

Resilient root systems for the Australian almond industry

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Horticulture Innovation Australia

SARMSII Administered: Government of South Australia, Primary Industries and Regions SA

Almond Board of Australia

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Resilient root systems

Limited knowledge around almond root systems/rootstocks and potential role in improving resource use efficiency.

Root systems provide:

- acquisition of both water and nutrients,
- barrier to soil stresses,
- signalling mechanisms that influence shoot growth and function.

Improved management and genetics of root system offer an opportunity to improve almond production and production efficiency.

CSIRO projects & major outputs

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

- Assessment of the resilience of *Prunus* rootstocks to abiotic stresses.
- Optimised management for root systems and rootstocks.

Resilient Rootstocks for Australian Almond Industry:

- Survey of root knot nematodes (RKN)
- Screen new rootstocks for resistance to RKN
- Tool kit for developing perfect markers to stress resilience for pre-screening new rootstocks

Better tree performance and water use efficiency through root system resilience

Everard J Edwards & Shuang-Xi Zhou

Rapid abiotic stress screening

Aim

Development of rapid screens for Prunus rootstocks:

- *tolerance of water stress,*
- *tolerance of high soil conductivity.*

Typical rootstock trials:

- very long-term,
- results may be specific to site (soils/climate)
- potentially impacted by orchard management decisions.

Rapid glasshouse screening with 1st year ungrafted material:

- 'agnostic' to growing conditions,
- can be repeated/compared across multiple sites/seasons,
- should represent 'inherent' response of genotype,
- *but* ignore scion interactions and risk not representing response of mature trees.

Screening principal used

Method chosen assesses the impact of stress on *relative growth rate* (RGR) to generate an index of stress tolerance.

- Growth is the ultimate measure of a plant's performance,
- but absolute growth rates (e.g. new biomass per day) are strongly affected by plant size (due ↑ leaf area).

RGR = increase in biomass per unit of biomass per day

- but even relative growth rates are affected by plant size (due to requirement for support tissue).

stress index = RGR under stress / RGR under control conditions

Glasshouse rootstock screening

- May-July 2015 – Brights Hybrid, Cornerstone, Felinem, GF677, Monegro, Nemaguard, Viking.
- 2015/16 - Barrier 1, Cadaman, Garnem, Atlas, K86.
- Jan-Feb 2017 - *Bright's Hybrid*, Controller 6, Controller 7, Controller 9.5, *Cornerstone*, *Garnem*, *GF677*, Rootpac 20, Rootpac 40.

Preliminary stress indices: *need validation!*

		Drought Index	Control RGR (d^{-1})
May-July 2015*	**Bright's Hybrid**		0.0021
	Cornerstone		0.0051
	Felinem		0.0022
	GF 677		0.0053
	Monegro		0.0059
	Nemaguard		0.0068
	Viking		0.0051
November – December 2015	**Barrier 1**		0.0266
January 2016-Febuary 2016	**Cadaman**		0.0488
	Garnem		0.0267
April 2016	**Atlas**		0.0572
May- June 2016	**K86**		0.0266
January-Feburary 2017	**Bright's Hybrid**		0.0231
	Controller 6		0.0207
	Controller 7		0.0208
	Controller 9.5		0.0204
	Cornerstone		0.0223
	Garnem		0.0176
	GF 677		0.0142
	Rootpac 20		0.0173
	Rootpac 40		*0.0085*

*Drought was 33% of control.

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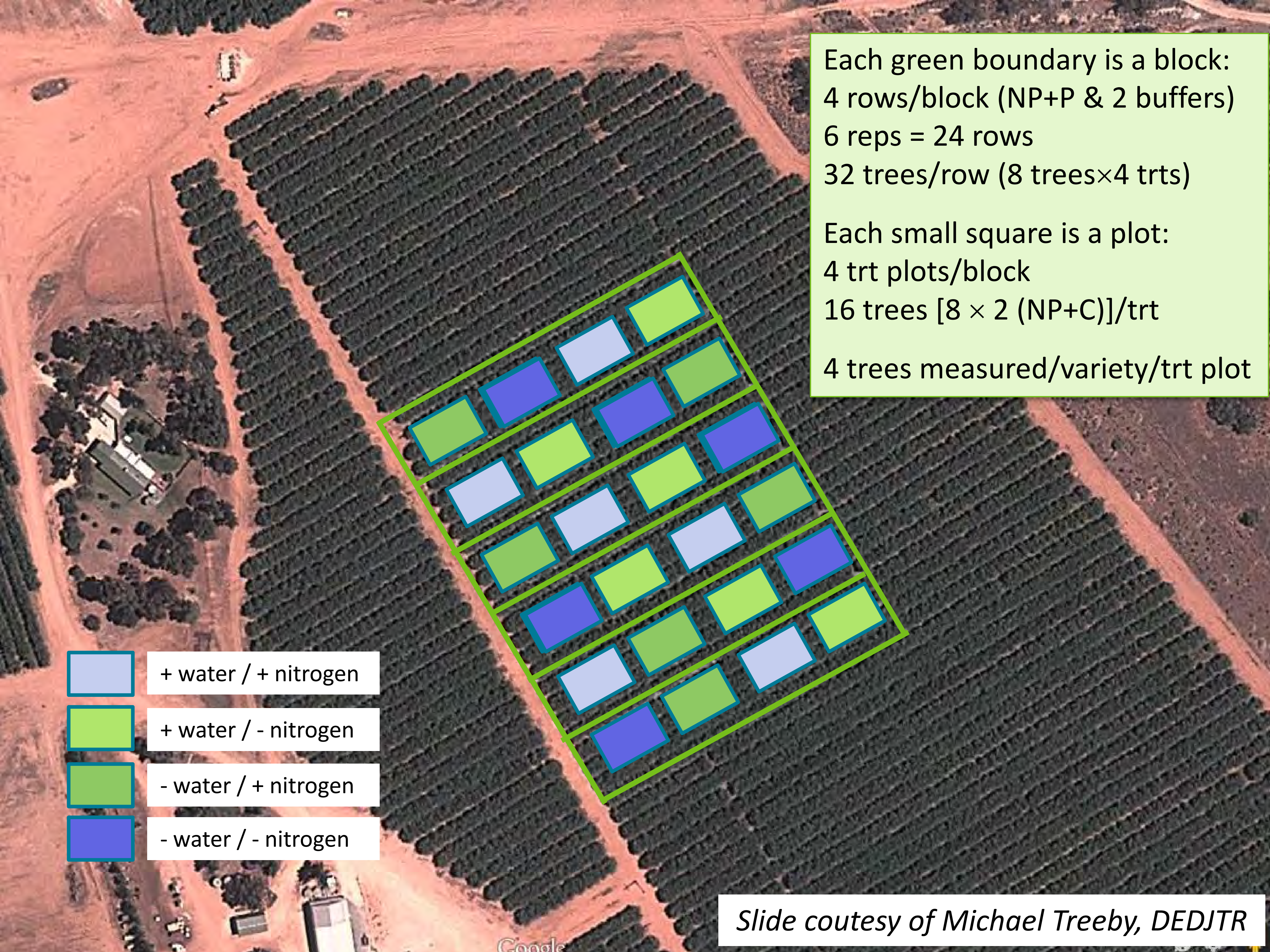
Understanding almond root systems

Utilising DEDJTR managed site established in mature orchard at CMV Farms Lindsay Point.

Reduced N and water application in factorial set-up (+N+H2O, -N+H2O, +N-H2O, -N-H2O).

Aims:

- establish phenology of root system (Nemaguard); timing of root growth, ageing of roots, root lifespan / demography / turnover, etc.
- determine the impact of reduced water and N application on root phenology and phenotype (mass, length, distribution),
- measure actual water uptake and impact of reduced irrigation, link to DEDJTR determined parameters (e.g. canopy size).


Each green boundary is a block:
4 rows/block (NP+P & 2 buffers)
6 reps = 24 rows
32 trees/row (8 trees×4 trts)
Each small square is a plot:
4 trt plots/block
16 trees [8 × 2 (NP+C)]/trt
4 trees measured/variety/trt plot
+ water / + nitrogen
+ water / - nitrogen
- water / + nitrogen
- water / - nitrogen
Slide coutesy of Michael Treeby, DEDJTR

Field experiment: methods

Minirhizotrons for root phenology & turnover

Field experiment: methods

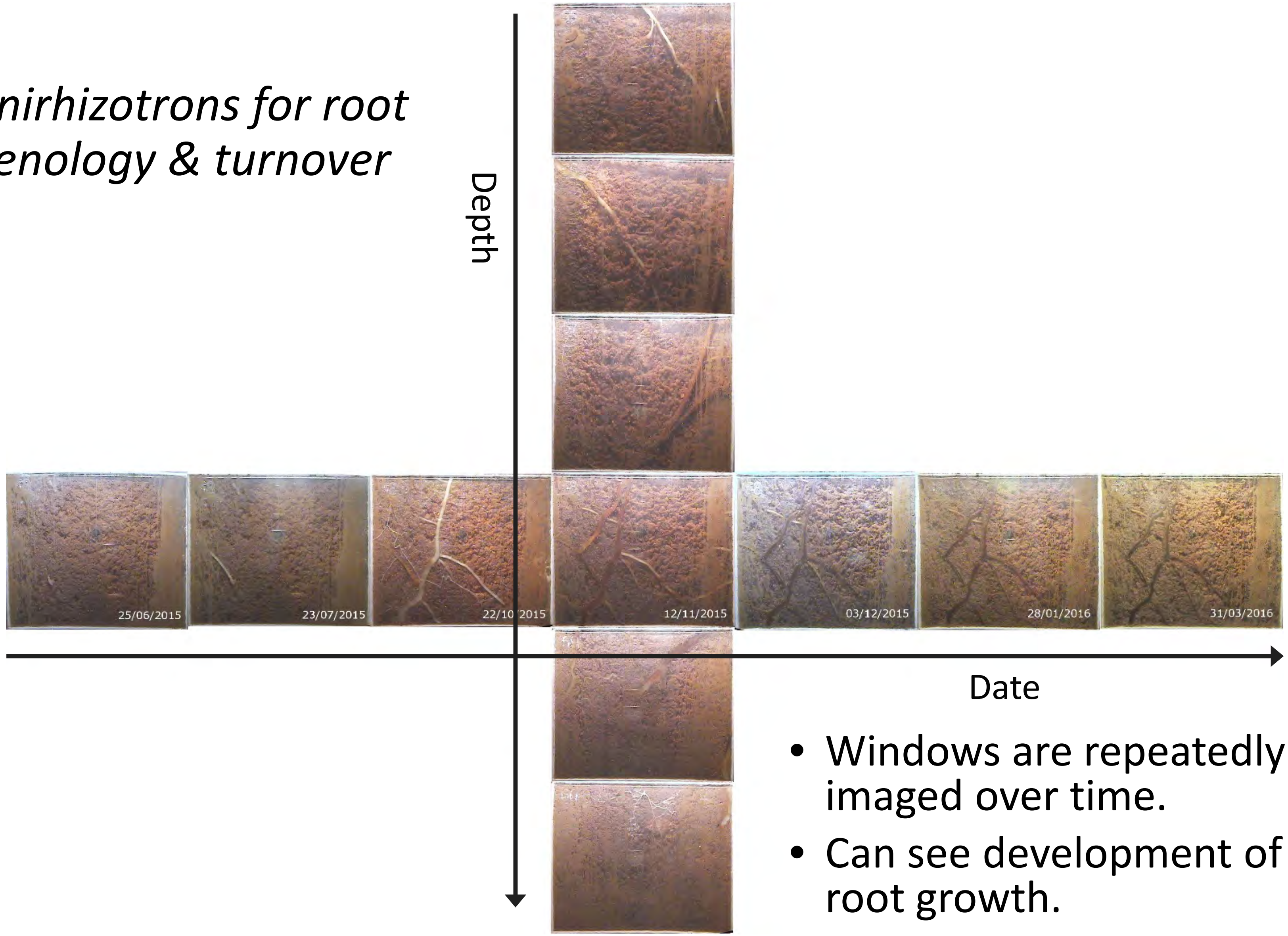
Minirhizotrons for root phenology & turnover

Imaging of 50+ windows per tube every three weeks.

Two tubes per rep per scion (96 total).

Field experiment: methods

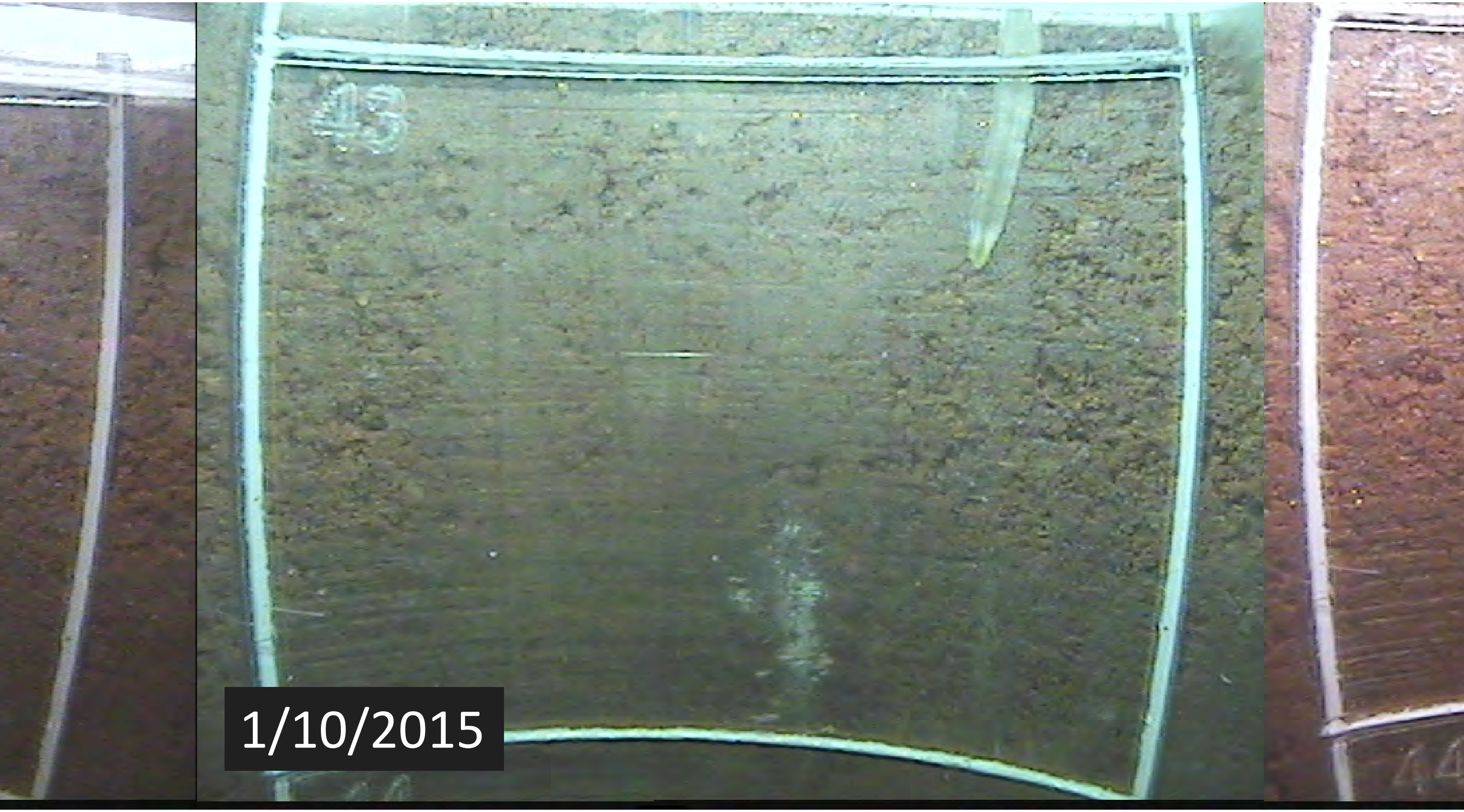
Minirhizotrons for root phenology & turnover



- Windows are repeatedly imaged over time.
- Can see development of root growth.

Field experiment: root phenology 60 cm

Field experiment: root phenology 60 cm



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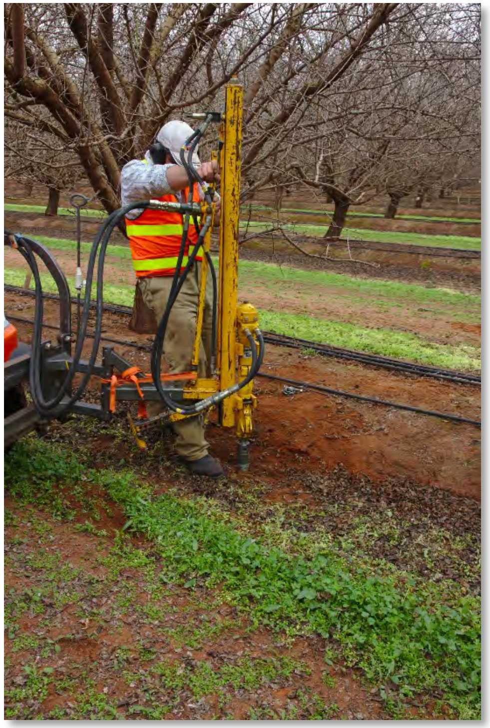
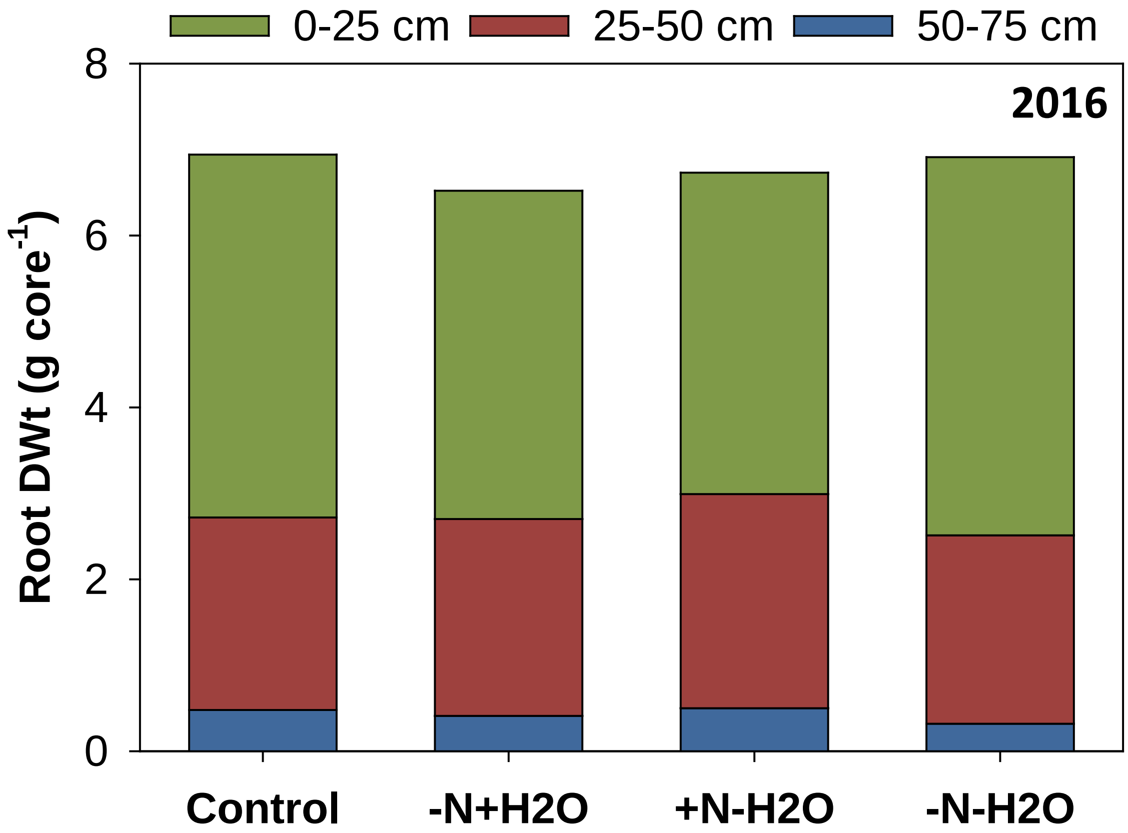
Field experiment: methods

Soil coring for root biomass



Field experiment: root biomass

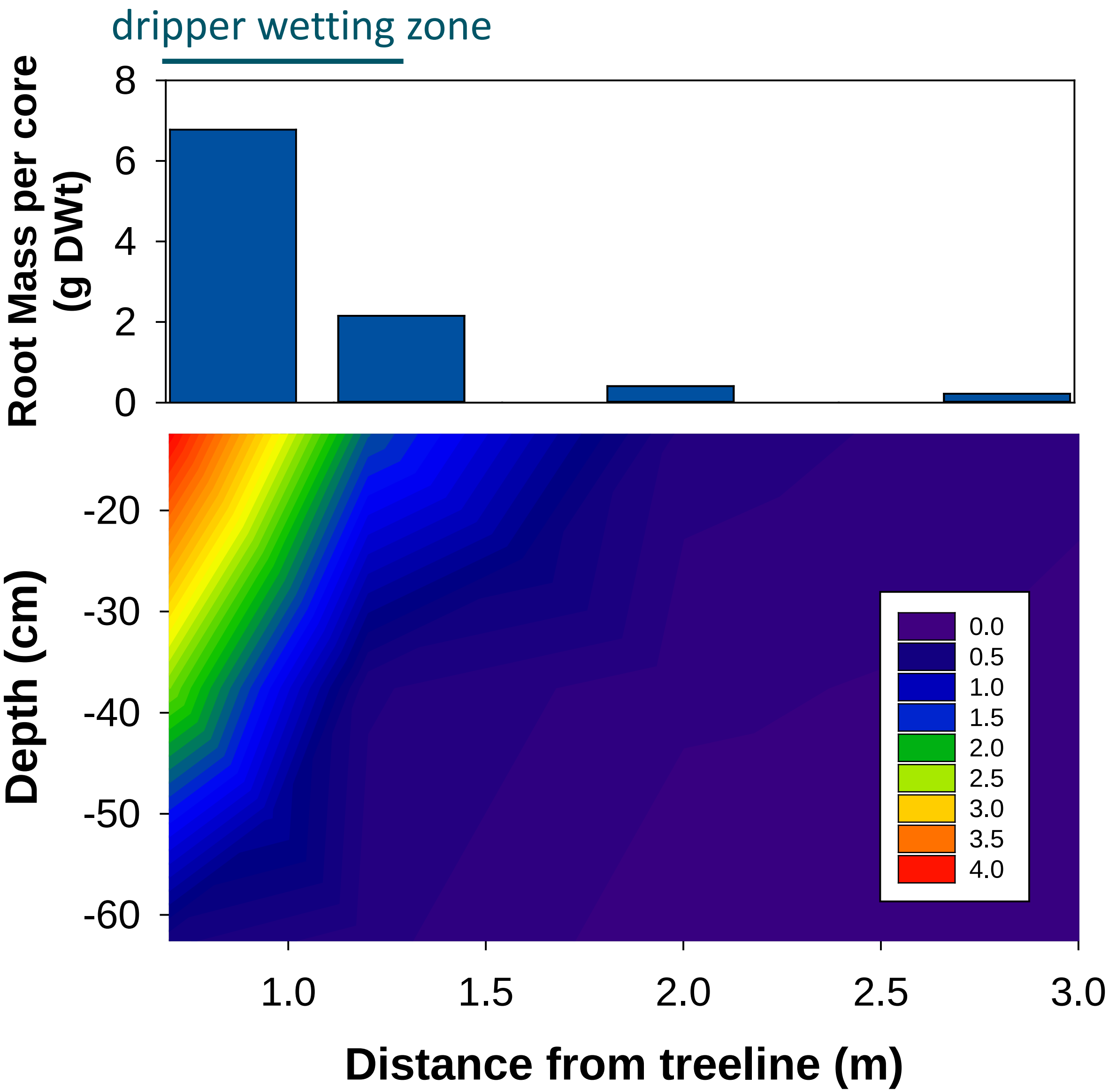


No significant effect on over-wintering root biomass after 1 yr.

Root length/morphology also determined.

Data collected in 2015 (treatments not yet applied) & 2016.

Field experiment: root biomass distribution



Summary

Rootstock screening:

- Eighteen rootstock genotypes screened + three repeats,
- Water deficit impacts typically larger than effects of (likely) high soil conductivities,
- Results will be published as 3-5 level tolerance index following validation.

Shared field experiment:

- Two+ seasons of minirhizotron images collected,
- Two+ sets of winter root cores collected (no treatment impact after one season),
- One+ season of whole tree water use monitoring (data not shown),
- Above will be combined with DEDJTR canopy data and ^{15}N tracer experiments to track N use efficiency.

Resilient Rootstocks for Australian Almond Industry

Mandy Walker & Harley Smith

Seed funding

Short term (10 month) project funded through South Australian River Murray Sustainability Program no. 2 (SARMSII) with industry support from the Almond Board of Australia

Concluded: 28th February 2017

Key objectives

Survey root knot nematodes across Murray Basin

Test new rootstocks for resistance to RKN

Develop rootstock identification method

Establish tool kit for marker development

Ultimate aim

Identify genes and markers to prescreen new rootstocks

Root knot nematodes cause extensive damage to susceptible *Prunus* root systems

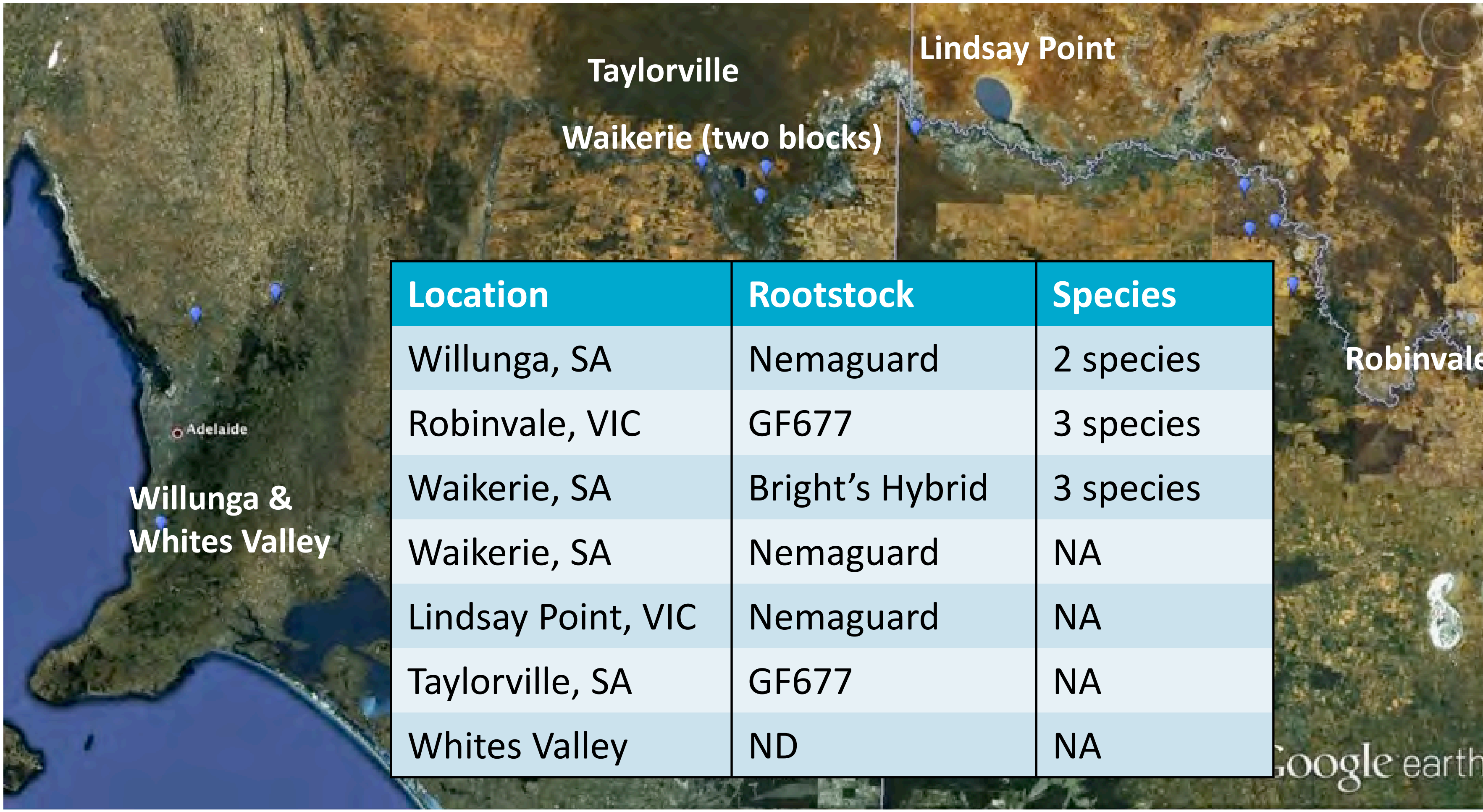
Root knot nematode

- **Galls decrease root function**
- **Decline in productivity**

Root knot nematode in the orchard

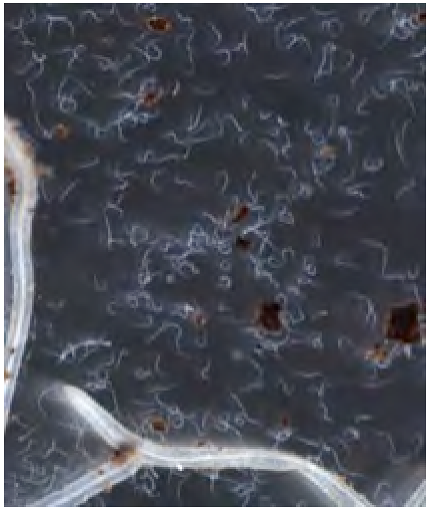
- ~15% decrease in yield & vigour in *Prunus*
- Rootstocks in replanting

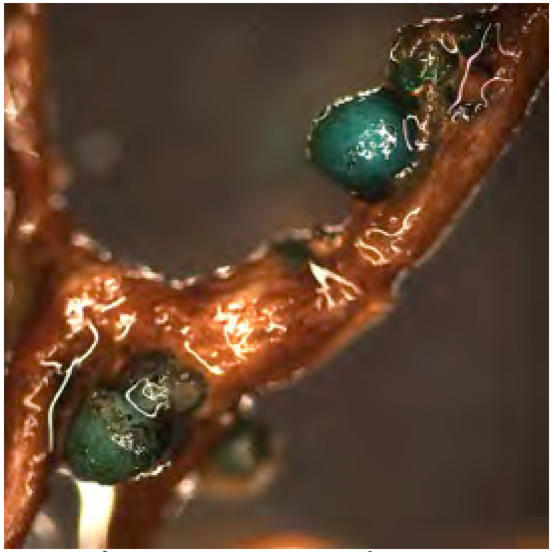
Root knot nematode survey



Location	Rootstock	Species
Willunga, SA	Nemaguard	2 species
Robinvale, VIC	GF677	3 species
Waikerie, SA	Bright's Hybrid	3 species
Waikerie, SA	Nemaguard	NA
Lindsay Point, VIC	Nemaguard	NA
Taylorville, SA	GF677	NA
Whites Valley	ND	NA

Root knot nematode screening assay





Optimized method to maintain and produce infectious root knot nematodes

Rootpacs less susceptible to a root knot nematode

Variety	Parentage	Classification
Garnem	*P. dulcis* x *P. persica*	Resistant
Rootpac 20	*P. besseyi* x *P. cerasifera*	Low susceptible
Nemaguard	*P. persica*	Low susceptible
Rootpac 40	*P. dulcis* x *P. persica*	Low susceptible
Cornerstone	*P. dulcis* x *P. persica*	Medium susceptible
Krymsk 86	*P. persica* x *P. cerasifera*	High susceptible
Barrier 1	*P. persica x P. davidiana*	High susceptible
Bright's Hybrid	*P. dulcis* x *P. persica*	High susceptible
GF677	*P. dulcis* x *P. persica*	High susceptible

New rootstocks (arrows pointing to Rootpac 20, Rootpac 40, Barrier 1)

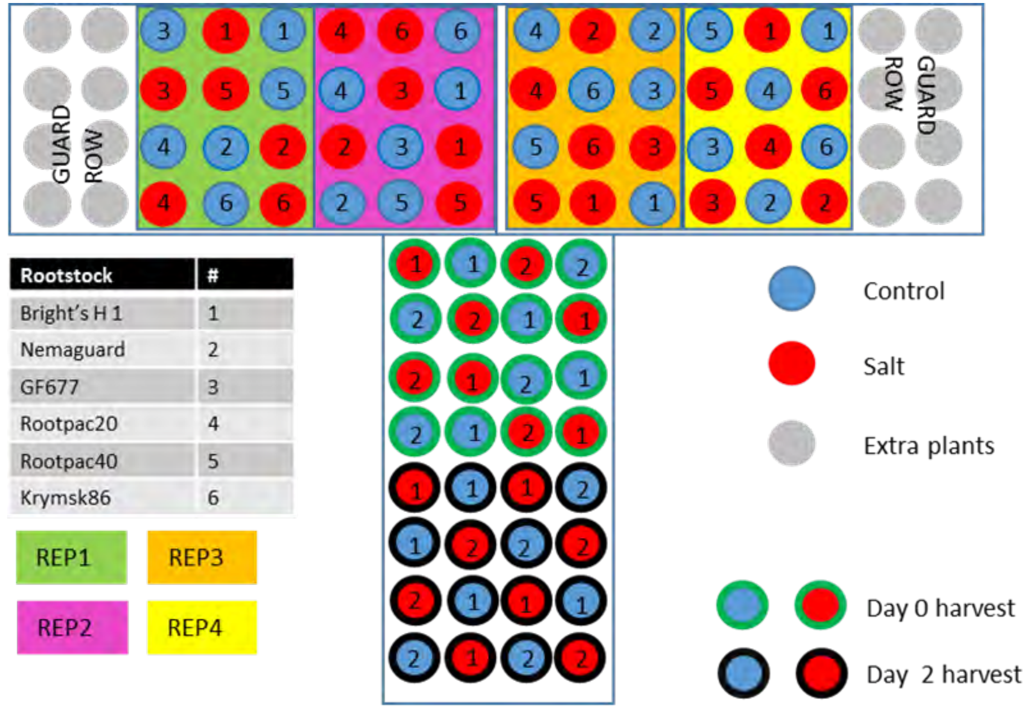
New methods for rootstock verification

- “Proof of concept” of rootstock identification
- DNA markers not linked to a particular trait
- Selected across different chromosomes
- Differentiates 16 tested cultivars

- Collaboration with Prof Diane Mather, Uof A

Ranking rootstocks for salt tolerance

- Ungrafted rootstock plants
- Planted in sand/perlite
- Given 21 days of mixed chloride
- 100mM chloride

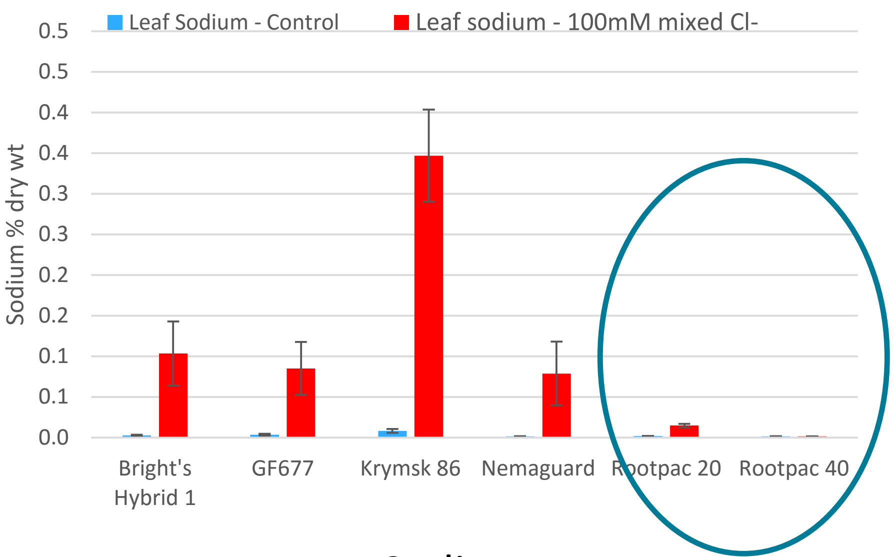


Rootstock	#
Bright's H 1	1
Nemaguard	2
GF677	3
Rootpac20	4
Rootpac40	5
Krymsk86	6

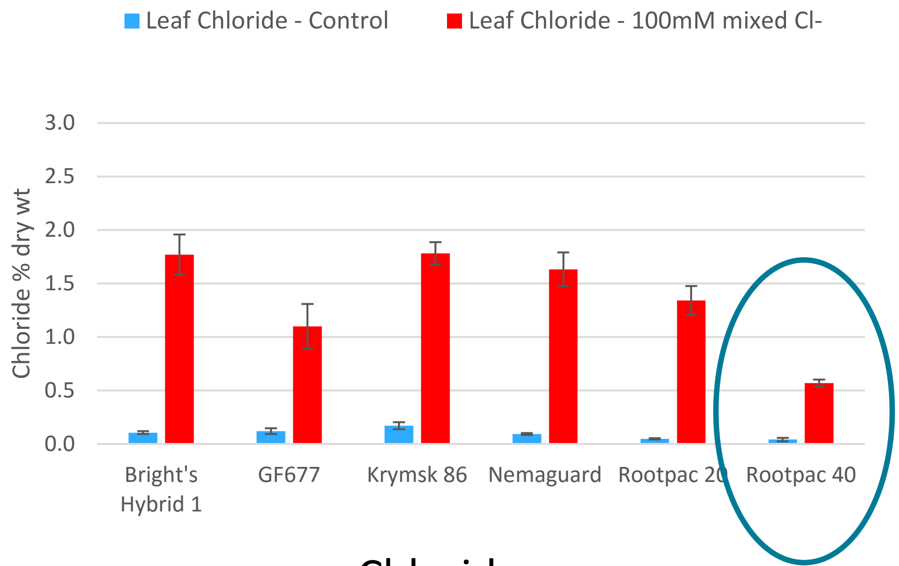
Layout of salinity experiment. Each variety was represented by a number, 4 reps for each line, harvest date and either salt (red) or control (blue).

Salinity experiment in glasshouse.

Rootpacs are good salt excluders



Sodium



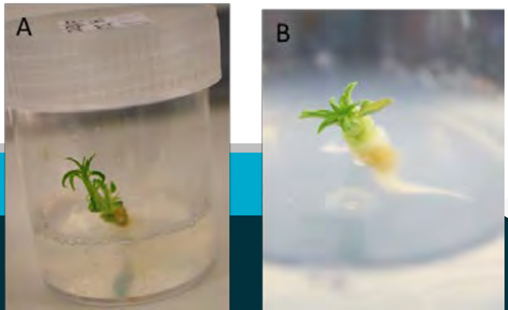
Chloride

Preliminary glasshouse study showed that Rootpac 20 & 40 have superior sodium exclusion and that Rootpac 40 has superior chloride exclusion compared to other widely-used rootstocks

Faster smarter markers for selected traits

- Important for decision-making on importing new rootstocks for Australia
- Important for predicting which rootstocks to plant/replant in a given location/conditions

Tool kit	Developed in SARMSII	Future project
• DNA libraries with large fragments of DNA	✓	
• Gene targets from other species	✓✓	✓
• Embryo rescue techniques	✓	
• Prunus transformation techniques		✓
• Mapping populations to check markers		✓



Future opportunities

1. **Screen** rootstocks with root knot nematode populations isolated from Willunga, Waikerie and Robinvale

2. Effect of **temperature** on root knot nematode **resistance**

3. **Ring nematode:** Ectoparasitic feeding reduces feeder roots, *P. syringae* predisposes peach trees to **“Peach Tree Short Life (PTSL)”**

4. Rootstock **verification** tool

5. Fast **smar**t technology to test **resilience** to interaction of abiotic stresses

6. **Perfect markers** for abiotic stress **resilience** and **resistance** to root knot nematode

Faster smarter screening for abiotic stress impacts and interactions

- Future Plant Accelerator screen
  - Rootstock/scion combinations
  - Different water, heat and salt conditions
  - Measures growth, inferred water use efficiency

Summary of outcomes

- Reliable, robust and resilient rootstocks selected in Faster Smarter ways
- Save on field evaluation costs and
- Faster commercial exploitation of superior rootstocks

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- Norma Morales
- Deidre Blackmore
- Debra McDavid

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