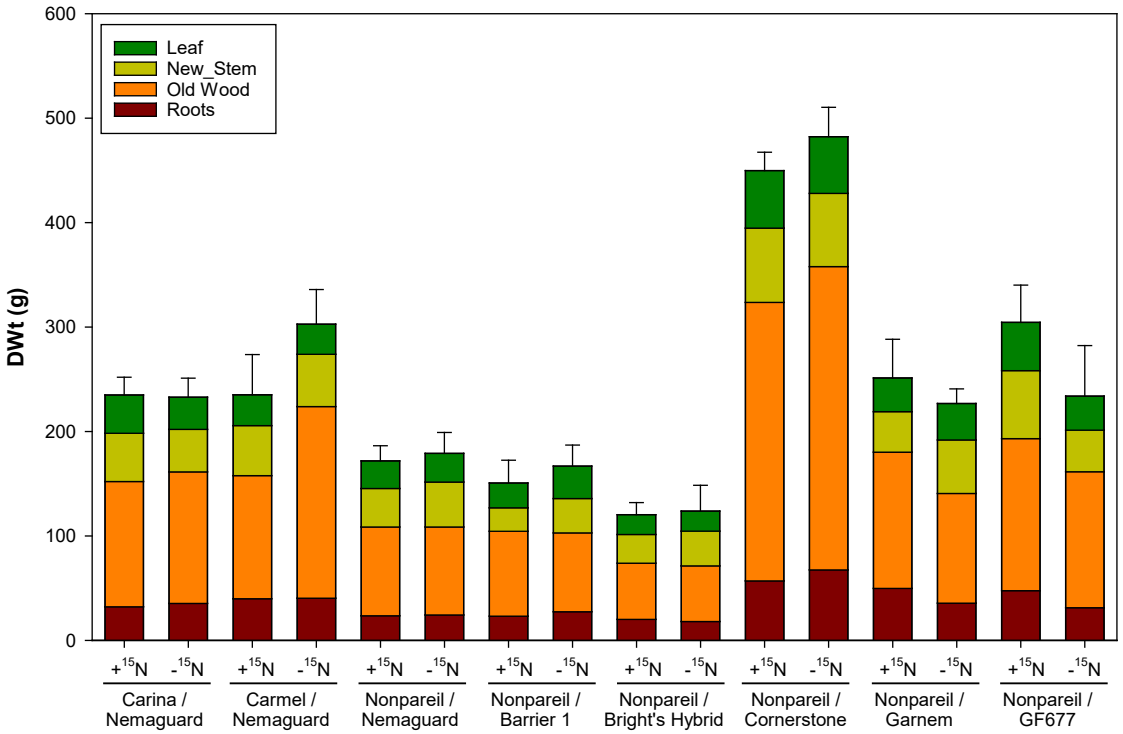


*Figure 23 – biomass allocation of eight rootstock:scion combinations fertilised with 10 g per plant of urea either labelled with 10 AT% of $^{15}N$ or unlabelled measured after nine days. Data are means +/- SE of the whole tree DWt.*

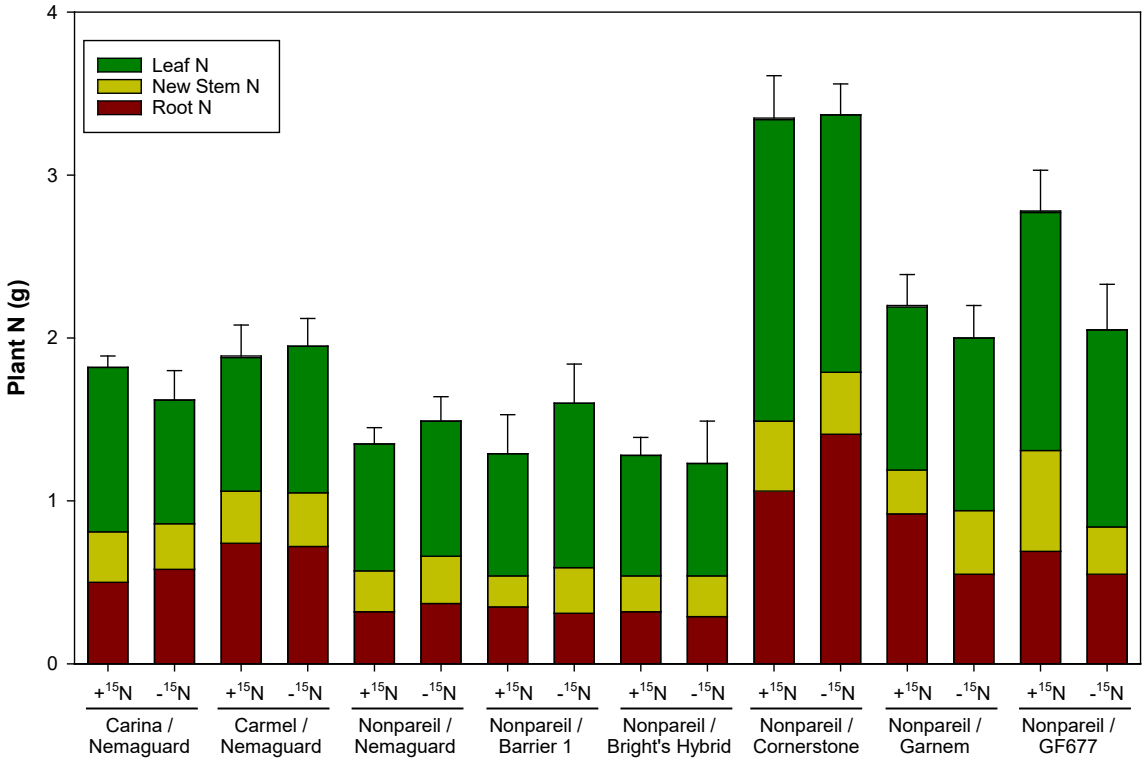


*Figure 24 – N allocation of eight rootstock:scion combinations fertilised with 10 g per plant of urea either labelled with 10 AT% of $^{15}N$ or unlabelled measured after nine days. Data are means +/- SE of the whole tree N.*

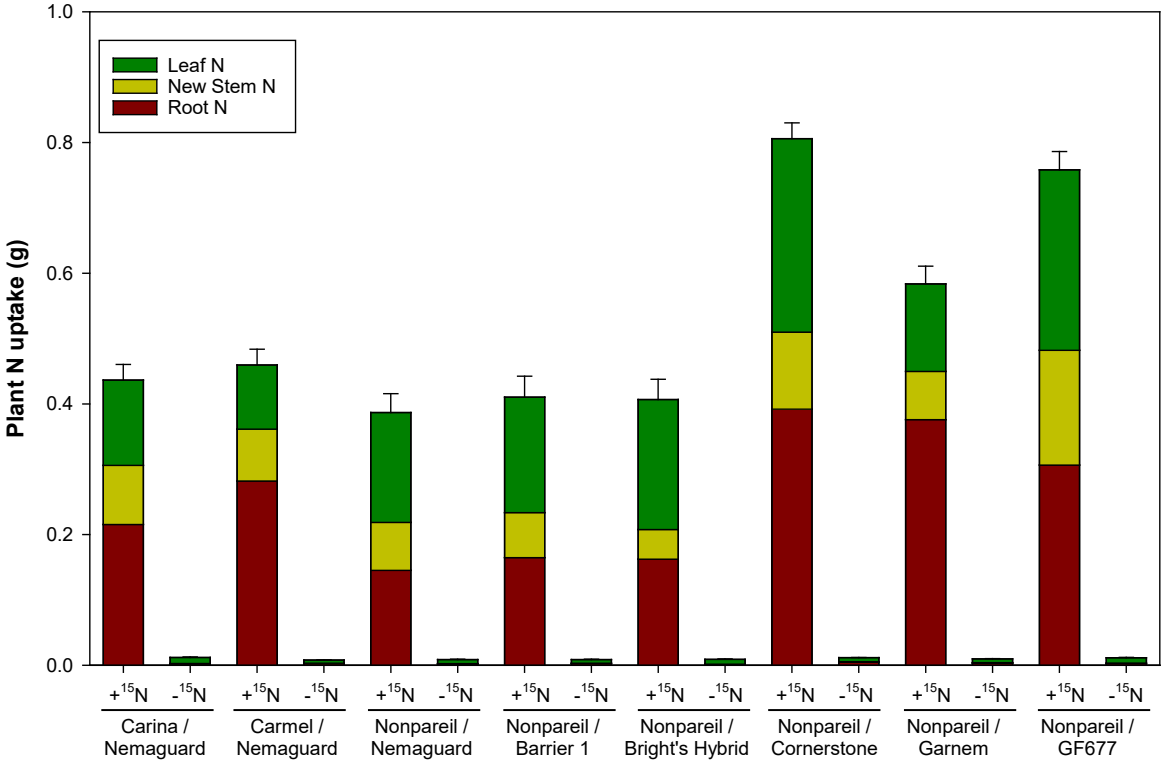


*Figure 25 – allocation of N uptake after nine days of eight rootstock:scion combinations fertilised with 10 g per plant of urea either labelled with 10 AT% of $^{15}N$ or unlabelled. Data are means +/- SE of the whole tree N.*

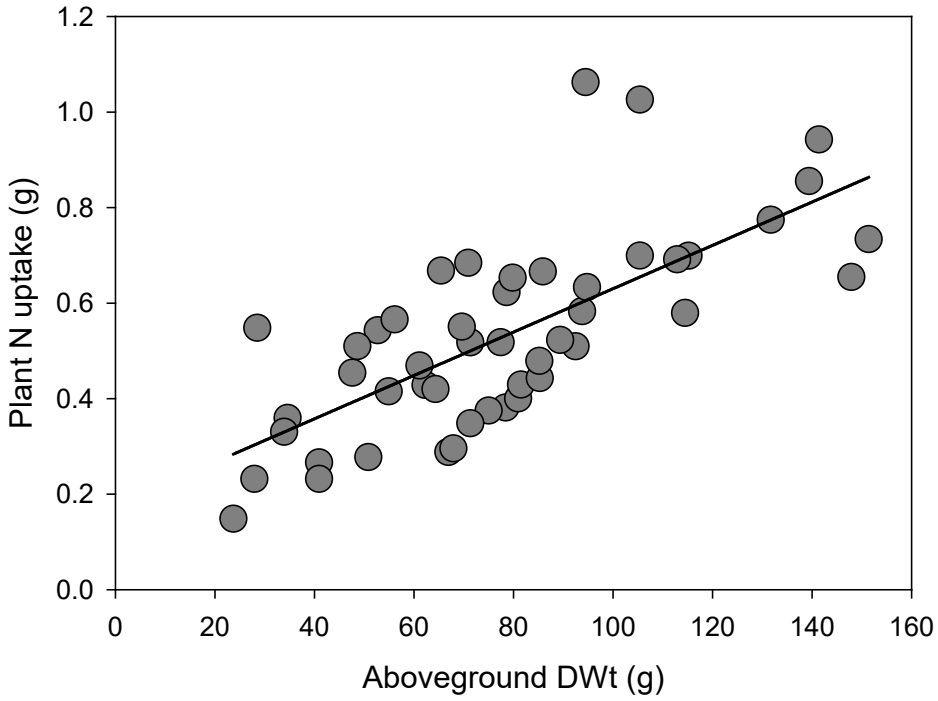


*Figure 26 – relationship between plant N uptake and plant size for eight rootstock:scion combinations. Data are individual plants.*

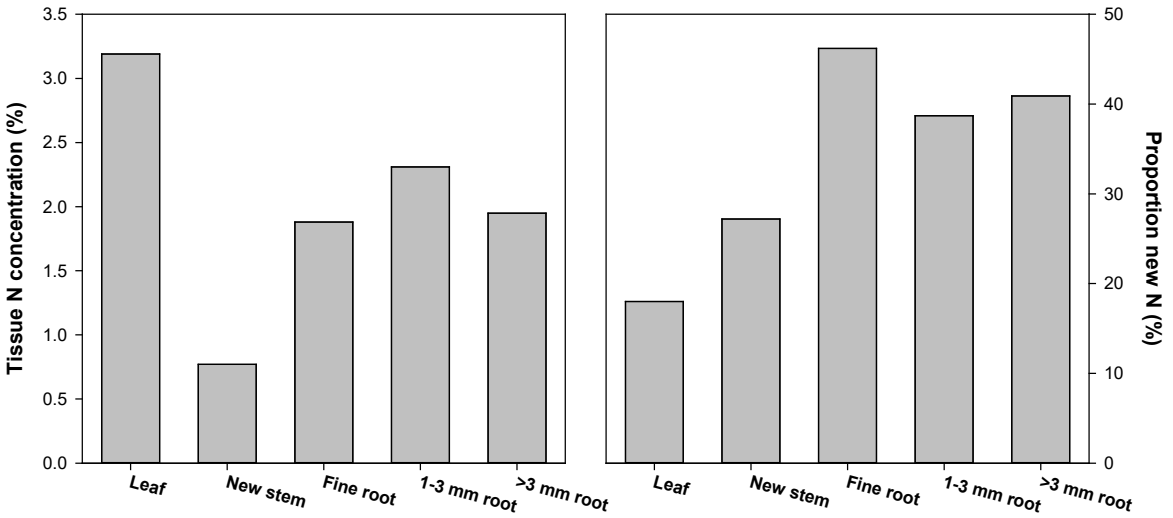


*Figure 27 – tissue N concentration (left) and proportion of N in tissue assimilated during a nine day experiment (right), averaged across all rootstock:scion combinations.*

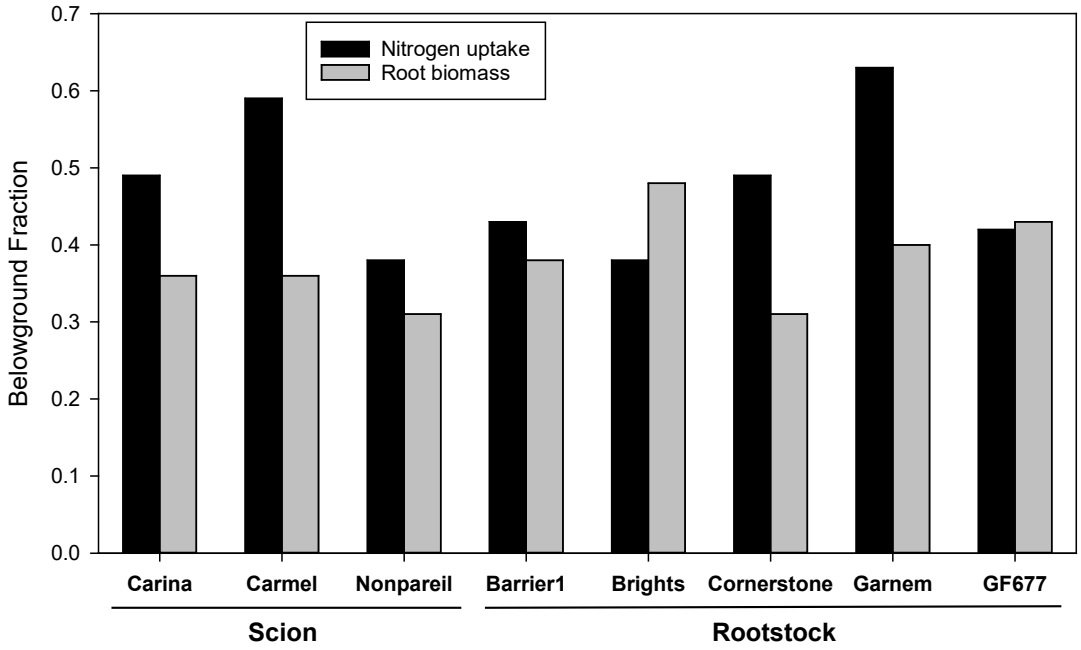


*Figure 28 – proportion of newly assimilated nitrogen allocated to the root system and proportion of total biomass allocated to the root system as affected by scion or rootstock genotype.*

Although the root samples were unable to be analysed from the orchard experiment using five-year old trees in 200L pots, the accumulation of labelled nitrogen in the leaves followed the same pattern as for the leaves in the glasshouse study, increasing over a few days before hitting a maximum (Figure 29). This helps to validate the applicability of the glasshouse study to older trees in a natural environment. The outdoor experiment was designed to determine the effect of phenology on nitrogen uptake, with most nitrogen applied from budburst until late December in commercial orchards. The uptake of labelled nitrogen was at a very similar rate for the January and March applications, but was significantly slower for the November application. There are a number of possible explanations, including differences in tree transpiration driving differences in N uptake, differences in tissue biomass resulting a in dilution effect, differences in allocation or differences in root uptake rate.

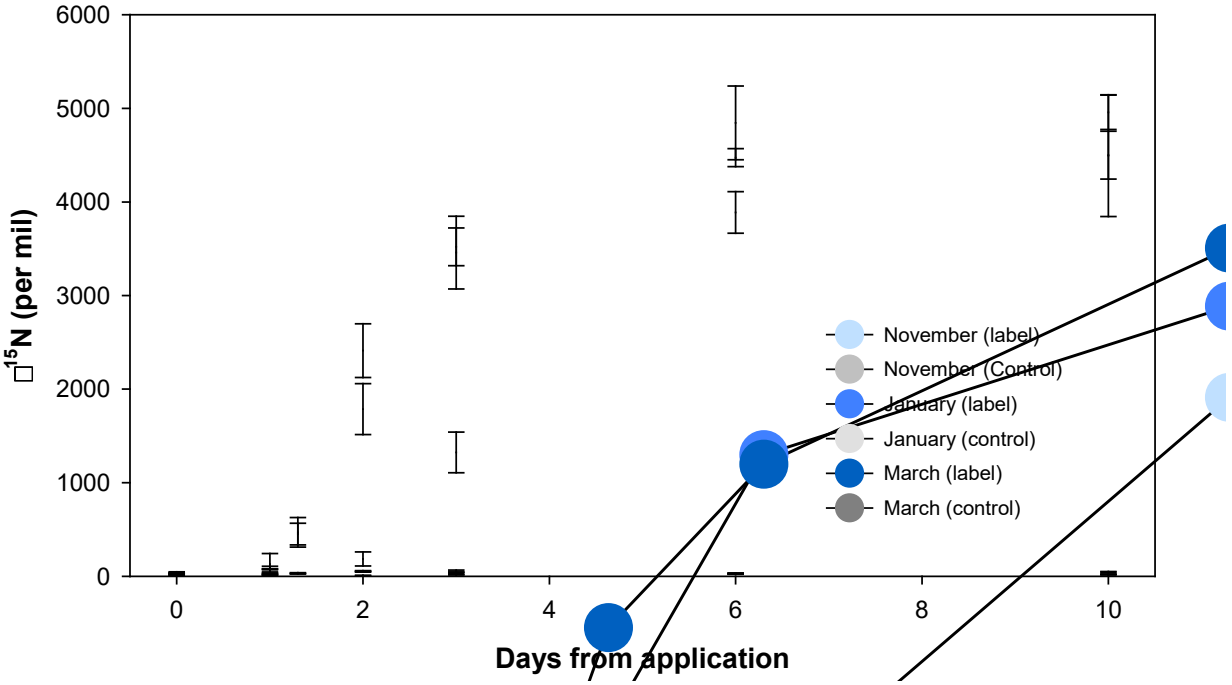


*Figure 29 – uptake of labelled nitrogen in the leaves of five-year old outdoor grown trees over 10 days at three different stages of the growing season (November, January and March). Data are means +/- SE (n=5).*

The weather conditions during the November 2019 application were cool and humidity moderately high, compared with January 2020, which included a 40°C day. However, the conditions for the March application were cooler than November, so it is unlikely that differences in tree water update explained the result. Although canopy size was not assessed, typically the canopy is barely fully grown in late November and certainly not larger than in January, so dilution of added N in a larger tissue mass in November is also unlikely. Accumulation of labelled nitrogen in the root systems of the glasshouse grown plants was always much more rapid than in the shoots, suggesting there could be a difference in the allocation of N in November, compared with later in the season. This requires further study to be confirmed however.

In summary, the nitrogen labelling work suggests that there is little difference between rootstocks in their ability to rapidly absorb available nitrogen from the soil. Whilst the glasshouse study suggested that total N uptake is strongly governed by shoot demand, it was unclear if this was the case in the field grown potted trees. Root class (defined by diameter) didn't affect assimilated N concentration in the greenhouse experiment, but as 85-90% of the root biomass was in the finest class (<1 mm in diameter) for these potted trees, the data from the outdoor experiment with mature trees is needed to confirm this. Despite shoot demand apparently being the largest driver of N uptake, there were clear differences in allocation between rootstock:scion combinations, Garnem:Nonpareil being the most extreme example. Understanding this effect and how to manipulate it offers the potential to more efficiently manage nutrient use in existing orchards and identifying the most efficient combinations would assist in genotype choice for new orchards.

## Belowground effect of soil organic matter amendment and reduced fertiliser application under commercial growing conditions

As part of the project aim to develop more efficient fertiliser management strategies, the interaction between compost and fertiliser application on soil nutrient content was examined at a commercial orchard at Robinvale, NE Victoria. Various soil nitrogen sources, P and K were assessed prior to compost application as well as soil chemical properties during the fertilisation part of the growing season (August to December).

The single modest compost application rate (4 t ha$^{-1}$), did not result in a measurable improvement in soil C or P, but the high potassium content of the compost, due to the inclusion of almond ash resulted in greater K being available four weeks after application (Figure 30). Although the compost was not aimed at replacing N fertiliser, there was also evidence of increased N availability after four weeks (Figure 31)

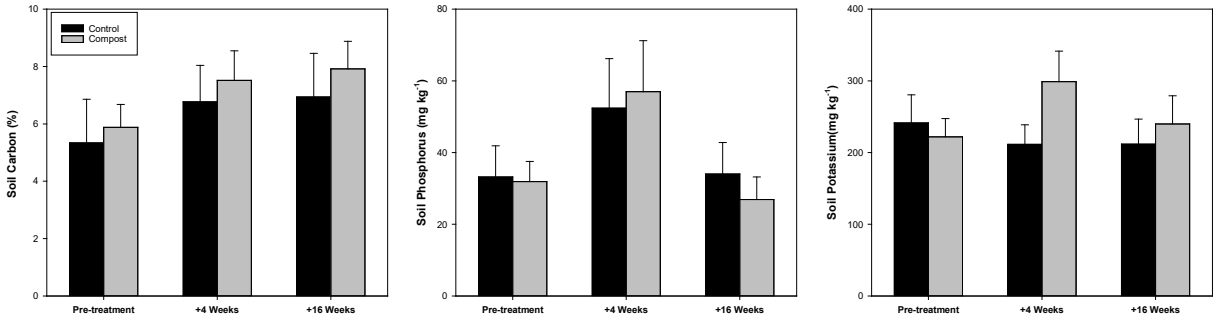


*Figure 30 – Soil carbon, phosphorus and potassium of the top 20 cm of soil before and following compost application. Compost values are the average of all four compost/fertiliser treatments.*

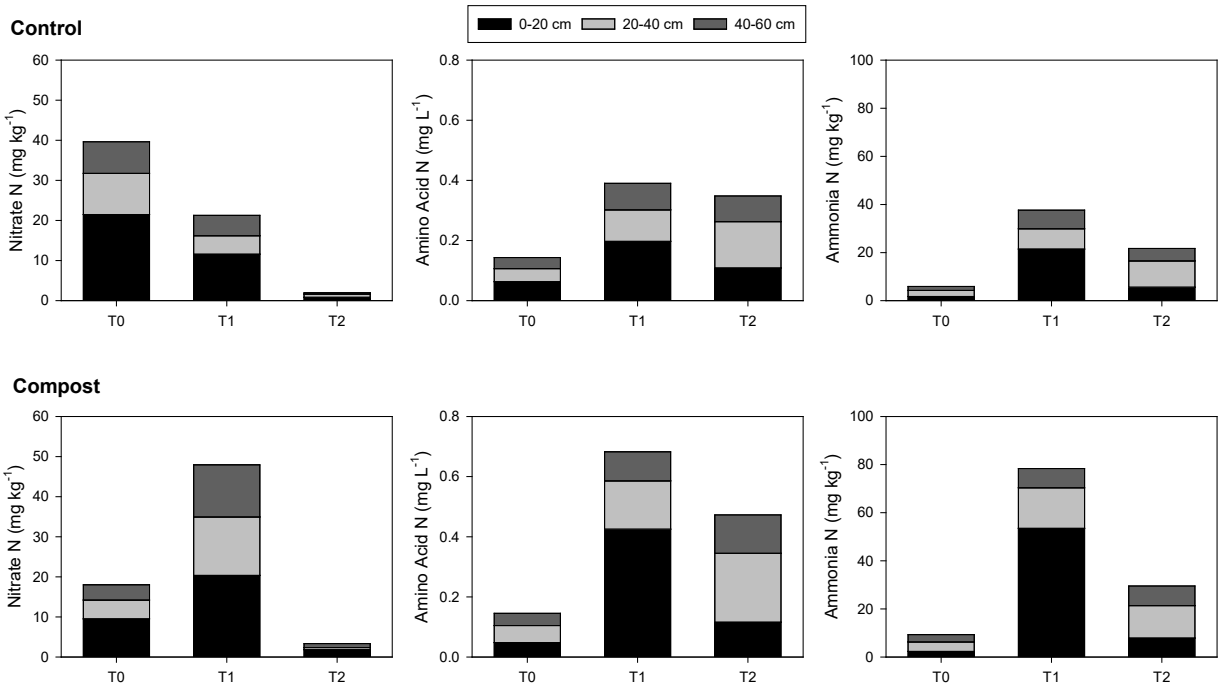


*Figure 31 – Soil nitrate, amino acid N and Ammonia to 60 cm before (T0), 4 weeks (T1) and 16 weeks following compost application. Compost values are the average of all four compost/fertiliser treatments.*

The ability of the high compost K content to replace fertiliser was examined with a reduced P&K fertiliser application (75% and 50% of standard). Four weeks after compost application K availability was improved in all the compost treatments, irrespective of fertiliser input and even after 16 weeks, K availability in all the compost treatments was equal to pre-treatment availability, whereas availability in the non-compost control was reduced, relative to the start of the season (Table 5).

*Table 5 – Colwell K (mg kg$^{-1}$) of the top 20 cm of soil before and following compost application.*

| | Pre-treatment (T0) | +4 Weeks (T1) | +16 Weeks (T2) |
|---|---|---|---|
| Control (standard P&K) | 242 | 212 | 212 |
| Compost + standard P&K | 254 | 276 | 242 |
| Compost + 75% P&K | 190 | 354 | 204 |
| Compost + 50% P&K | 198 | 260 | 198 |

The significant fertigation input in almond orchards has raised industry concerns of soil conductivity and was the reason for including soil conductivity in the rootstock screening described above. Typically, soil pH decreases during the irrigation season and soil conductivity increases, due to the fertigation required to maximise yield. Consequently, the potential for compost application to limit these within season changes was also examined. Whilst, the irrigation season decrease in soil pH was not affected, the increase in conductivity was halved (Figure 32), without any detriment in nutrient availability (see above), suggesting that improvements in soil chemistry due to compost application could occur beyond the potential value of fertiliser offset. The commercial viability of such compost application will depend on the availability of suitable compost and the labour cost of application.

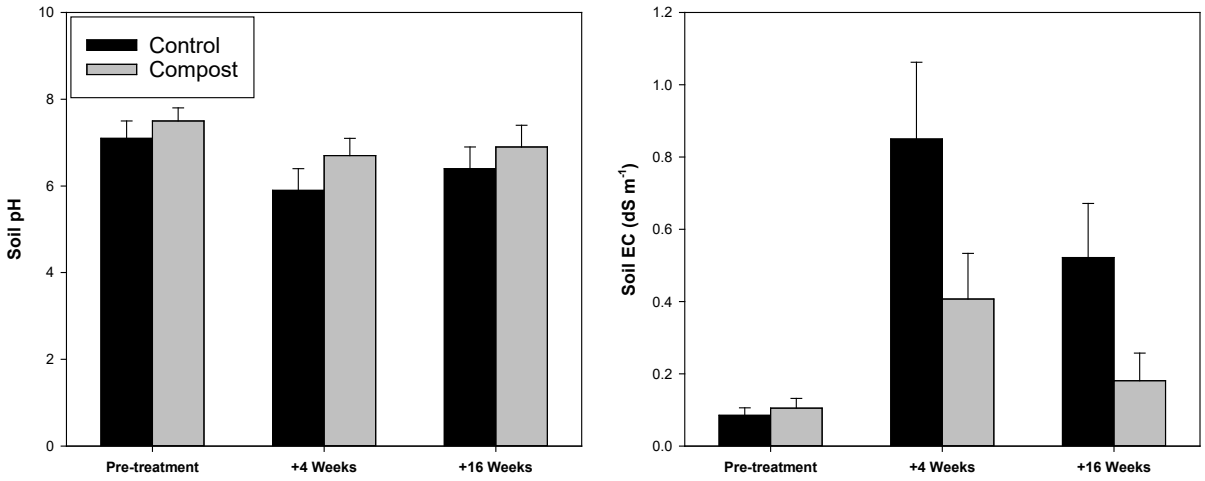


*Figure 32 – Soil 5:1 pH and electrical conductivity of the top 20 cm of soil before and following compost application. Compost values are the average of all four compost/fertiliser treatments.*

## Conclusions

A growth analysis based screening method for rootstock resilience to abiotic stress was developed and, when applied to most of the almond rootstocks available for Australian industry, demonstrated genotype variability in resistance to high soil conductivity and ability to maintain growth under reduced soil water availability.

Using an experimental trial at a commercial orchard root growth and development was little affected by significant reductions in irrigation (37%) and nitrogen (46%) application vs typical current practice. This was despite a reduction in tree water use (based on sap flow measurements) in the reduced irrigation treatment. The lack of any major impact on the root system was supported by the lack of any impact on kernel yield observed at the site (project AL14005).

Using labelled nitrogen fertiliser, it was found that roots of all genotypes appeared to rapidly maximise their nitrogen uptake (within 24 hours), but peak assimilation in the sapling canopies took several days, suggesting that nitrogen assimilation was shoot rather than root limited. Despite this, the allocation of nitrogen to shoot or root was dependant on rootstock-scion combination.

A modest application of a compost, rich in K, Ca and P didn't clearly affect soil carbon after a single dose, but did have an effect on soil available nutrients and properties such as pH and EC.

This project examined various aspects of the root system, its function and its influence on tree growth, highlighting the critical role that roots play in the orchard and raising further questions for future research.

# Appendix B: industry publications & conference posters

The field site in CMV almond orchard in Lindsay Point

## Better tree performance through root system resilience

Shuang-Xi Zhou; Everard Edwards; Rob Walker
CSIRO Agriculture and Food

**Summary**
The Australian almond industry has committed to enhance productivity and maintain profitability by creating a 'step-change' in the industry through optimising tree physiology and developing more efficient production systems. Abiotic stresses, such as soil water deficit, high soil solution salts or heat stress, can lead to dramatic impacts on crop productivity and quality. Plant root systems are a fundamental driver of plant performance and have the potential to increase water and nutrient use efficiency through a variety of mechanisms. Here we describe a new project titled 'Better tree performance through root system resilience' which aims to improve almond industry production and profitability through more informed rootstock choice, more efficient irrigation strategies and more efficient use of nutrition.

**Background**
The recent history of the Australian almond industry has been extremely positive, with the plantings increasing several folds since the year 2000 and production continuing to increase as a result. Meanwhile, the drought related difficulties faced in recent years by Californian growers, who provide over 80% of the world almond production, has helped to push up the value of almonds internationally. Despite this, there are concerns about profitability in the industry, with an Almond Board of Australia study suggesting that the costs of production are significantly higher in Australia than that in California, primarily due to labour, fertiliser and irrigation costs. Improving productivity and reducing costs provide a number of challenges for the industry, in particular, maximising the efficiency with which sunlight is converted into yield, the efficiency with which water is used by the tree, and the efficiency of fertiliser uptake by the tree. The root system of the tree plays a major role in improving these efficiencies, as both water and nutrient uptake occur through the root system, which also provides a barrier to adverse soil characteristics, such as high soil conductivity (whether through nutrient application or salinity) or soil water deficit. Furthermore, plant root systems contain a complex range of sensing and signalling mechanisms that can affect the shoot; for example, the role of rootstocks in determining shoot growth and architecture.

Despite their importance, there are significant gaps in our current knowledge of the mechanisms controlling root growth, development and function, including the signalling between root and shoot. Consequently, the three key aims of the project are to:
- provide growers with the information required to make an informed choice of rootstocks suitable to their orchard;
- provide the tools to ensure that new rootstocks provided to the Australian industry are suited to Australian growing regions and systems; and
- optimise the timing and positioning of water and nutrition to match root functioning, thereby improving uptake and utilization efficiency.

Fundamental knowledge and a better understanding of the functioning of the Prunus root system, including the signalling mechanisms between rootstock and scion, will also be developed.

**Project structure**
The project has three distinct, albeit overlapping, phases: rootstock characterisation, root-shoot interactions, and new management systems.

***Phase I Almond rootstocks screening for drought and salinity stress tolerance***
The first phase of the project is already underway and

The glasshouse experiment screens Prunus rootstocks for tolerance to water stress and high soil conductivity.

is to develop and use rapid glasshouse-based screens for abiotic stress tolerance in Prunus rootstocks, namely water stress and high soil conductivity (photo above). Declines in plant growth and productivity under abiotic stresses are well documented and often lead to a change in biomass allocation to the root system. These screens are being used to characterise all of the Prunus rootstocks currently available for use in the Australian almond industry.

The screens use ungrafted rootstock material allowing plants of a younger age and smaller size to be used, and assess the growth rate of plants under stress conditions relative to those grown without stress. This provides an index of stress response that can be used to compare genotypes. While field conditions are different, such screens in other studies have demonstrated differences between genotypes in response to stress that can be an indication of how trees respond in the field. In addition to this, a number of key above-ground and below-ground plant traits are being measured to improve our knowledge of how the rootstock genotypes differ in their stress responses, such as leaf photosynthetic rate, leaf area, root diameter, root length, and root surface area.

***Phase II: Improve understanding of root growth, development and function and root:shoot interactions in almonds***

As already noted, root systems contain a complex range of mechanisms, which influence key traits such as root growth and architecture, the uptake of water and mineral elements to the shoot and, in grafted trees, the vigour, precocity and architecture of the scion.

Glasshouse experiments will be undertaken to examine aspects of root:shoot communication in detail, but field experiments are also being used. One such experiment is underway in the Riverland region, where Agriculture Victoria has established an experiment in a current commercial planting to determine the interaction of water and nitrogen limitation on spur productivity (photo page 32). A system to monitor root growth and production throughout the growing season was installed ('minirhizotrons') together with sap-flow sensors, which will lead to an improved understanding of root activity (in respect to growth, water and nutrient uptake) in Prunus root systems (photo below). The sap-flow sensors were installed in Nonpareil trees only, while the minirhizotrons were installed both in the Nonpareil trees and one of the pollinators – Carmel, potentially allowing the effects of the scion on root growth to be determined.

A factorial layout of treatments has been developed, namely normal/low nitrogen application (normal practice versus 57% of normal practice) and normal/low irrigation treatments application (normal practice versus 70% of normal practice), and was first imposed in the 2015/16 season. This study will allow direct determination of crop water use index, nutrient use efficiency and measurement of root distribution, production, turnover and phenology under the different growing conditions.

***Phase III: Develop and trial irrigation and nutrient management strategies to improve nutrient and water use efficiency in new growing systems.***

The final stage of the project will develop new management options that utilise the knowledge of root:shoot signaling that we are developing to optimise water and nutrient use efficiency. It is expected that these would be options for existing plantings and would be based on improved timing and positioning of irrigation and fertigation. These new management strategies will be implemented and trialled within the project. In addition, the project would collaborate with other components of the program that implement novel plantings and/or management techniques.

**Outcomes**

This project will contribute to a better understanding of the function of Almond root systems, including signalling mechanisms between rootstocks and scion, an area of plant science with potential to produce significant impact. We expect to deliver: (1) an assessment of the resilience of available Prunus rootstocks to water stress and high soil-conductivity stress; (2) optimised management for root systems and rootstocks; and (3) practices to deliver significant gains in water use efficiency (yield per unit water applied) in almond orchards, potentially from higher density plantings involving scions on controlled vigour rootstocks.

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The researchers used the minirhizotron system to monitor the root production and turnover of almond rootstock, as part of the project already described. It is hoped that it will help develop improved management strategies for almond orchards, based on a better understanding of their root-systems. Using minirhizotrons to monitor root production and turnover in an almond orchard.

Photo 1: The Olympus videoscope

## Using minirhizotrons to monitor root production and turnover in an almond orchard

*Shuang-Xi Zhou; Everard Edwards; Rob Walker*

The water and nutrients required by a tree are obtained through its root system; consequently, the capacity of a tree to take up these resources is primarily controlled by factors such as the amount of root, the speed with which new roots are produced and grow and how long they live. Interactions with other organisms in the soil, particularly mycorrhiza, can also be important. The timing of root production is critical; active roots need to be present when water and nutrients are provided. Field observations of these parameters have the potential to greatly improve our understanding of how irrigation and fertiliser application can best be utilised by the tree and how this affects orchard performance. Unfortunately, observing roots in situ is difficult, but we are utilising a system known as a 'minirhizotron' to do just this, monitor the growth and production of roots without disturbance.

A minirhizotron is simply a clear tube that is inserted into the ground at an angle, within the root-system of the tree. A camera system is then used within the tube to image the roots, their growth and their disappearance (death) at regular intervals. We have installed 96 such tubes (1.5 m long x 3.5 cm diameter) in the project field site at Lindsay Point, Victoria, providing a view of the roots to a 1 m depth in the soil. Each tube is laser etched with 74 rectangular windows (2.5 x 2 cm) and these windows are imaged at three week intervals using an Olympus videoscope (Photograph 1). The images are then analysed using the open-source software 'Root Fly' (http://cecas.clemson.edu/~stb/rootfly/), which tracks individual roots over time, allowing root lifespan to be determined as well as length, diameter and, of course,

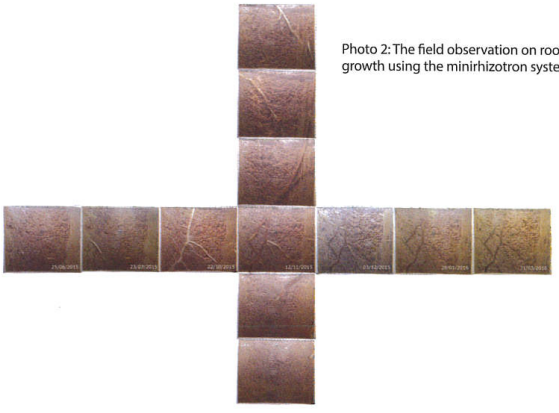

Photo 2: The field observation on root growth using the minirhizotron system.

depth. Knowing root lifespan allows root turnover, the proportion of the root system which is replaced by new roots over a given time, and root demography to be studied.

At present, a minirhizotron system is the only method available to non-destructively measure root growth in the field and, therefore, the only method that can be used to determine root lifespan. Taking soil cores or digging soil pits can only show how many roots are present at a given point in time and clearly affects subsequent root growth in the area where the soil has been disturbed. Minirhizotron imaging also minimises the amount of time required in the field.

The diameter of the minirhizotron is kept as small as possible to minimize any effect of its presence on root growth, but this also means that minirhizotrons are best used to track fine roots, as they rarely intersect larger, course roots. However, it is just these fine roots that are active in absorbing water and nutrients from the soil, with larger roots being used for transport, anchorage and storage.

Photograph 2 demonstrates what can be seen using our minirhizotron system, with differences in root density with soil depth visible by following consecutive windows down the tube and changes in roots over time visible by following a single window over multiple imaging dates. The effects of different orchard management regimes, such as changes to irrigation or fertiliser application, and of different almond genotypes (scion or rootstock) on the root system can then be determined, including effects that may only be present for short parts of the growing season.

The pattern, timing, and total amount of root production will potentially vary substantially from season to season. Identifying the cause of any such inter-annual variability, together with any relationship with water and nitrogen availability will assist in developing optimized irrigation and fertiliser application strategies. Continuing the minirhizotron observations over several years also allows the lifespan of longer-lived fine roots to be determined.
Our intention is to monitor root growth and its dynamics at the Lindsay Point orchard over four seasons, studying the impact of reduced irrigation and different nitrogen application rates on almond trees.

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1. Ungrafted rootstocks being screened for soil stress.

# Better tree performance through root system resilience – project update

In December 2016 Australian Nutgrower published an article which provided an introduction to a new project, based at CSIRO, which aimed to improve tree performance of almonds through better management of their root systems. The project is due to end in late 2020 and here the authors provide a progress report on the work to date.

**Rootstock screening**

The vast majority of almonds in Australia are grafted to Nemaguard rootstock. Whilst this is also the most commonly used rootstock in California, a wider range of rootstocks are used there, with soil type probably the largest factor behind rootstock choice. Currently, there is no breeding program used here for almond rootstocks in Australia, meaning that the Australian industry is reliant on imports for new genotypes.

In addition, the number of almond rootstock trials in Australia to date has been limited, such trials requiring many years and they cannot represent all soils and regions. Improving the availability of rootstock genotypes suitable for use with almonds and the information on their performance under Australian conditions for growers is likely to be a significant benefit to the industry.

To help address this situation, the first component of our project was to develop rapid, greenhouse based, rootstock screens against abiotic stresses (drought, high soil conductivity/salinity). These screens are intended to be a preliminary step, allowing many rootstock genotypes to be tested in a short time, prior to selection for traditional field trials or importation. The screens using ungrafted material (seedlings or cuttings), are completed in a few weeks and can be carried out by nurseries with little specialised equipment.

The principal is to use growth as the ultimate measure of production, but rather than use biomass per se, the screens use relative growth rate, the increase in biomass per unit biomass per day. This approach allows better comparison between trees of different sizes and between trees grown under non-identical greenhouse conditions. However, the approach can only be used for factors which affect the root system directly.

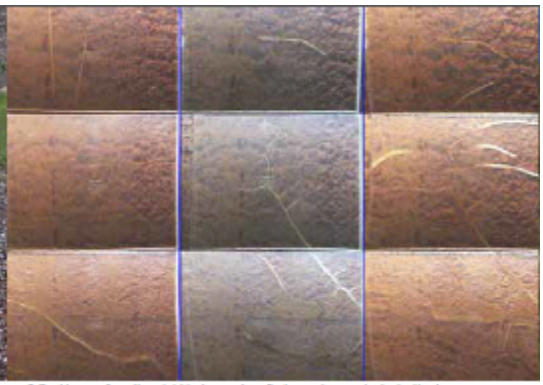
By comparing relative growth rate under control and stress conditions an index of stress tolerance is calculated. To date 17 rootstock genotypes have been screened against low soil water availability and against high soil conductivity (photo 1). We expect to publish these results in the coming months, following some additional validation work.

**Root phenology - the impact of reduced irrigation and nitrogen**

Both water and nutrients enter the tree primarily through the root system. Typically a fresh, white root is thought to have a much higher ability to absorb nutrients than a brown root and a woody root will absorb little water or nutrient. In almonds we have little idea how much difference there is between such roots, fine roots of different ages or between different classes (diameters) of fine root. We also have poor information on what proportion of fresh root is produced in spring relative to the rest of the season. We can use the term phenology for the root system, just as with the above-ground growth of the tree, and, by improving our understanding of fine root phenology in the orchard, we hope to improve the way that water and fertiliser is applied, resulting in an improvement in water and nutrient use efficiency.

To this end, the project has been collaborating with staff at the Department of Economic Development, Jobs, Transport and Resources (DEDJTR) Irymple and with CMV Farms. DEDJTR have been running a factorial trial with reduced water and nitrogen application in a CMV Farms orchard at Lindsay Point (photo 2).

2. Irrigation and nitrogen trials at CMV Farms Lindsay Point



3. Root images from the minirhizotron system. Each row shows a single depth whereas each column shows a different point in time.

As part of that trial we have been monitoring fine root production and growth using a minirhizotron camera system (see previous article in Dec 2016 issue), which allows us to track roots over time in the soil without disturbance.

Whilst the minirhizotron system provides information on root growth and phenology, it doesn't give us a good assessment of the actual amount of root present.

To establish this, the minirhizotron work has been paired with soil coring during tree dormancy. Roots are extracted from these cores, allowing root length density and biomass to be estimated per unit volume of soil. In addition we are monitoring whole tree water use with a sap flow measurement system. This data is then tied to the spur productivity and orchard production data being collected by DEDJTR.

The minirhizotron and soil coring work has been running at CMV farms for almost four seasons, with one season of data collected prior to the irrigation and nitrogen treatments being applied and the third consecutive season of treatments currently underway. This has resulted in tens of thousands of images, which the project team is currently working through to produce detailed information on root growth patterns and the effects of water and nutrient availability on those patterns (photo 3).

**Root function – the link with phenology**

Improving our knowledge of root phenology will tell us when the most active roots are present, the proportion of all fine roots which are active and to what extent active roots might appear post-harvest, but basing irrigation or fertigation decisions on that knowledge still relies on assumptions about which roots best take up water and nutrients. The third component of our project is linking more detailed investigation of root function, specifically how different roots vary in their ability to take up nitrogen, with phenology in the field to model whole system nutrient uptake.

This work is using short-term glasshouse experiments, where root systems can be dissected and their constituent parts examined, and whole season outdoor experiments using grafted trees in large pots (photo 4). At present all of these experiments are utilising a stable isotope of nitrogen (15N) to quantify nitrogen uptake in different parts of the root system and to trace the speed and transport of that nitrogen through the tree. 15N is a naturally occurring, harmless, non-radioactive isotope that makes up 0.4% of the nitrogen in the atmosphere and has the same chemical properties as the more common 14N, that makes up 99.6% of the nitrogen in the atmosphere. Nitrogen fertiliser with a high 15N content (10%) is fed to the trees, following which shoot and root samples are taken and analysed for their 15N content with a mass spectrometer.

At the time of writing, a large glasshouse experiment has been completed which utilised a range of rootstock:scion combinations and followed uptake of nitrogen over a period of ten days following a fertiliser application. The resulting tissue samples, including different root classes, are currently being processed and analysed. This experiment allows us to not only examine the rate and amount of N taken up by different roots, but also whether this differs by rootstock or is influenced by scion variety.

The outdoor pot experiment is also underway, with the second of three fertiliser applications high in 15N planned for the 2018/19 season having recently been completed. Again, root and leaf samples are currently being processed for analysis. The primary aim of this experiment is to examine how tree phenology alters root nitrogen uptake and the role that root age plays in this.

Ultimately, we will combine the knowledge gained on the potential nitrogen uptake of root classes at different times of the season with the knowledge of the proportion of the root system that falls into these classes from the minirhizotron work to produce predictions of whole tree potential nitrogen uptake at different times during the season, which can be used to further optimise current fertiliser regimes.

**Field application of the research**

The final component of the work is to apply all the knowledge gained as new management regimes

4. The outdoor pot experiment at bloom.

5. Manufacture of soil amendments from waste at Select Harvests Carina West.

in a commercially managed orchard. At present we are intending to centre this on nitrogen fertiliser use. To this end, the project is collaborating with Select Harvests at Robinvale who are trialling a soil amendment manufactured from orchard and processing plant waste (photo 5). The project team are monitoring any changes in the soil and their impact on the tree root systems.

**Acknowledgements**

*This project has been funded by the almond industry through Hort Innovation with co-investment from CSIRO and funds from the Australian Government.*

*We would like to thank Zelmarie Coetzee, her predecessor Dave Moinks, Michael Treeby and their staff at DEDJTR Irymple; Tim Preusker and his staff at CMV Farms; Upul Gunawardena at Select Harvests; Mark Ryland of AusBuds for offering almond rootstock plants and advice, and, finally, we acknowledge the Almond Board of Australia for their support.*

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# Hort Innovation new strategy

Hort Innovation is calling out to industry stakeholders to get involved and provide their input on the direction of its new corporate strategy.

Under the leadership of new CEO Matt Brand, a national roadshow of consultation workshops is now underway across Australia, visiting all states and territories, concluding in April.

Mr Brand said these workshops offer the opportunity to learn more about how Hort Innovation currently works to invest levy funds, and to freely discuss views and ideas on how it can be done better in the future to meet the needs and priorities of the sector.

"We are inviting our members, levy payers, industry bodies and horticultural growers nationwide to take part in consultation workshops that will help to determine our new strategic direction," he said.

"As the grower-owned Research and Development Corporation for the Australian horticulture industry, it is the task of Hort Innovation to invest levy funds into research, industry development and marketing projects that will grow the hort industry in both domestic and international markets.

"The new strategic plan will strengthen the way our organisation works from the top down – and we want to ensure that our growers are at the forefront of the new strategy."

The workshops are free to attend but registration is required. An online ideas portal has also been set up to allow anyone who cannot physically attend to provide insight and ideas into the strategy process.

The new corporate strategy will come into effect around mid-year, following the face to face workshops with growers, industry bodies, and incorporating online feedback.

All growers and industry stakeholders are encouraged to get involved and contribute.

Workshops began in early February and conclude on April 2 with one on the Sunshine Coast on April 2. You can participate by filling in the four question survey on the Hort Innovation website: www.horticulture.com.au

Hort Innovation

## Project Update

# Better tree performance through root system resilience

*By Everard Edwards, Alex Lawlor, Adelle Semmler, Nina Welti, Lynne Macdonald, Rob Walker.*

**Summary**
In previous issues of Australian Nutgrower we have published an introduction to and updates on our CSIRO based project, which aims to improve tree performance of almonds through better management of their root systems. The project will be completed in October 2020 and here we provide the final progress report on work to date.

**Rootstock screening**
As described in earlier articles, we are using differences in the relative growth rate (the change in whole tree dry mass per unit of existing dry mass per day) of various newly-propagated ungrafted almond rootstocks as a pre-screen for specific root driven traits. In our trials we have examined the resilience of the root system of 17 genotypes to soil water deficit and high soil conductivity (i.e. salinity). Currently we are undertaking final validation tests, comprising of repeated assessment of three rootstock genotypes at different plant sizes and periods of different intrinsic growth rates. The latter being simply being a result of growing under different daylengths. Following this we will release our results in conjunction with our assessment of the applicability of those results.
Root phenology - the impact of reduced irrigation and nitrogen

Determining almond root phenology in the field has been undertaken using a minirhizotron system to study changes in root growth in-situ (see Dec 2016 article for description of this system). The work was undertaken at CMV Farms, as part of a collaboration with Agriculture Victoria Irymple, which examined the tree response to reduced water and reduced nitrogen application in a factorial set-up. The practical component of this work was completed in July 2019. Our focus now is on analysing the tens of thousands of root images that have been collected.

Figure 1 provides an example of the simplest output of this data, average fine root length density over time. The results given are preliminary data for single replicates for each of the four treatments at the CMV Farms site and are limited to the first growing season during which treatments were applied. The very high amount of fine root growth was likely a response to the installation of the minirhizotron tubes, but differences in growth response to irrigation rate were already clear. Interestingly, this difference did not seem to remain for the over-wintering roots at the end of the growing season.

Although that observation is based on a small subset of the minrihizotrons and is only the first of four seasons of data, it matches what was seen in soil samples obtained during dormancy by coring. Soil coring was undertaken to i) to allow the results from the 2D image minirhizotron image data to be expressed in relation to the entire tree root system, ii) to independently validate what was seen in the minirhizotrons and iii) to obtain soil samples for basic characterisation.

Figure 2 includes data from two (winter 2017 & winter 2018) of the four years during which field treatments were applied (2016-2019) and suggests that as far as the over-wintering fine roots are concerned, any differences in root growth or root density during the growing season were not retained through dormancy. This data was typical of the data from the other three winters (2015, 2016 & 2019) during which soil cores were taken.

Both the minirhizotron and soil coring data provide much more information than just the root length density (minirhizotrons) and root mass density (cores) presented here, including root distribution, proportion of active (white) and transport (brown) roots, fine root growth rate and lifespan, the effect of time of season on these parameters (phenology) and, most importantly, the impact of the water and nitrogen treatments applied. As the data processing continues, we will be able to draw more of these details out.

In addition to the primary goal of understanding the effects of resource limitation on root growth and phenology, the Agriculture Victoria/CMV Farms collaboration included direct measurement of tree water use with sapflow sensors. Again, data processing is still in progress and the results will be linked with tree architecture data collected by Agriculture Victoria when complete, but early data demonstrates the interaction between canopy growth and water use (Figure 3).

The data suggests that by mid-September, at this site, any further canopy growth was not having a major impact on tree water use. Further, the slope of the relationship during the second part of the season

is effectively the tree driven component of kc, the crop factor which can be used to relate tree water requirements to weather station derived estimates of reference evapotranspiration (ETo) for irrigation decisions.

**Root function – characterising root nitrogen uptake**

As described in our previous update (Feb 2019), we have been using a naturally occurring and harmless stable isotope of nitrogen to investigate nitrogen uptake and allocation by the tree. We completed glasshouse and outdoor experiments (all in pots; unfortunately we estimated that to carry out the work, even at a very small scale, in a commercial orchard would cost $200,000 just for the nitrogen application!). Again, the experimental work is complete, and we are about half-way through analysing the more than 2,000 plant biomass samples we have generated.

The % atom enrichment (AT%, representative of nitrogen uptake) of white vs brown fine roots at day two clearly shows the difference in nitrogen uptake between active and transport roots (Figure 4). However, after five days the difference is small. These data don't tell us the relative contribution of these roots on a whole root system scale, nor changes through the season. These questions should be answered as we analyse the samples from the rest of the tree and from the season-long pot experiment.

**Conclusion**

With most field and practical experiments complete, the majority of the work from now until the end of the project in October is to complete sample and data analysis. As well as the new learnings about almond root systems from the various components of the project, the results will be synthesised to generate a simple model of the nitrogen uptake potential of an almond root system and how that potential changes through the growing season. This should assist growers in devising alternative nitrogen application regimes.

**Acknowledgements**

This project has been funded by the almond industry through Hort Innovation with co-investment from CSIRO and funds from the Australian Government. We would like to thank Zelmarie Coetzee, her predecessor Dave Monks, Michael Treeby and their staff at DEDJTR Irymple; Tim Preusker and his staff at CMV Farms; Upul Gunawardena at Select Harvests; Mark Ryland of AusBuds for offering almond rootstock plants and advice, and, finally, we acknowledge the Almond Board of Australia for their support.

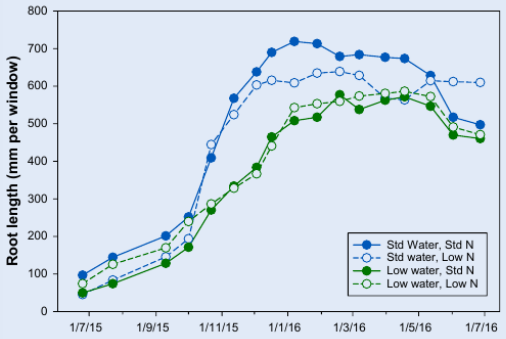



Figure 1 - fine root length density, as recorded from the minirhizotron tubes during the first season of reduced nitrogen and irrigation treatments at CMV Farms.

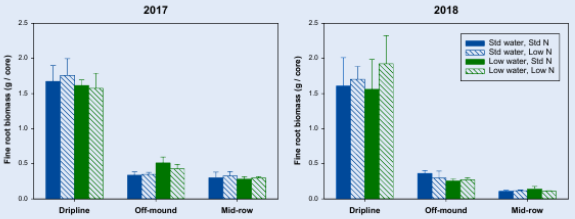



Figure 2 - fine root biomass density obtained by soil coring during dormancy, following the second and third seasons of reduced nitrogen and irrigation application at CMV Farms.

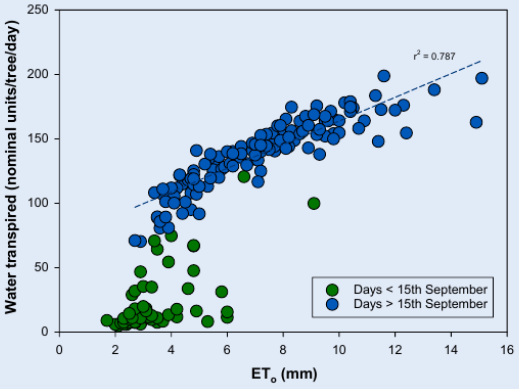



Figure 3 – The relationship between daily water use (transpiration) of a single tree, recorded using sapflow sensors during the 2018/19 growing season at CMV Farms, and calculated reference evapotranspiration.

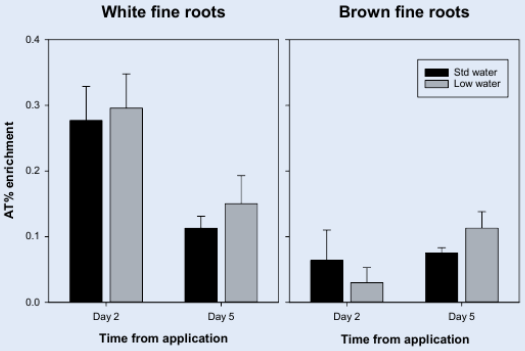



Figure 4 - 15N atom enrichment of root systems provided with labelled nitrogen fertiliser, under standard or reduced water availability.

RESEARCH
Better tree performance and
water use efficiency through
root system resilience
In A Nutshell - Summer 2020 Vol 20 Issue 4

RESEARCH

**Everard J. Edwards, Alex Lawlor, Adelle Semmler, Shuang-Xi Zhou, Nina Welti and Rob R. Walker |**

**CSIRO Agriculture and Food |**

**Back in 2014, CSIRO Agriculture and Food began work on a project to better understand almond root systems and use this knowledge to improve tree productivity and resource use efficiency. Co-funded through the almond levy, the project was part of the productivity program being developed by the Almond Board of Australia at the time and included collaboration with other research agencies at the CMV Farms Lindsay Point site. Originally intended to run for four years, staff changes led to project funds being stretched over five years to ensure the work was completed. The project concluded in November 2020 and a final report has been provided to Hort Innovation that will be available in due course.**

The key components of the project were to:

- monitor fine root development and phenology in-situ at the shared CMV Farms Lindsay Point trial site, including the response to reduced water and nitrogen supply
- track sapflow of those trees and link back to both the root system and canopy size (the latter through collaboration with the Agriculture Victoria work at Lindsay Point)
- determine the influence of root type and age on nitrogen uptake and whether this is affected by rootstock genotype
- combine the root phenology and nitrogen uptake information to estimate the potential for tree nitrogen uptake throughout the season and suggest optimisations to nitrogen fertiliser application.

The project also had a component that was to develop a rapid pre-screen method that could be used by nurseries or others to assess rootstock resilience to abiotic stress, which will be reported on elsewhere. The staffing changes and timing of achieving different parts of the work prevented a trial to test new fertiliser management strategies based on the work, but the project team did collaborate with Select Harvests in their trials to examine the potential to combine compost applications with changes to fertiliser application, including impacts on nitrogen availability .

The root phenology study at Lindsay Point involved more than 60 field trips over four years to image roots as they grew, using 90 minirhizotrons (transparent tubes buried in the rooting zone), resulting in nearly 100,000 individual images. The appearance, duration of activity (based on root colour), length, disappearance and, therefore, lifespan of individual roots was

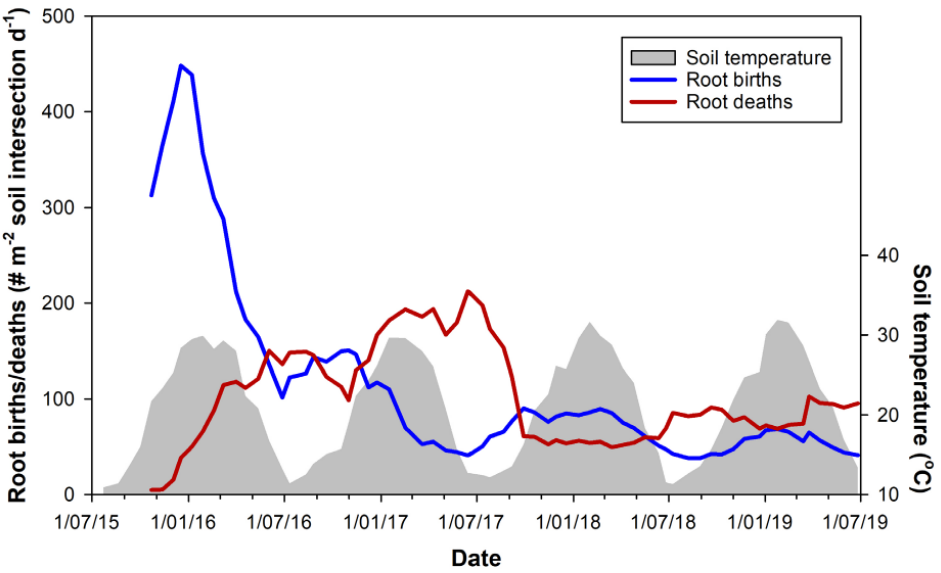



**Figure 1: The seasonality of both new root production and root death can be seen in the root imaging data, overlaid on a high initial rate of root production (and subsequent root death) as a result of the installation of the minirhizotron tubes.**

**Opposite page: Root imaging at CMV Farms.**

RESEARCH

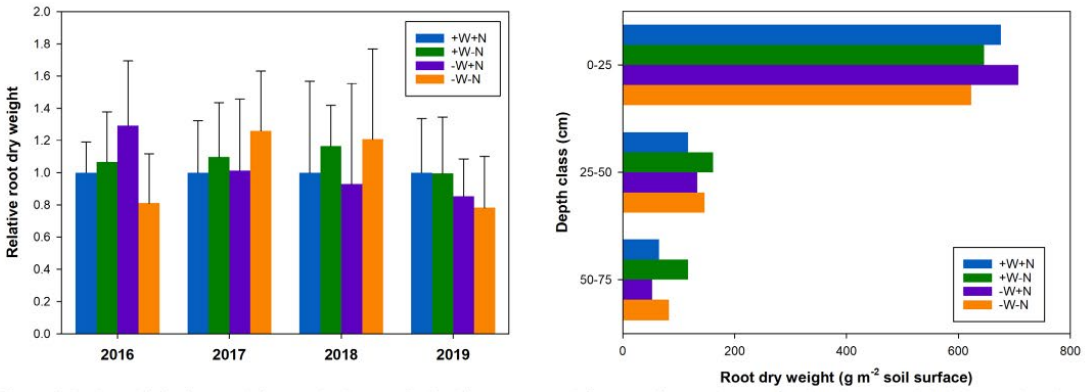



**Figure 2: Reduced irrigation and nitrogen had a very limited impact on total fine root biomass (normalised to the control, left), but reduced nitrogen resulted in a small redistribution of roots to further down the soil profile (right).**

Nitrogen uptake trial, Waite.

Soil coring at CMV Farms.

followed through the four seasons of data collection. Seasonality in the production, loss and the absolute amount of fine root present was clearly observed, but was less than expected, with some new roots appearing throughout the year and the majority of roots living for 2-3 seasons (Figure 1).

The root imaging was backed up by annual trips (pre-budburst) to determine over-wintering fine root length density and biomass by taking soil cores and extracting the roots. Overwintering fine root biomass was only marginally affected by the reduced water (-30 percent) and reduced nitrogen (-40 percent) treatments applied, although the reduced nitrogen treatments did cause a higher proportion of the root biomass to be located lower in the soil profile. Overall, two-thirds of the fine root biomass was located the top 25 cm of soil. (Figure 2).

Urea labelled with a stable isotope of nitrogen (15N), was used to determine the role of root age and time of season on nitrogen uptake by the root system. The cost of 15N labelled urea prevented this being used in a commercial orchard, but seasonality of nitrogen uptake potential was examined using potted trees outdoors and a separate glasshouse study looked at the interaction between rootstock and scion, with six rootstocks and three scions examined through eight individual combinations. These experiments demonstrated that fine root size class (diameter <1 mm or diameter 1-3 mm) made little difference to the nitrogen uptake and that whilst older, brown, roots were somewhat slower to take up nitrogen they still appeared to be able to do so at reasonable rates. Unfortunately, results on the effect of phenology were not available at the time of writing (Figure 3).

The final synthesis of the extensive results from this project will take more time and be communicated to growers in due course, but overall the work has demonstrated surprising resilience of almond root systems to water and nitrogen limitation, consistent with the lack of significant effects observed on yield by the Agriculture Victoria project (AL14005) that utilised the same CMV Farms trial.

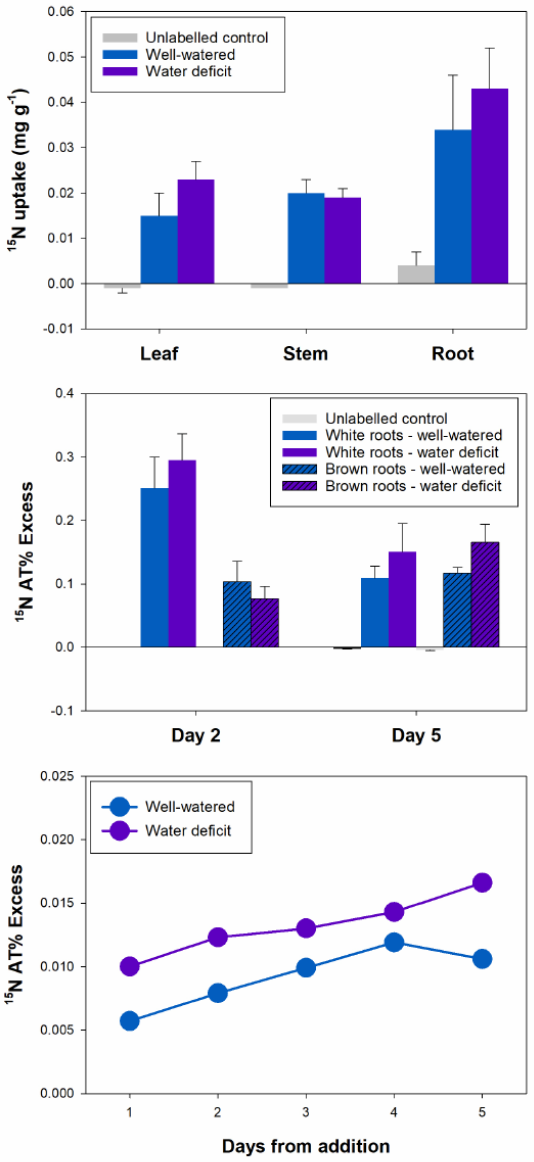



**Figure 3: Labelling nitrogen fertiliser with a non-radioactive isotope demonstrated a significant distribution throughout the plant five days after application (top), a higher initial rate of uptake in young, white roots (middle) and a higher uptake, relative to existing plant nitrogen, in plants subjected to water deficit (bottom).**

ALMONDS

# Understanding roots for greater efficiency

**Everard J. Edwards et al.***

The recent history of the almond industry has been extremely positive, with plantings rising from 5600ha in 2001, to over 45,000ha in 2018. During this period, production has risen from 9100t (2.2% of global) to approximately 80,000t (over 6% of global).

Despite this growth, production efficiency has not improved significantly and tonnes of kernel per hectare is below that of the USA. Furthermore, in 2013 the cash costs of production in Australia were estimated as almost 50% higher than the US, although the AU$ and US$ were at parity then.

Two of the largest differences in costs between the US and Australia are irrigation and fertilisation. Both of these inputs are taken up and transported to the above-ground parts of the tree by the root system.

Furthermore, the root system is also the tree's interface to a number of abiotic stresses, such as high soil conductivity, compaction, adverse pH, etc. Consequently, the resilience of the tree to these stresses can be influenced by rootstock choice, although over 90% of almond plantings are on a single rootstock, Nemaguard.

In 2014 CSIRO Agriculture & Food began work on a project to better understand almond root systems and use this knowledge to improve tree productivity and resource use efficiency.

Co-funded through the almond levy by Hort Innovation, the project (AL13009) was part of the productivity program developed by the Almond Board of Australia at the time. The work concluded in November 2020, with a final report provided to Hort Innovation which will be available once approved. It consisted of several components.

**Root dynamics**

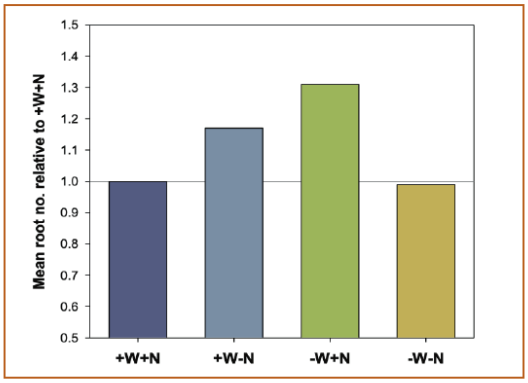
In collaboration with Hort Innovation project AL14005, work was undertaken over four seasons at a 2.2ha field trial at Lindsay Point in NE Victoria to examine root phenology and tree water use in response to a 30% reduction in irrigation, a 40% reduction in nitrogen application and a combination of the two.

Roots were studied *in-situ* using 1.5m long minirhizotrons (transparent acrylic tubes) buried in the rooting zone of the trees. Although the tubes were angled, they provided access to roots over 1m deep.

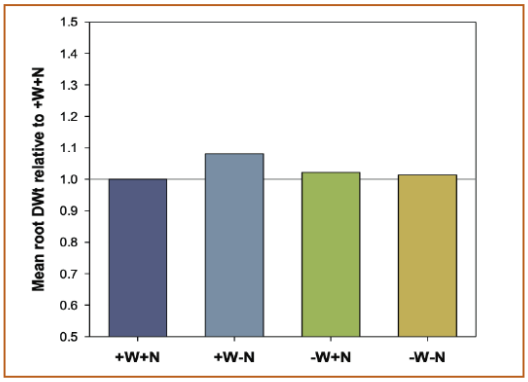
A videoscope was used to image the upper surface of the minrihizotrons almost 70 times, between June 2015 to June 2019, showing the changes in root growth through four seasons. Soil cores were taken during dormancy and the roots extracted to compare with the root dynamics results and sapflow sensors were installed to monitor tree water use.

Despite being fairly significant in scale, the reduced inputs had limited impacts on the root system or on tree water use, consistent with the lack of a statistically significant impact on yield seen by AL14005.

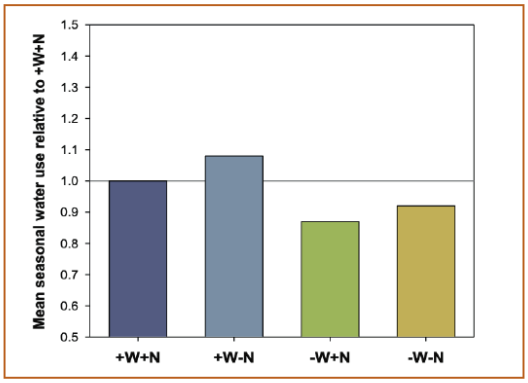
**A CSIRO trial has demonstrated a surprising resilience of almond root systems to water and nitrogen limitations.**




**Effect of reduced water and nitrogen input, alone or in combination on the average number of roots observed over four years of treatments.**




**Effect of reduced water and nitrogen input, alone or in combination on the average root dry mass obtained from soil cores during dormancy over four years of treatments.**




**Effect of reduced water and nitrogen input, alone or in combination on the average seasonal water uptake, estimated using sapflow sensors, relative to grower standard practice over three years of treatments.**

Under a single reduced input, root numbers seen in the minirhizotrons were increased, relative to standard inputs, but were no different to standard with the combined reduction of water and nitrogen. The same response was seen to a lesser extent in the root biomass during dormancy.

There was also a tendency for the low nitrogen treatments to have more root biomass lower in the soil profile. Water use decreased with reduced irrigation but appeared to increase under reduced nitrogen. This suggests that almond root systems are able to maintain adequate nutrition to the shoot in a broad range of input scenarios and improvements in resource use efficiency over current standard practice are possible.

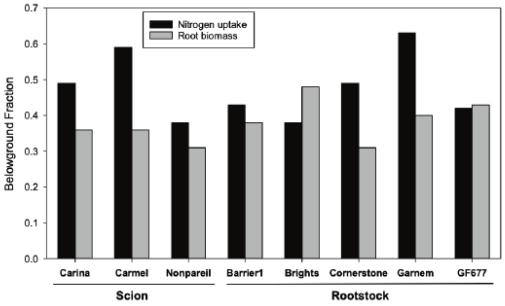
**Root function**

This field study was complimented with detailed studies using urea, labelled with the stable isotope $^{15}N$, which allowed nitrogen uptake to be quantified and tracked over as little time as a few hours. Five-year-old trees (Nonpareil on Nemaguard) were grown in the experimental orchard in 200L containers, with N uptake examined at multiple times through the season.

A second pot experiment using grafted trees was undertaken in a greenhouse environment. This examined N uptake across six rootstocks (Barrier 1, Brights Hybrid, Cornerstone, Garnem, GF677 and Nemaguard) all grafted with Nonpareil – and three scions (Nonpareil, Carina and Carmel) all grafted to Nemaguard. Both experiments separated different classes of fine roots.

There were two key learnings from this work. Firstly, that the fine root system was able to swiftly reach maximum nitrogen uptake rate (<24 hrs), whereas the concentration of labelled N in the leaves increased steadily over several days. This suggested that shoot demand for N, rather than the roots' ability to absorb it from the soil, was likely the major driver of whole plant nitrogen uptake. There was little difference between rootstocks in this either.

Secondly, that despite having little effect on N uptake rate, rootstock genotype did appear to influence whole tree nitrogen allocation. If this effect is also present in mature trees, it has implications for rootstock choice and tree management.




**Fraction of newly absorbed nitrogen (black bars) and of total biomass (grey bars) recovered from the root system of different rootstock:scion combinations.**

# ALMONDS

Soil core sample (surface is on the left, soil at 1m down on the right. Severed roots are visible in the image).

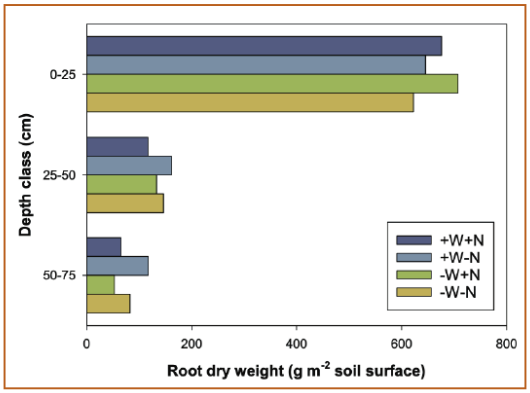
The project also had a component that developed a rapid pre-screen method that could be used by nurseries, importers or others to assess rootstock resilience to abiotic stress.

We were unable to develop and trial new fertiliser management strategies during the lifespan of the project, but the project team did collaborate with Select Harvests in their soil amendment trials using bioash-based compost with reduced fertiliser application. Both these areas of work are reported in the final report provided to Hort Innovation.

## Conclusion

The final synthesis of the extensive results from this project will take more time and be communicated to growers in due course, but overall, the work has demonstrated a surprising resilience of almond root systems to water and nitrogen limitations.

# ALMONDS




**Effect of reduced water and nitrogen input, alone or in combination on the depth distribution of root dry mass obtained from soil cores during dormancy over four years of treatments.**

**Acknowledgements**

This project has been funded by the almond industry through Hort Innovation with co-investment from CSIRO and funds from the Australian Government.

Thank you to **Zelmarie Coetzee**, her predecessor **Dave Monks**, **Michael Treeby** and their staff at DEDJTR Irymple; **Tim Preusker** and his staff at CMV Farms; **Upul Gunawardena** at Select Harvests; **Mark Ryland** of AusBuds for offering almond rootstock plants and advice, and, finally, we acknowledge the Almond Board of Australia for their support.

**Everard J. Edwards is a Research Team Leader at CSIRO Agriculture & Food. This article summarises a Hort Innovation project, recently completed by his team and colleagues at CSIRO including **Alex Lawlor**, **Adelle Semmler**, Dr **Shuang-Xi Zhou**, Dr **Nina Welti**, Dr **Lynne MacDonald** and Dr **Rob R. Walker**.*

**Alex Lawlor monitoring one of the minirhizotrons installed in the orchard at Lindsay Point that gave a firsthand view of root growth. Inset: A sapflow sensor used to monitor the movement of N through the almond trees.**

## Australian Olive Association seeks new Chief Executive Officer

The Australian Olive Association (AOA) is the peak industry body for the olive industry in Australia.

The AOA represents the industry and its members to all levels of government, regulators, NGOs and the international supply chain. Membership also includes key industry stakeholders such as processors, service providers, marketers, consultants and researchers.

Due to the imminent retirement of our current CEO, the AOA is seeking a suitably experienced person to provide executive leadership for the Association.

The role requires a person who can help provide a clear strategic focus for the Association, as it continues to grow and consolidate the position of Australian olive products with consumers.

You will be expected to provide strong and clear management control of the business activities of the Association, working with the Association's highly capable administration manager.

Background matters less than the skills you can bring but it is most likely that the role will best suit someone with experience in the agricultural sector and not for profit organisations.

Email president@australianolives.com.au for a detailed position description and desirable skillsets.

Applications close 5:00pm 15 January 2021

AUSTRALIAN OLIVE ASSOCIATION

## Australian Almond Conference 2014

# Roots and rootstocks

*Better tree performance and water use efficiency through root system resilience*

Everard J. Edwards & Rob R. Walker

CSIRO AGRICULTURE, WAITE CAMPUS
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Figure 1: Almond rootstock screening (top), necessities of grapevine roots in Sunraysia (bottom).

### Project summary

Tree root systems are a fundamental driver of plant performance. They are the tree's 'supply line' and 'front line' defence, as well as the source of root to shoot signals that help counter abiotic stress in the canopy.

A new CSIRO project, funded by the ABA through HAL, will improve the productivity and profitability of almond orchards by minimising the cost of using irrigation and fertiliser, through:

a) optimising the timing and positioning of water and nutrition to match root functioning,

b) providing growers with the information required to make informed choices of rootstocks suitable to their orchard and

c) providing the tools to ensure that new rootstocks provided to the Australian industry are suited to Australian growing regions and systems.

### Phase I: characterising rootstocks for Australian almonds

Rapid glasshouse based screens for rootstock resilience to water stress and high soil conductivity will be developed and used to characterise all of the *Prunus* rootstocks available for use in the Australian almond industry. In addition, the potential for such screens to determine tree water use efficiency, vigour and graft compatibility will be assessed. The screening will provide direct comparisons in root system performance between genotypes.

### Phase II: understanding root:shoot interactions in almonds

Root systems contain a complex range of sensing and signalling mechanisms that control root growth and architecture, the uptake of water and mineral elements to the shoot and, in grafted trees, the vigour, precocity and architecture of the scion. These signals can be chemical (e.g. ion concentrations, plant hormones), physical (e.g. hydraulic) or transcriptional (microRNAs). Determining how these signals operate in almonds will allow the development of new management techniques that manipulate these signalling mechanisms to optimise tree resource use.

### Phase III: new management for new growing systems

The final phase of the project will develop new management options, utilising the newly generated knowledge of root:shoot signalling to optimise resource use efficiency (water and nutrients). These will be primarily based on improved timing and positioning of irrigation and fertigation. In addition, the project will collaborate with the other components of the almond productivity program that implement novel plantings or management techniques.

### Measuring root activity in the field

One of the challenges for this project is to measure root activity in the field. This will be achieved using minirhizotrons, transparent tubes that allow the changes in root system to be monitored over a number of seasons, using a videoscope.

Figure 2: Installation of minirhizotron tube.

Figure 3: Example of minirhizotron images.

### Measuring tree water use in the field

A second challenge is to estimate the actual transpiration of trees, without which water use efficiency cannot be determined. Modern sapflow sensors provide an effective measurement system.

Figure 4: Installation of sapflow sensor.

FOR FURTHER INFORMATION
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This project is part of the almond industry 'Orchard Productivity' program.

ALMOND BOARD OF AUSTRALIA

HAL Horticulture Australia

## International Symposium on Root Research

# Assessing *Prunus* rootstocks for tolerance to water deficit and salinity

Shuang-Xi Zhou, Everard Edwards, Rob Walker
CSIRO Agriculture

www.csiro.au

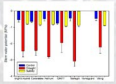
### Introduction

Declines in plant growth and productivity under water or salinity stresses often lead to a change in biomass allocation to the root system. Managing these responses and optimizing genotype choice and resource input in a horticultural planting requires studies exploring root:shoot interactions of grafted plants under abiotic stress. By imposing water and salinity stresses on seven *Prunus* rootstock varieties, this study aims to assess potential methods to screen genotypes for tolerance of drought and salinity.

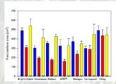
### Methods

7 rootstock varieties; 6 replicates;
2 months of treatments (harvest at three time points):
Control: RO water irrigated to the point of run-off daily;
Severe drought: 33% of evapotranspiration of well-watered plants;
Salinity: mixed chloride solution, electrical conductivity of 3.3 dS $m^{-1}$.

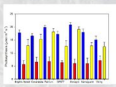
### Results



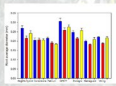
Drought ***
Salinity ***



Drought ***

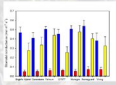


Drought ***
Salinity ***

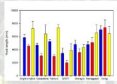


Drought ***
Speices:Drought 0.08

Salinity 0.069
Species:Salinity 0.06



Drought ***
Salinity **



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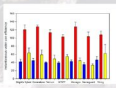
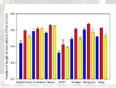


Drought ***
Salinity ***



Drought ***

Salinity *
Species:Salinity 0.098

### Conclusion

This study identified the coordinated responses between above-ground photosynthetic traits and below-ground root morphology traits under water and salinity stresses across seven almond rootstocks. However, these results are early data from a large growth analysis experiment and are only indicative of the final conclusion.

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ACKNOWLEDGEMENTS
This work is funded by the almond industry through Horticulture Innovation Australia Ltd. We thank Alexandra Lawlor for assisting on the experiment and Mark Ryland of AusBuds for offering almond rootstock plants and advices.

Horticulture Innovation Australia

ALMOND BOARD OF AUSTRALIA

## Australian Almond Conference 2016

# Better tree performance and water use efficiency through resilient root systems

Shuang-Xi Zhou, Rob Walker, Everard Edwards
CSIRO Agriculture and Food

www.csiro.au

### Introduction

Abiotic stresses, such as soil water deficit and high soil solution salts, can lead to negative impacts on crop productivity and quality. Plant root systems are a fundamental driver of plant performance and have the potential to increase water and nutrient use efficiency through a variety of mechanisms. This project, part of the ABA Productivity Program, aims to improve production and profitability through more informed rootstock choice and more efficient use of irrigation and nutrition.

### Methodology

Generate an index of stress tolerance using relative growth rate (RGR) of stressed and non-stressed rootstocks. Such an approach allows rootstocks to be assessed in a glasshouse under *similar* growth conditions, rather than requiring *identical* conditions (Figure 1).

To date, 11 rootstock genotypes used for grafting with almonds have been assessed for tolerance to:

- **water deficit:** irrigation at 33% (six genotypes) or 50% (five genotypes) of control evapo-transpiration;
- **high soil conductivity:** irrigation with water at 3.3 dS $m^{-1}$ (using mixed chloride solution).

Figure 1. The glasshouse experiment screens almond rootstock genotypes for tolerance to water stress and high soil conductivity.

Table 1. Index of stress tolerance using RGR of rootstocks.

| ROOTSTOCK | RGR OF CONTROL ($d^{-1}$) | DROUGHT EFFECT (% REDUCTION) | SALINITY EFFECT (% REDUCTION) |
|---|---|---|---|
| Atlas | 0.0572 | 43 | 28 |
| Krymsk 86 | 0.0266 | 63 | 1 |
| Garnem | 0.0278 | 38 | 24 |
| Cadaman | 0.0510 | 18 | 12 |
| Barrier 1 | 0.0266 | <0 | 3 |
| Bright's Hybrid | 0.0022 | 84 | 12 |
| Felinum | 0.0023 | 62 | 1 |
| GF677 | 0.0054 | >100 | 54 |
| Monegro | 0.0060 | 33 | 3 |
| Nemaguard | 0.0069 | 92 | 51 |
| Viking | 0.0052 | 8 | 23 |

### Project field component

In conjunction with the other Productivity Program participants, reduced irrigation and nitrogen are being trialled in the field (top left).

- minirhizotrons non-destructively monitor root growth and dynamics (top right and centre);
- sapflow sensors measure tree water use (bottom left).
- soil coring is used to assess root biomass and morphology (bottom right).

### Conclusions

The initial results (Table 1) should be treated as preliminary and need to be confirmed, however the results suggest that:

(1) there was considerable variation in stress tolerance between rootstock genotypes;

(2) the impacts of drought were generally larger than those of moderate salinity stress;

(3) tolerance to the two stresses appears to be independent.

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ACKNOWLEDGEMENTS
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Horticulture Innovation Australia

ALMOND BOARD OF AUSTRALIA

## US Almond Conference 2017

# Better tree performance and water use efficiency through resilient root systems

Shuang-Xi Zhou, Alexandra Lawlor, Rob R. Walker, Everard J. Edwards
CSIRO Agriculture & Food

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Plant root systems are a fundamental driver of plant performance. They not only supply water and nutrients, but act as a barrier to soil stresses and provide signalling mechanisms that influence shoot growth and function. A better understanding of almond root function and phenology offers an opportunity to improve orchard water and nutrient use efficiency through more appropriate management of the root system and more informed rootstock choice. Here we introduce a project, based in Australia, to address some of the limitations in our understanding of almond root systems.

### Rapid screening for rootstock abiotic stress tolerance

Rootstock trials are necessarily long-term, need to be replicated in multiple locations and can be compromised by changes in management strategies over time. Such trials can be complimented with rapid glasshouse screening under more controlled conditions. For traits that are directly driven or highly influenced by the soil or root system, screens with ungrafted plants are also a possibility (Fig. 1).

The first component of our work was to develop such a screen, providing an index of tolerance to water stress and salinity stress. Using an approach based on ratios of relative growth rate (RGR) minimised the impact of variations in tree size and growth environment, whilst utilising the ultimate measure of plant performance: accumulation of dry mass.

*RGR = increase in biomass per unit of biomass per day*

*stress index = RGR under stress / RGR under control conditions*

Figure 1: Ungrafted almond rootstocks during a glasshouse screening experiment for tolerance to water stress and salinity stress.

This screen has been used to assess a total of seventeen genotypes available in Australia, to a water stress of 50% ET, and a salinity stress of 3.3 EC, with a selection of the results below (Table 1). In general the water stress chosen had a greater impact on tree performance, with most rootstocks reasonably tolerant of the moderate salinity stress imposed.

Table 1: 'Heat map' of rootstock tolerance to drought and salinity during a rapid glasshouse screen, where dark green represents no effect of salt stress on plant performance and red represents complete loss of plant production. Results are preliminary and still subject to additional confirmation and further statistical analysis.

| | | Drought Index | Salinity Index | Control RGR ($d^{-1}$) |
|---|---|---|---|---|
| November – December 2015 | Barrier 1 | | | 0.0266 |
| January 2016-February 2016 | Cadaman | | | 0.0488 |
| | Garnem | | | 0.0267 |
| April 2016 | Atlas | | | 0.0572 |
| May- June 2016 | KB6 | | | 0.0266 |
| January-Feburary 2017 | Bright's Hybrid | | | 0.0231 |
| | Controller 6 | | | 0.0297 |
| | Controller 7 | | | 0.0208 |
| | Controller 9.5 | | | 0.0204 |
| | Cornerstone | | | 0.0223 |
| | Garnem | | | 0.0176 |
| | GF 677 | | | 0.0162 |
| | Rootpac 20 | | | 0.0173 |

### Understanding almond root system phenology

The project aims to optimise water and nutrient use efficiency by better linking the timing and positioning of application to root function through an improved understanding of root phenology and function.

This component of the project is utilising a shared field site with Agriculture Victoria, that is monitoring the effects of reduced water and nitrogen application, and combining this with a series of glasshouse and outdoor pot experiments on root nutrient uptake.

The field site is instrumented with 96 minirhizotron tubes, 24 sap flow sensors and soil coring is undertaken annually pre-budburst (Fig. 2). An example of the results from soil coring is given in Fig. 3, demonstrating no impact of reduced water and nitrogen on root biomass after one season.

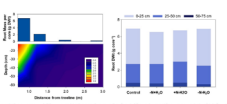

Figure 3: Root mass per 0.15 m core with distance from trunkline (top left), nutrient and/or water restriction (right) and root mass per 0.25 cm depth (bottom left).

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Hort Innovation Strategic levy investment

ALMOND FUND

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# Appendix C: refereed publications

## Better tree performance and water use efficiency through resilient root systems: rapid screening for drought and salinity stress tolerance using relative growth rate

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***Abstract***

**Abiotic stresses, such as soil water deficit and high soil solution salts, can lead to negative impacts on crop growth, productivity and quality, impacting industry profitability. The first contact of the tree with these stresses is the root system, providing the possibility of improving tree resilience by rootstock choice. Identifying almond rootstock genotypes with high stress tolerance is, consequently, of high importance in enabling the Australian almond industry to cope with these environmental challenges. This study developed and tested a potential tool to screen genotypes for tolerance to abiotic stresses, using the response of relative growth rate (RGR) to water deficit or salinity stress. Seedling plants of six almond rootstock cultivars were randomly allocated into three treatments (control, drought or salinity stress) for two months in a glasshouse. Plants in the well-watered treatment were irrigated to the point of run-off daily. The drought treatment was imposed by adding 33% of evapotranspiration of well-watered plants daily. Plants in the salinity treatment were supplied with mixed chloride solution (3.3 dS $m^{-1}$) to the point of run-off daily. Plants were harvested at three time points to calculate RGR. The percentage of RGR reduction in stressed plants, relative to the non-stressed plants, was calculated and used as an index of stress tolerance. Considerable variation for stress tolerance was found among the six almond rootstock genotypes, with some genotypes being able to maintain growth rates – whereas others stopped growing – under drought or salinity stress. The impacts of the drought treatment used were generally larger than those of salinity stress. The screening approach introduced in this study provides a relatively simple and robust method to compare rootstock genotypes, contributing to horticultural management decisions, whilst also being applicable to the generation of fundamental knowledge in this area.**

**Keywords:** almond, orchard management, rootstock, salt stress, stress tolerance, water stress

### INTRODUCTION

Australian almond production has increased rapidly since the year 2000, and is now the second-largest in the world. The Australian almond industry has committed to optimizing tree physiology in order to develop more efficient production systems, enhancing almond productivity and improving industry profitability. Doing so provides a number of challenges, because the current production costs – especially labour, fertilizer and irrigation – are significantly higher in Australia than overseas, particularly compared to California, which accounts for more than 80% of the world almond production.

As with other agricultural and horticultural industries, production can be significantly affected by environmental stress. In particular, abiotic stresses, such as soil water deficit and salinity stress, pose an enormous challenge worldwide (Forni et al., 2017), through impacts on plant growth and many biological processes, such as cell expansion, leaf expansion, photosynthesis, transpiration, etc. (Zhou et al., 2014, 2016a). For instance, in recent years the Californian almond industry has been seriously challenged with difficulties due to water

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Ed.: M.G. Wirthensohn

availability as a result of regional drought. There is the potential for more informed rootstock genotype choice to improve industry performance under these abiotic stresses, but rootstock field trials are expensive and long-term, as well as being limited in the number of genotypes that can be compared on a given site. A rapid screening system under easily replicated conditions with young trees (e.g., glasshouse or nursery), would not only allow many genotypes to be assessed together, but also provide a mechanism for the performance of new breeding material to be quickly related to existing material. Such a system would allow a rapid assessment for the resilience of *Prunus* rootstocks available in Australia and deliver key information to assist growers in preparing for future environmental challenges.

By imposing water and salinity stresses on six *Prunus* rootstock cultivars in Australia and observing the stress impacts on plant relative growth rate (RGR), this study aimed to develop and test a rapid and commercially feasible tool for practitioners to assess the stress tolerance of various genotypes. RGR – the dry weight increase in a plant per unit of biomass per unit of time – provides a measure of plant production that is not directly driven by plant size, although impacted by plant ontogeny. Further, by determining the proportional effect of a given stress (e.g., drought or salinity) on RGR, different plant species or genotypes with different inherent growth rates or ontogeny, can be compared and assessed in imperfectly controlled conditions (Poorter, 1989). Using the RGR of stressed and non-stressed rootstocks, this study introduces an index of stress tolerance and assesses the potential for such a screen to determine the stress response of different genotypes.

**MATERIALS AND METHODS**

In February 2015, one-year-old ungrafted plants of six *Prunus* taxa – 'Bright's Hybrid' (*P. amygdalus* × *P. persica* hybrid), 'Felinem' (*P. amygdalus* × *P. persica* hybrid), 'GF677' (*P. persica* × *P. amygdalus* hybrid), 'Monegro' (*P. amygdalus* × *P. persica* hybrid), 'Nemaguard' (*P. persica* × *P. davidiana*, or a pure *P. persica* cultivar, based on contradictory reports), and 'Viking' (a cross involving 'Nemaguard', *P. amygdalus* 'Jordanolo', and *P. blireiana*) – were transplanted into 2.4-L pots with 2 L of sandy loam soil which was evenly mixed with slow-release fertilizer. The plants were grown in the glasshouse under a 27°C/20°C diurnal temperature cycle and maintained in a moist condition – 100% field capacity – for three months to allow establishment. Six plants of 'Bright's Hybrid', 'Felinem', 'Monegro', and 'Nemaguard', and five plants of 'GF677' and 'Viking' were randomly assigned to six blocks in the glasshouse to be assessed for tolerance to soil water deficit or high soil solution conductivity (salinity).

Plants were subjected to one of the three watering treatments – control, drought or salinity stress – for up to 60 days in the glasshouse during May-July 2015. Two large halogen lights were used to extend the daily light length for three additional hours, ensuring that short day-length didn't induce dormancy. Plants in the control treatment were irrigated to the point of run-off daily. The drought treatment was imposed by adding only 33% of evapotranspiration of the well-watered plants daily – which were quantified before the watering treatments based on pot weighing. The salinity treatment was imposed by adding mixed chloride solution (3.3 dS $m^{-1}$) to the point of run-off daily. 3.3 dS $m^{-1}$ is a high salinity level limiting orchard yield in Murray Darling Basin in Australia (Walker et al., 2002; Zhang et al., 2002). The stress treatments were maintained whilst plants were harvested at three time points (Figure 1). The leaves, stem, and roots were oven-dried separately and the total dry biomass was used to calculate RGR.

RGR values were calculated as the slope (R) in linear regression models of the form: $\log W_t = \log W_0 + R * t$, where $W_0$ is dry mass at the start of the treatment and $W_t$ is the dry mass after treatment duration (t). The percentage of RGR reduction in plants from stress treatment compared to the control treatment was calculated and used as an index of stress tolerance.

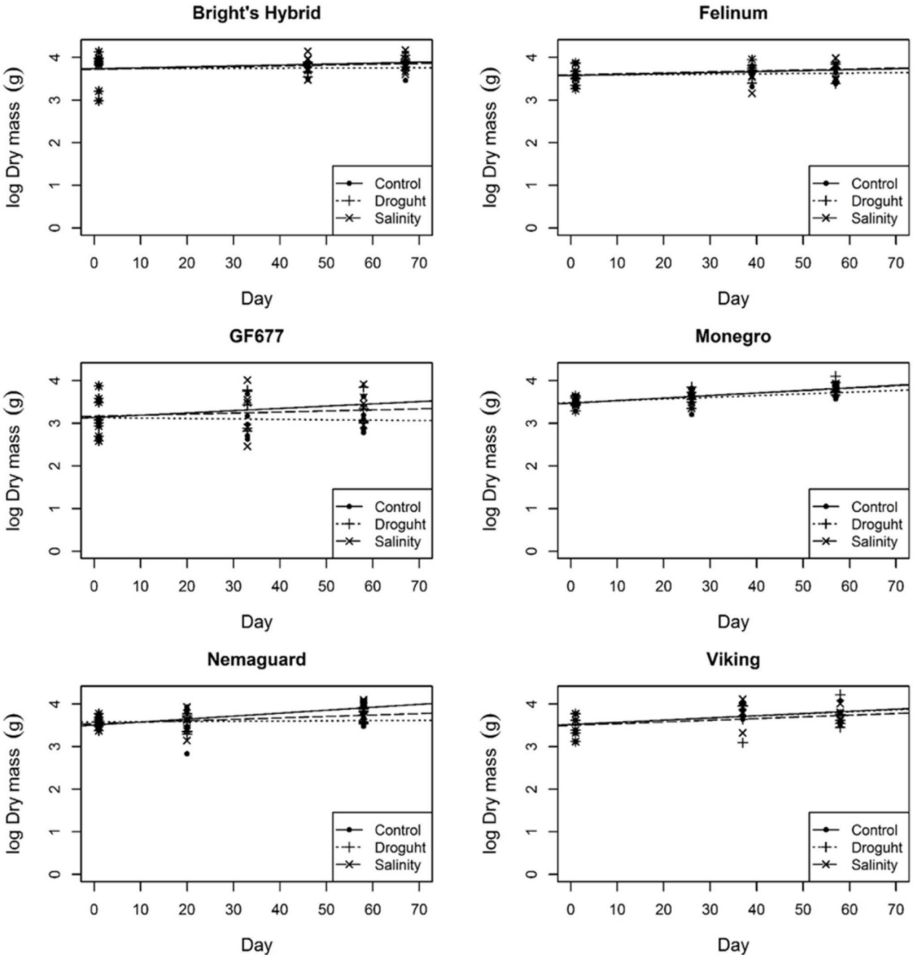


Figure 1. Plant dry mass at three harvest time points of six *Prunus* genotypes exposed to watering (100% FC vs. 33% ET of well-watered plants) or salinity (0 vs. 3.3 dS $m^{-1}$) treatments, respectively (*n*=6 for 'Bright's Hybrid', 'Felinem', 'Monegro', and 'Nemaguard'; *n*=5 for 'GF677' and 'Viking').

**RESULTS AND DISCUSSION**

We tested this rapid glasshouse-based screen approach for stress tolerance in six *Prunus* rootstock genotypes, which differed in the extent of RGR response to drought or salinity stress (Figure 1, Table 1). Drought led to a large reduction of RGR in 'Bright's Hybrid', 'Felinem', 'GF677' and 'Nemaguard', and salinity led to a large reduction of RGR in 'GF677' and 'Nemaguard' (Figure 1, Table 1). In particular, 'GF677' plants under drought were inhibited in growth so severely that it showed >100% RGR reduction in stressed plants compared to non-stressed plants (Figure 1, Table 1). For five of the six genotypes – except 'Viking' – the impacts of drought were generally larger than those of salinity stress (Figure 1, Table 1). Species with high RGR are usually more competitive in acquiring soil resources, while species with low RGR are more conservative in resource acquisition (Reich et al.,

2003). Interspecific variation in RGR is related to differential plant strategies in biomass allocation and construction of plant organs – for plants to adapt to their environmental conditions (Poorter and Remkes, 1990; Tomlinson et al., 2014). Stress-tolerant species generally have lower RGR than stress-intolerant species, and many studies have reported the trade-off between RGR and stress-tolerance indicative traits (e.g., cavitation resistance) (Reich et al., 2003).

Table 1. Relative growth rate (RGR, $d^{-1}$) and index of stress tolerance (as percentage of RGR reduction in stressed plants compared to non-stressed plants) in six *Prunus* genotypes exposed to watering (100% FC vs. 33% ET of well-watered plants) or salinity (0 vs. 3.3 dS $m^{-1}$) treatments, respectively.

| Rootstock genotype | RGR of control | RGR of drought | RGR of salinity | Drought effect (% reduction) | Salinity effect (% reduction) |
|---|---|---|---|---|---|
| Bright's Hybrid | 0.0022 | 0.0003 | 0.0019 | 84.02 | 11.99 |
| Felinem | 0.0023 | 0.0009 | 0.0023 | 62 | 1.22 |
| GF677 | 0.0054 | -0.001 | 0.0025 | >100 | 54.2 |
| Monegro | 0.006 | 0.004 | 0.0058 | 33.44 | 3.39 |
| Nemaguard | 0.0069 | 0.0005 | 0.0034 | 92.12 | 50.91 |
| Viking | 0.0052 | 0.0048 | 0.004 | 7.8 | 23.06 |

The screen approach developed uses a comparison of RGR between control and stressed plants to determine genotype differences in response to stress. The whole plant growth used to calculate RGR is the ultimate measure of plant productivity and amenable to use by non-experts. Meanwhile, it also allows more sophisticated growth rate models to be utilised for further assessment and does not interfere with measurement of additional plant traits, such as biomass allocation, leaf gas exchange (Zhou et al., 2016b) or root morphology (Zhou et al., 2016c). The rootstock assessment tool generated by this study can be implemented by nurseries and breeders and could provide valuable information for potential importers before introduction of new rootstocks from overseas. The tool is currently being utilised by the authors to characterize *Prunus* rootstocks available for use in the Australian almond industry.

The capacity for early assessment of potential rootstocks for selected key traits – such as RGR in the present study – can save significant resources by identifying only the best selections for expensive, long-term evaluation thereby creating cost efficiencies and maximizing chances of success. By identifying a genotype of higher tolerance against a specific environmental stress, this tool is expected to improve the productivity and profitability of almond orchards, through (1) ensuring that the new rootstocks provided to the Australian industry are suited to Australian growing regions and systems, and (2) providing growers with the information required to make informed choices of rootstocks suitable to the orchard. In the longer-term, planting with rootstock genotypes assessed against a cultivar of abiotic stresses has the potential to reduce production costs in many areas and combined with transformational production practices could be expected to provide a significant improvement in orchard profitability and sustainability. Furthermore, the improved profitability and sustainability of such almond production systems will improve the global competitiveness of Australia's almond industry, which will benefit national and regional economies.

## CONCLUSIONS

A rapid screen approach based on RGR for assessing the performance of ungrafted *Prunus* rootstock cultivars under abiotic stress conditions has been developed. The results highlight considerable variation in stress tolerance among *Prunus* rootstock genotypes in the extent of growth responses to water and salinity stresses. The impacts of drought were generally larger than those of salinity stress. The results also have significant implications for interspecific differential adaptations under drought or salinity stress, useful for both

plant physiologists and almond industry practitioners.

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## Literature cited

Forni, C., Duca, D., and Glick, B.R. (2017). Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. Plant Soil 410 (1-2), 335–356 https://doi.org/10.1007/s11104-016-3007-x.

Poorter, H. (1989). Interspecific variation in relative growth rate: on ecological causes and physiological consequences. In Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants, H. Lambers, M.L. Cambridge, H. Konings, and T.L. Pons, eds. (The Hague: SPB Academic Publishing), p.45–68.

Poorter, H., and Remkes, C. (1990). Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. Oecologia 83 (4), 553–559 https://doi.org/10.1007/BF00317209. PubMed

Reich, P.B., Wright, I.J., Cavender-Bares, J., Craine, J.M., Oleksyn, J., Westoby, M., and Walters, M.B. (2003). The evolution of plant functional variation: traits, spectra, and strategies. Int. J. Plant Sci. 164 (S3), S143–S164 https://doi.org/10.1086/374368.

Tomlinson, K.W., Poorter, L., Bongers, F., Borghetti, F., Jacobs, L., and van Langevelde, F. (2014). Relative growth rate variation of evergreen and deciduous savanna tree species is driven by different traits. Ann. Bot. 114 (2), 315–324 https://doi.org/10.1093/aob/mcu107. PubMed

Walker, R.R., Blackmore, D.H., Clingeleffer, P.R., and Correll, R.L. (2002). Rootstock effects on salt tolerance of irrigated field-grown grapevines (Vitis vinifera L. cv. Sultana): 1. Yield and vigour inter-relationships. Aust. J. Grape Wine Res. 8 (1), 3–14 https://doi.org/10.1111/j.1755-0238.2002.tb00206.x.

Zhang, X., Walker, R.R., Stevens, R.M., and Prior, L.D. (2002). Yield-salinity relationships of different grapevine (Vitis vinifera L.) scion-rootstock combinations. Aust. J. Grape Wine Res. 8 (3), 150–156 https://doi.org/10.1111/j.1755-0238.2002.tb00250.x.

Zhou, S., Medlyn, B., Sabaté, S., Sperlich, D., Prentice, I.C., and Whitehead, D. (2014). Short-term water stress impacts on stomatal, mesophyll and biochemical limitations to photosynthesis differ consistently among tree species from contrasting climates. Tree Physiol. 34 (10), 1035–1046 https://doi.org/10.1093/treephys/tpu072. PubMed

Zhou, S.-X., Medlyn, B.E., and Prentice, I.C. (2016a). Long-term water stress leads to acclimation of drought sensitivity of photosynthetic capacity in xeric but not riparian Eucalyptus species. Ann. Bot. 117 (1), 133–144 https://doi.org/10.1093/aob/mcv161. PubMed

Zhou, S.-X., Edwards, E., and Walker, R. (2016b). Better tree performance through root system resilience. Australian Nutgrower 30 (4), 32–34.

Zhou, S.-X., Edwards, E., and Walker, R. (2016c). Using minirhizotron system to monitor the root production and turnover of almond rootstock. Australian Nutgrower 30 (4), 34–35.

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# Decoupled drought responses of fine-root versus leaf acquisitive traits among six *Prunus* hybrids

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## Abstract

***Aims*** Predicting drought consequences on forests and fruit crop plantings requires improved understanding of drought responses of both leaf and fine-root resource acquisitive traits (specific leaf area—SLA, specific root surface area—SRA and specific root length—SRL). We hypothesize their responses are coordinated towards integrated plant resource conservation under severe drought.

***Methods*** We tested the hypothesis with a greenhouse-based drought experiment on saplings of six *Prunus* hybrids with *a priori* known contrasting drought sensitivity. Saplings were subjected to either control (100% field capacity) or severe drought stress treatment (33% evapotranspiration of hybrid-specific control plants). Sample collections were carried out at 30 and at 60 days after the start of treatments, for both control and stressed saplings.

***Important Findings*** No hybrid showed concurrent significant decrease of SLA and SRA (or SRL) under severe drought. The fine-root traits of the six hybrids showed two major drought-response scenarios, in particular: (i) increased root tissue density (RTD) and decreased average root diameter without significant change of SRL and (ii) increased RTD and decreased SRL without significant change of average root diameter. Drought responses of leaf gas exchange, SRA, SRL and RTD were closely correlated along a gradient towards resource conservation from control to drought-stressed plants in all hybrids, which was orthogonal to another gradient characterized by a hybrid-dependent decrease of SLA. These findings highlight (i) the multi-dimensionality of root-trait drought responses, (ii) the decoupling between leaf economics and leaf hydraulics and (iii) the covariation of leaf and root hydraulics in terms of trait drought responses. The study contributes to identifying the origin of the multi-dimensionality of root-trait drought response at intraspecific scale, and highlights differential drought–response combinations of leaf and fine-root traits among hybrids to survive under severe soil drought stress.

***Keywords:*** multi-dimensional drought response, soil water stress, specific leaf area, specific root length, specific root surface area

**摘要：**要预测干旱对森林和果树种植的影响，就需要更好地了解干旱胁迫对叶片和细根的资源获取性状(比叶面积SLA、比根面积SRA以及比根长SRL)的影响。本研究试图验证以下科学假设：在重度干旱胁迫下，叶片与细根的资源获取性状具有协同效应，使得植物能够采取整合的资源节约策略。我们收集了不同干旱敏感性的六种李属砧木的幼树，通过在温室中对它们进行干旱试验来验证我们的科学假设。这些幼树被分为两组进行水分处理试验，即对照处理组(浇水量为100%田间持水量)与重度干旱处理组(每种砧木的浇水量为该砧木对照组植物每日蒸散量的33%)。在处理开始后的第30天和第60天，分别对两组处理下的六种砧木的植物叶片与细根进行取样。结果表明，在重度干旱胁迫下，没有任何一种植物同时显著降低了SLA和SRA(或SRL)。六种李属砧木植物的细根性状表现出两种主要的干旱响应组合：(1)根组织密度(RTD)增大，同时平均根径减小，而SRL没有显著变化；(2) RTD增大，同时SRL减小，而平均根径没有显著变化。六种砧木植物性状的干旱响应展现出两个相互垂直的变化梯度，这两个梯度均以从对照处理组到干旱处理组植物的资源节约性变化为特征，其中一个梯度展现了叶片气体交换，SRA，SRL以及RTD的干旱响应之间的密切相关关系，而另一个梯度以SLA的降低为特征。这些发现突出了(1)根系性状干旱响应的多维性；(2)重度干旱胁迫下，叶片经济性状与叶片水力性状之间的解耦联关系；(3)重度干旱胁迫下，叶片水力

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性状与根系水力性状之间的协同变化关系。这项研究有助于在种内尺度上确认根系干旱响应多维性的起源，并重点突出了不同植物如何通过不同的叶片与细根性状的干旱响应组合而得以在重度干旱胁迫下存活。

**关键词**：多维度干旱响应，土壤水分胁迫，比叶面积，比根长，比根表面积

## INTRODUCTION

Severe drought has been reported to affect forest function and induce forest mortality across the globe (Allen *et al.* 2010; Phillips *et al.* 2016). The drought vulnerability of woody species is related to drought intensity and frequency, time scale, plant developmental stage and the species-specific capacity of drought resistance and adaptation (Maseda and Fernández 2006; Phillips *et al.* 2016; Zhou *et al.* 2016, 2018). Plants have evolved a wide range of above- and below-ground strategies in adaptation to soil water deficit (e.g. Bardgett *et al.* 2014; Markesteijn and Poorter 2009; Maseda and Fernández 2006; Zhou *et al.* 2014). By characterizing above- and below-ground plant traits, the variation in functional roles among different plant species and organs—as constrained by evolutionary and environmental drivers—can be described (Keddy 1992; Liu *et al.* 2010; Reich *et al.* 2003; Woodward and Diament 1991).

In a resource-limited environment, the survival and growth of different plant species are influenced by their species-specific inherent capacity for resource acquisition (light, water, nutrients, etc.). For leaves, specific leaf area (SLA)—the ratio of leaf area to leaf dry mass—is the principal leaf trait that connects plant mass investment with light interception (Craine *et al.* 2001). Leaves with a pronounced resource-acquisition strategy—which facilitates high light interception and carbon fixation—are usually characterized with low lifespan but high SLA, and leaves with a conservative strategy are characterized by the reverse. For roots, specific root surface area (the ratio of fine-root surface area to mass, SRA) and—more commonly measured and reported—specific root length (the ratio of fine-root length to mass, SRL) provide the ratio of a standard unit of resource acquisition (root surface area or length) to mass investment in fine-roots (Craine *et al.* 2001; Lõhmus *et al.* 1989; Olmo *et al.* 2014; Pérez-Harguindeguy *et al.* 2013; Withington *et al.* 2006).

Like leaves, roots typically exhibit a high degree of plasticity in response to changes in environmental conditions (Kong *et al.* 2014; Lõhmus *et al.* 1989). SRA and—more frequently measured and reported—SRL have been used as indices to compare root benefit (resource acquisition) against root cost (construction and maintenance) among plant species, genera and biomes under different environmental conditions (e.g. Eissenstat 1992; Lõhmus *et al.* 1989; Ostonen *et al.* 2007a, 2007b). Root system plasticity to soil nutrient and water availability is typically exhibited by modifying four fine-root traits—SRA, SRL, average root diameter and/or the trait describing plant investment into root tissue (e.g. dry weight per unit volume—RTD) (Olmo *et al.* 2014; Ostonen *et al.* 2007a), whose responses can vary considerably among species (Chapman *et al.* 2012; Chen *et al.* 2013; Eissenstat and Yanai 1997; Hodge 2004; Valverde-Barrantes *et al.* 2013).

SLA, SRA, SRL and average root diameter are closely related to the whole-plant economic strategy (Reich 2014), which assumes that the above- and below-ground traits are coordinated in resource acquisition and investment to fit the environments (Ostonen *et al.* 2007a; Reich 2014). Traits associated with root resource acquisition (SRL and SRA) and leaf light absorption (SLA) are usually strongly correlated for woody species (e.g. Withington *et al.* 2006), and SRA and/or SRL are often viewed as below-ground analogues of SLA (Eissenstat *et al.* 2000; Pérez-Ramos *et al.* 2012). This may contribute to the expectation that stress responses of SLA and SRL (or SRA) would mimic each other. If the analogy is true, then drought response of SRA (or SRL) should be predictable from that of SLA, such that drought-induced lower SLA and lower SRA (or lower SRL) should be common for drought-stressed plants. Simultaneously examining leaf and fine-root acquisitive traits—and their coordination in response to drought stress—is important for understanding plant–water relations and resource-acquisition strategies under current and future droughts (Cheng *et al.* 2016).

Studies simultaneously examining difference in drought responses of leaf and fine-root acquisitive traits governing plant resource acquisition and investment are limited (Cheng *et al.* 2016; Fort *et al.* 2013). Moreover, studies investigating congeneric hybrids for their concurrent drought responses of leaf and fine-root traits are rare. If adjustments of both leaf and fine-root acquisitive traits are necessary for plants to survive severe drought, then lower SLA and lower SRL (or SRA) (Barkaoui *et al.* 2016) should be both common for plants under severe drought. We hypothesized that drought responses of leaf and fine-root acquisitive traits are coupled towards integrated plant resource conservation for plants to survive severe drought stress. We tested the hypothesis using a greenhouse experiment on saplings of six *Prunus* rootstock hybrids with *a priori* known contrasting drought sensitivity (Company and Gradziel 2017; Felipe 2009). The six hybrids are utilized by growers in the world's top two almond planting areas, California and Australia, which provide the majority of the world almond production while both regions face severe drought challenges in recent years. Performance of almond plantings under drought is potentially determined, to a large extent, by *Prunus* rootstock hybrids which vary greatly in their drought tolerance (Company and Gradziel 2017). Saplings were imposed with either control [100% field capacity (FC)] or severe drought stress treatment [33% of evapotranspiration (ET) of hybrid-specific control plants] every day for 30 and 60 days, respectively.

Particularly, we investigated the drought responses of key variables associated with the hypothesis and the interrelationships among them, including (i) leaf gas exchange, (ii) the most commonly studied leaf trait (SLA) associated with leaf acquisitive capacity of light, (iii) two fine-root traits (SRA and SRL) associated with absorptive capacity of the root system, (iv) two fine-root traits (RTD and average root diameter) governing the morphological modification of SRL and (v) the distribution of root length according to root diameter classes (hereafter referred to as root diameter distribution) and particularly the percentage of root length within the finest diameter class. SLA and SRA (or SRL) are key traits that reflect plant resource acquisition and investment strategies (Cheng *et al.* 2016). The four fine-root traits—SRA, SRL, average root diameter and percentage of root length within the finest diameter class—are proxies of the fineness of the root system (Bergmann *et al.* 2017). Root diameter can predict root lifespan (Kong *et al.* 2019), and RTD is a proxy for the plant investment trade-off between building cheap but fragile roots and building expensive but resistant roots (Craine *et al.* 2001). Our goal was to test the hypothesis and thereby contribute to a better understanding of drought responses of leaf and fine-root traits.

## MATERIALS AND METHODS

### Choice of hybrids

We collected six *Prunus* hybrids which have a documented gradient of sensitivity to drought stress. The six hybrids included the



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drought-sensitive 'Nemaguard' (*P. persica* × *P. davidiana*, or a pure *P. persica* cultivar, based on contradictory reports) and 'Viking' (a cross involving 'Nemaguard', *P. amygdalus* cv. Jordanolo and *P. blireiana*), the drought-tolerant 'Felinem' (*P. amygdalus* × *P. persica* hybrid) and 'Monegro' (*P. amygdalus* × *P. persica* hybrid), and two intermediate hybrids—'Bright's Hybrid' (*P. amygdalus* × *P. persica* hybrid) and 'GF677' (*P. persica* × *P. amygdalus* hybrid) (Company and Gradziel 2017). 'Felinem' and 'Monegro' can adapt to Mediterranean drought conditions, with higher drought tolerance in 'Monegro' (Felipe 2009).

### Plant material, growth conditions and experimental design

In February 2015, 1-year-old ungrafted saplings of the six *Prunus* hybrids were transplanted into 2.4 L pots with 2 L of sandy loam soil which was evenly mixed with slow-release fertilizer. The plants were grown in the greenhouse transparent to sunlight under a 27/20°C diurnal temperature cycle and maintained in a moist condition—100% FC—for 3 months to allow establishment. Two night-break lights were used to extend the daily light length for three more hours to prevent short-day responses as the season was entering into autumn. Within each hybrid, plants of similar sizes were randomly assigned to one of the two watering treatments—control treatment or severe drought stress treatment—in the greenhouse during May–July 2015. Plants in the control treatment (100% FC) were irrigated to the point of run-off every day. Plants under severe drought stress treatment were watered every day with 33% hybrid-specific ET (33% ET) of control plants of the same hybrid—which were quantified before the watering treatments based on pot weights. Plants were harvested at three time points—the day before the beginning of treatments, 30 and 60 days of treatments, respectively. Six plants of 'Bright's Hybrid', 'Felinum', 'Monegro' and 'Nemaguard', and five plants of 'GF677' and 'Viking' from each block were harvested at each time point.

### Tissue morphology

At both 30 and 60 days of treatments, all leaf, stem and root tissues were harvested, respectively, and key leaf and fine-root traits were measured. Total leaf area was determined by detaching all leaves and measuring leaf area using the LI-3000C Portable Area Meter. After removing the stem by cutting at the soil surface, the entire contents (roots and 2 L of soil) were removed from the pot and then cut longitudinally from the middle. A radial segment representing approximately one-eighth of the soil volume was taken as a subsample and stored in the dark at 3°C until processed. The roots were gently washed from the subsample using water and a fine mesh sieve (0.2 mm). There was no taproot for any plant sampled in this study. Root traits (root length, surface area, average diameter and volume) were then determined using a scanning and digital image analysis system (WinRhizo; Régent Instruments, Quebec, Canada). Total root length was determined from the root length at each radial segment sampling divided by the ratio between the sampled root dry mass and the plant total root dry mass. In addition, root length was apportioned into 10 root diameter classes (0–0.075, 0.075–0.13, 0.13–0.2, 0.2–0.3, 0.3–0.4, 0.4–0.5, 0.5–0.75, 0.75–1, 1–2 and more than 2 mm)—based on the pattern of root order distribution at the initial scanning and analysis using WinRhizo—to investigate the root diameter distribution.

Following trait measurements, the leaf and root samples were oven-dried at 60°C for at least 48 h and weights were recorded after samples were verified with a constant weight. SLA ($cm^2\ g^{-1}$) was calculated as the ratio between fresh leaf area and leaf dry mass. SRA ($m^2\ kg^{-1}$) was calculated as the ratio between root surface area and dry mass (<2 mm). SRL ($m\ g^{-1}$) was calculated as the ratio between root length and dry mass (<2 mm). RTD ($g\ cm^{-3}$) was calculated as the ratio between root dry mass and fresh volume (<2 mm). Percentage of root length within each diameter class to the total root length was also calculated.

### Stem water potential and leaf gas exchange

At 60 days of treatments, prior to the morphological trait determination, measurements of midday stem water potential ($\Psi_{stem}$) and leaf gas exchange were also conducted. Mature leaves were enclosed in reflective, opaque, plastic bags (PMS Instrument Company, Corvallis, OR, USA) for 1 h before the midday $\Psi_{stem}$ measurements were determined using a pressure chamber (Soilmoisture Corp, Santa Barbara, CA, USA). $\Psi_{stem}$ measurement was not determined for 'Nemaguard' due to its small petiole size. Leaf gas exchange measurements were performed on the same day using young, fully expanded, sun-exposed leaves, with a portable photosynthesis system (LI-6400, Li-Cor Inc., Lincoln, NE, USA). Before each measurement, the leaf was acclimated in the chamber for 5–10 min to achieve stable gas exchange readings, with leaf temperature maintained at 25°C, reference $CO_2$ concentration controlled at 400 µmol $CO_2$ $mol^{-1}$ air, and a saturating photosynthetic photon flux density ($Q$) of 1800 µmol photon $m^{-2}\ s^{-1}$. Vapour pressure deficit ($D$) was held as constant as possible during the measurement ($D$ = approximately 1.5 kPa). After the leaf acclimated to the cuvette environment, the light-saturated net $CO_2$ assimilation rate ($A_{sat}$) and stomatal conductance ($g_s$) were recorded. The intrinsic water use efficiency ($WUE_i$) was calculated as the ratio between $A_{sat}$ and $g_s$ to represent the instantaneous balance between photosynthesis and transpiration.

### Statistical analyses

All statistical analyses were conducted in R (R Core Team 2014, Version 3.1.2). Homogeneity test and normality test were conducted before the analysis of variance. The package lm() was used to fit linear models and the package anova() was used to compute analysis of variance for the fitted models to assess the effects of hybrid, treatment, treatment duration and their interactions. The package HSD.test() was used to make multiple means comparisons by means of Tukey's HSD test. Principal components analysis (PCA) was conducted on nine traits—SLA, SRA, SRL, RTD, average root diameter, percentage of root length within the finest diameter class, $A_{sat}$, $g_s$ and $WUE_i$—in six *Prunus* hybrids after 60 days of watering treatments (100% FC versus 33% ET of control plants) to investigate the trait drought–response relations.

## RESULTS

### Effects of severe drought stress on leaf physiology and growth

Severe drought stress had a significant effect on $\Psi_{stem}$, $A_{sat}$, $g_s$ and $WUE_i$ ($P < 0.001$; Table 1). Compared with plants kept under 100% FC for 60 days, plants of the six *Prunus* hybrids kept under 33% ET consistently showed lower $A_{sat}$ ($P < 0.05$), lower $g_s$ ($P < 0.05$), higher $WUE_i$ ($P < 0.05$) and more negative $\Psi_{stem}$ (not significant for 'GF677'; measurement not applicable on 'Nemaguard') (Fig. 1a–d). The two hybrids with smaller plant sizes—'GF677' and 'Viking'—showed relatively less reduction of $\Psi_{stem}$ in drought-stressed plants compared with the other four hybrids (Fig. 1).

The six hybrids developed few new leaves after 60 days of severe drought stress, while none of them showed significant net leaf or root tissue loss (Fig. 1e and f, Supplementary Figs. S1 and S2). However, after 60 days of 100% FC treatment there was a net increase in total leaf area only for 'Monegro' and 'Nemaguard' plants ($P < 0.05$; Fig. 1e) and a net increase in total root length only for 'GF677', 'Monegro' and 'Viking' plants when compared with plants before treatment ($P < 0.05$; Fig. 1f).

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**Table 1:** *P*-values of Tukey's HSD test to compare plant traits across six *Prunus* hybrids exposed to two watering treatments (100% FC versus 33% ET of control plants) for 30 and 60 days

| | $\Psi_{stem}$ | $A_{sat}$ | $g_s$ | $WUE_i$ | SLA | SRA | SRL | RTD | Average root diameter | Percentage of root length within 0.075–0.13 mm |
|---|---|---|---|---|---|---|---|---|---|---|
| Hybrid | 0.4926 | 0.4854 | 0.5531 | 0.3324 | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| Treatment | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| Time | NA | NA | NA | NA | **<0.001** | 0.2939 | **<0.001** | **0.0465** | **<0.001** | **<0.001** |
| Hybrid × Treatment | 0.1928 | 0.2854 | 0.4973 | 0.6487 | 0.2075 | 0.3147 | 0.3797 | 0.1790 | **0.0421** | **0.0467** |
| Hybrid × Time | NA | NA | NA | NA | **0.0188** | **0.0142** | **0.0131** | **0.0153** | **0.0421** | 0.1633 |
| Treatment × Time | NA | NA | NA | NA | **0.0121** | 0.2612 | **0.0419** | 0.9959 | **<0.001** | **0.0085** |
| Hybrid × Treatment × Time | NA | NA | NA | NA | 0.2937 | 0.2947 | 0.3138 | 0.1769 | **0.0445** | **0.0159** |

The traits include: $\Psi_{stem}$ = stem water potential, $A_{sat}$ = leaf net photosynthesis at saturating light, $g_s$ = stomatal conductance; $WUE_i$, intrinsic water use efficiency. Data are, respectively, shown in Figs 1 and 2 and Supplementary Figs S1–S3. NA is not applicable because data were not able to be collected at 30 days due to resource limitations. Bold values indicate significant effect where $P < 0.05$.

### Effects of severe water stress on SLA, SRA and SRL

Severe drought stress had a significant effect on SLA, SRA and SRL ($P < 0.001$; Table 1). There was a significant interaction between hybrid and time effects on SLA, SRA and SRL ($P < 0.05$; Table 1). There was also a significant interaction between treatment and time effects on SLA and SRL ($P < 0.05$; Table 1), indicating that the impact of drought stress on SLA and SRL changed over time (Fig. 2a and c).

After 30 days of severe drought stress, no hybrid showed a modification in SLA or SRL, and 'Felinum' was the only hybrid which showed significantly lower SRA (Fig. 2a–c). After 60 days of severe drought stress, 'Monegro' was the only hybrid which showed significantly lower SLA, and 'GF677' was the only hybrid which showed significantly lower SRA and SRL (Fig. 2a–c).

### Multi-dimensionality of root-trait drought response

Severe drought stress had a significant effect on RTD, average root diameter and the percentage of root length within finest diameter class (0.075–0.13 mm) ($P < 0.001$; Table 1). For RTD, there was a significant interaction between hybrid and time ($P < 0.05$; Table 1). For average root diameter, there were significant interactions between hybrid and treatment ($P < 0.05$; Table 1), between hybrid and time ($P < 0.05$; Table 1), between treatment and time ($P < 0.001$; Table 1) and between hybrid and treatment and time ($P < 0.05$; Table 1). For the percentage of root length within finest diameter class, there was a significant interaction between hybrid and treatment ($P < 0.05$; Table 1), between treatment and time ($P < 0.01$; Table 1) and between hybrid and treatment and time ($P < 0.05$; Table 1). Results of root diameter distribution showed that the majority of roots were within the diameter classes 0.075–0.13 mm (except 'GF677' whose majority of total root length was within the diameter classes 0.13–0.2 mm) (Supplementary Fig. S3).

Higher RTD was observed in 'Bright's Hybrid', 'Felinum' and 'Monegro' ($P < 0.05$ in each case) after 30 days of severe drought stress, and in 'GF677' ($P < 0.05$) and 'Viking' ($P < 0.05$) after 60 days of severe drought stress (Fig. 2d). Thinner average root diameter was observed in 'Bright's Hybrid' ($P < 0.05$), 'Felinum' ($P < 0.05$), 'Monegro' ($P < 0.05$) and 'GF677' (marginally significant) after 30 days of severe drought stress, and in 'Viking' ($P < 0.05$) after 60 days of severe drought stress (Fig. 2e). Higher percentage of root length within the finest diameter class (0.075–0.13 mm) was observed in 'Felinum' ($P < 0.05$), 'Monegro' ($P < 0.05$) and 'Bright's Hybrid' (marginally significant) after 30 days of severe drought stress, and in 'Viking' after 60 days of severe drought stress ($P < 0.05$) (Fig. 2f and Supplementary Fig. S3).

Average root diameter and the percentage of root length within the finest diameter class showed similar response, except 'GF677'—whose root length within this diameter class did not account for the majority of total root length (Fig. 2f and Supplementary Fig. S3).

The drought-induced increase of RTD found at 30 days on 'Bright's Hybrid', 'Felinum' and 'Monegro', and the reduction of average root diameter found at 30 days on 'Bright's Hybrid', 'Felinum', 'GF677' and 'Monegro', were mitigated at 60 days. Similarly, the reduction of percentage of root length within the finest diameter class (0.075–0.13 mm) found at 30 days on 'Bright's Hybrid', 'Felinum' and 'Monegro' was mitigated at 60 days. 'Nemaguard' was the only hybrid which showed no modification of any root trait at either time point (Fig. 2b–e).

No hybrid showed concurrent significant modifications of the four fine-root traits depicting root fineness—SRA, SRL, average root diameter and percentage of root length within the finest diameter class. Severe drought stress only caused significant decrease of SRA and SRL in 'GF677' at 60 days, due to a significant increase of RTD but not root diameter (Fig. 2b–e). Compared with control plants, 'Bright's Hybrid', 'Felinum' and 'Monegro' produced roots with equal SRL but significantly higher RTD and thinner average root diameter after 30 days of severe drought stress (Fig. 2c–e), and 'Viking' showed the same modifications—but only after 60 days of severe drought stress (Fig. 2c–e).

### Leaf and fine-root trait drought–response relations

PCA showed strong dominance by the first principal component (PC1), which explained 56.9% of the total variation (Fig. 3). PC1 showed a gradient from control to drought-stressed plants for all six hybrids, characterized by the positive correlation between $WUE_i$ and RTD, and by their negative correlations with $A_{sat}$, $g_s$, SRA and SRL. Drought-stressed plants of all six hybrids showed this combination of trait responses and were aligned to the right part of Fig. 3, while control plants of all six hybrids under were aligned to the left part (Fig. 3).

The second principal component (PC2) explained 31.23% of the total variation. PC2 tended to be mainly driven by lower SLA in drought-stressed plants of 'Monegro' ($P < 0.05$) and 'Nemaguard' (not significant) (Figs 2a and 3). Besides, the drought responses of average root diameter and percentage of root length within the finest diameter class were negatively correlated, contributing to both PC1 and PC2 (Figs 2e, f and 3). The *a priori* known gradient of drought sensitivity of the six hybrids was not reflected along either PC1 or PC2.

## DISCUSSION

This study presents important empirical evidence that (i) the drought response of SRA, SRL and RTD—and leaf gas exchange—is orthogonal to that of the SLA, while both gradients showed a trend towards resource conservation in drought-stressed plants and (ii) congeneric hybrids can display diverse above- and below-ground trait drought–response combinations to survive severe drought stress.



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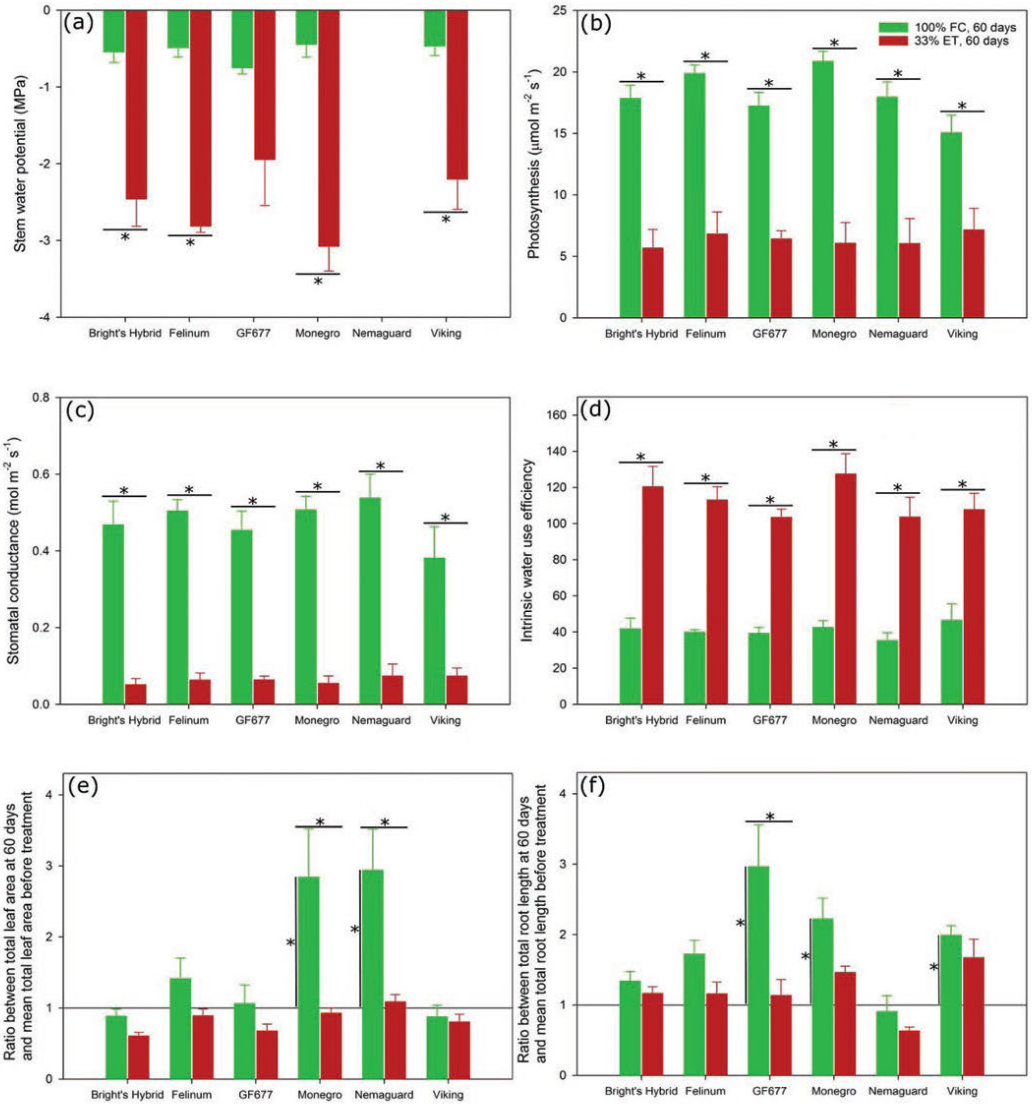


**Figure 1:** Stem water potential and components of leaf gas exchange in six *Prunus* hybrids exposed to watering treatments (100% FC versus 33% ET of control plants), after 60 days of treatments, with (**a**) stem water potential (measurement not applicable for 'Nemaguard' due to small leaf size); (**b**) leaf net photosynthesis at saturating light ($A_{sat}$); (**c**) stomatal conductance ($g_s$); (**d**) intrinsic water use efficiency ($WUE_i$); (**e**) the ratio between the plant total leaf area at 60 days and the mean total leaf area before treatment (horizontal background line represents that the ratio equals to 1) and (**f**) the ratio between the plant total root length at 60 days and the mean total root length before treatment (horizontal background line represents that the ratio equals to 1). Values are means ± SE ($n$ = 6 for 'Bright's Hybrid', 'Felinum', 'Monegro' and 'Nemaguard'; $n$ = 5 for 'GF677' and 'Viking'). Horizontal lines and asterisks denote significant differences between 100% FC and 33% ET treatment after 60 days. Vertical lines and asterisks in subplot (**e**) and (**f**) denote significant differences between plants before and after 60 days of treatment for a given treatment. Significant differences are indicated as *$P < 0.05$.

### Multi-dimensionality of root-trait drought response

Root function could be maximized using a more diverse combination of traits which can achieve equal evolutionary success during selection (Valverde-Barrantes and Blackwood 2016). Plant species can take advantage of diverse root trait combinations to maximize root resource acquisition, such as reducing SRL and RTD while increasing root diameter at the same time (Valverde-Barrantes and Blackwood 2016). This study identifies two major different combination scenarios of root-trait drought responses. The first major combination scenario is characterized by a concurrent significant increase of RTD and decrease of average root diameter without a significant change of SRL—found in 'Bright's Hybrid', 'Felinum' and 'Monegro' after 30 days and in 'Viking' after 60 days (Fig. 2c–e). The second major combination scenario is characterized by a concurrent significant increase of RTD and decrease of SRL, whilst average root diameter remained unchanged—found in 'GF677' plants after 60 days of severe drought stress (Fig. 2c–e).

RTD, average root diameter and SRL are three key fine-root traits defining plant–water relations in terms of root absorption and foraging potential under drought (Barkaoui *et al.* 2016). Higher RTD can cause longer root lifespan for species with thin roots (Kong *et al.* 2019). Meanwhile, thinner root diameter can be an important



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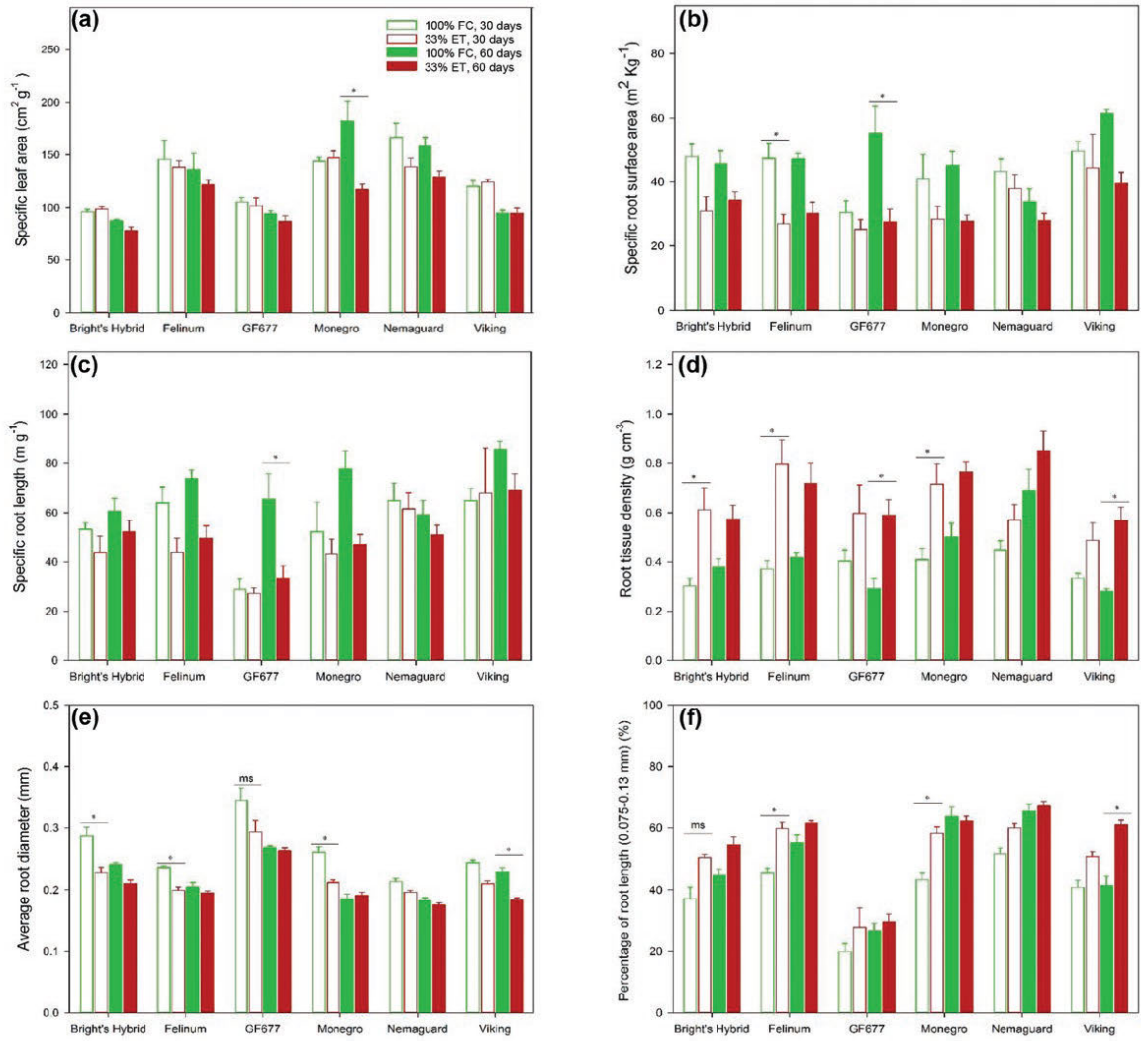


**Figure 2:** Leaf and fine-root traits in six *Prunus* hybrids exposed to watering treatments (100% FC versus 33% ET of control plants) after 30 and 60 days of treatments, with (**a**) SLA, (**b**) SRA, (**c**) SRL, (**d**) RTD, (**e**) average root diameter and (**f**) percentage of root length within the finest diameter class (0.075–0.13 mm). Values are means ± SE ($n = 6$ for 'Bright's Hybrid', 'Felinum', 'Monegro' and 'Nemaguard'; $n = 5$ for 'GF677' and 'Viking'). Significant differences are indicated as $^{ms}P < 0.1$ (marginal significance); $^{*}P < 0.05$.

fine-root trait enabling effective water acquisition under drought stress (Bardgett *et al.* 2014). Thinner roots have greater plasticity in growth proliferation (Eissenstat 1991), and greater flexibility in root construction under severe drought stress conditions (Chen *et al.* 2013). Thinner roots require less construction cost (Chen *et al.* 2013; Eissenstat 1992), in contrast to lower SRL and lower SRA in roots—or lower SLA in leaves—which are theoretically more expensive for plants to construct and maintain in terms of mass investment per unit of length and surface area with potential for resource acquisition. Both decreased root diameter and increased RTD—as found in the first major combination scenario—could potentially enhance hydraulic cavitation safety, which will increase plant tolerance to drought (Wang *et al.* 2016). Besides, fine-root with lower SRL can also reflect a resource-conservative strategy—lower growth rate but longer life span (Cheng *et al.* 2016; Freschet *et al.* 2015)—especially when soil water and leaf photosynthesis are limited. The root environment with limited soil water and soil depth, such as pot in this study, could render fine-root with limited foraging potential, decreased SRL and increased RTD (Barkaoui *et al.* 2016)—as found in the second major combination scenario.

This study reflects important hybrid-specific below-ground strategies to survive severe drought stress. The evidence of varied combinations of fine-root trait drought response found in this study is significant for root researchers in (i) supporting recent studies calling for a multi-dimensional view to consider root trait plasticity (e.g. Weemstra *et al.* 2016) and (ii) identifying the origin of the multi-dimensionality of root-trait drought response—at least at intraspecific scale.

### Drought–response relations of leaf and fine-root traits

No hybrid showed concurrent significant reduction of SLA and SRA (or SRL) at either time point (Fig. 2a–c). The lack of evidence across the



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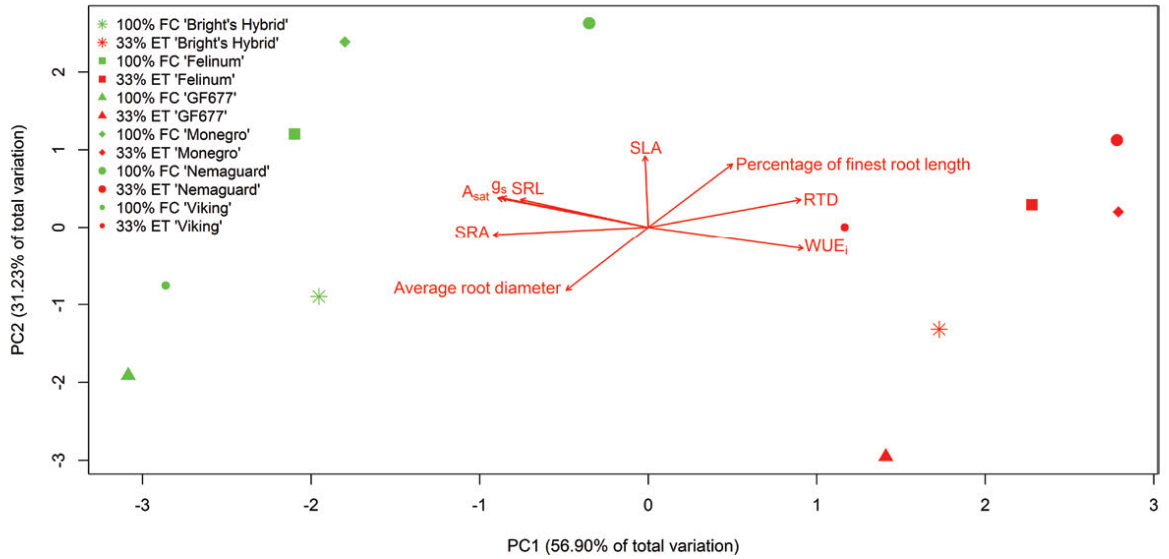


**Figure 3:** Differential drought responses of fine-root and leaf traits depicted by PCA conducted on nine traits, SLA, SRA, SRL, RTD, average root diameter, percentage of root length within the finest diameter class, $A_{sat}$, $g_s$ and $WUE_i$ in six *Prunus* hybrids after 60 days of watering treatments (100% FC versus 33% ET of control plants). The PC1 explained 56.9% of the total variation and the PC2 explained 31.23% of the total variation.

studied six *Prunus* hybrids for a common significant drought response between SLA and SRA (or SRL) may be due to the fact that root traits are shaped by below-ground environmental constraints which are different from the constraints shaping leaf traits (Bergmann *et al.* 2017; Kembel and Cahill 2011).

The drought responses of leaf gas exchange ($A_{sat}$, $g_s$ and $WUE_i$) and fine-root traits associated with root absorptive capacity (SRA and SRL) and root investment trade-off (RTD) were closely correlated along a gradient from control plants (resource acquisition) to drought-stressed plants (resource conservation) for all six hybrids (Fig. 3). The drought response of SLA was orthogonal to that of leaf gas exchange, SRA, SRL and RTD, reflecting a hybrid-dependent gradient towards resource conservation (Fig. 3). The gradient along PC1 in Fig. 3 was mainly driven by the treatment effect, while the gradient of drought responses of SLA along PC2 tended to be driven more strongly by the hybrid effect (Fig. 3). These findings on leaf trait drought responses support previous studies reporting decoupled leaf economics—depicted by SLA in this study—and leaf hydraulics—depicted by $g_s$ in this study (e.g. Li *et al.* 2015). Given the root traits in this study are closely related to root hydraulics (e.g. average root diameter and RTD; Rieger *et al.* 1999; Wang *et al.* 2016), the results also suggest the covariation of leaf and root hydraulics in response to severe drought (Fig. 3)—underscoring the divergence between leaf and fine-root trait drought response.

The difference between the drought responses of fine-root traits versus SLA may be associated with the degree to which the treatment and hybrid interact, such that drought responses of fine-root traits are similar across all hybrids while the extent to which SLA responds to treatment is affected by hybrid. The drought–response relations displayed along PC1 and PC2 are not likely to be driven by the inclusion of 'GF677' and 'Viking' of smaller plant sizes (and/or less daily ET and less reduction of $\Psi_{stem}$) compared with the other four hybrids (Fig. 3). This is because, within each hybrid, the drought-stressed and control plants were of similar plant sizes and were randomly assigned to one of the two treatments, and the drought-stressed plants were watered with 33% hybrid-specific ET of control plants every day.

The orthogonal drought–response relationship between SLA and SRA (and SRL) goes against the long-standing expectation that drought responses of SLA and SRA (or SRL) would mimic each other (Eissenstat *et al.* 2000; Pérez-Ramos *et al.* 2012). The notable interspecific variation in drought responses of fine-root traits and SLA suggests the existence of a wide range of combinations of above- and below-ground trait-based drought responses for different hybrids to survive severe drought stress. The diverse combinations of these trait modifications could be crucial for plant survival under severe drought stress—especially when carbon assimilation was significantly limited (Fig. 1 and Table 1).

This pot-based study provides a meaningful perspective on the integrated drought response of leaf and fine-root traits for future experiments exploring drought–response interrelationships among plant traits. The findings derived from the present pot experiment under controlled conditions remain to be tested in field conditions, where the soil volume available to the root system is considerably larger than in the pot (Poorter *et al.* 2012), and the impact of the increased mechanical impedance associated with declining soil water availability is difficult to simulate within pots (Cairns *et al.* 2011). This study cannot answer to what extent the observed change of characteristics were associated with existing or later-developing leaves and fine-roots, although plants under drought conditions can modify SLA and root traits in both existing and new leaves and roots (Poorter *et al.* 2009). Given all drought-stressed plants in this study scarcely developed any new leaves during the treatments, the drought response of SLA tended to be mainly associated with existing leaves.

This study contributes to better understanding of drought resilience of tree saplings, which in particular requires more research efforts as sapling performances are vital for understanding of drought consequences on forests and fruit crop plantings. This study advances our understanding on intraspecific tree drought responses which—if combined with findings from studies on interspecific drought–response variation (e.g. that on congeneric species within the *Eucalyptus* and *Quercus* genera, respectively; Zhou *et al.* 2014)—would be very useful for prediction of drought consequences at larger scale. The differential drought response among six *Prunus* hybrids found at their sapling stage remains to be tested at other developmental stages in the plant life cycle, as values of traits such as SLA or SRL can change more rapidly at the sapling stage. The duration of the present drought experiment



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is shorter than many drought manipulation experiments in the field, although drought-induced modifications in plant morphology and/or physiology could occur in a relatively short period in young plants (e.g. Guarnaschelli *et al.* 2006; Zhou *et al.* 2016) compared with mature trees studied in the field (e.g. Limousin *et al.* 2013; but see Binks *et al.* 2016 for limited trait modification after long-term drought). It also remains to be tested whether the findings in this study would occur among congeneric hybrids or species of other genera or plant functional types—as the relationships among fine-root traits under environmental constraints may be associated with the phylogenetic or functional group studied (Valverde-Barrantes and Blackwood 2016). Meanwhile, cultivated tree species may not evolve in the same way as wild tree species in terms of drought resilience. Future work on entire root systems of adult trees incorporating information on root orders might also enhance our interpretation of the functional and structural trade-offs underlying drought-induced below-ground morphological differences (Kong *et al.* 2014). Besides, explicit representations of drought responses of key leaf and fine-root traits within models at community scale may help researchers and practitioners better predicting drought consequences on forests and fruit crop plantings.

## CONCLUSIONS

This study provides important insight on how different congeneric hybrids can survive severe drought stress through diverse combinations of leaf and fine-root trait drought responses towards integrated plant resource conservation. A multi-dimensional perspective which integrates both leaf and fine-root traits—and particularly their differential trait drought response—may be key to reduce uncertainty in predicting drought consequences on woody species, fruit crop plantings and forest ecosystems.

### *Supplementary Material*

Supplementary material is available at *Journal of Plant Ecology* online.

Figure S1: The ratio between the plant total leaf area at 30 days and the mean total leaf area before treatment (horizontal background line represents that the ratio equals to 1).

Figure S2: The ratio between the plant total root length at 30 days and the mean total root length before treatment (horizontal background line represents that the ratio equals to 1).

Figure S3: Percentage of root length within 10 root diameter classes (0–0.075, 0.075–0.13, 0.13–0.2, 0.2–0.3, 0.3–0.4, 0.4–0.5, 0.5–0.75, 0.75–1, 1–2 and more than 2 mm) at 60 days of treatments.

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## REFERENCES

Allen CD, Macalady AK, Chenchouni H, *et al.* (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For Ecol Manage* **259**:660–84.

Bardgett RD, Mommer L, De Vries FT (2014) Going underground: root traits as drivers of ecosystem processes. *Trends Ecol Evol* **29**:692–9.

Barkaoui K, Roumet C, Volaire F (2016) Mean root trait more than root trait diversity determines drought resilience in native and cultivated Mediterranean grass mixtures. *Agric Ecosyst Environ* **231**:122–32.

Bergmann J, Ryo M, Prati D, *et al.* (2017) Root traits are more than analogues of leaf traits: the case for diaspore mass. *New Phytol* **216**:1130–9.

Binks O, Meir P, Rowland L, *et al.* (2016) Limited acclimation in leaf anatomy to experimental drought in tropical rainforest trees. *Tree Physiol* **36**:1550–61.

Cairns JE, Impa SM, O'Toole JC, *et al.* (2011) Influence of the soil physical environment on rice (*Oryza sativa* L.) response to drought stress and its implications for drought research. *Field Crop Res* **121**:303–10.

Chapman N, Miller AJ, Lindsey K, *et al.* (2012) Roots, water, and nutrient acquisition: let's get physical. *Trends Plant Sci* **17**:701–10.

Chen W, Zeng H, Eissenstat DM, *et al.* (2013) Variation of first-order root traits across climatic gradients and evolutionary trends in geological time. *Glob Ecol Biogeogr* **22**:846–56.

Cheng J, Chu P, Chen D, *et al.* (2016) Functional correlations between specific leaf area and specific root length along a regional environmental gradient in Inner Mongolia grasslands. *Funct Ecol* **30**:985–97.

Company RS, Gradziel TM (2017) *Almonds: Botany, Production and Uses*. Boston, MA: CABI, 220–1.

Craine JM, Froehle J, Tilman DG, *et al.* (2001) The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. *Oikos* **93**:274–85.

Eissenstat DM (1991) On the relationship between specific root length and rate of root proliferation: a field study using citrus rootstocks. *New Phytol* **118**:63–8.

Eissenstat DM (1992) Costs and benefits of constructing roots of small diameter. *J Plant Nutr* **15**:763–82.

Eissenstat DM, Wells CE, Yanai RD, *et al.* (2000) Building roots in a changing environment: implications for root longevity. *New Phytol* **147**:33–42.

Eissenstat DM, Yanai RD (1997) The ecology of root lifespan. *Adv Ecol Res* **27**:1–60.

Felipe AJ (2009) 'Felinem', 'Garnem', and 'Monegro' almond × peach hybrid rootstocks. *HortScience* **44**:196–7.

Fort F, Jouany C, Cruz P (2013) Root and leaf functional trait relations in *Poaceae* species: implications of differing resource-acquisition strategies. *J Plant Ecol* **6**:211–9.

Freschet GT, Swart EM, Cornelissen JH (2015) Integrated plant phenotypic responses to contrasting above- and below-ground resources: key roles of specific leaf area and root mass fraction. *New Phytol* **206**:1247–60.

Guarnaschelli AB, Prystupa P, Lemcoff JH (2006) Drought conditioning improves water status, stomatal conductance and survival of *Eucalyptus globulus* subsp. *bicostata* seedlings. *Ann For Sci* **63**:941–50.

Hodge A (2004) The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytol* **162**:9–24.

Keddy PA (1992) Assembly and response rules: two goals for predictive community ecology. *J Veg Sci* **3**:157–64.

Kembel SW, Cahill JF Jr (2011) Independent evolution of leaf and root traits within and among temperate grassland plant communities. *PLoS One* **6**:e19992.

Kong D, Ma C, Zhang Q, *et al.* (2014) Leading dimensions in absorptive root trait variation across 96 subtropical forest species. *New Phytol* **203**:863–72.

Kong D, Wang J, Wu H, *et al.* (2019) Nonlinearity of root trait relationships and the root economics spectrum. *Nat Commun* **10**:2203.

Li L, McCormack ML, Ma C, *et al.* (2015) Leaf economics and hydraulic traits are decoupled in five species-rich tropical-subtropical forests. *Ecol Lett* **18**:899–906.

Limousin JM, Bickford CP, Dickman LT, *et al.* (2013) Regulation and acclimation of leaf gas exchange in a piñon-juniper woodland exposed to three different precipitation regimes. *Plant Cell Environ* **36**:1812–25.

Liu G, Freschet GT, Pan X, *et al.* (2010) Coordinated variation in leaf and root traits across multiple spatial scales in Chinese semi-arid and arid ecosystems. *New Phytol* **188**:543–53.

Lõhmus K, Oja T, Lasn R (1989) Specific root area: a soil characteristic. *Plant Soil* **119**:245–9.

Markesteijn L, Poorter L (2009) Seedling root morphology and biomass allocation of 62 tropical tree species in relation to drought- and shade-tolerance. *J Ecol* **97**:311–25.

Maseda PH, Fernández RJ (2006) Stay wet or else: three ways in which plants can adjust hydraulically to their environment. *J Exp Bot* **57**:3963–77.

Olmo M, Lopez-Iglesias B, Villar R (2014) Drought changes the structure and elemental composition of very fine roots in seedlings of ten woody tree species. Implications for a drier climate. *Plant Soil* **384**:113–29.



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Ostonen I, Lõhmus K, Helmisaari HS, *et al.* (2007a) Fine root morphological adaptations in Scots pine, Norway spruce and silver birch along a latitudinal gradient in boreal forests. *Tree Physiol* **27**:1627–34.

Ostonen I, Püttsepp Ü, Biel C, *et al.* (2007b) Specific root length as an indicator of environmental change. *Plant Biosyst* **141**:426–42.

Pérez-Harguindeguy N, Díaz S, Garnier E, *et al.* (2013) New handbook for standardised measurement of plant functional traits worldwide. *Aust J Bot* **61**:167–234.

Pérez-Ramos IM, Roumet C, Cruz P, *et al.* (2012) Evidence for a 'plant community economics spectrum' driven by nutrient and water limitations in a Mediterranean rangeland of southern France. *J Ecol* **100**:1315–27.

Phillips RP, Ibáñez I, D'Orangeville L, *et al.* (2016) A belowground perspective on the drought sensitivity of forests: towards improved understanding and simulation. *For Ecol Manage* **380**:309–20.

Poorter H, Bühler J, van Dusschoten D, *et al.* (2012) Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Funct Plant Biol* **39**:839–50.

Poorter H, Niinemets Ü, Poorter L, *et al.* (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytol* **182**:565–88.

R Core Team (2015) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Reich PB (2014) The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. *J Ecol* **102**:275–301.

Reich PB, Buschena C, Tjoelker MG, *et al.* (2003) Variation in growth rate and ecophysiology among 34 grassland and savanna species under contrasting N supply: a test of functional group differences. *New Phytol* **157**:617–31.

Rieger M, Litvin P (1999) Root system hydraulic conductivity in species with contrasting root anatomy. *J Exp Bot* **50**:201–9.

Valverde-Barrantes OJ, Blackwood CB (2016) Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum: commentary on Kramer-Walter *et al.* (2016). *J Ecol* **104**:1311–3.

Valverde-Barrantes OJ, Smemo KA, Feinstein LM, *et al.* (2013) The distribution of below-ground traits is explained by intrinsic species differences and intraspecific plasticity in response to root neighbours. *J Ecol* **101**:933–42.

Wang Z, Xie J, Li Y (2016) Safety-efficiency trade-offs in the cotton xylem: acclimatization to different soil textures. *J Arid Land* **8**:443–52.

Weemstra M, Mommer L, Visser EJ, *et al.* (2016) Towards a multidimensional root trait framework: a tree root review. *New Phytol* **211**:1159–69.

Withington JM, Reich PB, Oleksyn J, *et al.* (2006) Comparisons of structure and life span in roots and leaves among temperate trees. *Ecol Monogr* **76**:381–97.

Woodward FI, Diament AD (1991) Functional approaches to predicting the ecological effects of global change. *Funct Ecol* **5**:202–12.

Zhou SX, Medlyn BE, Prentice IC (2016) Long-term water stress leads to acclimation of drought sensitivity of photosynthetic capacity in xeric but not riparian Eucalyptus species. *Ann Bot* **117**:133–44.

Zhou S, Medlyn B, Sabaté S, *et al.* (2014) Short-term water stress impacts on stomatal, mesophyll and biochemical limitations to photosynthesis differ consistently among tree species from contrasting climates. *Tree Physiol* **34**:1035–46.

Zhou SX, Prentice IC, Medlyn BE (2018) Bridging drought experiment and modeling: representing the differential sensitivities of leaf gas exchange to drought. *Front Plant Sci* **9**:1965.

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