Final Report

Project title:

Innovating new virus diagnostics and planting bed management in the Australian Sweetpotato Industry

Project leader:

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Delivery partner:

Australian Sweetpotato Growers (Incorporated)

Project code:

VG13004

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Innovating new virus diagnostics and planting bed management in the Australian Sweetpotato Industry VG13004

Disclaimer:

Funding statement:

This project has been funded by Hort Innovation, using the vegetable research and development levy and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Publishing details:

ISBN 978 0 7341 4383 9

Published and distributed by: Hort Innovation

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www.horticulture.com.au

Content

Summary

The provision of disease free planting material is a key driver of Australia's burgeoning sweetpotato industry, which currently achieves the highest commercial yields in the world. Australian Sweetpotato Growers Inc. (ASPG) investigated how to improve productivity of on-farm multiplication nurseries (plant beds). Their four-year project also studied virus occurrence and threats to the Australian industry and explored new techniques for detecting viruses. The project worked closely with commercial sweetpotato growers in Queensland and Northern New South Wales, representing 95% of Australia's production.

Queensland scientists monitored grower plant beds over four years, assessing production of planting material (sprouts), and problems, such as plant bed breakdown, that arose during the season. They investigated management options such as sweetpotato root size, plant bed nutrition, irrigation and soil temperatures, in detailed experiments at research facilities and with on-farm collaborators.

The virology team surveyed viruses present in the Australian industry, and how they varied geographically and across the season. They compared different diagnostic techniques, including herbaceous indexing, NCM-ELISA and qPCR for accuracy and cost-efficiency.

Research demonstrated sprout multiplication could be improved 25% by constructing higher, well-drained plant beds, only covering bedding roots with 3-5 cm of soil, irrigating sparingly early, and keeping soil temperatures between 17-26^{o}C in spring by careful use of plastic covers. The dominant issue was premature plant bed breakdown, particularly with the new, nematode-resistant cultivar *Bellevue*. The project investigated physiological and pathogenic causes of breakdown and developed guidelines to reduce risk.

Surveying found only two main viruses, sweetpotato feathery mottle virus (SPFMV) and sweetpotato leaf curl virus (SPLCV) in commercial cropping areas. North Queensland has two more viruses at least, mainly in home/market garden situations. The Australian industry planting material scheme is free of viruses, and by using pathogen-tested material, virus infections have negligible impact on yields.

Herbaceous indexing with *Ipomoea setosa* is still the most reliable method of detecting sweetpotato viruses but is very time consuming. Molecular technologies such as qPCR proved accurate for two viruses, however for several endemic and exotic viruses, current assays produced too many false negatives, particularly testing asymptomatic sweetpotato material. The molecular technologies are very cost-effective and are rapidly improving. The project developed new virus assays for endemic and exotic viruses not currently in Australia.

The project team prepared plant bed and virus management guides, as well as fact sheets and experimental reports, available on ASPG and Hort Innovation websites. They had excellent engagement with well over 85% of current Australian sweetpotato growers, through on-farm visits, and 24 field days and workshops during the project. The project also developed exciting collaborative relationships with sweetpotato researchers at several Australian Universities, neighbouring Pacific countries, and particularly scientists from Louisiana State University. The latter were involved in several reciprocal visits over the four years.

Further research into the causes and management of premature breakdown of plant beds would certainly benefit the Australian industry, as would improvement in molecular diagnostic assays for virus detection, to support sweetpotato industry biosecurity.

Keywords

sweetpotato; ipomoea; batatas; ASPG; pathogen tested; plant bed; sprouts; seedbed; virus; qPCR; NCM-ELISA; SPFMV; SPLCV

Introduction

Background

As outlined in the 2013 Australian Vegetable Industry Strategic Investment Plan (page 43), since the early 2000s, the Australian Sweetpotato Industry underwent massive expansion and productivity improvement. Its farm gate value was estimated at $80-90M in that year. The dramatic production increase of around 17% per annum over a decade had been associated with significant investment in research, development and extension (RDE). The seminal work done by Eric Coleman *et. al.* (2006), demonstrated that using high quality, virus-free planting sprouts, of consistent length and condition, planted precisely, improved marketable sweet potato yields by 80%.

Sweetpotato viruses and phytoplasmas are of particular concern: Currently more than 30 viruses infecting sweetpotato, assigned to 9 families, have been identified (Clark *et. al.* 2012). Sweetpotato viruses are associated with significant yield decline (Loebenstein *et. al.* 2009, Clarke and Valverde 2009). Several world authorities have attributed viral diseases as the main cause of yield decline and therefore decline of cultivars globally (Salazar and Fuentes 2000).

Research in the last two-three decades has consistently demonstrated viruses in planting material was the major agronomic factor limiting production of even-shaped, smooth-skinned (i.e. easy to peel), high yielding sweetpotatoes. A consumer study clearly identified that ease of use and reliability of quality was one of the key factors persuading consumers to preferentially purchase sweetpotatoes (Gething *et. al.* 2012).

One of the most significant RDE outcomes in the last decade was been the development and commercialisation of the system for supplying pathogen-tested (PT), 'virus-free' planting material to growers, via a Quality Assured, structured process. This PT planting material system has been so successful that 100% of the current Australian commercial sweetpotato production utilises this scheme. It is also currently being adapted and promoted for use internationally, even in developing countries such as Papua New Guinea (Dennien *et. al.* 2013). The situation is very similar in the USA, where they have moved to PT planting material schemes in the late 1990's (Labonte *et. al.* 2004).

The virus disease situation is far from resolved. Most research to date had focussed on Sweetpotato Feathery Mottle Virus (SPFMV). There was very limited knowledge of other sweetpotato viruses that may or may not be present in Australia's commercial sweetpotato crops. In addition, some viruses have the unfortunate capacity to mutate and recombine genetic material quite readily and become less detectable by ultra-specific diagnostic methods. It was important that clean planting material programs use up to date virus testing protocols, to guarantee freedom from virus. The current Australian PT program tests for SPFMV and nine other viruses using a standard NCM ELISA procedure, backed up by virus indexing.

A critical component of the PT process is the conversion of PT storage roots from the scheme into commercial cuttings for planting. Most growers purchase their PT roots from February through to October, although many were chasing a year-round supply. These roots are then planted into designated planting beds. These beds are not large in area, in the order of 10-1000 square metres. Some larger growers may have bigger areas. High quality sprouts are produced from these storage roots. They are harvested once they reach ideal planting length (around 40 cm); they are cut by hand and transferred for planting in commercial sweetpotato fields. Some growers may be forced to use second generation cuttings once removed from the initial PT roots, due to lack of PT roots in proportion to their prospective planting areas.

Opportunities for improvement

Sweetpotato virus diagnostics

Firstly, there was a constant need to keep abreast of continually evolving sweetpotato viruses especially those in the Geminivirus family. This requires on-going assessment of sweetpotato virus diagnostic techniques in use globally, to improve field detection methods in Australia and identify where there are gaps in these technologies. As new viruses are identified, purified antisera can be developed, and detection methods are improved for rapid analysis of the national situation.

The pathogen tested sweetpotato planting material scheme relies on the capacity to detect (and then remove) viruses. Because viruses are constantly changing (recombinants), up to date virus testing is essential to guarantee freedom from important sweetpotato viruses.

There were identified biosecurity risk concerns around our sweetpotato protocols. The Australian Sweetpotato industry required knowledge of (a) which viruses are already present in Australia; (b) which viruses are present in significant trading partners likely to supply product to Australia and (c) how can we effectively test for this suite of viruses and virus complexes.

This project RDE would support an ASPG long term plan to improve quarantine (although development of such a plan was not an intention of this project) and assist in future pathogen and vector management in the main commercial sweetpotato cropping areas. It will also inform and provide guidelines for the development of contingency plans in the event of a new incursion.

Planting bed management

This project aimed to:

- Investigate the causes of planting bed loss; issues such as anaerobic conditions, pathogen infection, and the role of irrigation management. The drive is to minimise the risk of these catastrophic losses.
- Describe the practices that impact on the capacity of the planting bed to generate physiologically hardened, yet rapid-growth capable sweetpotato cuttings, which can provide high numbers of marketable sweetpotato roots per cutting throughout the sweetpotato cropping season.
- Describe how to determine the balance between sequential cutting of existing planting beds, and when to switch to a new planting bed.
- Outline the desirable equipment and land resource attributes that can best deliver optimal planting bed conditions; e.g. row covers, irrigation infrastructure, soil condition and nutritional status, PT root planting arrangements (orientation, planting depth, root size and density).

Methodology

Project target audience

The project was primarily aimed at growers, consultants, industry service organisations and businesses that work with the Australian sweetpotato industry. The industry is very concentrated, with 95% of Australia's commercial production coming from within 100 km of the coast between Rockhampton (Central QLD) and Cudgen (Northern NSW) – see Appendix 1, although North Queensland is starting to regain a presence at the time of writing this report. The three core members of the project team (Craig Henderson, Sandra Dennien and Rachael Langenbaker) had extended conversations with at least 85% of sweetpotato growers and relevant industry services, either at industry field days, or on their farms, several times during the project. Growers planting more than 10 ha of sweetpotatoes per year represent around 50% of the grower population but produce nearly 90% of Australia's sweetpotatoes. We engaged with those growers as our prime target audience, and specifically visited them several times per annum.

A secondary target audience for the virus diagnostics component of the project was scientists, regulators and service providers supporting biosecurity at regional and national levels. The Australian industry is committed to ensuring Australia has capacity, knowledge and intent to maintain freedom from several important viruses currently not found in this country.

Improving sweetpotato plant bed productivity

Baseline industry practice survey and sweetpotato plant beds literature review

Between April-September 2014, we surveyed 44 Australian sweetpotato growing businesses on their use of Pathogen-Tested (PT) planting material in their production systems (Appendix 1). Each face to face interview contained 27 questions identifying their current practices, as well as their key issues, and thoughts on research priorities for plant bed improvement. The businesses covered represent approximately 95% of Australia's commercial sweetpotato production.

Early in the project we reviewed the scientific and extension literature on management issues and best practice in sweetpotato plant bed systems (Outputs p 13).

The gathered information was used to prioritise grower monitoring and detailed experimental activities associated with the plant bed management component of the project. The research also formed the basis for the initial iteration of the published plant bed management guide (Outputs p 13).

Seasonal monitoring of grower plant beds (**Appendix 2**)

In each sweetpotato production season (assumed to commence with July installation of plant beds), the project team monitored performance and production issues with growers' plant beds in Cudgen, Lockyer and Bundaberg districts. Monitoring involved recording growers' plant bed layout, structures and management practices, benchmarking their sprout productivity through sampling, and recording any obvious productivity issues. At the same time, we discussed the growers' views on seasonal performance, and any issues they felt were impacting on their plant beds. The monitoring focussed on 3-5 key growers in both Cudgen and Bundaberg, and 2 growers in the Lockyer District, visiting them at least every 2 months. However, we additionally took advantage of regular district visits for experimental or other extension activities to collect information on plant bed performance and productivity issues.

Targeted experimental investigations into plant bed performance

We conducted seven major field experiments during the project; three at the Gatton Research Facility and four on collaborating grower properties in Bundaberg. These studies investigated the impacts of the following issues on plant bed productivity (both quantity and quality of sprouts) and longevity:

2014/15 Bedding root size and grade, cultivar *Beauregard* (Gatton, Bundaberg) Appendix 3.

2015/16 Nitrogen nutrition of plant beds, cultivars *Beauregard, Bellevue, Orleans* (Gatton, Bundaberg) Appendix 4.

2016/17 Rates of organic and inorganic nitrogen fertiliser, cultivars *Beauregard, Bellevue* (Gatton). Robin Scarffe Honours Thesis (Outputs p13).

2017/18 Irrigation strategies and subsequent soil water status, cultivar *Bellevue* (Bundaberg) Appendix 5.

These experiments comprised six treatments replicated four times, using a total of 60 m of plant bed. We assessed sweetpotato sprout production and quality by sampling, usually 6-7 cuts every 3-4 weeks over a period of 6 months.

We conducted two demonstration-style studies in Cudgen in 2017/18, using large commercial grower plots to further evaluate the impacts of bedding root size on premature breakdown of cultivar *Bellevue* in plant beds (Appendix 6).

In January/February 2018, we implemented a pilot study exploring the use of growth cabinets to investigate the physiological and pathological causes of premature breakdown of *Bellevue* bedding roots. We placed roots in tubs of sand and exposed them to controlled temperature and humidity conditions for a period of 6 weeks. We then assessed their condition and sampled for pathogenic organisms (Appendix 7).

Assessing sweetpotato sprout potential

We reviewed the literature on key drivers of storage root initiation from sweetpotato planting material. We also reviewed what experimental techniques could be useful to index the potential yields of sweetpotato sprouts. We extensively planted out sprouts from our experiments, however found results extremely variable (Appendix 8). After the project mid-term review, we focussed our efforts on controlled environment systems (aeroponics, sand culture systems) to be able to index sprout quality, particularly a rhizotron-based system to sequentially observe root development from sprouts (Appendix 9).

Virus management and diagnostics

Virus incidence and diagnostics literature review

Early in the project we reviewed the scientific and extension literature on sweetpotato viruses throughout the world (including Australia), as well as best practice diagnostic and sampling techniques (Outputs p13).

This information was used to prioritise monitoring strategies for viruses and diagnostic techniques associated with the virus component of the project. The research also formed the basis for the initial iteration of the published virus management and diagnostic guides (Outputs p13).

Virus surveys and diagnostics (**Appendix 10**)

From January 2014 to January 2018, sweetpotato samples and *Ipomoea* weeds were collected from the major Australian sweetpotato growing regions of Australia. Vine cuttings and/or storage roots were allocated an identification number and entered into the sample database with collection details.

Seeds of the biological indicator plant, *I. setosa* seeds were potted within an insect proof glasshouse and graft inoculation was performed each summer from October 2014 to January 2018. Sweetpotato vine sections containing at least one node were grafted onto *I. setosa* plants, as per Love *et al*. (1987), Beetham and Mason (1992), Fuentes (2010) and Dennien *et al*. (2013). Previously tested known positive control plants for a range of viruses and Phytoplasma were also grafted. All plants were observed, with data recorded weekly until 42 days post grafting.

At 14-21 days after grafting, tissue from graft inoculated plants was then subjected to a Nitrocellulose membrane, enzyme linked immunosorbent assay (NCM-ELISA), a specific antibody assay purchased from the International Potato Centre (CIP). DNA and RNA were also extracted and quantified at this time and stored at -20^{o}C for downstream qPCR. All qPCR assays were performed on a StepOnePlus^{TM} (Applied Biosystems) real time quantitative PCR system.

The following published qPCR protocols were used in the detection of sweetpotato viruses with GRF 100 (PT Beauregard) used as a negative control:

- Sweetpotato leaf curl virus: published protocols as per Barkley (2011) were used with positive control plants GRF 300 and GRF 003.
- Sweetpotato feathery mottle virus along with the closely related Sweetpotato virus 2 syn: *Ipomoea* vein mosaic virus: published protocols as per Kokkinos and Clark (2006), SPFMV positive control plants GRF 300, GRF 001.
- The exotic Sweetpotato virus G (SPVG) qPCR was run using published protocols as per Kokkinos and Clark (2006). Imported samples previously testing positive to SPVG in NCM-ELISA as part of ACIAR project HORT 2014/097 were used as positive controls.
- Published protocols for the detection of the exotic Sweetpotato chlorotic stunt virus (SPCSV) as per Kokkinos and Clark (2006) and Fuentes (2012) were used with samples previously imported as part of ACIAR project PC 2011/053.
- Phytoplasma qPCR protocols were obtained from DAF Mareeba and assays were run using positive control plants GRF 357, GRF 356 and GRF 1008.
- Positive control plants for Sweetpotato collusive virus (SPCV) were sent for next generation sequencing (NGS) to facilitate development of qPCR primers and probes in conjunction with Amit Sukal (QUT). Protocols are currently undergoing review and efficacy testing prior to publication. Positive control plants GRF 069 and GRF 85 and negative control plant PT Beauregard were used in this assay.
- SPCFV: In the absence of any published qPCR protocols to detect Sweetpotato chlorotic fleck virus this project facilitated the development of a specific qPCR primer/probe set based on published sequence data on identified isolates in conjunction with Dr Amit Sukal (QUT). Assays were run with known positive control plant GRF 323.

Ipomoea investigations

Current virus indexing relies on *I. setosa* as the graft plant, as it displays the broadest and most consistent range of virus symptoms. Emma Coleman and Sandra Dennien investigated the potential for alternative *Ipomoea* spp. to host sweetpotato viruses. The intent was to increase the indexing window (*I. setosa* can only be grown for 8 months of the year in Gatton) and determine if other *Ipomoea* species could offer different virus segregation opportunities to *I. setosa* (Outputs p13).

In conducting the above study, it became obvious there was confusion about national and international trade in *Ipomoea spp*. and the potential threat to the Australian sweetpotato industry. Mary Firrell produced a report detailing the status of *Ipomoea spp*. in Australia (Outputs p13).

Industry engagement

Direct grower visits

Project team experience meant we knew regular, direct contact with individual growers was key to establishing effective extension pathways. Project team members met with sweetpotato growers at least monthly throughout the project, as part of monitoring, experiment implementation, or issue identification activities (see Outputs p 21).

Industry events

The project team conducted 22 major industry field days or workshops during the project; ten in Bundaberg, eleven in Cudgen, and one on the Atherton Tableland. The workshops comprised presentations on project results to date, information on updated best-practice components, and advice on future activities. They also often involved presentations by allied sweetpotato researchers, from Australia, or international sweetpotato scientists.

Field days focussed on specific plant bed issues, such as the impacts of bedding root size, or premature breakdown of *Bellevue*.

In addition, we conducted a tour of the Gatton Research Facilities, focussing on virus diagnostics and plant bed research, in November 2014. This tour included hands-on grafting experiences, and laboratory demonstrations, and was attended by 30 growers and associated businesses from Bundaberg and Cudgen, as well as four Lockyer growers.

We advertised events by mail, email, SMS, phone and direct contact.

Best practice guides and factsheets

Using the information developed through monitoring, diagnostics and experimental activities, as well as industry consultation, the project team bi-annually updated Best Practice Guides for plant bed management, virus management and virus diagnostics. Sandra Dennien prepared a factsheet on Sweetpotato Chlorotic Stunt Virus, one of the most damaging sweetpotato viruses currently not in Australia (but probably present in close Pacific neighbours) in June 2016. These were lodged on the ASPG website and with Hort Innovation (Outputs p13), and growers were regularly advised of their existence at industry days and in email updates (see Outputs p19).

Broader community engagement

The project focus was on servicing levy payers; however, the project team gave presentations to broader farmer and community groups on several occasions. The project team also developed articles for general horticultural and rural media, which were carried in at least 8 publications (see Outputs p20).

Collaboration and developing industry capacity

National

During the project, the team regularly interacted with other sweetpotato researchers, particularly from Central Queensland University (CQU) and University of Southern Queensland (USQ), sharing information, and providing advice on industry issues. The team conducted collaborative activities around *Bellevue* breakdown (Appendix 7) and investigating sprout productivity (Appendix 8). The virus team enlisted the meta data analysis expertise of PhD student and SPC, Fiji virologist Amit Sukal with Queensland University of Technology (QUT), Northern Australia Quarantine Strategy (NAQS) and USQ (Appendix 10). The project also mentored and co-supervised internships and honours projects for two students from the University of Queensland (UQ) (Outputs p13).

International

Sandra Dennien continued close relationships with Segundo Fuentes from CIP Peru, and diagnosticians at SPC Fiji. The project team developed collaboration with USA sweetpotato researchers and facilitated visits to Cudgen and Bundaberg by several scientists attending the 2014 International Horticultural Congress in Brisbane (Appendix 11).

The project supported and co-funded visits by Sandra Dennien and Rachael Langenbaker (2015), and Craig Henderson (2017) to USA sweetpotato growing regions, and particularly Louisiana State University scientists Professor Arthur Villordon, Dr Chris Clark, and Dr Don Labonte. It also funded and hosted a return visit by Prof. Villordon to Cudgen, all Queensland sweetpotato regions, Sydney markets and Hort Innovation (Appendices 12-14).

Project management

The project team had nine project teleconferences with the ASPG Executive, to regularly provide updates and receive feedback on project activities. Mr Henderson had annual project reviews with the Hort. Innovation Program Manager, generally accompanied by a key ASPG grower. These reviews were usually around a major milestone report.

In February 2016, Professor Calum Wilson from the University of Tasmania conducted a mid-term review of the project. In response to his report, the project team added several new project activities around virus diagnostics, sprout productivity and collaborative international visits (Appendix 15).

Outputs

Sweetpotato industry management guides, literature reviews and fact sheets

The two management guides for growers, the sampling and diagnostic protocol, and the CSV fact sheet are the enduring written outputs from the project. They synthesise the research knowledge, project team and grower experiences, and collaborator wisdom gathered during the project. Apart from representing the current state of knowledge, they are also foundational blocks on which future technologies, research and experiences can improve.

The grower guides were updated annually during the project, and the April 2018 versions stand as the most up to date representations of industry best practice at the time of publication. Because there is current work on plant bed management and sweetpotato virus diagnostics (though somewhat limited in ongoing projects, it is probable that the management guides and diagnostic protocols will continue to be annually updated for several years.

Additionally, literature reviews on sweetpotato plant bed management and virus detection, a report on *Ipomoea* spp. trade and importation, as well as two student honours theses on herbaceous indexing and plant bed nutrition, were also completed as project outputs.

The full list of guides, factsheets and reports are:

- Managing sweetpotato plant beds guide 2018
- Managing sweetpotato viruses guide 2018
- Australian sweetpotato pathogen testing procedures 2018
- SPCSV fact sheet 2016
- Alternate *Ipomoea* species as a diagnostic tool 2017 (Emma Coleman Honours Thesis)
- Fertiliser impacts on sweetpotato plant beds 2017 (Robin Scarffe Honours Thesis)
- *Ipomoea* species importation implications 2018
- Sweetpotato virus detection review 2018
- Sweetpotato plant beds literature review 2015

All of these project documents, as well as this Final Report, are available on the ASPG website at:

http://www.aspg.com.au/past-projects/

Similarly, Hort Innovation also lodge some of these project materials on their sweetpotato website at:

http://horticulture.com.au/grower-focus/sweetpotato/

Industry workshops and field days

The focus for project extension was always about providing face to face contact with growers and industry. As can be seen below, the project team held major industry events in the key sweetpotato growing centres (Bundaberg and Cudgen) every 6 months.

Notes for interpretation of Output Tables.

* Denotes guest presentations at VG13004 organised extension event

** Denotes VG13004 presentation at event organised by other groups

+++ Denotes Topics NOT accompanied by available Presentation files. All other Topic presentations available on request from ASPG.

2014

DATE	LOCATION	PRESENTER	TOPICS	AUDIENCE	ATTENDEES
28 MAR 2014	Cudgen Leagues Club, Cudgen	Craig Henderson	Introduction to Hort Innovation project VG13004	Growers and industry	39
		Sandra Dennien	VG13004 virus research component		
		Craig Henderson	VG13004 plant bed research component		
04 APR 2014	CQU, Bundaberg	Craig Henderson	Sweetpotato innovation partnerships**	Growers, industry and community	>100
13 MAY 2014	Old Bundy Tavern, Bundaberg	Sandra Dennien	VG13004 virus research component	Growers and industry	48
		Craig Henderson	VG13004 plant bed research component		
19 AUG 2014	Cudgen Leagues Club, Cudgen	Craig Henderson	Optimising plant bed performance in sweetpotato	Growers and industry	37
		Arthur Villordon	Sweetpotato root architecture*		
		Christie Chang	Sydney sweetpotato market*		
		Mike Hughes	Sweetpotato research in Papua New Guinea*		
		David Picha	Sweetpotato post-harvest care*		
		Don Labonte	Sweetpotato cultivars from LSU*		
21 AUG 2014	Brisbane Convention Centre, Brisbane	Craig Henderson	Optimising plant bed performance through adaptive research with the Australian Sweetpotato Industry**	International sweetpotato scientists, growers and industry	31
25 AUG 2014	Old Bundy Tavern, Bundaberg	Craig Henderson	Optimising plant bed performance in sweetpotato	Growers and industry	62
		Arthur Villordon	Sweetpotato root architecture*		
		Theresa Arnold	Sweetpotato nutrition*		
		Mike Hughes	Sweetpotato research in Papua New Guinea*		
		Theresa Arnold	Sweetpotato plant bed densities*		
		Don Labonte	Sweetpotato cultivars from LSU*		
13 NOV 2014	Gatton Research Facility, Gatton	Sandra Dennien	Virus diagnostics (herbaceous indexing, ELISA, qPCR) $^{+++}$	Growers and industry	34
		Craig Henderson	Plant bed research and management^{+++}		
4 DEC 2014	Farm field walks, Bundaberg	Craig Henderson	Plant bed experimental work and early results^{+++}	Growers and industry	22
4 DEC 2014	Old Bundy Tavern, Bundaberg	Craig Henderson	Plant bed research update	Growers and industry	33
		Sandra Dennien	VG13004 virus research update		

2015

DATE	LOCATION	PRESENTER	TOPICS	AUDIENCE	ATTENDEES
23 MAR 2015	Cudgen Leagues Club, Cudgen	Craig Henderson	VG13004 plant bed management component update	Growers and industry	19
		Sandra Dennien	VG13004 virus diagnostics and management component update		
		Mike Hughes	Sweetpotato research in Papua New Guinea*		
18 JUN 2015	Bundaberg Research Facility, Bundaberg	Craig Henderson	VG13004 plant bed management component update	Growers and industry	39
		Sandra Dennien	VG13004 virus diagnostics and management component update		
		Mike Hughes	Sweetpotato research in Papua New Guinea*		
08-JUL 2015	CQU University, Bundaberg	Craig Henderson	Sweetpotato plant bed management research**	CQU researchers	15
03 AUG 2015	Cudgen Leagues Club, Cudgen	Craig Henderson	VG13004 plant bed management component update	Growers and industry	19
		Sandra Dennien	VG13004 virus diagnostics and management component update		
		Mike Hughes	Sweetpotato research in Papua New Guinea*		
29 OCT 2015	Cudgen Leagues Club, Cudgen	Sandra Dennien, Rachael Langenbaker	Sweetpotato trip to Louisiana, Alabama and California	Growers and industry	21
07 DEC 2015	Old Bundy Tavern, Bundaberg	Sandra Dennien, Rachael Langenbaker	Sweetpotato trip to Louisiana, Alabama and California	Growers and industry	34

2016

DATE	LOCATION	PRESENTER	TOPICS	AUDIENCE	ATTENDEES
11 MAR 2016	Gatton Research Facility, Gatton	Sandra Dennien	Sweetpotato diagnostic facilities and research	DAF Management	8
22 MAR 2016	Gatton Research Facility, Gatton	Sandra Dennien	Sweetpotato virus indexing: 'The unknown unknowns in sweetpotato'	QLD Horticulture Industry Leaders	14
18 APR 2016	Farm field walks, Cudgen	Craig Henderson	Factors impacting performance of plant bed cuttings in commercial fields	Growers and industry	19
21 APR 2016	Farm field walks, Bundaberg	Craig Henderson	Factors impacting performance of plant bed cuttings in commercial fields	Growers and industry	29
27 JUL 2016	National Horticultural and Innovation Expo, Gatton	Sandra Dennien, Rachael Langenbaker	Sweetpotato science**	Expo visitors	30 (full tent)
12 DEC 2016	Cudgen Leagues Club, Cudgen	Craig Henderson	Nitrogen fertiliser rates on plant beds have minimal impact on sprout production or performance	Growers and industry	16
		Sandra Dennien	Sweetpotato virus diagnostics and management		
		Mike Hughes	Sweetpotato on the Atherton Tablelands – a snapshot*		
		Emma Coleman	Alternate *Ipomoea* species as a diagnostic tool for virus detection in sweetpotato		
		Rob Scarffe	Nitrogen application to seedbeds		
		Bree Wilson	Biopesticides for sweetpotato pests*		
15 DEC 2016	Bundaberg Research Facility, Bundaberg	Craig Henderson	Nitrogen fertiliser rates on plant beds have minimal impact on sprout production or performance	Growers and industry	16
		Sandra Dennien	Sweetpotato virus diagnostics and management		
		Mike Hughes	Sweetpotato on the Atherton Tablelands – a snapshot*		
		Andy Mead	Identifying the opportunities and impediments to growing the export of sweetpotatoes into ASEAN markets*		
		Jady Li	CQU sweetpotato research*		
		Bree Wilson	Biopesticides for sweetpotato pests*		

2017

DATE	LOCATION	PRESENTER	TOPICS	AUDIENCE	ATTENDEES
17 OCT 2017	University of Queensland, Gatton	Robin Scarffe	Impacts of organic and inorganic nitrogen fertilisers on sweetpotato (*Ipomoea batatas*) sprout production**	UQ scientific staff and visitors	40
		Emma Coleman	Alternate *Ipomoea* species as a diagnostic tool for virus detection in sweet potato**		
17 OCT 2017	Bundaberg DAF Conference Facility, Bundaberg	Craig Henderson	Key factors for managing sweetpotato plant beds	Growers and industry	39
		Craig Henderson	Reducing breakdown risks in *Bellevue* plant beds***		
		Sandra Dennien	Virus diagnostics and management		
		Bree Wilson	Use of LAMP for soil biota analytics*		
		Craig Perry	Sweetpotato marketing program*		
		Brian Ramsay	Sweetpotato Strategic Investment Plan*		
		Craig Henderson	Sweetpotato Research Coordination*		
		Craig Henderson	Sweetpotato Biosecurity Activities*		
19 OCT 2017	Cudgen Leagues Club, Cudgen	Craig Henderson	Key factors for managing sweetpotato plant beds	Growers and industry	29
		Craig Henderson	Reducing breakdown risks in *Bellevue* plant beds+++		
		Sandra Dennien	Virus diagnostics and management		
		Bree Wilson	Use of LAMP for soil biota analytics*		
		Craig Perry	Sweetpotato marketing program*		
		Brian Ramsay	Sweetpotato Strategic Investment Plan*		
		Craig Henderson	Sweetpotato Research Coordination*		
		Craig Henderson	Sweetpotato Biosecurity Activities*		
19 OCT 2017	Farm field walks, Cudgen	Craig Henderson	Best practice in sweetpotato plant beds	Growers and industry	29
11 NOV 2017	Louisiana State University Field Station, Chase, Louisiana, USA	Craig Henderson	A snapshot of the Australian sweetpotato industry, including research activities and outcomes in VG13004**	Researchers and sweetpotato growers	18
15 NOV 2017	University of Queensland, Brisbane	Emma Coleman	Alternate *Ipomoea* species as a diagnostic tool for virus detection in sweet potato**	Bell Medal Awards night	>100
30 NOV 2017	Cudgen Leagues Club, Cudgen	Emma Coleman	Alternate *Ipomoea* species as a diagnostic tool for virus detection in sweet potato+++	ASPG AGM	20
		Rachael Langenbaker	Impacts of organic and inorganic nitrogen fertilisers on sweetpotato (*Ipomoea batatas*) sprout production+++		

2018

DATE	LOCATION	PRESENTER	TOPICS	AUDIENCE	ATTENDEES
13 MAR 2018	Cudgen Leagues Club, Cudgen	Sandra Dennien	Virus diagnostics and management	Growers and industry	22
		Craig Henderson	Breakdown stories in sweetpotato plant beds		
		Arthur Villordon	Fifty shades of phosphorus: A sweetpotato story*		
15 MAR 2018	Old Bundy Tavern, Bundaberg	Sandra Dennien	Virus diagnostics and management	Growers and industry	37
		Craig Henderson	Breakdown stories in sweetpotato plant beds		
		Arthur Villordon	Fifty shades of phosphorus: A sweetpotato story*		
15 MAR 2018	Bundaberg Research Facility, Bundaberg	Rachael Langenbaker	Aeroponics and rhizotron studies of early sweetpotato root development+++	Sweetpotato researchers and consultants	12
		Jady Li	Nematode research in sweetpotatoes*+++		
		Tham Dong	Hydroponic and pot studies of sweetpotato storage root initiation and development*+++		
19 MAR 2018	Kairi Hotel, Kairi	Mike Hughes	Sweetpotato research and development in Australia: a quick overview	Growers and industry	19
		Arthur Villordon	Fifty shades of phosphorus: A sweetpotato story*		

Sweetpotato industry updates

Regular project updates were circulated to growers and industry representatives for the duration of VG13004. In the industry feedback survey following the December 2016 industry meetings, growers confirmed they preferred information via personal contact (visit, phone call or SMS), rather than web or email. The project team concentrated on one-one connection as the preferred form of extension engagement. Because of the small and geographically contained production area of the industry, this proved both achievable and successful. Our team conducted numerous visits to farms or small neighbour groups, combining experimental activities with casual discussions about industry issues, problems and innovations. Growers were also reminded of the availability of updated or new materials on the ASPG and Horticulture Innovation websites, which are freely accessible to all.

DATE	DISTRIBUTION	AUTHOR	SUBJECT	AUDIENCE	RECIPIENTS
28 APR 2014	Email and postal list	Craig Henderson	VG13004 project introduction	National sweetpotato industry contact list	61
13 NOV 2014	Event attendees	Craig Henderson	VG13004 MS102 pdf	Growers and Industry	34
02 APR 2015	Email	Craig Henderson	VG13004 project update pdf	National sweetpotato industry contact list	73
30 JUN 2015	Email	Craig Henderson	VG13004 project update pdf and links to sweetpotato management guides	National sweetpotato industry contact list	75
17 JUN 2016	Email and postal list	Craig Henderson	VG13004 MS105 summary notes and pdf	National sweetpotato industry contact list	73
1 JUL 2016	Email and postal list	Craig Henderson	Sweetpotato chlorotic stunt virus fact sheet and summary notes	National sweetpotato industry contact list	73
06 DEC 2016	SMS, email and postal lists	Craig Henderson, Rachael Langenbaker, John Maltby	Sweetpotato industry field day notices (including VG13004 project items)	National sweetpotato industry contact list	81
03 OCT 2017	SMS, email and postal lists	Craig Henderson, Rachael Langenbaker, John Maltby	Sweetpotato industry field day notices (including VG13004 project items)	National sweetpotato industry contact list	81

ASPG Executive updates

DATE	LOCATION	PRESENTER	TOPICS	AUDIENCE	ATTENDEES
19 JUN 2014	Teleconference	Craig Henderson	VG13004 progress update	ASPG Executive	12
18 SEP 2014	Teleconference	Craig Henderson	VG13004 progress update	ASPG Executive	12
14 APR 2015	Teleconference	Craig Henderson, Sandra Dennien	VG13004 progress update	ASPG Executive	12
15 OCT 2015	Teleconference	Craig Henderson, Sandra Dennien	VG13004 progress update	ASPG Executive	12
14 JUL 2016	Teleconference	Craig Henderson	VG13004 progress update	ASPG Executive	12
01 FEB 2017	Teleconference	Craig Henderson	VG13004 progress update	ASPG Executive	12
27 JUL 2017	Teleconference	Craig Henderson	VG13004 progress update	ASPG Executive	12
12 OCT 2017	Teleconference	Craig Henderson	VG13004 progress update	ASPG Executive	12
07 FEB 2018	Teleconference	Craig Henderson, Sandra Dennien	VG13004 progress update	ASPG Executive	12

General media publications

15 December 2014 - New virus diagnostics and planting bed management for Australian sweetpotato. *Vegetable Advisory Committee Annual Report* **2013/14**, p 36.

17 August 2015 – Sweetpotato growers enthusiastic about supporting focussed research. *Hortlink* **Spring edition 2015**, online at http://hortlink.horticulture.com.au/Spring2015/4/

19 August 2016 – Sweetpotato research looks to healthy roots. *Good Fruit and Vegetables* **August 2016**; online at http://www.goodfruitandvegetables.com.au/story/4105363/sweetpotato-research-looks-to-healthy-roots/?cs=4917

18 September 2016 – Sweetpotato industry tackles viruses through science. *Good Fruit and Vegetables* **September 2016**; online at http://www.goodfruitandvegetables.com.au/story/4167538/sweetpotato-industry-tackles-viruses/?cs=4928

19 September 2016 - Science takes on sweet potato viruses. *Fresh Plaza* http://www.freshplaza.com/article/163638/Science-takes-on-sweet-potato-viruses

28 September 2016 – Sweetpotato project showing steady progress. *Vegetables Australia*, **September/October 2016,** pp 52-53.

15 December 2017 - Sweetpotato industry clues up for the future. *Queensland Country Life.* http://www.queenslandcountrylife.com.au/story/5110142/sweetpotato-industry-clues-up-for-the-future/

15 December 2017 - Sweetpotato industry clues up for the future. *Good Fruit and Vegetables.* http://www.goodfruitandvegetables.com.au/story/5110142/sweetpotato-industry-clues-up-for-the-future/?cs=4928

Targeted grower visits

Rachael Langenbaker, Sandra Dennien and to a lesser extent Craig Henderson, regularly visited Bundaberg, Lockyer Valley and Cudgen growers, at least weekly in Rachael's case (because of the larger grower numbers), as part of their project activities. This provided ongoing opportunities for extension and two-way communication between the project team and growers. In North Queensland, Mike Hughes visited the increasing number of sweetpotato growers in that region and kept them apprised of VG13004 activities and results. Because of his regular connections with the VG13004 team, he was well positioned to do so.

2-7 March 2015 – Craig Henderson, Rachael Langenbaker and Sandra Dennien visited Cudgen, Bundaberg and Rockhampton sweetpotato growers and discussed their virus, plant bed and general agronomic issues. They developed notes from this visit for future extension activities.

15-17 February 2016 – Craig Henderson, Rachael Langenbaker, Sandra Dennien and ASPG representatives visited Cudgen and Bundaberg sweetpotato growers, accompanying University of Tasmania Professor Calum Wilson on his mid-term project review. We discussed all aspects of project activities. Prof. Wilson's review is outlined in more detail in Appendix 15.

19-26 August 2014 – Craig Henderson, Rachael Langenbaker, Sandra Dennien and associated researchers visited Cudgen, Gatton and Bundaberg, sweetpotato growers, accompanying scientists from Louisiana and North Carolina State Universities on their industry tour. We discussed general industry agronomy and disease issues, as well as specific project activities. This visit is outlined in more detail in Appendix 12.

22-24 November 2016 – Rachael Langenbaker and Sandra Dennien visited North Queensland sweetpotato growers and discussed their virus, plant bed and general agronomic issues. They developed notes from this visit and presented their findings to growers at the December 2016 Sweetpotato Industry Days.

14-19 August 2017 – Rachael Langenbaker and Sandra Dennien visited Cudgen and Bundaberg sweetpotato growers and discussed their virus, plant bed and general agronomic issues. They developed notes from this visit, with their findings used at the October 2017 Sweetpotato Industry Days.

12-14 September 2017 – Craig Henderson, Rachael Langenbaker and Sandra Dennien visited Cudgen and Bundaberg sweetpotato growers and discussed their virus, plant bed and general agronomic issues. They developed notes from this visit, with their findings used at the October 2017 Sweetpotato Industry Days.

12-22 March 2018 – Craig Henderson, Rachael Langenbaker, Sandra Dennien and associated researchers visited Cudgen, Bundaberg, Rockhampton and North Queensland sweetpotato growers, accompanying Louisiana State University Professor Arthur Villordon on his industry tour. We discussed plant bed management and general agronomic issues. Prof. Villordon's visit is outlined in more detail in Appendix 14.

Outcomes

Substantial sweetpotato plant bed practice change and improved productivity

At the start of the project in 2014, we were monitoring values of around 200 sprouts/m^2 as a common plant bed productivity value, and this was usually for the most common cultivar, *Beauregard* (Appendix 1).

In the four seasons monitoring growers' plant beds, we observed the following issues and consequences (Appendix 2).

- Widescale replacement of *Beauregard* with *Bellevue* and *Orleans*. The shift to *Bellevue* has seen a dramatic rise in:
    a) problems with sprouting, particularly in cooler weather, and
    b) problems with premature plant bed breakdown, after only 1-2 cuts.
- Plant beds rotting due to being waterlogged, or because bedding roots were covered by too much soil (more than 10 cm in some instances).
- Slow recovery of plant beds, after sprouts being cut too close to the soil surface.
- Sporadic, but not unusual outbreaks of disease in plant beds.

The premature breakdown of plant beds, usually associated with *Bellevue*, was by far the most widespread and difficult problem (see Appendix 5 Introduction for more detailed discussion). This was not an anticipated problem at the start of the project.

Industry practice change between 2014 and 2017/18

Bedding root supply

The specifications for PT bedding roots have both tightened and become more nuanced. The supplier is generally harvesting his crop earlier, so there are more small-medium roots, and fewer large and oversize roots. Roots larger than 9 cm in diameter are very rarely supplied, unless there is a shortage, and the commercial grower accepts the risks. *Bellevue* in particular is usually supplied in the 4-8 cm range. Research showed within the 4-9 cm diameter range, there was no difference in sprout productivity/m^2, or the quality off sprouts produced. The smallest bedding roots may be a few days slower to produce premium quality shoots (Appendix 3, Appendix 6). The project developed a rule of thumb for plant bed requirements of bedding roots (Appendix 3):

Quantity of bedding roots required (kg) = 3 * Area of plant bed (m^2) * median diameter of bedding roots (cm)

For example, given a 100 m long plant bed 1 m wide; a bedding root supply of median diameter 6 cm, the grower would need approximately 1.8 t of bedding roots to install that bed.

The most recent consideration is avoiding supply of aged *Bellevue* bedding roots. Even with good storage conditions; current thinking is that roots more than 4 months old probably only have a short plant bed life, independent of their field management.

Close attention is paid to temperature management in storage at the bedding root production facility. Heating treatment is no longer done of any roots, unless a grower specifically requests it, and is clearly informed of, and accepting the risks. Any heat treatment is no more than 28-30^oC, and generally for less than 24 hrs.

Where possible, the transport of roots is via direct supply to the growers, to avoid issues with sub-optimal storage in distribution warehouses.

Plant bed timing

We have seen several growers shift from 1-2 plant bed installations per year, to four, and even five separate supplies. This is potentially because they are concerned about virus reinfection of their plant beds through the season, but also because feel they are unable to maintain plant bed productivity, or the quality of their sprouts, past about 4 cuts. It is unclear the value of this strategy, however the fact that several large growers are doing it, suggests it is at least cost neutral for them.

Our plant bed research and indexing has shown sprout generation can potentially continue at highly productive levels (around 230 sprouts/m^2) for at least 5 cuts if bed conditions are good. We found sprout quality (apart from virus load) did not decline as the season progressed, just the capacity of the plant bed to produce high numbers of sprouts, if there is substantial breakdown.

Plant bed construction

One of the biggest changes has been the heights of grower plant beds. Almost all growers would now have beds of at least 25 cm high, and some narrower beds are closer to 45 cm high. Low beds were often associated with premature breakdown (Appendix 2). Well-drained plant beds appear the key factor in coping with adverse weather (Appendix 5).

Most growers are very careful with their depth of soil coverage over their bedding roots. Early in the project, poor sprout emergence and root breakdown was often associated with deep burial (Appendices 2-3). Growers encourage their workers to press the larger roots deeper into the soil, to have a level height along the tops of the roots. Alternatively, some are grading their roots for size, and installing them in separate areas, to achieve the same effect. Most growers are looking at 2-4 cm of soil coverage. Ideally, 3-5 cm is probably optimum in most circumstances, to encourage independent rooting of the developed sprouts (Appendix 4, Appendix 5, Appendix 7). Deeper coverage may be more helpful in hot conditions. In the latter seasons, we were seeing more problems with insufficient coverage, due to rain or slumping uncovering roots.

Plant bed nutrition

Growers have started using more complete fertilisers (organic and inorganic), applying them both as a basal, and regularly during the production season. They are less fearful of over-fertilising and understand the importance of a sprout with high nutrient levels (Appendix 4, Outputs p13). This was further emphasised by results presented during Arthur Villordon's recent visit to Australia, which showed that sprouts with the best nutrient levels and well-grown foliage initiated the most storage roots (Appendix 14).

Plant bed irrigation

Growers have become much more aware of not overwatering their plant beds, either through over-irrigating early in the production season, or having extremely non-uniform irrigation systems. They have also moved more to overhead irrigation, except some growers, who still use drip irrigation under their plastic. Most producers now use solid-set systems specifically designed for their plant beds.

In the final project season, most growers kept their plant beds relatively dry, until sprouts were well shot. This was probably a reaction to *Bellevue* breakdown in previous seasons. The optimal balance may be a little more water early on, to promote some bedding root system development (Appendix 5, Appendix 7). Certainly, the guide of relatively dry under plastic is still a good option.

Plastic management

The other substantial change has been how growers use plastic to heat their plant beds. Temperature certainly appears to play a key role in premature bedding root breakdown and poor plant bed performance (Appendix 5, Appendix 7). In the final project year, growers sacrificed early *Bellevue* production by not using any plastic at all. Other growers used plastic, but preferred to use a hooped system, that provided more air flow, and slightly less extreme heat build-up. The other change is growers being much more conscious of soil temperatures, rather than using air temperature, or amount of burn on the sprouts, as the signal for plastic removal. There has also been a shift to row-cover floating meshes, which are also associated with less rapid heat build-up.

Because it appears temperature management is going to be one of the key issues for successful plant bed longevity, it is likely that soil temperature loggers and warning systems will become much more common.

In terms of practice, growers are much more aware of plastic manipulation, and will regularly move plastic on and off their plant beds, in response to either weather predictions, perceived or real temperature fluctuations.

Sprout harvesting and sorting

Sprout harvesting crews are now more careful in the precision with their cutting, starting at around 3 cm for the first cut, and generally cutting above the scars in ensuing cuts.

A significant proportion of growers are now doing a field or shed sort, to provide higher quality, and more uniform planting pieces for their planting crews. This is particularly the case for machinery-planted operations. Where possible, growers are going for a longer planting piece (35-45 cm), to try and maximise their yield potential.

The project used the following criteria to grade sweetpotato sprout (Appendix 8):

Acceptable sprouts

No visible damage or disease, an intact and vigorous tip, at least 20 cm long, at least 3 mm diameter at the cut end, and at least 1 node within 15 cm of the cut end.

Premium sprout (increased resilience in difficult planting conditions, yield advantage of around 10%)

No visible damage or disease, an intact and vigorous tip, at least 28 cm long, at least 4 mm diameter at the cut end, and 2-5nodes within 15 cm of the cut end.

Timing of harvesting

Premature sprout harvesting can reduce mean sprout length, and the proportion of premium sprouts. Extremely premature sprouting can reduce acceptable sprouts. Delaying harvesting beyond the optimal window can increase tip damage and harvesting/processing time, due to tangling and excessive length (Appendix 3 Discussion).

Productivity result

During the life of the project, we have seen sprout productivity from plant beds jump to around 250 sprouts/m^2 (a 25% improvement), with some growers achieving an even higher benchmark. This is notwithstanding the introduction of *Bellevue* as a cultivar, which is a particularly problematic plant bed performer. With optimum timing of sprout harvesting, at least 80% of those sprouts can achieve premium grade, although this is a little more difficult with *Bellevue*.

This is an outstanding improvement in industry performance.

Specific focus on managing cultivar *Bellevue* in plant beds

Monitoring, and experimental observations generated the following recommendations for cultivar *Bellevue* in plant beds (Appendix 2, Appendix 4, Appendix 5, Appendix 7).

- Where possible, use small-medium bedding roots, preferably no more than a few months old, and keep in consistently cool storage conditions (16°C) prior to installation.
- Don't use roots with unhealed wounds or apparent disease.
- If using plastic to heat beds, make sure the plastic structure is well ventilated, and monitor soil temperatures. Ideally keep soil temperatures below 30°C, and perhaps even around 25-26°C. If temperatures are likely to rise above that level, take the plastic off. Similarly for row covers.
- Avoid installing *Bellevue* into plant beds in circumstances likely to experience hot temperatures. Established plant beds can potentially survive, but it's possible new beds are more vulnerable.
- Ensure any irrigations at installation, and before sprouts are established, are even and light.
- Good drainage is essential.
- Avoid installing plant beds in ground with a known history of diseases, particularly bacterial.

Improved sweetpotato planting material research techniques

Sand culture systems in a controlled environment appears the most effective way to investigate the quality and potential performance of sweetpotato planting material (Appendices 8-9, Appendices 13-14).

Crucial factors for consideration are:

- Consistent, high light levels, to maximise photosynthate accumulation of developing shoots. This requires the use of artificial light sources, rather than relying on natural light, particularly with the shading in most glasshouse structures. Recommended, a minimum 400 uM m^{-2} s^{-1} of photosynthetically active radiation.
- Use of a standard nutrient solution (e.g. Hoagland's) in either an aeroponic or sand culture system.
- Avoidance of aeration issues, by manipulating solution-spraying intervals (aeroponics) or solution-addition intervals (sand culture).
- Avoidance of compaction/compression issues in growing media, by loose addition of sand, and ensuring large enough pots to not restrict root development.
- Regulation and standardisation of growing temperatures where possible.
- Use of standard control cultivars, of known productivity, for comparison.

Reduced sweetpotato virus incidence in commercial sweetpotato growing areas

Potentially due to a higher prevalence of domestic sweetpotato gardens, and more historical sweetpotato interaction with neighbouring countries, North Queensland appears to have greater presence of multiple virus infections and less common viruses (Appendix 10). In the future it will be important to maintain a virus watch presence in this region.

Sweetpotato Leaf Curl Virus (SPLCV) and Sweetpotato Feathery Mottle Virus (SPFMV), were by far the most common viruses detected in commercial production areas, with no sense that other viruses are present in regions where PT systems are used.

Testing suggests virus incidence is initially low in spring, and then builds to a peak in summer, as plant beds age and vector numbers increase. Symptoms decline as the weather cools.

Fortunately, the source of pathogen tested bedding roots never returned any detectable viruses through indexing, ELISA and qPCR testing of collected samples.

Ipomoea species as regional virus reservoirs and potential herbaceous indexing test plants

Several *Ipomoea* species were shown to be susceptible to graft transmitted sweetpotato viruses and Morning glory (*Ipomoea plebia*) weeds collected from commercial areas tested positive to SPFMV and SPLCV, indicating that that they provide a virus reservoir within commercial cropping regions (Appendix 10). Also, many home garden *Ipomoea* spp. also proved very susceptible to SPFMV infection. Some species appeared asymptomatic, however the samples are still to be tested to see if they were infected, which would be a biosecurity concern, as they could carry and spread current or new viruses (Outputs p13).

Although many *Ipomoea* spp. e.g. *I. purpurea, I. coccinea* and *I. nil* cv. Pink Morning Glory. did provide symptoms of SPFMV, none proved as reliable at expressing the range of symptoms as *I. setosa* (Outputs p13).

Enhanced Grower awareness of virus management and biosecurity

A majority of Australian sweetpotato growers are familiar with virus occurrence and diagnostic procedures, through participating in the GRF Sweetpotato Field Day in November 2014, and reinforcement at workshop presentations. Growers demonstrated effective virus knowledge, by often pointing out symptoms in plant beds or commercial crops for sampling.

The Australian sweetpotato industry is on alert for unusual symptoms that may indicate a potentially damaging incursion of a new virus (e.g. sweetpotato chlorotic stunt virus) see SPCSV fact sheet (Outputs p13).

Particular biosecurity risks are the range of *Ipomoea* spp. being traded through regulated and unregulated channels, as well as endemic populations of native and escaped species. Information on many of these species is provided in the *Ipomoea* import implications report (Outputs p13).

Improved virus diagnostics

Throughout the project, diagnostic protocols have been refined, and the most recent methodologies are captured in the Australian sweetpotato pathogen testing procedures 2018 (Outputs p13).

qPCR assays produced accurate results comparable to existing diagnostics for the detection of the exotic SPVG virus and the endemic Phytoplasma. Results for other endemic sweetpotato viruses such as SPFMV, SPCFV, SPCV, SPLCV and V2 using qPCR and qRT-PCR suggest that more work is needed to optimise assays to improve accuracy (Appendix 10).

Whilst every attempt was made to sample plants concurrently and to use the same individual symptomatic leaves in NCM-ELISA and qPCR/qRT-PCR assays, this was not always practical. It is possible that movement of virus particles within indicator plants between sampling times, unknown distribution of virus particles within plants and sampling of different leaves as well as low titres (common to some sweetpotato viruses) all contribute to differing accuracy levels between diagnostic tests. Sweetpotato plants are mostly asymptomatic, but although indicator plants produce foliage symptoms readily, symptoms are often confined to a limited number of leaves which need to be utilised for DNA and RNA extraction, NCM-ELISA and tissue preservation, hence asymptomatic leaves from virus infected plants are sometimes used.

Initial qRT-PCR assays for SPV2 and the exotic SPCSV unfortunately did not produce any amplification and as such were null and void. This could be due to several factors such as non-viable controls, quality of primers and experimental error. New positive controls for qRT-PCR assays for SPV2 and the exotic SPCSV would need to be obtained and assays retested.

Tissue from sweetpotato plants and indicator plants yielded similar results in qPCR for SPLCV. However, sweetpotato plant tissue yielded negative results in initial SPFMV qRT-PCR assays, whilst tissue from indicator plants (*I. setosa*) yielded positive results from both dried and fresh tissue. This could be influenced by inhibitors in sweetpotato sap, the area of the plant that was sampled (differing distribution of virus particles within plants), the time of year that plants were sampled (stress may affect virus particle distribution and titre), and whether plants were symptomatic or asymptomatic (affecting titres).

qPCR is cost effective and labour saving and has the potential to speed up routine virus diagnostic capability and is a valuable addition to the suite of complimentary methods used in sweetpotato virus detection at GRF. Further work needs to be done on primers and protocols to optimise accuracy and efficiency.

For this reason, the current Australian PT system relies on a combination of complimentary diagnostic techniques, Phyto-diagnostics, NCM-ELISA and the development of qPCR.

Phyto-diagnostics can detect the presence of virus infection including new or “novel” viruses using biological indicator plants. As indicator plants are highly sensitive to most sweetpotato infecting viruses, leaf symptoms produced are more apparent than those observed on sweetpotato plants. This sensitivity of indicator plants to virus infections also facilitates rapid increases in virus titres, enhancing downstream diagnostics such as NCM-ELISA and PCR. Phyto-diagnostic indexing as part of the Australian PT protocol is conducted by experienced, skilled staff at Gatton Research Facility (GRF) as some virus symptoms can be transient (Potyviruses), difficult to discern at lower titres (SPLCV, SPCV) and mixed infections influence symptom expression. The lack of symptoms on some rounds indicates the need for repetition as recommended in Dennien *et al.* (2013). However, this process is time consuming and requires considerable glasshouse space.

The (NCM- ELISA (CIP) test is able to detect 10 known sweetpotato infecting viruses, (C-6, CMV, SPCaLV [now called SPCV], SPCFV, SPCSV, SPFMV, SPLV, SPMMV, SPMSV and SPVG).

These virus detection technologies offer high levels of repeatability and reproducibility. Boonham *et al.* (2013) suggests that qPCR will become the most widely used diagnostic technology in testing laboratories around the world. NGS technologies are rapidly becoming more affordable and are a promising platform for developing future portable devices for plant pathogen detection devices. While there is a need to adopt new molecular technologies, the use of herbaceous indicators remains essential, due to their wide-ranging ability to detect all sweetpotato viruses.

Initial results sequencing

Further analysis and assembly of sequencing mega data is ongoing, and identification of specific strains present in country could facilitate the design of specific primers for accurate detection of viruses reported from Australia and the production of specific SPLCV antisera to be added to the NCM-ELISA suite of tests (Appendix 10).

There are no antisera as yet developed for SPLCV in NCM-ELISA, but negotiations are underway to initiate development, based on strains identified in next gen sequencing.

Enhanced sweetpotato industry integration with Australian biosecurity services

Sandra Dennien and Rachael Langenbaker met with members of the Northern Australia Quarantine Survey (NAQS) officers in Cairns in November 2016. These officers are tasked to collect samples on a regular basis from northern Queensland, including the Torres Strait islands and into southern, and central Papua New Guinea. Guidelines were given on what to look for when collecting samples; most effective methods for tissue conservation and transport; and the new movement restrictions for plant material from Torres Strait islands to the mainland.

Sandra Dennien has developed a strong network of diagnosticians, as well as good working relationships with surveillance practitioners, including the Northern Australian Quarantine Strategy. The Australian industry virus diagnostic protocols are being standardised for partners in Oceania and are very similar to protocols more recently developed in the USA.

Laboratory visits

Sandra Dennien has visited the following laboratories: DAF virology laboratory at Eco sciences precinct, Brisbane; the DAF plant pathology laboratory in Mareeba; the USQ crop health laboratory in Toowoomba; the LSU sweetpotato virology laboratory in Louisiana, USA; and the NAQS quarantine pathogen diagnostic laboratory in Cairns.

Information was gathered, and learnings were made on protocols and time saving tips on processes for a range of detection techniques, new learnings on the use of NCM-ELISA, qPCR work flow planning, suitable equipment required and the advantages and disadvantages of different makes and models, laboratory maintenance and classification, primer design and master mix concentrations, cataloguing and storing of samples, and safe handling and operating procedures.

To further her knowledge on qPCR and as part of LAMP development, Ms Dennien hopes to visit the centre for Agri Bioscience at LaTrobe University in Melbourne and the NSW DPI Elizabeth Macarthur institute (Centre of Excellence in Plant and Animal Health), in NSW later this year.

Better access to information

During the project, sweetpotato growers, allied industry, collaborators and interested people have been able to access reference information on:

- occurrence, importance, diagnostics and current management protocols for viruses
- plant bed management

via ASPG and Hort Innovation websites (Outputs p13), as well as attending ASPG and project events (see Outputs p14-18).

Successful sweetpotato industry engagement

At the commencement of the project, the baseline survey (Appendix 1) identified current plant bed management systems, productivity, and issues. The productivity gaps and issues raised informed the initial experimental and monitoring focus for the plant bed activities. The willingness of growers producing 95% of Australia's sweetpotatoes to participate in the project continued for its duration.

The ready adoption of practice change, constant requests for advice, regular attendance and support of industry events, and enthusiasm for face to face visits by project personnel all indicate successful industry engagement (see Outputs). A good example of engagement was the November 2014 showcase at Gatton Research Facility, where over 30 growers attended, most driving 3-6 hours, and staying overnight.

Growers provided positive feedback to most events, rating as either good or very good. They were certainly willing to critique aspects they felt required improvement (see Monitoring and evaluation).

Improved grower and scientist awareness of international sweetpotato systems and research

Through the visits from sweetpotato scientists from the USA to Australia, and the core project team to the USA, both the scientists and subsequently the Australian industry, became aware of research and commercial production technologies and trends in the USA. Of particular value were understanding the nuances of Louisiana sweetpotato cultivars that are now the mainstay of the Australian industry, virus diagnostic techniques and priorities, the physiology driving storage root initiation and development, and sweetpotato investigative techniques in controlled environments (Appendices 11-14).

Improved industry capacity and research involvement

The project experience has enhanced the skills and knowledge of the core research team. The quality of Ms Dennien's research work within VG13004 has been recognised by her acceptance into a Master's Degree at the University of Queensland. She has achieved promotion, in recognition of her skills, experience, achievement, and international status as a sweetpotato virologist.

Rachael Langenbaker's role is being reclassified in recognition of her skills, and performance within the project. Ms Langenbaker completed a Diploma of Horticulture, based on Recognition of Prior Learning achievements within the project. She is performing an on-ground, development role within the sweetpotato industry, based on her experiences and knowledge improvement within the project.

The project team mentored two University of Queensland Honours students. Emma Coleman received a Distinction for her initial internship within the project. Both Emma Coleman and Robin Scarffe completed Honour's studies within VG13004 and received their Honour's degrees (Outputs p13). Emma was a finalist for the UQ Bell Medal for Best Honours Thesis, a recognition of her challenging work. It emphasises the value in supporting additional research activities within projects such as VG13004.

Emma Coleman and Robin Scarffe gained sweetpotato industry experience within the project. Emma continues to work in sweetpotato diagnostics, and with Jean Bobby and Mary Firrell, have enhanced virus diagnostic and management capacity within the industry. Through collaboration in VG13004, and an allied ACIAR sweetpotato project, the University of Southern Queensland (especially Dr Bree Wilson), have become significantly engaged with the sweetpotato industry. This has brought a major increase in laboratory diagnostic skills and resources into the potential industry service space. Similarly, nematode and plant physiology studies at the Central Queensland University have readily engaged with the core project team and are enhancing R&D resources within the sweetpotato industry (Appendix 14).

Enhanced international collaboration

Through international exchange visits, and constant communications, project team members have enhanced strong international collaborative networks in sweetpotato disease diagnostics and agronomy. Sandra Dennien has very strong virology networks through Oceania (National Agricultural Research Institute PNG, Secretariat for Pacific Community Fiji), USA (Louisiana State University) and with the Center for International Potato Research in Peru. The reciprocal visits between Australian and USA sweetpotato researchers (Appendices 11-14) have exchanged knowledge and expertise. In addition, there is increased interest in collaborative research, which can only benefit the Australian sweetpotato industry. These issues were specifically discussed in Prof. Villordon's recent visit to Australia.

Monitoring and evaluation

Even though this project pre-dated Hort Innovation Monitoring and evaluation requirements, the project team addresses the following key evaluation questions (Table 1)

Table 1. Project key evaluation questions.

Key evaluation questions	Project-specific questions
Effectiveness	
1. To what extent VG13004 achieved its expected outcomes?	*To what extent has VG13004 increased the adoption of the industry Best Practice Guidelines in plant bed and virus management?* *To what extent has VG13004 increased the productivity of sweetpotato plant beds, and improved potential productivity of commercial sweetpotato plantings?* *To what extent has VG13004 improved our understanding of the viruses currently present in the Australian sweetpotato industry, and potential biosecurity virus threats?* *Has VG13004 developed new virus diagnostic technology that is now available for industry uptake?*
Relevance	
2. How relevant was the project to the needs of intended beneficiaries?	*To what extent has VG13004 met the needs of sweetpotato industry levy payers?*
Process appropriateness	
3. How well have intended beneficiaries been engaged in the project?	*To what extent were the target engagement levels of sweetpotato industry levy payers achieved?* *Have regular project updates been provided through linkage with the industry communication project?*
4. To what extent were engagement processes appropriate to the target audience/s of the project?	*Did the project engage with sweetpotato industry levy payers through their preferred learning style?* *How accessible were extension events to sweetpotato industry levy payers?*
Efficiency	
5. What efforts did VG13004 make to improve efficiency?	*What efforts did the VG13004 project team make to improve efficiency?*
Other (if any)	
6. What key aspects in the conduct of VG13004 can be improved in future projects?	*What key aspects in the conduct of VG13004 can be improved in future projects?*

To what extent has VG13004 increased the adoption of the industry Best Practice Guidelines in plant bed and virus management?

Extensive practice change has occurred with Australian sweetpotato growers' management of their plant beds, as outlined in Outcomes. Clear examples are the change in the heights of plant beds, and the much shallower covering of bedding roots, since the project began. We have also monitored more growers doing field or shed sorts of cut sprouts, to try and maximise plantings of premium planting material. We do notice growers targeting different sprout lengths, to suit their mechanical planting equipment or hand-planting styles.

Probably the clearest examples have been around managing cultivar *Bellevue* in plant beds. During experiences with premature plant bed breakdown in the 2015/16 and 2016/17 seasons, we had ongoing discussions with growers and the bedding root supplier about potential causes and solutions. As a result, we recommended smaller bedding roots, limited or no- pre-heat treatment, reduced early irrigation and careful management of bedding plastic. In the 2017/18 season, all these recommendations were taken on board by most *Bellevue* growers.

This reactive practice change comes about through regular industry engagement, and good relationships with the bedding root supplier and key growers. Reinforced by presentations and conversations at field days and workshops, where growers readily take part in the discussions.

Growers have demonstrated excellent understanding of sweetpotato viruses and keys to successful management. Most growers can identify virus symptoms when they are clearly expressed, although as sweetpotatoes are often asymptomatic unless stressed, this is not reliable. Their widespread adoption, and increased frequency of use of PT materials, shows they know the importance of regular renewal of planting material. Growers also regularly spray to manage vectors, and actively manage volunteer crops that could be virus reservoirs.

The project team assesses there has been very rapid, interactive adoption of best practice guidelines (Managing sweetpotato plant beds guide 2018; Managing sweetpotato viruses guide 2018 - See Outputs p13), based on our monitoring observations and grower discussions during the project.

To what extent has VG13004 increased the productivity of sweetpotato plant beds, and improved potential productivity of commercial sweetpotato plantings?

During the life of the project, we have seen sprout productivity from plant beds jump to around 250 sprouts/m^2 (25% improvement), with some growers achieving an even higher benchmark. This is notwithstanding the introduction of *Bellevue* as a cultivar, which is a particularly problematic plant bed performer. With optimum timing of sprout harvesting, at least 80% of those sprouts can achieve premium grade, although this is a little more difficult with *Bellevue*. Because of *Bellevue*, fewer growers persist with long-term plant beds, although we still see some plant beds lasting 6 months, if well managed by the grower, and with favourable weather conditions.

Because of the shift to a completely new suite of cultivars, it is difficult to compare productivity of commercial plantings between the start and end of the project. What we can say is that the project has supported the successful transition to plant bed production of new cultivars, which in themselves have dramatically lifted commercial productivity. Without the nematode resistant *Bellevue*, the industry would have really struggled with few nematode control options. *Orleans* has delivered a higher quality sweetpotato, which has supported sales in periods of oversupply. We have also seen growers shift their focus to better quality sprouts, through more careful cutting and sorting, which research suggests should improve potential yields by around 10%.

To what extent has VG13004 improved our understanding of the viruses currently present in the Australian sweetpotato industry, and potential biosecurity virus threats?

The extensive survey and sampling of sweetpotato crops, weeds, home gardens and markets through the project has given a very clear picture of the extent of sweetpotato viruses, both geographically and temporally. It has confirmed that the advent and widespread use of PT systems by growers has reduced the range of viruses found in the main production areas of Central and Southern Queensland, and Northern NSW. It is evident that endemic populations of SPFMV and SPLCV are the main viruses in those areas.

The project has identified North Queensland as a hotspot of a broader range of viruses, and multiple virus complexes. In addition to the two viruses mentioned above, SPCFV and SPCV were also detected in this region. The project encouraged biosecurity services to focus on sampling and monitoring in that area, which is an ongoing activity.

Has VG13004 developed new virus diagnostic technology that is now available for industry uptake?

qPCR technology proved successful for detecting phytoplasma and exotic SPVG. Although we got 65-85% positive detections with qPCR from samples detected as positive for SPFMV, SPLCV or SPCV with other methods, that is too many false negatives to be considered a reliable replacement for current testing protocols. The project team is hopeful that with refinement of assays, this detection rate can be improved. The capacity to detect SPLCV directly from sweetpotato plants was promising, as there is currently no NCM-ELISA test for this virus. Next gen sequencing may lead to such a test in the near future.

qPCR and allied methods have the potential to rapidly speed up the process of virus testing, reducing return times from around 50 days to one-two days, as well as being available all year round. This is an exciting prospect for the Australian sweetpotato industry, provided reliability can be enhanced through refined assay techniques.

To what extent has VG13004 met the needs of sweetpotato industry levy payers?

The team judges the project has met the needs of levy payers using the following measures:

- Rapid adoption of guidelines, suggestions and even potential ideas into widespread industry practice change.
- Consistent, high levels of attendance at industry workshops and field days (see Outputs and Engagement levels).
- Enthusiastic welcomes for project team members to visit farms, conduct monitoring, sampling or experiments, and have discussions with growers and their production teams.
- Positive formal feedback. For example, of the 34 growers who travelled to Gatton Research Facility for the November 2014, 42% rated it as Very Good, and 58% as Excellent.

To what extent were the target engagement levels of sweetpotato industry levy payers achieved?

The project team intentionally targeted engagement with the 50% of the growers producing 90% of Australia's sweetpotatoes. We can confidently say that all those growers were aware of and involved in the project, with most being visited several times a year for field activities and discussions. Most of those growers would have attended at least 70% of the industry events in their region.

The project well exceeded engagement targets, as many smaller growers also participated in the project, and attended industry events. In Cudgen and Lockyer districts, we regularly got at least 80% of commercial growers and associated businesses attending (22-27 people). In Bundaberg it was usually between 70-80% (35-40 people). The quality of that engagement is reflected in the dot points mentioned in response to the previous question.

Have regular project updates been provided through linkage with the industry communication project?

The industry communications project did not exist at the time of project initiation. Project updates were regularly supplied to growers, associated businesses and collaborators on our sweetpotato mailing list (see Outputs p19). Project updates were also provided to *Vegetables Australia* and *Good Fruit and Vegetables* magazines, as well as Hort Innovation to publish through their media (Outputs p20).

Did the project engage with sweetpotato industry levy payers through their preferred learning style?

Very early in the project, it was clear from discussions that growers wanted one to one contact, and industry events, as their primary communication pathways. They mentioned they seldom read emails, or extensive literature. They would go to reference materials to address emerging problems, or as a refresher to what they had heard or seen at an event.

In response to this feedback, the project team deliberately focussed on regularly visiting growers, conducting experiments and demonstrations on their farms, and holding at least two major events in each region each year.

The December 2016 events were probably our least successful, and an excellent improvement opportunity from the feedback received. Key learnings were:

- Experienced growers want specific detail and new technologies they can directly apply to their businesses. They are less interested in generalities, or information they have heard before.
- Newer growers can benefit from basic introductory materials and can find information targeted at experienced growers difficult to follow.
- We should provide summary information on what is being presented before the event.
- Use SMS as the preferred notification system, rather than email.

We applied those principles for future events. The most successful events were where we:

- Had interactive presentations, or even better, grower participation
- Had new, immediately applicable information, addressing a timely problem or opportunity
- Had guest presenters of interest to growers, e.g. our USA sweetpotato colleagues

How accessible were extension events to sweetpotato industry levy payers?

The project team coordinated with key growers and ASPG, to determine the most suitable times and venues for extension events. We also provided transport for the Bundaberg growers to visit Gatton Research Facility. The good-excellent attendance we had at most events suggests they were accessible to most sweetpotato growers and businesses. We never received feedback to suggest event accessibility was an issue.

What efforts did the VG13004 project team make to improve efficiency?

The project team regularly consulted with key growers to get their opinions on project objectives and methodology, and their assistance with project activities, as a way of reducing costs, and enhancing engagement. In conducting the project, the team worked with University students, providing mentoring and building industry capacity, in exchange for experimental assistance. We also engaged with other institutions, such as USQ and UQ, to build connections and cross-pollinate expertise, as well as build industry capacity and access to new people and resources.

The project team committed to the Project Mid-Term Review and redesigned the final 12 months of the project to reprioritise the main activities in the review (Appendix 15).

What key aspects in the conduct of VG13004 can be improved in future projects?

Engage growers more intimately in the design and implementation of the activities. This would enhance the prospect of addressing the highest priority issues, as well as create more ownership of the process.

Develop a platform where project team members across institutions could more easily share data and information, Current institutional firewalls and policies are very restrictive.

Realise how much time data analysis and reporting requires. Allocate sufficient time and resources to those tasks.

Provide timely information back to growers when they have participated in an activity. Allocate this as a priority task, as important as organisational reporting.

Resist the urge to overcommit to activities given the resources and available funding.

Recommendations

Plant bed management

- Define optimal soil temperature and moisture regimes for 8 weeks after plant bed installation, to promote sprouting without causing bedding root breakdown.
- Investigate strategies for reducing the impacts of pathogenic organisms in plant beds, particularly *Fusarium spp*., *Erwinia spp*. and *Sclerotium rolfsii*.
- Investigate characteristics of cultivar *Bellevue* that cause it to be slow sprouting and more vulnerable to premature breakdown.
- Explore collaboration with Prof. Villordon to definitively characterise premium sweetpotato plant material.

Virus management and diagnostics

- Further improve virus indexing capacity, allowing industry to make informed decisions on scheduling plant bed replenishment.
- Maintain virus monitoring capacity to identify potential virus threats and aid future sweetpotato industry biosecurity plans.
- Expand virus surveys of home/market gardens in North Queensland, as well as investigate home/market gardens closer to the main sweetpotato commercial cropping areas.
- Continue to refine modern technologies, such as qPCR and operational protocols, to provide accurate sweetpotato virus detection for the Australian sweetpotato pathogen testing system.
- Provide ongoing enhanced, standardised national virus detection protocols for the Australian sweetpotato industry and integrate with international researchers, particularly Pacific neighbours.
- Investigate improved tissue sampling methods for accurate virus detection.
- Conduct Next Generation sequencing of retained suspect samples, providing genetic information on virus species/strains, to facilitate primer design and accurate detection.

Industry development

- Build collaborative relationships with international researchers, and particularly the sweetpotato scientists at Louisiana State University.
- Formulate and support an RDE capacity building strategy for the Australian Sweetpotato Industry, so current project momentum is not lost.
- Develop a strategy to integrate levy players even more intimately in new project activities.

Refereed scientific publications

Conference poster

Dennien, S.E., Hughes, M.J., Coleman, E.A., Sukal, A.C., Henderson, C.W.L, Langenbaker, R.J., Persley, D.M., Galea, V.J., 2017. *Ipomoea setosa;* an effective and reliable tool for the detection of SPLCV in sweetpotato. *Science Protecting Plant Health.* Brisbane, 26-28 September 2017.

Coleman, E.A., Dennien, S.E., Bobby, J., Galea, V.J., Henderson, C.W.L, 2017. Alternate *Ipomoea* species as a diagnostic tool for enhancing virus detection in sweetpotato. *Science Protecting Plant Health.* Brisbane, 26-28 September 2017.

Conference paper

Dennien, S.E., Hughes, M.J., Coleman, E.A., O'Donnell, W., 2016. A list of historical virus detections in sweetpotato from Australia's east coast – a decade of testing 2004 to 2014. *ISHS International Symposia on Tropical and Temperate Horticulture*. Cairns, 20-25 November 2016.

Journal article

Henderson, C., Dennien, S., Langenbaker, R., Coleman, E., Prichard, M., Brown, P., Villordon, A., Best, T., 2016. Optimising plant bed performance through adaptive research with the Australian sweetpotato industry. *Acta Horticulturae* **1118**, 103-108. https://doi.org/10.17660/ActaHortic.2016.1118.15

Sukal, A., Dennien, S., Kidanemariam, D.B., Harding, R.M., James, A.P, (in prep.) Australian isolate of Sweet potato collusive virus (SPCV) infecting Sweet potato.

Thesis

Coleman, E.A., 2017. Alternate *Ipomoea* species as a diagnostic tool for enhancing virus detection in sweetpotato. Honours Thesis, University of Queensland, Brisbane.

Scarffe, R.., 2017. Impacts of organic and inorganic nitrogen fertilisers on sweetpotato (*Ipomoea batatas*) sprout production. Honours Thesis, University of Queensland, Brisbane.

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Intellectual property, commercialisation and confidentiality

There are no project IP, project outputs, commercialisation or confidentiality issues to report.

Acknowledgements

The core project team comprised Craig Henderson, Sandra Dennien and Rachael Langenbaker. They would like to thank all their colleagues for their efforts in the extensive range of project activities undertaken in the last four years. Jean Bobbie and Emma Coleman, in addition to being excellent laboratory scientists, readily and expertly assisted with field and extension activities. Their dedication to the job at hand was commendable, whether it be at sunrise cutting seedbeds, countless hours of grafting, or a long day in the lab performing numerous ELISA tests or extractions. Emma continued her enthusiasm throughout her internship, and subsequent honours projects, within the broader scope of VG13004. She delivered high quality work and hopefully gained valuable industry experience working with us and the growers. Robin Scarffe similarly performed good field science and investigative roles during his honours project, for which the project team was grateful.

Thanks to Mary Firrell, who joined the project team in 2016, for assisting with generic project administrative roles, mentoring of the Honours students and her report on Ipomoea biosecurity issues, associated with imported and indigenous plants. Thanks also to Michael Hughes, DAF sweetpotato team leader, for his project advice, continual support to the project team, and for providing another linkage to ACIAR project activities.

Bill O'Donnell, Peter Templeton, Renee Liddle, Jesse Thomas and Jordan Cram; UQ students - Laura Firrell, Jess Harper, Emily Pattison, James Dalton, Matthew Rogan and Dawson Henricks; and CQU students - Karli Groves, Paramullage Upamali, Sandaruwan Peiris, Tham Hong Dong and Jake Hogan were all excellent assistants in harvesting and assessing plant beds. Without them, the days would have been even longer and harder. Thanks for putting up with the dawn starts. A special thanks to GRF manager Gavin Berry for willingly assisting with laboratory infrastructure improvements and to farm supervisor Ron Hermann, who was always there to assist with plant bed, igloo and glasshouse maintenance. And thank you to DAF plant pathologist John Duff, for ad hoc identification of fungal and bacterial pathogens in sweetpotato roots.

Late in the project, Dr Bree Wilson was a keen collaborator with the project team, and brought a fresh perspective to laboratory, diagnostic, and pest management issues. She was an enthusiastic and friendly colleague during field monitoring and extension activities, for which we sincerely thank her.

The project team would especially like to thank the sweetpotato growers who collaborated during the project. Cudgen growers - Matthew and Henry Prichard; Doug, Jim and Steve Paddon; Stuart, Chanel and Kevin Kennedy; Esk growers - Michael, Aaron and Nick Jess; Bundaberg growers - Mal and Cindy Beutel; Darren and Linda Zunker; Russell and Shana Mortimer; Troy and Daniel Prichard were just a pleasure to work with. Their unselfishness in providing experimental sites on their farms, resources, staff, advice and encouragement for our activities is truly acknowledged and appreciated. We are amazed at their ability to maintain their smiles and good nature, and still have time for a chat, despite our regular disruptions to their days.

Many other growers enthusiastically participated in project activities by providing ideas and suggestions, farm access and assistance with extension activities. The project team acknowledges Sam Tully, Ken Small, Russ McCrystal, Mike Prichard, Adam Galea, Eugenio Mizzi and Zac Hayward for their friendly and informative conversations. Thanks to everyone who provided us with samples for virus diagnostics, or who allowed us to collect samples on their properties. We would also like to thank all the growers we have not named; no matter how small a role you have played, it has been invaluable to the success of the project.

A very special thanks to key ASPG grower committee members Rodney Wolfenden, Matthew Prichard and Dean Akers for their advice, support, and keen industry leadership. The enthusiasm of ASPG and the whole industry for project VG13004, and general research/extension engagement, makes working with them exciting, rewarding and usually even joyful.

Enthusiastic international collaboration with our fellow sweetpotato scientists in the USA has been a privilege and highlight during this project. Thanks particularly to sweetpotato physiologist Prof. Arthur Villordon for his engagement through multiple trips to Australia, as well as hosting the project team in Louisiana on several occasions. His fellow Louisiana State University colleagues Dr Chris Clark and Dr Don Labonte have also been very interested and helpful, with their extensive experience and knowledge of sweetpotato viruses and breeding respectively. We are very thankful and fortunate to have strong relationships with such an excellent group of friendly people. The team is also appreciative of the continued expert advice and assistance from applied sweetpotato virologist Segundo Fuentes of the International Potato Centre, Peru, and Amit Sukal, QUT and SPC CePact (Fiji) for his assistance with primer design, and analysis of our next gen sequencing data.

The staff at Hort Innovation have always been helpful in their discussions on project management, and reviews of project activities. Thanks, in particular to the two Program Managers, Drs Ben Callaghan and Brenda Kranz, who had oversight of VG13004. Your support and forbearance when things got tough, as they always do in such extensive undertakings, was particularly appreciated. It was a pleasure to deal with people professionally, with a mind-set of getting things done. Reviewer Prof. Calum Wilson was a pleasure to work with and explore opportunities for improving VG13004 during its implementation.

The project team sincerely thanks ASPG Secretary, John Maltby, who always ensured that forms got signed, notices got sent out, contracts got returned, and most importantly, invoices got paid. John certainly helps run a tight ship, with a minimum of fuss. John was always helpful with a friendly word, a voice of experience, and a gentle reminder when things needed to happen.

Finally, Craig, Sandra and Rachael would like to give their heartfelt thanks to Eric Coleman and his wife Kristie for their support, assistance, encouragement, knowledge, and amazing mentoring of the entire sweetpotato team, and our broader project family. Eric is without doubt the godfather of recent sweetpotato research and development in this country, and an enthusiastic champion of the industry. His knowledge and capacity to think through issues, to continue to turn up and support his colleagues is outstanding. Both Eric and Kristie are always generous with their time and hospitality to us all. We will never underestimate the value of that relationship and those friendships.

Thank you to all.

Appendices

Appendix 1

Baseline grower planting bed management survey 2014

Executive Summary

Between April-September 2014, we surveyed 44 Australian sweetpotato growing businesses on their use of Pathogen-Tested (PT) planting material in their production systems. Each face to face interview contained 27 questions identifying their current practices, as well as their key issues, and thoughts on research priorities for plant bed improvement. The businesses covered represent approximately 95% of Australia's commercial sweetpotato production.

The growers reported planting just over 1100 ha of sweetpotatoes; 900 ha of the Gold cultivar *Beauregard*, another 100 ha of alternate Gold cultivars, and 100 ha of Red or Purple cultivars. Fifty percent of respondents were from Bundaberg, with another Very Large Rockhampton business, making up the Central Queensland (CQ) region. Another 25% were from Cudgen/Tweed district (NSW), with the remainder spread amongst Lockyer/Esk/Gold Coast (SQ) and North Queensland (NQ) regions. CQ dominates planted area (75%), with NSW and SQ at 10% each.

Very Large (>50 ha), Large (25-50 ha) and Medium (10-25 ha) growers made up half the respondents but accounted for 90% of the planted area.

There is a single supplier of PT sweetpotato planting material, based just outside of Rockhampton, in Central Queensland. All sweetpotato planting material from the supplier is distributed as sweetpotato 'bedding roots'. The responding growers indicated a total of around 550 t of PT bedding roots were supplied in 2014. To date, independent testing has continued to show the PT bedding root production system free of known sweetpotato viruses.

Survey results from 2014 suggest 80% of the commercially planted sweetpotato area was from direct G1 sprouts cut from PT plant beds (beds established directly from supplied PT bedding roots), with a further 12% of plantings from G1 field cuttings (tip cuttings from plants established from the aforementioned G1 sprouts). Just over 85% of growers use the recommended flat-top plant beds to generate their sprouts. Median reported plant beds were 0.9 m wide, using 20 kg of bedding roots per square metre of installed plant bed. Smaller growers usually had a winter, or winter and spring pattern of plant bed installations. Medium or larger growers from Central Queensland, had 3-4 plant bed installation occasions, spread across the entire year, including a late summer/autumn installation for overwintering the plant beds.

Interpolating from grower estimates, the median quantity of sprouts produced from the plant bed systems (per cut) was 190 sprouts m^{-2} of plan bed, or 9.5 sprouts kg^{-1} of bedding roots installed. There was a general trend for larger growers to report slightly lower sprout productivity. This may have been an artefact of their generally more specific record keeping!

All growers reported cutting plant beds several times, with a median value of 5 cuts. The number of cuts reported by growers seemed independent of any other agronomic or business factors. Most growers stated that cuts 2-5 were most productive, with plant beds generating fewer and less vigorous sprouts after that number.

Introduction

It was important at the start of the project to have a sense of how growers were currently managing their multiplication of Pathogen Tested (PT) planting material utilisation within the Australian sweetpotato industry. One purpose was to indicate opportunities for improvement of practices within the provision, distribution and use of PT material on-farm. The other main purpose was for comparison with PT systems as the project progresses, to aid evaluation of project impact.

Methodology

This report uses data collected within an Australian Sweetpotato grower survey, involving face to face interviewing with 50 growers on their properties, between April and September 2014. Each interview contained 27 questions identifying their current practices, as well as their key issues, and thoughts on research priorities for plant bed improvement. See the end of the Appendix for a list of the questions. The interviews generally took between 30 minutes to an hour per sweetpotato grower.

We have combined answers from the same business, giving a total of 44 individual responses. The businesses covered represent approximately 95% of Australia's commercial sweetpotato production. We have amalgamated the information into like groups, to de-identify individuals and businesses.

Growers answered the questions with a very large range of styles and detail. We have had to interpolate or estimate answers, particularly those relating to planting areas and plant bed productivity, based on details provided in companion questions.

Results and Discussion

Industry Demography

In 2014, the 44 respondents planted a total of around 1000 ha of Gold sweetpotatoes (approx. 90% cv. *Beauregard*) with a further 100 ha of other Red or Purple cultivars (predominantly *Northern Star* and *WSPF*). Of the 23 growers who planted non-Gold sweetpotatoes, 5 had less than 1 ha, 14 planted areas of 1-7 ha, and only 4 growers planted more than 7 ha. Eight growers could be considered Red/Purple specialists, devoting more 25% of their plantings to non-Gold cultivars, and growing more than 1 ha of these sweetpotato types. The rest of this report will focus on sweetpotatoes as a group, rather than discuss cultivar types independently.

Geography

More than 50% of our respondents were from Central Queensland (CQ), including the main production centres of Bundaberg and Rockhampton, as shown in Fig. 1a. Around a quarter of the respondents were from Cudgen/Tweed region in New South Wales (NSW), with the remainder from the Esk, Lockyer and Gold Coast regions of South Queensland (SQ), and two respondents from the Atherton Tableland in North Queensland (NQ).

CQ dominates production by planted area at 75% of total plantings, with NSW and SQ around 10% each (Fig. 1b).

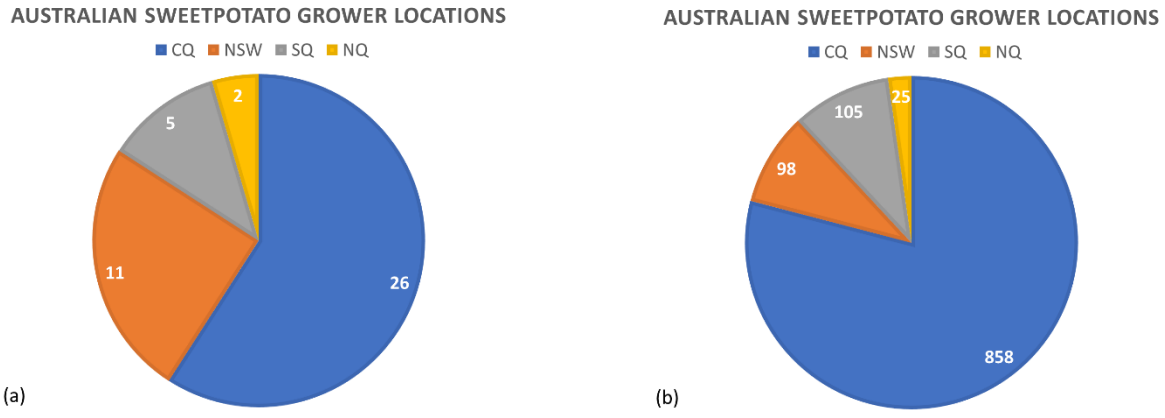


Fig. 1. Central Queensland has over half of Australia's (a) commercial sweetpotato growers; and more than three quarters of Australia's (b) commercial area (ha) planted to sweetpotatoes.

Sweetpotato plantings

We categorised growers as Very Large (>50 ha), Large (25-50 ha), Medium (10-25 ha), Small (3-10 ha) and Very Small (<3 ha). On that basis, about one quarter of our respondents were Large or Very Large growers (Fig. 2a), however they accounted for around three quarters of the area planted to sweetpotatoes in 2014 (Fig. 2b). Including the Medium growers as well, half the growers accounted for 90% of the planted area.

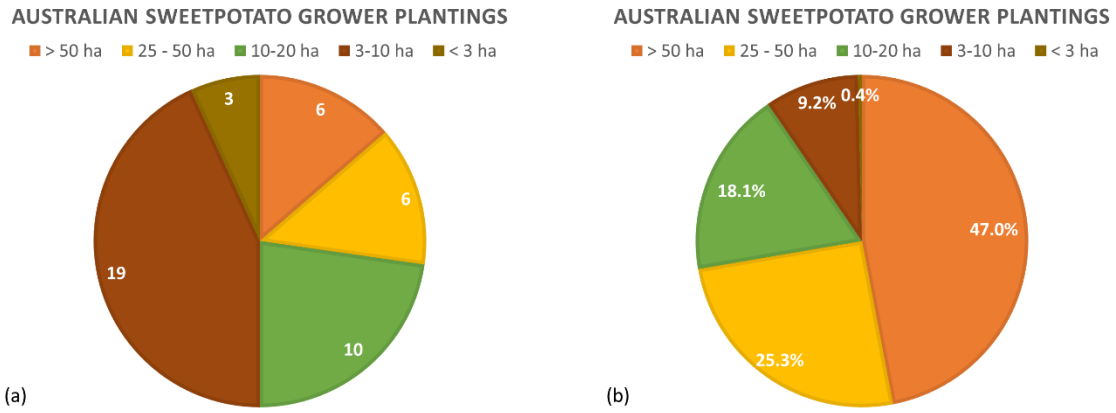


Fig. 2. A small number of (a) Medium (10-20 ha planted) to-Very Large (>50 ha planted) sweetpotato growers grow more than 90% of (b) the area planted to Australian sweetpotatoes.

Commercial planting material distribution chain

There is a single supplier of PT sweetpotato planting material, based just outside of Rockhampton, in Central Queensland. The production facility is around 45 km (as the crow flies) from the nearest commercial sweetpotato farm. Core stock is held in a bio-secure tissue culture laboratory. Each year this mother stock is multiplied via insect-proof tunnels, with a final generation of commercial PT roots in the field. These fields are regularly sprayed for virus-vector control and inspected to (as reasonably as possible) maintain virus-free status. Plant samples are regularly sent for virus indexing by an independent laboratory. All results so far have indicated the PT material generated by this supplier is indeed free of known virus infections.

All sweetpotato planting material from the supplier is distributed as sweetpotato roots. The majority of roots are supplied in half-tonne bins, although an occasional small order may be filled in 18 kg cartons. Commercial sweetpotato growers place their main orders for this planting material (bedding roots) in January/February. Orders are freighted to growers to meet their specific delivery dates. There is very limited capacity to supply roots that are not pre-ordered.

Bedding roots are field harvested by the supplier through most of the year, and stored in controlled temperature/humidity rooms until required. In 2014, there was limited modification or pre-sprouting of roots in storage, apart from skin-hardening (curing), to reduce the risk of premature bedding root breakdown.

The responding growers indicated a total of around 550 t of PT bedding roots were supplied in 2014.

Sweetpotato grower generation of commercial planting material

In 2014, growers indicated they produced planting material for their commercial sweetpotato fields from four sources.

- **G1 sprout production**. Installing the PT bedding roots in plant beds, taking sprouts from those plant beds over several cuts during their planting season.
- **G1 field cutting**. Taking tip cuttings from commercial fields planted using the above G1 sprout production method.
- **G2 sprout production**. Harvesting sweetpotato storage roots from commercial fields planted with G1 sprouts, and using those G2 roots to generate sprouts via the plant bed process.
- **G2+ field cutting**. Taking tip cuttings from commercial fields not planted with G1 sprouts.

Survey results from 2014 suggest 80% of the commercially planted sweetpotato area was from direct G1 sprouts cut from PT plant beds (Table 2), with a further 12% of plantings from G1 field cuttings. Seventeen of the 22 Medium to Very Large growers reported using G1 sprouts exclusively, compared to 7 of the 22 Very Small to Small growers. Three growers accounted for all the plantings of G2 sprouts, while only two growers used G2+ field cuttings.

Table 2. Planting material source for Australian sweetpotato plantings in 2014

Source	G1 sprouts	G1 field cuttings	G2 sprouts	G2+ field cuttings
Percentage of total planting area	80	12	5	3

Plant bed implementation

Most surveyed growers (38) used raised, flat-top plant beds to generate their sprouts. See *Managing sweetpotato plant beds guide* (Outputs p13) for a description of the general style of this system. The beds are generally raised above ground level for drainage, with heights varying from 5-35 cm. Wider beds generally produce more high-quality sprouts, as there are less edge effects (causing long, thin 'runners'; frequently less productive in the field). However, bed width is constrained by machinery requirements, as well as the ability of workers to reach and cut central sprouts, without trafficking the bed surface. Median reported plant bed width was 0.9 m, with 90% of growers reporting in the range 0.6-1.4 m across. There was no identifiable relationship between business size and the width of beds they implemented.

In Australia, growers hand place the bedding roots on the plant bed, either parallel or perpendicular to the bed axis. Generally, they aim for around 1-2 cm space between the roots. Density of bedding roots varies, mainly due to the size of the roots supplied. Larger roots have a greater kg m^{-2} on the bed surface. Growers reported a median bedding root installation density of 20 kg m^{-2}, with 90% of growers in the range 15-25 kg m^{-2}. As above, there was no identifiable relationship between business size and bedding root installation density.

Plant beds were usually watered by overhead sprinkler irrigation on an as-needs basis. A range of synthetic or organic complete fertilisers were broadcast at installation, with side dressings after each one or two cuts. Plant beds installed in July through September were often covered in clear plastic to increase soil temperatures. The plastic was usually 'hooped' once sprouts had emerged. Plastic covers were removed in September/October, to prevent over-heating and plant bed breakdown.

Four growers used commercial sweetpotato-styled hills to generate their G1 sprouts. These hills had a single, or in one case a double row of bedding roots laid end to end along the hill axis. Apart from one medium-size grower, these growers tended to be small operations, often focussed on producing Red or Purple cultivars. A common issue with this style of multiplication is a greater proportion of long, thin sprouts. These growers will often use 'backcuts' i.e. create several planting pieces from one, long sprout, with only on piece having the original growing tip. Research suggests 'backcuts' have lower yield potential than tip cuttings, all other aspects being equal.

Two Very Small growers used novel sprout multiplication systems; very labour intensive and which seemed to reflect their machinery and farming systems, rather than any inherent productivity advantage. These will not be further considered.

Plant bed timing

There was a very broad range of strategies for the timing of plant bed installations. Fourteen of the smaller growers installed all their plant beds on one occasion in winter (June, July or August), with this strategy reported across all the growing districts. Another 6 Small to Medium growers had one installation occasion in spring. They indicated they used field cuttings from over-wintered sweetpotatoes for their early plantings of commercial sweetpotatoes.

Thirteen growers separated their plant beds into winter and spring installations, a strategy particularly common in NSW. The remainder of the growers (11), predominantly large or very large producers from Central Qld, had 3-4 plant bed installation occasions, spread across the whole year, including a late summer/autumn installation for overwintering the plant beds.

Plant bed productivity

Depending on time of year, it can take 5-10 weeks for plant beds to produce useable sweetpotato sprouts for planting. The longest interval is for the mid-winter installations, whilst obviously the shortest is for mid-summer beds. Once plant beds are producing, the interval between cuts varies from 18-28 days, primarily depending on weather. Growers may go early or late depending on planting schedules. Growers are targeting planting materials 28-40 cm long, although they will plant pieces as short as 20 cm when desperate.

In 2014, beds were all cut by hand, with teams of cutters removing sprouts with secateurs or knives, and gathering them into uniformly graded planting bundles. These planting materials were either planted out that day, or stored in a cool, moist environment for no more than two days before planting.

For the flat-topped, wide plant beds, growers reported a wide range of values for sprouts collected at each cut. Note that we often calculated these values from other information provided by the grower, and are subject to large estimation errors. The median value was 190 sprouts m^{-2}, with 90% of growers indicating productivity between 130 and 260 sprouts m^{-2}. Four small growers suggested they were averaging more than 300 sprouts m^{-2}. There was a general trend for larger growers to report slightly lower sprout productivity. This may have been an artefact of their generally more specific record keeping!

We've also calculated sprout productivity based on kg of bedding roots installed, to enable the in-row plant bed method to be included in the comparisons. As previously, these values were often calculated from other information provided by the grower, and are similarly subject to large estimation errors. The median value was 9.5 sprouts kg^{-1}, with 90% of growers indicating productivity between 5.6 and 15.0 sprouts kg^{-1}. There was a general trend for larger growers to report slightly lower sprout productivity.

Plant bed cutting longevity

All growers reported cutting plant beds several times, with a median value of 5 cuts, and 90% of growers indicating they cut between 4 and 9 times (Fig. 3). The number of cuts reported by growers seemed independent of any other factor. Most growers stated that cuts 2-5 were most productive, with plant beds generating fewer and less vigorous sprouts after that number.

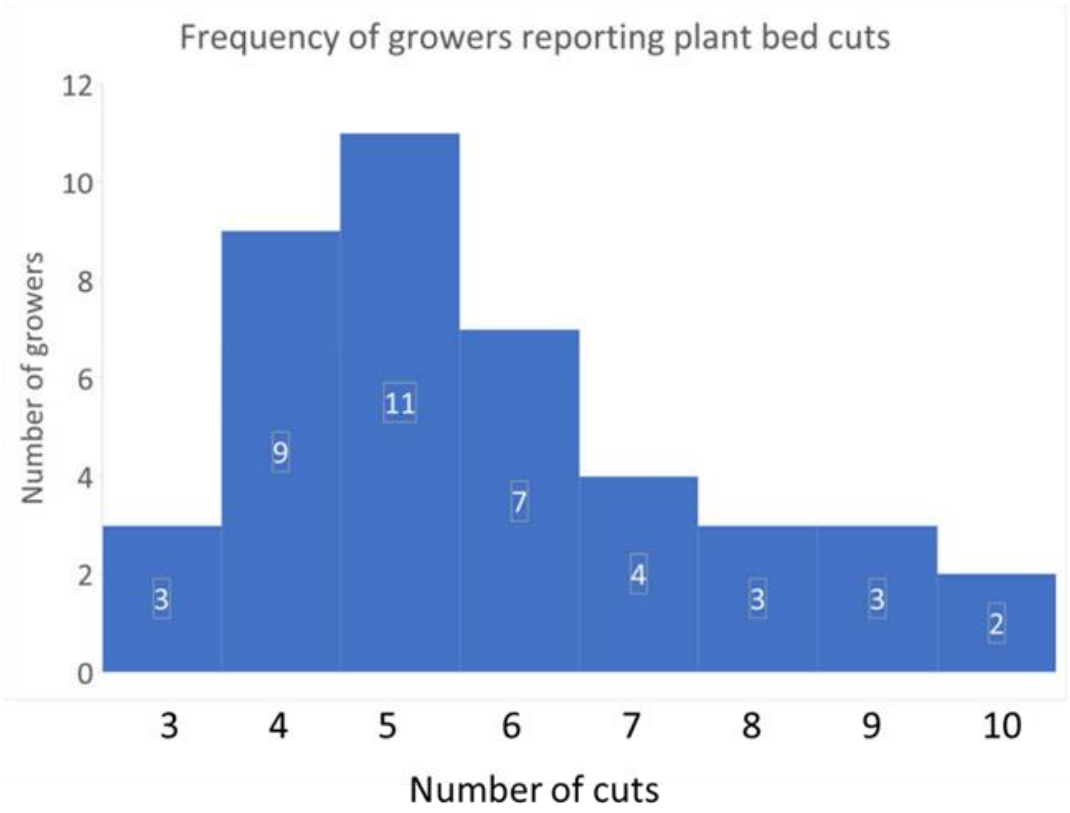


Fig. 3. Sweetpotato growers reported cutting their plant beds a variable number of times in 2014.

Commercial sweetpotato planting

Growers PT planting material is planted into commercial fields at a fairly consistent inter-row spacing, median value of 1.5 m, with 90% of growers between 1.4-1.6 m. In contrast, their intra-row spacings varied appreciably, generating a broad range of planting densities. These had a median value of 33,000 sweetpotato plants per hectare, with 90% of growers in the range 25,000-48,000 plants per hectare.

Grower questions

1. Where is your main farm? Includes geographic location, latitude longitude (use Google Maps)
2. Describe the farm, what crops are grown, what types and cultivars of sweetpotato. What soil type is cropped to sweetpotato?
3. Where do you get your storage roots from that you use in your planting beds? When do you order the roots? When do you get them?
4. Where are the planting beds located, both absolute, and in relation to other commercial sweetpotatoes?
5. How do you decide how much planting bed you are going to construct?
6. When do you start constructing your planting beds?
7. What does your planting bed look like when you construct it (e.g. width, height, total length)?
8. When do you plant your planting beds?
9. How do you lay out your storage roots in your planting bed? How many roots, or how many kg of roots, do you plant per square metre, or length of row?
10. What is your planting bed planting procedure?
11. What is your planting bed agronomy; fertilisers, chemicals, irrigation, cultivation practices?
12. When do you cut your first slips?
13. What is your slip cutting procedure – cutting method, cutting height, how are the slips collected, are they sorted?
14. Do you store your slips before planting; if so how, and for how long?
15. What do your slips look like? Do you modify your slips (e.g. remove leaves) after cutting?
16. What is your slip yield (how many slips per square metre for example)? What proportion of these slips do you use?
17. How much commercial crop do you think your slips will plant, e.g. hectares of crop planted per metre of planting bed?
18. How do you commercially plant your sweetpotatoes? What is your row and plant spacing?
19. What do you estimate your 'average sweetpotato yields are from your planting bed generated slips?
20. How many times do you sequentially harvest your planting beds? What is the time between planting bed cutting?
21. Does the number or standard of slips you get from your planting beds change as the season progresses?
22. Do you notice any change in the performance of the slips that you cut as the season progresses – e.g. do the slips produce less productive commercial plants as the season goes on?
23. Do you notice any other changes in your planting beds as the season progresses?
24. Do you do anything special with your planting beds at certain times of the year?
25. When do you normally finish using a planting bed – is it after so many harvests, or a certain time of the year, or when the planting bed declines to a certain performance level?
26. Are there any other issues about your planting beds that you would like to mention?
27. What are the top three things you would like to know or to change about how you operate your planting beds?

Appendix 2

Monitoring performance and issues with sweetpotato grower plant beds

Production season 2014/15

The 2014/15 plant bed season was a challenging one for sweetpotato growers.

Sweetpotato plant beds in the Lockyer region grew very well, with little signs of virus or general deterioration. This was true of plant beds on Gatton Research Facility, as well as growers in the district. Well performing plant beds were producing 210-230 sprouts m^{-2} of plant bed per cut, with cuts every 3 weeks during the peak of summer. Plant bed performance was maintained for at least 5 cuts (at GRF less than 10% drop off on sixth cut).

In contrast, plant beds in Cudgen and Bundaberg had substantial fungal disease incidence (mainly *Sclerotium rolfsii* during wet periods) from summer onward. Around Christmas, both Cudgen and Bundaberg had very heavy and extended periods of rain. Observations were that apart from dramatically increasing disease incidence, it also impacted on nutrition and insect levels in plant beds and commercial crops. Virus symptoms were evident in plant beds after four, and even three cuts. This was particularly the case after the heavy rain, when both plant beds and commercial crops were stressed. By March 2015, many plant beds in the main Cudgen and Bundaberg growing regions were displaying virus symptoms. Many growers had observed large populations of whiteflies and aphids during the summer period, particularly post-Christmas; i.e. a 'bad' vector year.

Benchmarking Bundaberg plant beds showed spring sprout production around 180-200 sprouts m^{-2} of plant bed per cut, with cuts every 3 weeks. By third and fourth cuts in mid-summer, production declined to around 150 sprouts m^{-2}. Late March performance declined to 35, 73 and 95 sprouts/m^2 on the 3 monitoring sites respectively. Certainly, soil borne diseases were a major constraint to maintaining plant bed performance – areas of sprouts were rotted from a combination of *Sclerotium*, *Fusarium spp.* and potentially bacterial rots during the wetter periods.

Depth of bedding roots was obviously critical. Where bedding roots were more than 7 cm below the soil surface, we observed slow establishment and emergence of sprouts. There was also much increased disease incidence in the deeper buried roots and sprouts. The project suggests 1.5-5 cm of soil covering appears best. Perhaps at least 2.5 cm of covering soil may aid sprouts to develop independent root symptoms?

Aligned with this was the need for good plant bed drainage, as the most crucial factor with plant bed shape for longevity of production.

Monitoring yield performance of planted sprouts

Measuring yield performance of the sprouts planted out from the monitored plant beds did not really tell us much about the productivity potential of the sprouts. We observed that the agronomic practices of the growers, as well as seasonal conditions, had a much bigger impact on crop yields and sweetpotato quality than anything we could detect as due to sprout quality.

For example, the poorest sprout production for Bundaberg Grower 1 was at the 7th cut, planted out on 9 May 2015 (Table 3). Note that although the number of sprouts produced was low, the performance of those sprouts was still high, with the best proportion of premium sweetpotatoes they achieved that season (of their crops that we monitored).

Similarly, compare the results for the Cudgen growers, whose sprouts were sourced from the same plant beds cut on the same day. Note their different harvest dates, and the relative proportions of size grades as a consequence.

Table 3. Examples of sweetpotato yields (t/ha) across time, region and growers in 2014/15

	Planting date	Harvest date	Total yield (t/ha)	Premium (t/ha)	Small (t/ha)	Large (t/ha)	Seconds (t/ha)
Bundaberg Grower 1	26/11/14	07/04/15	44	26	15	4	-
	16/12/14	07/07/15	71	36	10	22	3
	08/01/15	10/09/15	64	27	10	21	4
	30/01/15	12/10/15	62	26	8	19	8
	21/02/15	04/12/15	51	22	13	7	9
	09/05/15	16/01/16	86	53	13	15	5
Cudgen Grower 1	23/12/14	06/05/15	90	51	24	8	7
	12/01/15	28/07/15	88	59	20	6	3
	03/02/15	17/09/15	76	44	16	12	5
	04/03/15	29/10/15	73	47	20	3	3
Cudgen Grower 2	23/12/14	01/07/15	89	43	27	7	12
	12/01/15	20/08/15	93	56	24	12	2
	03/02/15	-	-	-	-	-	-
	04/03/15	30/10/15	77	38	26	10	4

For the remainder of the project, we deemed it more useful to focus less on final yields from sprouts as a measure of sprout potential, and to rather develop an index of sprout potential, based on morphological characteristics at time of cutting.

Production season 2015/16

Installation of 2015/16 commercial plant beds commenced in sweetpotato growing districts in July 2015. This season there was a major increase in planting of new sweetpotato cultivars, with large installations of Gold cultivars *Orleans* and *Bellevue*, with smaller plantings of Red *Southern Star*, Purples *Eclipse* and *Philippino White*, White *Bonita*, and a few even newer cultivars. Even at this early stage, it was evident that different plant bed strategies will be required for different cultivars. In particular, *Bellevue* was demonstrably sensitive to wet or hot conditions, exacerbating bedding root breakdown.

Other observations during that season included:

- Ventilation in plastic covering of beds is very important. Perhaps growers need to sacrifice temperature improvement for better ventilation in 'risky' disease or drainage conditions.
- Recovery after cutting is dependent on cutting height (short cuts = longer to re-grow), as well as availability of nutrients. If using composts, there may be low N in situations after heavy rain. Growers were advised to consider dosing with potassium nitrate to re-boost plant bed soil nutrition levels.
- Growers and scientists discussed whether quarantining small diseased patches of plant beds would reduce the spread of disease between cuts? This has since proven to be unlikely.
- In this season it became obvious that we needed to investigate what's going on below ground. Some plant beds were still functioning well, despite bedding roots having broken down and disappeared. Sprouts were surviving on root systems alone. In these cases, we suspected cutting height was even more critical, to retain capacity to produce sugars in residual stems and leaves.

Premature plant bed breakdown

The most obvious issue with sweetpotato plant beds in 2015/16 was how to manage the relatively new, nematode-resistant cultivar *Bellevue*. The problem was that *Bellevue* is very slow to sprout, particularly in cold conditions. In the USA, where the cultivar originated, they often use heat treatment of the bedding roots before installation, to initiate sprouting. The issue was that *Bellevue* appears particularly prone to root breakdown / rotting in the plant bed (and even in storage), which appears to be exacerbated by heat treatment. This is probably less an issue in the USA, where they are only looking for 1-2 cuts from their plant beds.

Growers lost substantial production from plant bed after two-three cuts, due to the *Bellevue* bedding roots breaking down and disappearing, and subsequent diseases such as *Sclerotium rolfsii* or *Fusarium spp.* killing the sprouts in the bed. In an extreme example, a Cudgen grower lost 65% of his bedding roots by the time of the second cut, even though he achieved 240 sprouts/m^2 on his first cut. These beds had been covered with plastic, to promote sprouting, and achieved the first cut 9 weeks after the beds were installed in mid-July. In contrast, another Cudgen grower who only used floating row covers, rather than heating plastic, didn't cut until 11 weeks after installation in late July (warmer conditions). There is definitely a trade-off between heat (shed or field) to promote early sprouting (and therefore cutting), and enhanced risk of rots.

To a **much** lesser extent there were also issues with some *Beauregard* plantings. The early breakdown appeared to be mainly due to *Fusarium spp*. infection, although there was rapid transition to *Erwinia* infection once the initial breakdown started. It appeared that early breakdown was worse if: (i) storage roots had been pre-treated with a heat cycle to promote early sprouting; (ii) storage roots were large-medium or bigger (iii) plant beds were consistently wet, or even worse, waterlogged. There have been several discussions with root supplier Eric Coleman about this issue. He was intending to adapt his pre-treatment and supply timing for next season, as a consequence of this season's experiences. Current recommendations at the time were to minimise the amount of heat treatment to just encourage initial sprouting, and confine heat treatment to those roots that were going to be installed in winter and early spring. Also, by preference to use small-medium storage roots for those early installations. Most importantly, and this is a general consideration, build up plant beds so they were well above general ground level, so they drain freely, and to err on the side of under-watering until sprouts are well established.

As the industry is becoming extremely reliant on this cultivar (because of increasing problems with nematode management), developing a better program for using *Bellevue* in plant beds became a critical industry focus.

Industry discussions with root supplier Eric Coleman about this issue continue. Ideas at the time were to minimise or even eliminate heat treatment, and to try and reduce the physiological age of the *Bellevue* bedding roots.

A substantial proportion of growers were still not getting sufficient height above ground level in their plant beds. Generally, these were the beds that suffer greatest losses of bedding roots, and deterioration in plant bed productivity.

Shallow coverage of roots in the plant bed was critical for early and prolonged plant bed productivity. We still observed poor or uneven early sprout emergence, and increased storage root breakdown, where coverage of storage roots was too deep (greater than 7 cm, and preferably 3-5 cm), even where this deep burial was just in patches.

Several growers were trying out mechanical cutting of sprouts using hedge trimmers or similar equipment. This could save on initial sprout harvesting costs. However, there was also a much greater risk of uneven management of sprout cutting height, and subsequent sprout recovery and growth. We observed instances of very variable sprout quality from beds that had previously been machine cut. We suggested that growers could experiment with their machine cutting techniques on later cuts in the plant bed season, when ongoing plant bed performance and productivity is less critical, until they finely tuned their techniques.

Apart from where there were major losses from plant bed rots (mainly *Bellevue*), most of the evaluated growers achieved 210-250 sprouts/m^2, for at least the first 3 cuts from their plant beds, particularly with *Beauregard*, *Orleans*, or the red (e.g. *Northern Star*) or purple (e.g. *WSPF* or *Eclipse*) cultivars.

Compared to 2014/2015, there appeared to be less virus vector pressure, however more incidence of leaf pest species such as hawk moth, leaf miner and mites. Several of the evaluated growers felt comfortable in using their plant beds for 6-7 cuts and were confident that they were not risking virus impacts in their commercial crops. Some of these growers maintained sprout productivity above 200 sprouts/ m^2 for that entire period, whilst others accepted production drops to 150-170 sprouts/m^2 in their later cuts. Many growers installed a second round of plant beds in mid-late summer, to carry over winter for early spring production.

As expected, we saw very little relationship between plant bed sprout productivity and commercial yields. Growers were generally only using reasonable quality sprouts from their plant beds, and there are so many other factors that impact on yields achieved (see discussion in previous section). For example, a bed of *Orleans* providing 210-220 optimal quality sprouts m^{-2} from consecutive cuts yielded 76 t/ha or 101 t/ha respectively of marketable sweetpotatoes.

Production season 2016/17

Following the 2015/16 season, we emphasised with the industry the importance of good plant bed structure to promote plant bed longevity. Particular focus was building high beds and ensuring shallow coverage of the bedding roots, to maximise drainage and maintain good air exchange in the soil.

With the collaboration of regional growers, the project team visited plant bed installations in Cudgen, Lockyer, Bundaberg and one visit to Atherton Tableland. Emphasis was on assessing plant bed breakdown, as we tried to get on top of this critical issue.

Probably the biggest change in sweetpotato plant bed installations and management had come about because of a major shift in cultivars. There was an ongoing and substantial move to replace *Beauregard* plantings with *Bellevue* (mainly for nematode management), and to a lesser extent *Orleans* (shape and uniformity). *Bellevue* is much more difficult to manage in plant beds, due to its reticence to sprout in cool conditions, and its propensity for rotting in certain circumstance.

Rotting with *Bellevue* appeared to be associated with physiologically old bedding roots; large bedding root size or damaged roots; wet, poorly drained or aerated plant beds; or overheating under plastic. Because of the 2015/16 season's experience with bedding root breakdown, particularly with *Bellevue*, growers were conservative with their plant bed management. We identified several practices changed from previous seasons. Many growers requested their roots not receive sprouting stimulation heat treatment in pre-delivery storage. A few growers also did not use plastic covers for their winter plant bed installations. One result was delayed and sporadic sprouting for their early season, spring sweetpotato planting.

Even so, some plant beds still suffered large proportions of bedding root rots in the winter/spring installations. Although many growers were concerned it was due to properties of the roots, separate growers had very different experiences with the same root lots from the supplier. Increased amounts of rot losses were usually associated with:

- Low bed heights, and the subsequent impacts on poor drainage and aeration.
- Overwatering, through rain; excessive or uneven irrigation. Insufficient attention to water management was one of the main agronomic areas where we could envisage improvement.
- There may potentially have been an interaction with elevated levels of organic fertiliser and plastic covering, although at that stage this was only circumstantial correlation.

Around 20% of growers were also cutting their sprouts off too low, between ground level and only 2-3 cm above the surface. We were seeing increasing levels of disease (*Sclerotium rolfsii*) in those beds, as well as slower sprout recovery rates between cuttings.

Most growers were getting their soil coverage right, with only a handful having more than 5 cm of soil over their bedding roots. In fact, we were seeing more growers with exposed roots, probably because of rain/irrigation washing away soil and insufficient attention allocated to re-covering between cuts.

Probably because of the much more widespread use of the more difficult to manage *Bellevue*, overall sprout production from planting beds in 2016/17 was probably 20-25% down on average, at 180-200 sprouts/m^2, compared to the 2015/16 season figures.

Apart from focussing on practices we knew were essential (good plant bed height; even, shallow coverage of bedding roots; minimum cutting height of 4-5 cm), the main plant bed productivity issues going forward were:

- Management nuance for different cultivars, particularly difficult ones such as Bellevue.
- Managing bedding root breakdown through options such as: reduced bedding root size and/or physiological age; appropriate use of organic fertilisers and amendments.
- Irrigation precision for productivity and bed longevity.

Provided we have at least 230-250 virus-free sprouts/m^2, each 28-45 cm long and with at least three, well-spaced nodes within 15 cm of the cut end, the plant bed has done its job. Commercial grower yields from then on are influenced by the suitability of the cultivar to forecast growing conditions, crop nutrition, and almost certainly planting density. We were certainly seeing big variance in sweetpotato yields and quality between growers, however this seems much more due to issues unrelated to inherent plant bed sprout *potential*.

Production season 2017/18

With the collaboration of regional growers in the 2017/18 season, the project team surveyed plant bed installations in Cudgen, Lockyer Valley and Bundaberg every month since August 2017. Emphasis continued to be on assessing plant bed breakdown.

Following major plant bed losses with cv. *Bellevue* in 2016/17, none of the bedding roots supplied by Aus. Sweetpotato Seeds P/L were pre-heat treated for the 2017/18 growing season. The supplied bedding roots were generally substantially smaller grades. This was primarily due to unfavourable growing conditions and flood losses, however there is also an increasing general preference by growers for smaller roots. Project research has shown that small-medium roots give the greatest number of sweetpotato sprouts per kg and are therefore the most cost-effective bedding roots for growers (provided all size grades are priced the same). Our project team also theorises that small-medium roots are the least vulnerable to physiological breakdown. In our field demonstration in Cudgen, we found that the range of sizes currently supplied are not inherently conducive to excessive breakdown (see Appendix 3, Appendix 6).

As our project progressed, most growers got on board with the importance of maximising bed heights; shallow root covering; and not overwatering; to avoid poor drainage and aeration. Most growers were also watching their sprout-harvesting practices, to avoid spreading diseases, as well increasing the return rate of new sprouts between cuts.

Because of trying to avoid too much soil coverage, we were seeing more growers with exposed roots, probably because of rain/irrigation washing away soil and insufficient attention allocated to re-covering between cuts.

A substantial proportion of growers, particularly in the warmer Bundaberg region, have gone away from using plastic to heat their *Bellevue* plant beds. Even those growers who still use the transparent plastic are keeping a close eye on temperatures under that plastic, to try and reduce the risk of over-heating and initiating bedding root breakdown. This season, as in past years, we have seen major plant bed losses where growers have left the plastic on too long, and overheated the beds, which confirms the importance of managing temperatures to avoid catastrophic losses. Most growers are aware of using hooped plastic, and enhancing ventilation by slitting the plastic, or removing and reinstalling during very hot weather. Manipulating plastic covers remains a critical component of successful plant bed management.

Many growers have also markedly reduced the amount of irrigation they apply to the plant beds. Many leave them dry until a substantial proportion of the sprouts have emerged. Certainly, it is important not to overwater, however it is still not known whether sprout emergence is marginally promoted by some moisture and consequent development of new roots from the bedding roots. We have certainly seen substantial adventitious root development from bedding roots, although the importance in sprout initiation and development is unclear (see Appendix 7).

Our surveys suggest that irrigation management is another critical factor in establishing and maintain good plant bed productivity. Several of the surveyed growers have irrigation systems for their plant beds that are unlikely to be able to deliver the uniformity and sophistication of irrigation required for optimal plant bed performance.

Because of all the conservative treatments of the plant beds, many growers are experiencing delays in emergence of *Bellevue* sprouts. It is likely that growers will have to employ different strategies for managing *Bellevue* in plant beds (compared to other dominant cultivars), including purchasing more bedding roots, intensive management of plastic covers, and potentially accepting high rates of physiological breakdown on beds targeted for early planting.

Interestingly, some growers also had issues in this season with breakdown of *Bellevue* beds planted in mid-summer. It is possible this is due to a combination of elevated temperatures causing excessive respiration of the bedding roots (see Appendix 7), excessive moisture from rain or irrigation, and/or high inherent disease loads in the plant beds.

Following implementation of the mid-term review recommendations, and the increased workload in other activities, there was limited widespread measurement of grower's plant bed performance. However, those beds that were monitored showed very high rates of productivity, well into the life cycle of the plant beds. In Cudgen, we saw *Bellevue* and *Orleans* plant beds producing 260 sprouts/m^2 and 280 sprouts/m^2 respectively at the 4th cut. In Bundaberg, we saw a well-managed *Bellevue* bed peak at 300 sprouts/m^2, at its 4th cut. Heavy rains in mid-January 2018 were associated with breakdown in the plant beds, with productivity declining to 200 sprouts/m^2 by the 5th cut, and a low of 115 sprouts/m^2 by the 7th cut. Also, in Bundaberg, a similarly well-managed *Bellevue* plant bed produced 275 sprouts/m^2 at only its 2nd cut. Once the January rain fell, it's productivity also declined, although not as rapidly as the older plant bed. Its final 5th cut averaged 135 sprouts/m^2.

Discussion

At the start of the project in 2014, we were monitoring values of around 200 sprouts/m^2 as a common plant bed productivity value, and this was usually for the most common cultivar, *Beauregard*.

Most growers were implementing 1-2 plant beds per year, in winter and/or spring. The plant beds were usually raised, although many growers only had them 5-10 cm above the ground surface. Bedding roots were installed by hand, and then covered by hand (shovel) or machine. Cooler season plant beds were covered with plastic, and beds were usually well irrigated before covering. Plastic was usually left on until sprouts had emerged and were pressing against the plastic. Sometimes growers would then remove the plastic, while others would change to hooped plastic covers. Plant beds would be irrigated by either drip irrigation, hand shift or solid set sprinklers, usually similar to those they used in commercial fields. Once plant beds were in production, growers would cut sprouts by hand, pack them crudely shaken to remove debris, store in the cool for a few hours to a few days, and then plant.

In the four seasons monitoring growers plant beds, we observed the following issues and consequences.

- Widescale replacement of *Beauregard* with *Bellevue* and *Orleans*. The shift to *Bellevue* in particular has seen a dramatic rise in:
    c) problems with sprouting, particularly in cooler weather, and
    d) problems with premature plant bed breakdown, after only 1-2 cuts.
- Plant beds rotting due to being waterlogged, or because bedding roots were covered by too much soil (more than 10 cm in some instances).
- Slow recovery of plant beds, after sprouts being cut too close to the soil surface.
- Sporadic, but not unusual outbreaks of disease (*Sclerotium rolfsii*) in plant beds.

The premature breakdown of plant beds, usually associated with *Bellevue*, was by far the most widespread and difficult problem (see Appendix 5 and Appendix 7 for more detailed discussion). This was not an anticipated problem at the start of the project.

Industry practice change between 2014 and 2017/18

Bedding root supply

The specifications for PT bedding roots have both tightened and become more nuanced. The supplier is generally harvesting his crop earlier, so there are more small-medium roots, and fewer large and oversize roots. Roots larger than 9 cm in diameter are very rarely supplied, unless there is a shortage, and the commercial grower accepts the risks. *Bellevue* in particular Is usually supplied in the 4-8 cm range.

The most recent consideration is avoiding supply of aged *Bellevue* bedding roots. Even with good storage conditions; current thinking is that roots more than 4 months old probably only have a short plant bed life, independent of their field management.

Close attention is paid to temperature management in storage at the bedding root production facility. Heating treatment is no longer done of any roots, unless a grower specifically requests it, and is clearly informed of, and accepting the risks. Any heat treatment is no more than 28-30^oC, and generally for less than 24 hrs.

Where possible, the transport of roots is via direct supply, to the growers, to avoid issues with sub-optimal storage in distribution warehouses.

Plant bed timing

We have seen a number of growers shift from 1-2 plant bed installations per year, to four, and even five separate supplies. This is potentially because they are concerned about virus reinfection of their plant beds through the season, but also because they are unable to maintain plant bed productivity, or the quality of their sprouts, past about 4 cuts. It is unclear the value of this strategy, however the fact that several large growers are doing it, suggests it is at least cost neutral for them.

Plant bed construction

One of the biggest changes has been the heights of grower plant beds. Almost all growers would now have beds of at least 25 cm high, and some narrower beds are closer to 45 cm high.

Most growers are very careful with their depth of soil coverage over their bedding roots. They encourage their workers to press the larger roots deeper into the soil, to have a level height along the tops of the roots. Alternatively, some are grading their roots for size, and installing them in separate areas, to achieve the same effect. Most growers are looking at 2-4 cm of soil coverage. Ideally, 3-5 cm is probably optimum in most circumstances. Deeper coverage may be more helpful in hot conditions. In the latter seasons, we were seeing more problems with insufficient coverage, due to rain or slumping uncovering roots.

Plant bed nutrition

Growers have started using more complete fertilisers, applying them both as a basal, and regularly during the production season. They are less fearful of over-fertilising and understand the importance of a sprout with high nutrient levels. This was further emphasised by results presented during Arthur Villordon's recent visit to Australia, which showed that sprouts with the best nutrient levels and well-grown foliage initiated the most storage roots.

Plant bed irrigation

Growers have become much more aware of not overwatering their plant beds, either through over-irrigating early in the production season, or having extremely non-uniform irrigation systems. They have also moved more to overhead irrigation, except some growers, who still use drip irrigation under their plastic. Most producers now use solid-set systems specifically designed for their plant beds.

In the final project season, most growers kept their plant beds relatively dry, until sprouts were well shot. This was probably a reaction to *Bellevue* breakdown in previous seasons. The optimal balance may be a little more water early on, to promote some bedding root system development. Certainly, the rule of relatively dry under plastic is still a good option.

Plastic management

The other substantial change has been how growers use plastic to heat their plant beds. In the final project year, growers sacrificed early *Bellevue* production by not using any plastic at all. Other growers used plastic, but preferred to use a hooped system, that provided more air flow, and slightly less extreme heat build-up. The other change is growers being much more conscious of soil temperatures, rather than using air temperature, or amount of burn on the sprouts, as the signal for plastic removal. There has also been a shift to row-cover floating meshes, which are also associated with less rapid heat build-up.

Because it appears temperature management is going to be one of the key issues for successful plant bed longevity, it is likely that soil temperature loggers and warning systems will become much more common.

In terms of practice, growers are much more aware of plastic manipulation, and will regularly move plastic on and off their plant beds, in response to either weather predictions, perceived or real temperature fluctuations.

Sprout harvesting and sorting

Sprout harvesting crews are now more careful in the precision with their cutting, starting at around 3 cm for the first cut, and generally cutting above the scars in ensuing cuts.

A significant proportion of growers are now doing a field or shed sort, to provide higher quality, and more uniform planting pieces for their planting crews. This is particularly the case for machinery-planted operations. Where possible, growers are going for a longer planting piece (35-45 cm), to try and maximise their yield potential.

Productivity result

During the life of the project, we have seen sprout productivity from plant beds jump to around 250 sprouts/m^2 (a 25% improvement), with some growers achieving an even higher benchmark. This is notwithstanding the introduction of *Bellevue* as a cultivar, which is a particularly problematic plant bed performer. With optimum timing of sprout harvesting, at least 80% of those sprouts can achieve premium grade, although this is a little more difficult with *Bellevue*.

This is an outstanding improvement in industry performance.

Appendix 3

Impact of bedding root size and grade on sweetpotato sprout production

Introduction

In our initial survey with growers, we were often discussing their preferences for the size of pathogen-tested (PT) bedding roots they received. There was a diversity of opinions, with some growers favouring large roots, as they felt they sprouted more readily, and produced more vigorous sprouts. Others preferred smaller roots, because they needed fewer bins to install their plant beds.

The main PT supplier was wanting to be able to offer growers advice on what they could expect from supplied roots, as well as manipulate their root production, harvesting and grading practices to deliver the best quality product to their grower clients. For both the supplier and the growers, it was important to determine the productivity potential of the range of potential sweetpotato bedding root sizes and grades, so they could both make cost-effective decisions about their plant bed requirements.

These experiments were implemented to examine the sprout productivity of sweetpotato bedding roots in plant beds, both in immediate production for the key first cuts, and then whether there were longer-term impacts on plant bed sustainability.

Methodology

We established detailed experimental sites to bedding root grades in plant beds. One was established at Gatton Research Facility (GRF), the other used a collaborating grower's designated plant bed area in Bundaberg (BRG). These provided different soil types and weather conditions.

Bedding root collection and grading

Craig Henderson and Rachael Langenbaker visited the bedding root supplier (Rockhampton) in September 2014, and collected three tonnes of cultivar *Beauregard* PT bedding roots, as they were being harvested from a production field. We graded them into six 500 kg lots, corresponding to the six treatments we intended implementing at our two research sites.

Plate 1. Collecting and grading bedding roots from the PT supplier.

The six treatments were:

a) **Oversize** – usually graded out by the supplier, and sold as a commercial sweetpotato to a local market

b) **Medium-large** grade - would be included in a mixed commercial lot

c) **Small-medium** grade - would be included in a mixed commercial lot

d) **Undersize** - would not normally be included in a commercial lot, and usually disposed of on farm

e) **Distorted** - roots with irregular shapes, or severe bends, considered unsuitable for a commercial lot

f) **Commercial** - a standard commercial lot, comprising a mixed grade, provided by the supplier

The grades were halved, with 1.5 t of roots sent to each of our two experimental locations.

Plate 2. Sweetpotato bedding root experiment at GRF.

Plate 4. Installing PT sweetpotato roots at GRF.

GRF

This experiment was located on a black, alluvial clay-loam at Gatton Research Facility. Beds were raised to 16 cm high, and 100 cm wide. We designated a single 60 m length of plant bed as our experimental area, dividing it evenly into 24 plots each, with the central 1 m of each plot as the datum area. The experimental design was a randomised complete block, comprising our six bedding root grade treatments replicated four times.

Installation

On 14 October 2014, we weighed out 30 kg of the appropriate bedding root grade for each plot. From that 30 kg, we took a random sample of 20 roots, and measured their length, maximum diameter and weight, then replaced them in the 30 kg lot.

Plate 3. Assessing PT sweetpotato roots at GRF.

The following day, we took the 30 kg for each plot, and placed them in the central datum area of the plot, installing them across the plot, separating each root by 1-2 cm. We measured the area of plant bed covered by the 30 kg of roots. Once we had installed all the datum areas, we then used the remaining roots to fill in the residual plot areas within the experimental beds.

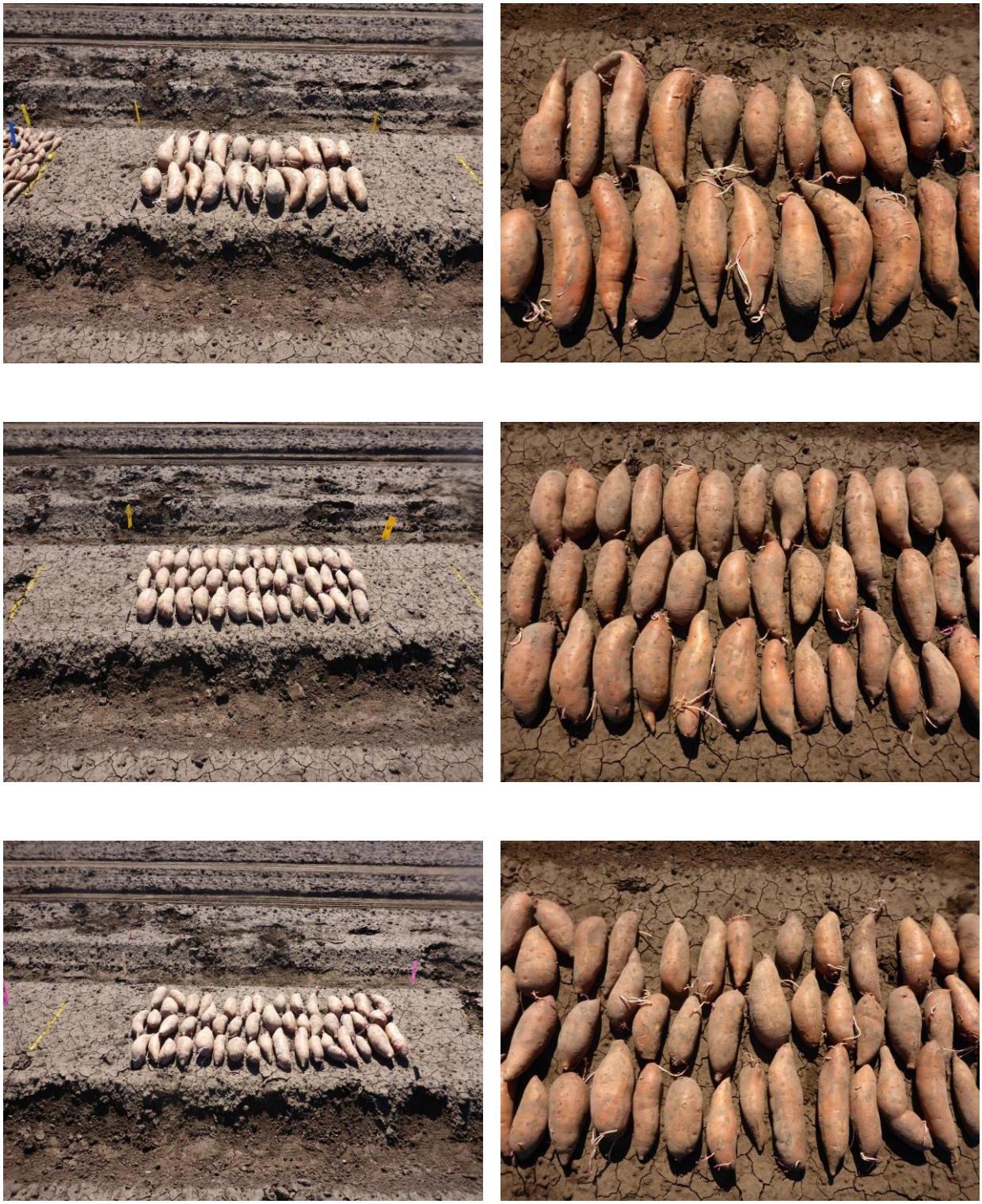


Plate 5. **Oversize (top), Medium-large (centre) and Medium (bottom) bedding root grades installed at GRF.**

Plate 6. Small (top), Distorted (centre) and Commercial (bottom) bedding root grades installed at GRF.

We shovelled 3-5 cm of soil over the roots, and then spread 270 g/m^2 of compound fertiliser (Nitrophoska) and 830 g/m^2 of gypsum over the installed beds. We irrigated with 75 mm of irrigation using hand-shift sprinklers. For the remainder of the experiment, we alternated between mini-sprinklers and hand-shift irrigation, until we installed two rows of drip lines in mid-December 2014.

We irrigated, weeded the plant beds, and sprayed for insects to maintain the plant beds as per grower best practice. We also recovered the plant bed edges that got washed away during heavy rainfall.

We trimmed the plant beds back once to increase sprout uniformity, on the 10 November 2014.

Plate 7. Covering PT sweetpotato roots at GRF.

Plate 8. Sweetpotato bedding root experiment at BRG.

BRG

The second experiment was located on a collaborating grower's property south-west of the Bundaberg Research Facility. It was a red kraznozem soil, regularly used for commercial sweetpotato production and plant beds.

The grower raised plant beds around 15 cm high, and 120 cm wide. The experimental layout and design the same as at GRF.

Installation

The assessment of the roots lots and laying of roots on the bed were the same as at GRF, and all occurred on 8 October 2014. The grower covered the roots using a mechanised plough and leveller, which furrowed the area between the beds, covered the main bed, and attempted to level the surface.

Plate 9. Covering PT sweetpotato roots at BRG.

This was only partially successful and resulted in uneven burial of the bedding roots. We tried to even this out, however it appeared that many areas were covered with more than 7-10 cm of soil.

The grower applied 200 g/m^2 of compound fertiliser ((GF 451) over the installed beds. The plant beds were irrigated with a solid-set irrigation system, delivering approximately 12 mm/hr of irrigation, with a uniformity of around 75%.

The collaborating grower managed nutrition, weeding and pests along with their normal plant beds. Rachael Langenbaker would advise the grower if she saw any issues arising from her regular field inspections (usually once, and often twice a week).

The collaborating grower trimmed the plant beds back once to increase sprout uniformity, on the 3 November 2014.

Experimental data collection.

At both experiments, when sprouts were ready for harvesting, we would sample the central 1 m of each plots for measurement. These samples were collected using standard commercial harvesting practices, cutting around 4 cm above the soil surface. We would collect all the material, and place in crates for storage and assessment. The remaining buffer areas were also cut, and the sprouts supplied for commercial planting, or disposed of if there was no ready use for the material.

Plate 10. Harvesting sprouts from BRG plant beds.

The collected sprout samples were processed in the following manner. We weighed the total fresh biomass, and then separated the material into:

- Damaged or diseased sprouts
- Undersized sprouts (<20 cm long) and loose, leaf material
- Acceptable sprouts (28-50 cm long). Sprouts longer than 50 cm were trimmed back to this length.
- Back cuttings (the trimmed excess of sprouts longer than 50 cm)

Plate 11. Assessing sprouts for productivity and quality at Bundaberg.

We weighed each of the four categories and counted the number of acceptable sprouts. We randomly selected 20 acceptable sprouts, measured their length, width at the cut end, and counted the number of nodes in the 15 cm interval closest to the cut (distal) end.

After commencing sprout cutting in late November 2104 (BRG) and early December 2014 (GRF), we completed 6 cuts at GRF by early April 2015, whilst we conducted 7 cuts at the BRG site, the last one in early May 2015.

Results

Bedding root grade analysis

The six grades of roots were readily differentiated at both sites. A good segregator was root diameter, which is also the most readily assessed character in a grading activity (Fig. 4).

In understanding the graphic, use the GRF Oversize grade (dark blue) as the example. The lower 'whisker' (value 70 mm), represents the lowest standard diameter for bedding roots in that grade. The lower boundary of the box (value 90 mm) indicates that 25% of roots were between 70-90 mm in diameter. The upper boundary of the box (value 107 mm) shows 50% of the roots were 90-107 mm in diameter. And the top 'whisker (value 120 mm) shows the maximum standard diameter for that bedding root grade, with 25% of the roots between 107-120 mm diameter. The x (value 96 mm) marks the mean diameter, while the line across the box marks the median diameter. The few points above and below whisker plot show outlier values that were considered unusual, and unrepresentative within the sample.

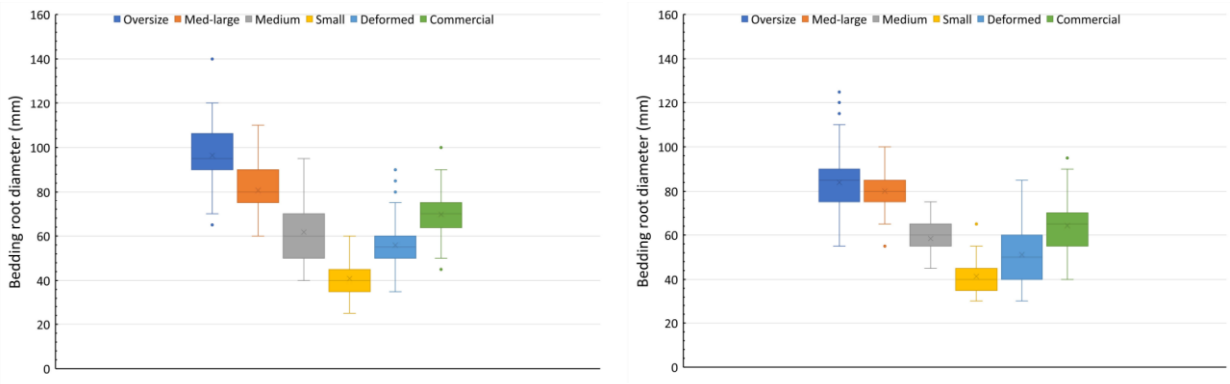


Fig. 4. Distribution of bedding root diameters for the six treatments in the bedding root quality experiments at Gatton (left) and Bundaberg (right).

The Commercial grade was similar for both sites, between 40-90 mm, with a median of 70 mm. The Deformed grade at both sites had more narrow roots, with 75% of roots less than 60 mm diameter, and a median of 50 mm. Three-quarters of the Undersize grade (referred to as Small in the graphics) were less than 44 mm in diameter. The Small-medium grade really sized out at Medium and is referred to as such for the rest of this report. Although the median of the Medium grade was 60 mm in both instances, the spread was greater at the GRF site. Similarly the spread of the Medium-large grade was greater at GRF, although both GRF and BRG had medians around 80 mm. The Oversize at GRF were definitely wider diameter bedding roots than at BRG.

The bedding root lengths for the grades demonstrated several interesting features (Fig. 5). The Oversize roots at BRG were at least as long as the GRF roots, indicating they probably made that grade on a length basis. Similarly, the Med-large grade at BRG tended to be longer than the GRF counterparts. Most of the Deformed roots were at least as long as the Med-large roots. This could be previously seen in the photographs of the roots at installation.

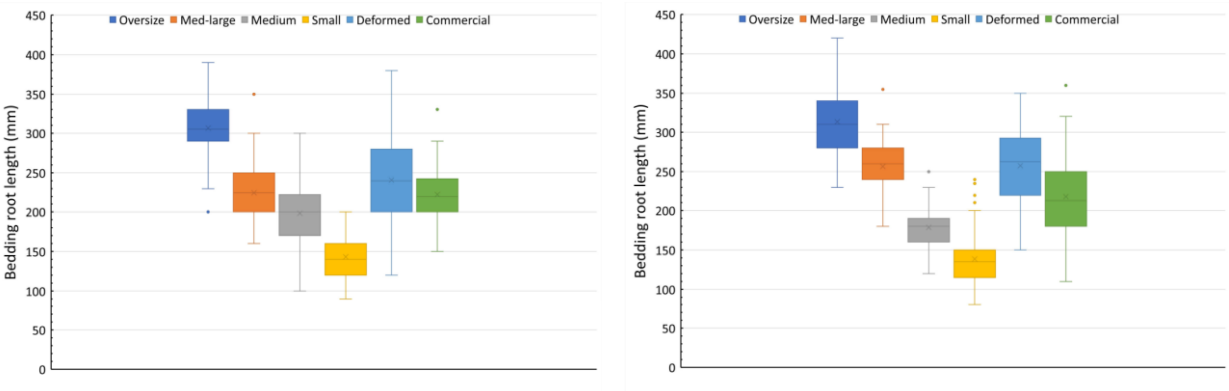


Fig. 5. Distribution of bedding root lengths for the six treatments in the bedding root quality experiments at Gatton (left) and Bundaberg (right).

Bedding root weight grades (Fig. 6) demonstrate clear distinctions between the four size-based grades at both sites. Both the Deformed and Commercial lots had a distribution of bedding root weights similar to the combination of the Medium and Medium-large grades.

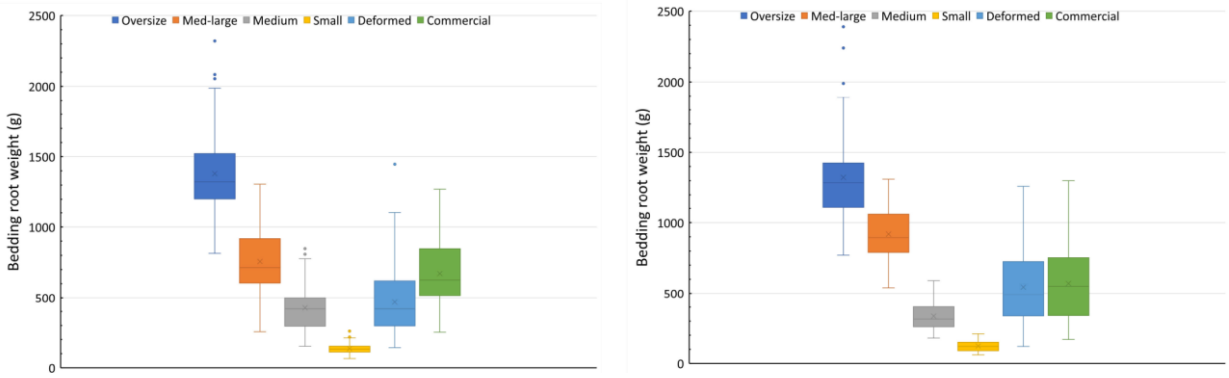


Fig. 6. Distribution of bedding root weights for the six treatments in the bedding root quality experiments at Gatton (left) and Bundaberg (right).

Predicting plant bed requirements

In laying out the roots, it was obvious that a much greater weight of larger roots was required to fill up the designated area of plant bed. We analysed the relationship between root size and the kg required to fill a square metre of plant bed, by looking at the data from both GRF and BRG. There was a very clear relationship between root diameter and bedding root requirement (Fig. 7). The relationship failed once roots were more than 80 mm diameter. This was probably because large and oversize roots were very hard to pattern onto the bed, particularly laying across the bed. As an example of the usefulness of this relationship, if we know a bedding root lot has a median diameter of 7 cm, we can quickly estimate we'll need 21 kg of bedding roots for every square metre of plant beds we're installing.

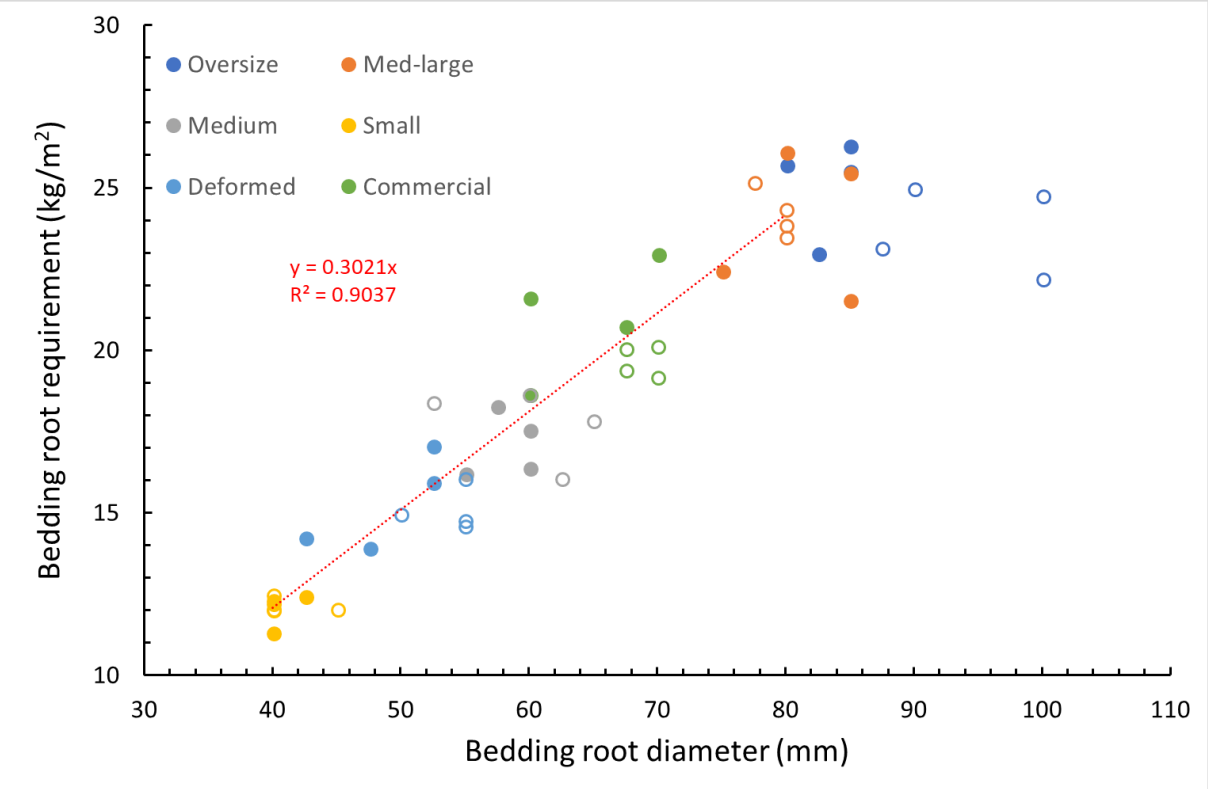


Fig. 7. Relationship between mean bedding root diameter and the quantities of roots required to fill a plant bed area, for plots at Gatton (closed circles) and Bundaberg (open circles).

Plant bed productivity and sprout quality

GRF

It took 7 weeks from bed installation for the GRF plant bed to reach size for the first cut, although the first harvest was only 21 days after the initial trim (Fig. 8).

Cuts 2-4 were on average 22 days apart, while the later cuts were extended as the temperatures cooled. Although there was no significant difference in sprout production between the bedding root grades at each cut, here was a consistent trend for the Med-Large grade to produce slightly more sprouts for the first few cuts. The total amount of sprouts produced by the Med-large roots across all cuts was 10% more than the other grades, averaging 240 sprouts/m^2 at each cut. The Oversize roots had the lowest productivity at both later cuts. Sprout production improved through the season, peaking at Cut 4, before declining in the last two sprout harvests.

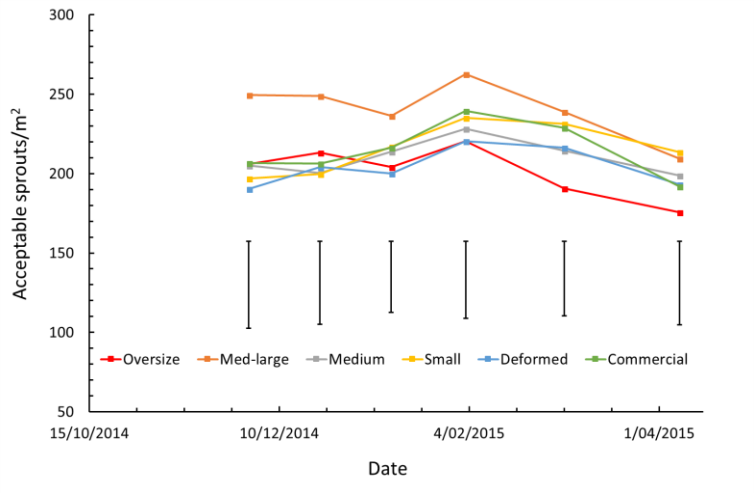


Fig. 8. Sprout productivity at GRF only slightly affected by bedding root size grade – bars show the 95% confidence intervals for the respective Cut means.

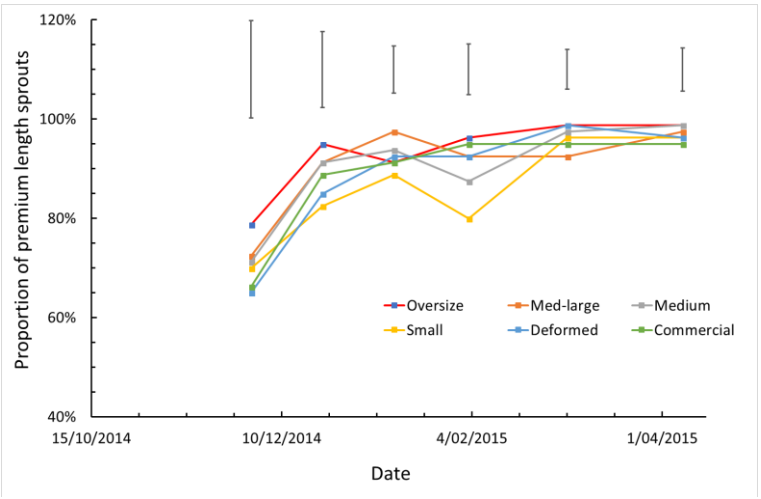


Fig. 9. The proportion of premium sprouts at GRF slightly lower for undersize bedding roots – bars show the 95% confidence intervals for the respective Cut means.

Apart from the first Cut, all the ensuing harvests produced a very high proportion of premium length sprouts (Fig. 9). We categorise sprouts 28 cm or longer to be of a premium length (see Appendix 8). This was an excellent result. The slight dip at Cut 4 suggests we may have been a few days early with that harvest. The Small grade was the poorest performer, particularly in the mid-season cuts.

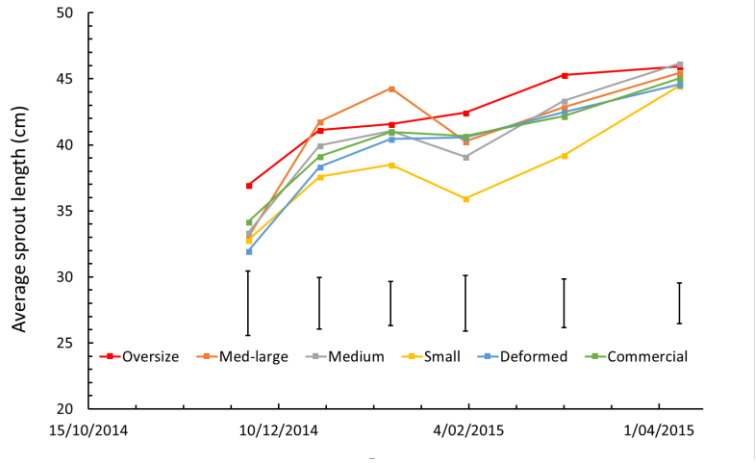


Fig. 10. Larger bedding roots produce significantly longer sprouts during mid-season Cuts – bars show the 95% confidence intervals for the respective Cut means.

The average length of sprouts tells a similar story, although the poorer performance of the smallest bedding roots is much clearer here (Fig. 10). Those small roots were still producing an acceptable sprout, just significantly shorter than the larger grades. In comparison, the Oversize roots consistently produced long sprouts at each Cut.

Across all the Cuts, the mean sprout width was 3-3.5 mm, with 3-5 nodes in the distal 15 cm of the sprout. The number of nodes was inversely related to the length of the sprout. We did not detect any consistent relationship between sprout width or node number and bedding root size grade, although there was a marginal trend for the smallest roots to have more nodes close to the cut end of the sprout.

BRG

As at GRF, it took 7 weeks from bed installation for the BRG plant bed to reach size for the first cut, with the first harvest 22 days after the initial trim (Fig. 11). This 3 week interval was constant, except for Cut 5 and Cut 7, which were both delayed by rain.

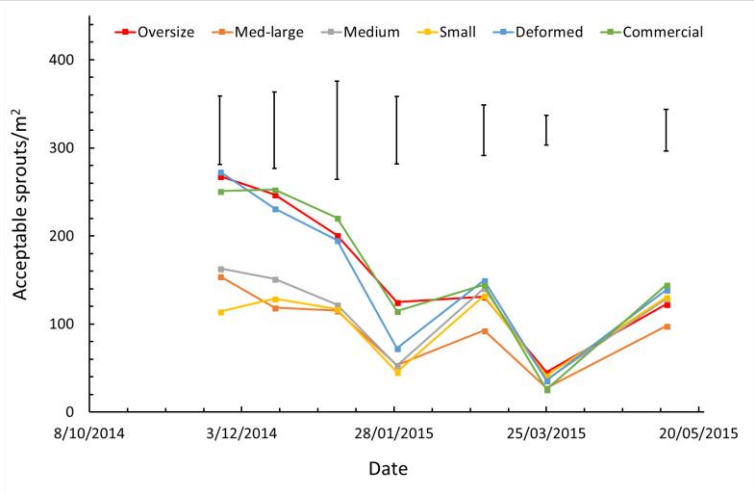


Fig. 11. Sprout productivity at BRG affected by emergence failure, plant death, and premature cutting – bars show the 95% confidence intervals for the respective Cut means.

Unfortunately, we had very poor sprout emergence from several of the plots, probably due to burial of the roots being too deep in some plots. This created substantial variability across the experiment, making comparisons between the treatments difficult. Certainly, the Small, Medium and Med-large treatments were worst affected, reflected in their poor sprout production. Although the production of the other three treatments was initially very high, they dropped by nearly 50% over the two months from early December to early February. Rather than reflecting on the impact of bedding root size, this experiment shows rapidly declining plant bed performance through root and sprout death, and the impact of premature harvesting on sprout production (Cuts 4 and 6).

Plate 12. BRG plot with good sprout emergence (left), compared to poor sprout emergence (right).

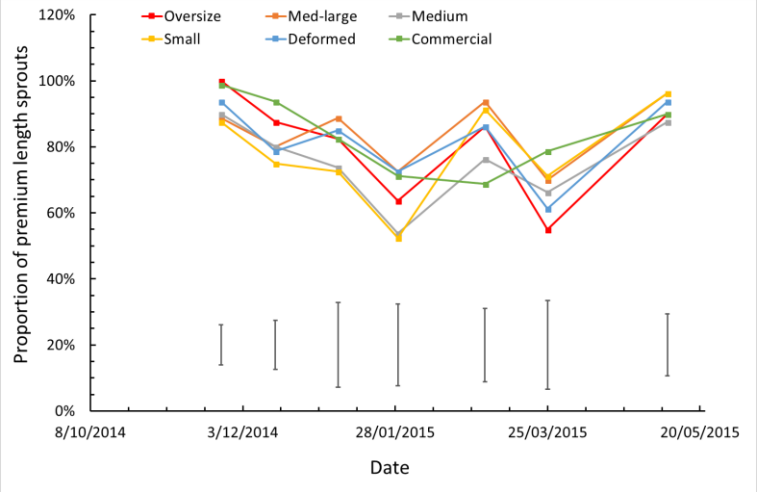


Fig. 13. The proportion of premium sprouts at BRG variable and impacted by harvest timing – bars show the 95% confidence intervals for the respective Cut means.

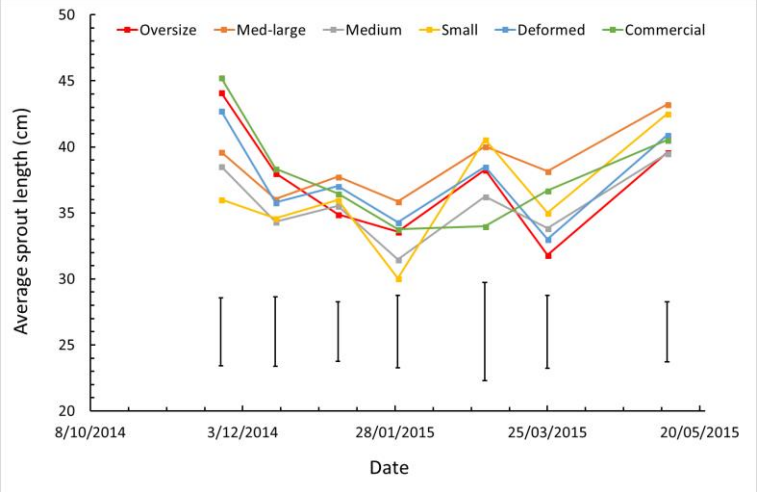


Fig. 14. Larger bedding roots produce significantly longer sprouts during mid-season Cuts – bars show the 95% confidence intervals for the respective Cut means.

In contrast to GRF, Cut 1 at BRG produced a good proportion of premium sprouts (Fig. 13). Thereafter it was very variable, and particularly poor at Cuts 4 and 6. There was no clear association with root grade, although the smaller roots did appear to produce fewer premium length sprouts during the mid-season. The proportion of premium sprouts always improved when cutting was delayed.

In terms of sprout length (Fig. 14), there was a clear segregation at the first cut between the three poor productivity treatments (Small, Medium and Med-large) and the remaining treatments. The former obviously had delayed sprout emergence, as well as many sprouts not emerging at all. This difference in sprout length had disappeared by Cuts 2 and 3. The smallest roots again performed less well at Cut 4, although experimental variability clouds this result. Delayed harvesting for Cut 7 allowed all bedding root grades sufficient time to produce longer sprouts.

Across all the Cuts, the mean sprout width was very consistent at 3.2-3.6 mm, with absolutely no effect of bedding root size. All sprouts had at least 3 nodes in the distal 15 cm of the sprout. The number of nodes was inversely related to the length of the sprout, so on occasions and in those treatments where length was down, node number were closer to 5. Interestingly, at Cut 7, even though the sprouts were long, they still had 4-5 nodes in their distal region.

Discussion

The rule of thumb of multiplying the bedding root diameter (cm) by three to calculate the kg/m^2 of bedding roots required will be a very useful planning tool for growers and the bedding root supplier. It will enable communications of requirements, order volumes, and expectations in that relationship to be much clearer, particularly when the root sizes may be different from initially anticipated.

The problem with uneven root depth coverage at the BRG site was disappointing, however it was an excellent learning exercise for us early in the project. It also reflected issues that commercial growers had that same year. It was definitely a stimulus to really press the point of the importance of precise plant bed coverage throughout the rest of the project. In terms of commentary on the impact of bedding root size and grade, the focus will mainly therefore be on the GRF experiment.

It is unclear how much to read into the slightly superior performance of the Med-large bedding roots, compared to the other treatments. It was consistent across the first four Cuts. The fact that the Commercial treatment had a very similar size distribution of roots, yet performed similarly to the other grades, suggests it was probably just a chance occurrence. All the other treatments performed similarly, so we suggest that within the bedding root grades currently received by growers, there is probably very little impact on sprout production potential, in terms of numbers of sprouts per area of plant bed.

The distorted roots performed equally as well as the other grades. The main problem is the care of handling, as they break easily, which may exacerbate disease issues. If they intact, they are very difficult and time-consuming to install in plant beds. So, they will mainly not be included for practical reasons, although the odd root in a lot is probably no issue.

Looking at sprout quality, it is possible that in the mid-season cuts (6-12 weeks after cutting commences), sprouts from small roots (40-50 mm diameter) may be slower to recover and regrow. Perhaps in high productivity plant beds, they more quickly exhaust the nutrient and sugar supplies from the host bedding root, than do sprouts from larger bedding roots. Whether this effect can be overcome by higher nutrient levels in the plant bed, as well as really encouraging independent sprout root systems, is unclear.

As the plant bed season progresses, the relative nutrient and sugar contributions from bedding roots to sprouts, compared to independent root systems (nutrients) and sprout leaf area (photosynthate) would be very interesting to determine. Our net conclusion is that in a plant bed with predominantly small bedding roots, the time between cuts may need to be delayed for a few days.

Sprout quality, primarily length, is very much a function of appropriate harvest intervals. A well-managed plant bed can probably turn around 90% premium length sprouts in around 20-22 days in mid-summer, however this will be weather dependent. Low or extreme temperatures, extended rainy, cloudy days, poor irrigation, or extremely poor nutrition, will extend this interval. Most growers are aware of this issue, however practical farming circumstances, or planting demands, can mean that harvesting may take place too early, or too late. This optimal window is obviously shorter in peak growing conditions and may only be 3-4 days in mid-summer.

A cut that is a few days early will result in a lower proportion of premium sprouts, and a shorter overall mean sprout length, e.g. GRF Cut 4. A cut even more premature will start to reduce the actual numbers of acceptable sprouts, for example as occurred in Cuts 4 and 6 at BRG. Cutting sprouts off too close to ground level will delay regrowth, as well as potentially actually reduce sprout numbers. Unless extremely premature, the timing of the cuts is unlikely to affect sprout width.

Letting plant beds grow past their optimal harvest date is usually less problematic, unless the delay is appreciable. An appreciable delay can be as short as 4-5 days in mid-season, or several weeks during cooler periods. Probably the main issue is that sprouts become tangled, and much more difficult to harvest. Not only does tangling mean cutting is more time consuming, there is also much greater chance of damaging the critical sprout tips. A damaged tip means the sprout has much lower yield potential. Depending on their planting systems, growers have a maximum sprout length they can handle. Delayed cutting means more sprouts have to be trimmed, another time expense. In some instances, the rapidly expanding sprout can have insufficient nodes in the distal 15 cm, due to extended internode distances.

Apart from the variability issues, the decline in the BRG plant bed as the season progressed was much more noticeable than at GRF. The best plots did start from a very high base, of around 270 sprouts/m^2. This decline was fundamentally associated with sprout death, as even when cutting was delayed, allowing spouts to reach optimal size, the numbers were still well down, compared to early in the season. Cuts 5 and 7 suggest more than half the sprouts had been lost, compared to Cuts 1 and 2. This plant bed rotting is associated with both physiological breakdown of the bedding root, as well as pathological attack by fungi (e.g. *Fusarium spp*.) or bacteria (e.g. *Erwinia spp.*). This plant bed breakdown is more common in vulnerable cultivars such as *Bellevue*.

In this instance, it is likely that the deep root placement impacted the three poor treatments at BRG. There was very heavy rain during January, which would certainly have impacted, and increased rots in the plant beds, particularly of those roots that were marginal, in terms of soil coverage.

The results from these experiments suggest that most root sizes in the commercially supplied lots will generate similar sprout densities and quality. There is no problem with a few out of specification (e.g. small or distorted roots) in a supply. A well-maintained plant bed can maintain benchmark production (230-250 sprouts/m^2) of premium sprouts for at least 5 cuts, although this is potentially cultivar dependent.

Because production from a square metre of plant bed is basically independent of bedding root size, the most productive bedding root grade on a per kg basis is a small-medium root (around 50-60 mm diameter), although they may take a few days longer to produce a premium sprout than a medium root (60-70 mm).

The greatest risk to plant bed performance and longevity is premature breakdown, usually associated with pathogenic attack on the bedding root, spreading to the sprouts. It is possible that bedding root size plays a role, with large roots, greater than 80 mm diameter being more vulnerable. This is discussed at greater length in Appendix 5 and Appendix 7. However, it is just one of the risk factors, and potentially others, such as temperature, aeration and water logging, bedding root age and pathogen load are more important.

Appendix 4

Assessing the impact of nitrogen nutrition on sweetpotato sprout production

Introduction

Generating sweetpotato planting material as sprouts from bedding roots in the Australian system is a highly intensive process, which extends over 4-6 months, and often even longer. Some growers look for 7-8 cuts from their beds, with each cut removing up to 10 kg/m^2 of stem and leaf material at each cut. In the initial stages, nutrition for the sprouts is provided by the bedding roots, however sprouts should establish independent root systems to support nutrient requirements for later cuts, as the bedding roots degrade. Bedding roots also re-establish feeder root systems after installation, although that role in supporting sprout development is unknown.

Growers regularly apply around 100 g/m^2 of complete inorganic fertilisers, or 300 g/m^2 of chicken-manure based organic fertilisers, as a basal application at plant bed installation. They follow this with regular side-dressings of fertilisers during the production period, usually after every cut. These side-dressings are nitrogen focussed, targeting the equivalent of 30-50 kg/ha of N.

The experiments reported here sought to determine appropriate levels of side-dressing fertiliser addition to maintain sprout productivity from sweetpotato plant beds. Of interest was whether requirements were similar for different cultivars, whether excessive nitrogen could cause sprout quality issues, and whether the amount of nitrogen applied as side-dressings affected the incidence of plant bed breakdown.

Methodology

We established detailed experimental sites to evaluate nitrogen nutrition in plant beds. One was established at Gatton Research Facility (GRF), the other used a collaborating grower's established plant beds in Bundaberg (BRG). These provided different soil types, weather and cultivar conditions.

GRF

This experiment was located on a black, alluvial clay-loam at Gatton Research Facility. Soil testing of the site showed an initial nitrate level of 32 ppm. Prior to forming the plant beds, we spread the equivalent of 10 t/ha of gypsum to provide high levels of calcium and sulphur, as well as reduce the risk of surface crusting. Beds were raised to 16 cm high, and 100 cm wide. The formed beds were sprayed with a solution of MicroZB plus (containing 10% boron, 15 % zinc and 1% molybdenum), at a rate of 1 L/ha, before the bedding roots were installed.

We designated two 30 m lengths of plant beds as our experimental area, dividing evenly into 12 plots each, with the central 1 m of each plot as the datum area. The experimental design was a randomised complete block, comprising six treatments replicated four times. The structure was a factorial, comprising two cultivar treatments by three nitrogen fertiliser treatments.

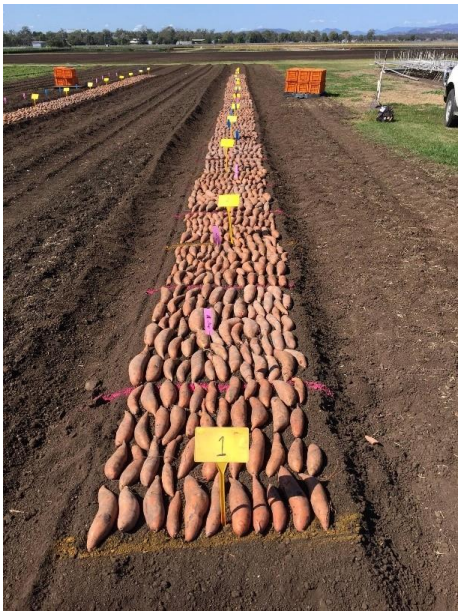


Plate 13. Installation of PT sweetpotato roots into plant beds into the GRF nitrogen experiment.

We used two cultivars, *Beauregard* and *Bellevue*. The three nitrogen treatments were:

1. **Low** – the equivalent of 10 kg N/ha; adding 7.7 g potassium nitrate per square metre, balanced with 40.8 g potassium sulphate to maintain a constant potassium level for each plot. Considered one third standard grower practice.
2. **Med**, – the equivalent of 30 kg N/ha; adding 23.1 g potassium nitrate per square metre, balanced with 24.5 g potassium sulphate to maintain a constant potassium level for each plot. Considered standard grower practice.
3. **High** - the equivalent of 60 kg N/ha; adding 46.2 g potassium nitrate per square metre. Considered double standard grower practice.

The nitrogen fertiliser treatments were applied by dissolving the required amounts of potassium nitrate and potassium sulphate in 18 L of water and irrigating them on using a watering can. We applied the treatments on five occasions, immediately after bed installation, and subsequently one or two days after the first four sprout harvests.

We installed around 17 kg/m^2 of graded *Bellevue* and 16 kg/m2 *Beauregard* PT bedding roots on top of the formed beds, and then manually covered the roots with 3 cm of soil. Prior to supply, the *Beauregard* and *Bellevue* bedding roots were pre-sprouted at the Rockhampton production facility, by heat treating at 25-30^{o}C (90% RH) for one and three weeks respectively.

The plant beds were installed on 10 September 2015 and irrigated with 50 mm of irrigation the following day. A hand-shift irrigation system, delivering approximately 25 mm/hr of irrigation, with a uniformity of around 75%, was initially used for this experiment. Because of issues with wind and irrigation uniformity, a drip system was installed to assist with irrigation on difficult days.

Plate 14. Plant bed irrigation system.

We irrigated, weeded the plant beds, and sprayed for insects to maintain the plant beds as per grower best practice. We also recovered the plant bed edges that got washed away during heavy rainfall.

BRG

The second experiment was located on a collaborating grower's property south-east of the Bundaberg Research Facility. It was a red kraznozem soil, regularly used for commercial sweetpotato production and plant beds. The plant beds had already been installed by the grower in mid- September 2015. When we installed our plots, the beds were around 14 cm high, and 80 cm wide. Soil testing of the site showed it had a nitrate level of 48 ppm on 3 October 2018.

We implemented the same experimental treatments as at GRF, with the exception that the *Beauregard* cultivar was replaced with *Orleans*. Because of the grower's layout, all the *Orleans* plots were in one plant bed, and all the *Bellevue* plots in a second bed. As at GRF, the individual plots were 2 m long, and we used the central 1 m as the datum area. The plant beds were irrigated with a high precision solid-set irrigation system, delivering approximately 5 mm/hr of irrigation, with a uniformity of around 85%.

The collaborating grower managed nutrition, weeding and pests along with their normal plant beds. Rachael Langenbaker would advise the grower if she saw any issues arising from her regular field inspections (usually once, and often twice a week). The grower had a history of high performance plant beds, so we were very confident they were managed in line with their concept of best practice.

Ms Langenbaker applied the fertiliser treatments (same as at GRF) on four occasions, immediately after a first preliminary bed trim on 5 November 2015, and subsequently one or two days after the first three plant harvests.

Experimental data collection.

Before the bedding roots were installed at GRF, we took four 10 kg samples of *Bellevue* and four 10 kg samples of *Beauregard* bedding roots from the commercial lots, to assess their grade characteristics. For each sample, we measured the length, maximum diameter, weight and extent of sprouting of each root in the sample.

Plate 15. Samples of *Beauregard* (left) and Bellevue (right) for bedding root quality assessment.

At both experiments, when sprouts were ready for harvesting, we would sample the central 1 m of each plots for measurement. These samples were collected using standard commercial harvesting practices, cutting around 4 cm above the soil surface. We would collect all the material, and place in crates for storage and assessment. The remaining buffer areas were also cut, and the sprouts supplied for commercial planting, or disposed of if there was no ready use for the material.

Plate 16. Assessing sprouts for productivity and quality at Gatton (left) and Bundaberg (right).

The collected sprout samples were processed in the following manner. We weighed the total fresh biomass, and then separated the material into:

- Damaged or diseased sprouts
- Undersized sprouts (<20 cm long) and loose, leaf material
- Short acceptable sprouts (20-28 cm long)
- Optimal sprouts (28-40 cm long). Sprouts longer than 40 cm were trimmed back to this length.
- Back cuttings (the trimmed excess of sprouts longer than 40 cm)

We weighed each of the five categories and counted the number of short and optimal sprouts. We randomly selected 10 sprouts from each of the short and optimal groups, measured their width, and counted the number of nodes in the 15 cm interval closest to the cut (distal) end.

We intermittently sampled the surface soils to check surface nitrogen concentrations in the plant beds at both sites.

By the end of February 2016, there had been 5 cuts at GRF and 4 cuts at BRG.

Results

Analysis of GRF bedding roots

As discussed, we assessed root grades at GRF, before the beds were installed.

See Appendix 3 p60 for interpretation of the box/whisker plots. The analysis of the root size distributions for the two cultivars shows very consistent grades for both (Fig. 15). Whilst their lengths were similar, the *Bellevue* tended to be slightly larger diameter than *Beauregard*. They were both regular, medium size bedding root grades.

Almost all roots were within 4-8 cm in diameter, with 50% between 4-7 cm.

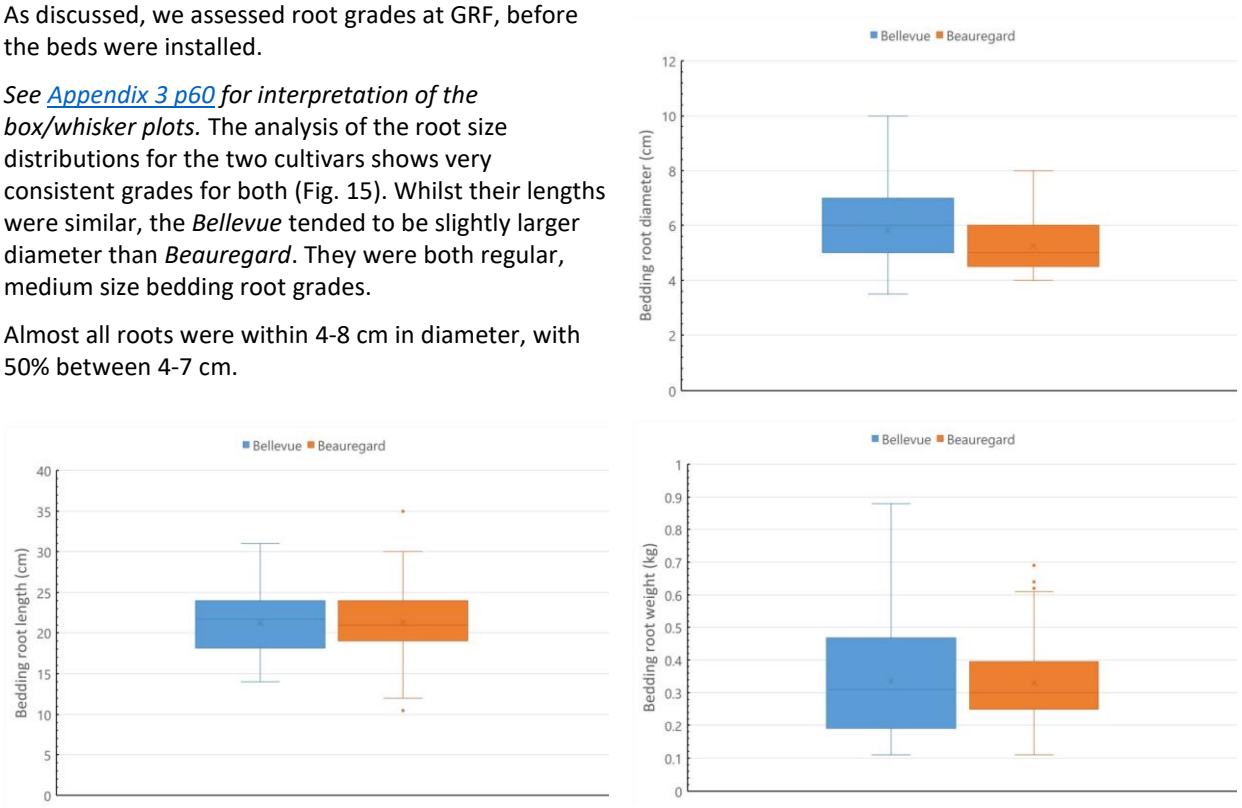


Fig 15. Diameter (top right), length (left) and weight (right) of individual bedding roots from the 2015/16 plant bed nutrition experiment at Gatton Research Facility.

The heating pre-treatment resulted in 50% of both cultivars having visible sprouts, those roots averaging 1-2 sprouts per root. Most of the *Beauregard* sprouts were only 1-2 mm long. In contrast, while 75% of *Bellevue* sprouts were 3 mm or less, the remaining 25% were up to 7-9 mm in length.

Plant bed productivity

It took 8 weeks from bed installation for the GRF plant bed to reach size for the first cut (Fig. 16). Cuts 2-5 were on average 26 days apart, although Cuts 3 and Cut 4 were taken early, due to logistics around holiday arrangements. Productivity at this site was consistently around 230-250 sprouts/m^2, except for the early harvest at Cut 3, where our harvest averaged 170 sprouts/m^2. At Cuts 2-4, *Bellevue* produced 10% more sprouts than *Beauregard*. At no cut did the amount of nitrogen fertiliser applied affect the number of acceptable sprouts harvested.

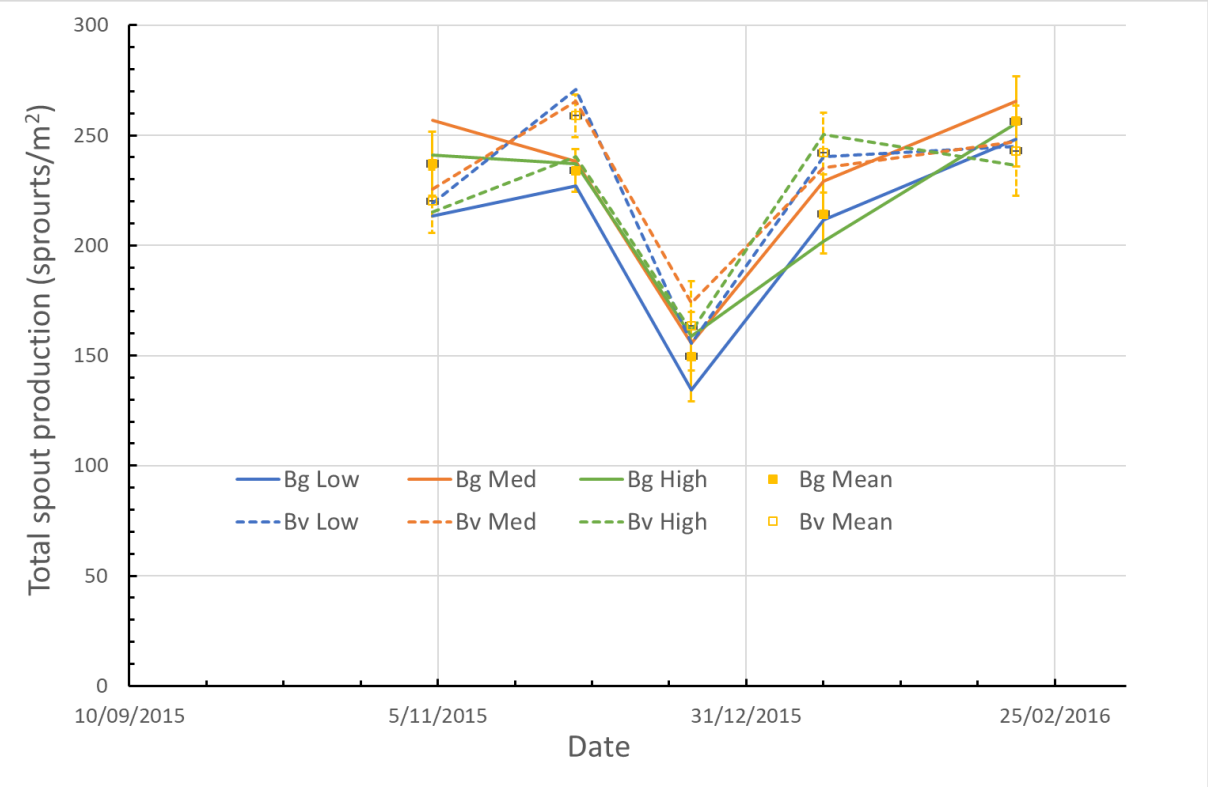


Fig. 16. Sprout productivity at GRF affected by cultivar and days between cuts, but not nitrogen nutrition treatments – bars show the 95% confidence intervals for the respective Cut means for *Beauregard* (Bg) and *Bellevue* (Bv).

At BRG, it was nearly 12 weeks from bed installation until sprout harvesting. The grower trimmed the plant beds in early November to improve uniformity, so the first harvest was around 26 days after that event (Fig. 17). The grower removed the *Bellevue* plant beds after 16 weeks, as they were not planting that cultivar after January, due to perceived unacceptable cracking and discolouration risk. *Bellevue* averaged around 215 sprouts/m^2 at both of its cuts. *Orleans* produced appreciably more sprouts than *Bellevue* at both those early cuts, consistently generating over 250 sprouts/m^2. *Orleans* productivity did decline by Cut 4. Productivity at this site was more variable, reflected in the larger confidence intervals. As at the GRF location, nitrogen did not impact the number of acceptable sprouts harvested on any occasion.

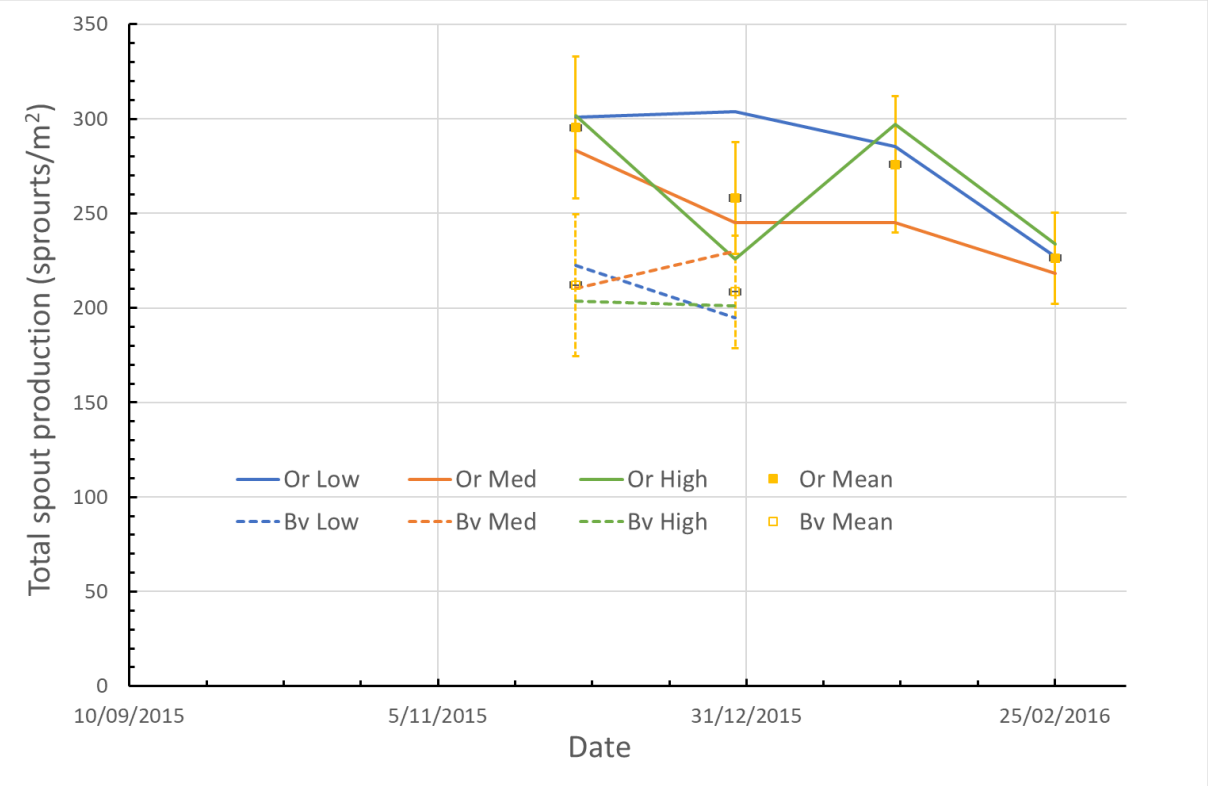


Fig. 17. Sprout productivity at BRG affected by cultivar, but not nitrogen nutrition treatments – bars show the 95% confidence intervals for the respective Cut means for *Orleans* (Or) and *Bellevue* (Bv).

Sprout quality

The nitrogen fertiliser strategy did *not* impact any sprout quality trait, for any cultivar, at either site.

At GRF, for both *Beauregard* and Bellevue cultivars, provided harvesting was delayed until the beds were ready, both cultivars were capable of providing around 85% premium grade sprouts. This is shown at Cuts 1, 2 and 5 (Fig. 18). Harvesting early at Cuts 3 and 4 resulted in a much greater proportion of short sprouts. *Bellevue* had a lower proportion of premium sprouts than *Beauregard* on both those occasions.

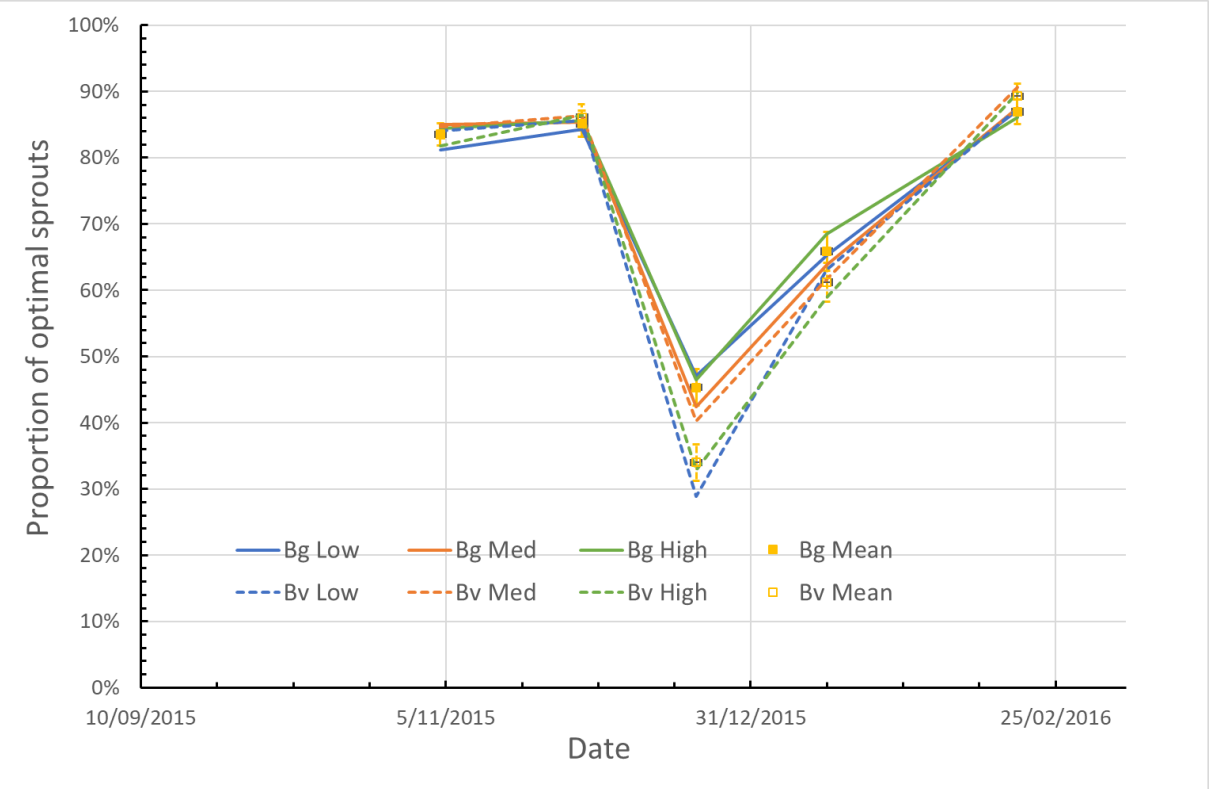


Fig. 18. The proportion of premium sprouts at GRF affected by cultivar and days between cuts, but not nitrogen nutrition treatments – bars show the 95% confidence intervals for the respective Cut means for *Beauregard* (Bg) and *Bellevue* (Bv).

At BRG, *Bellevue* was consistently around 80% premium sprouts on both cutting occasions. *Orleans* was lower at 70% and declined to 60% by Cut 4 (Fig. 19).

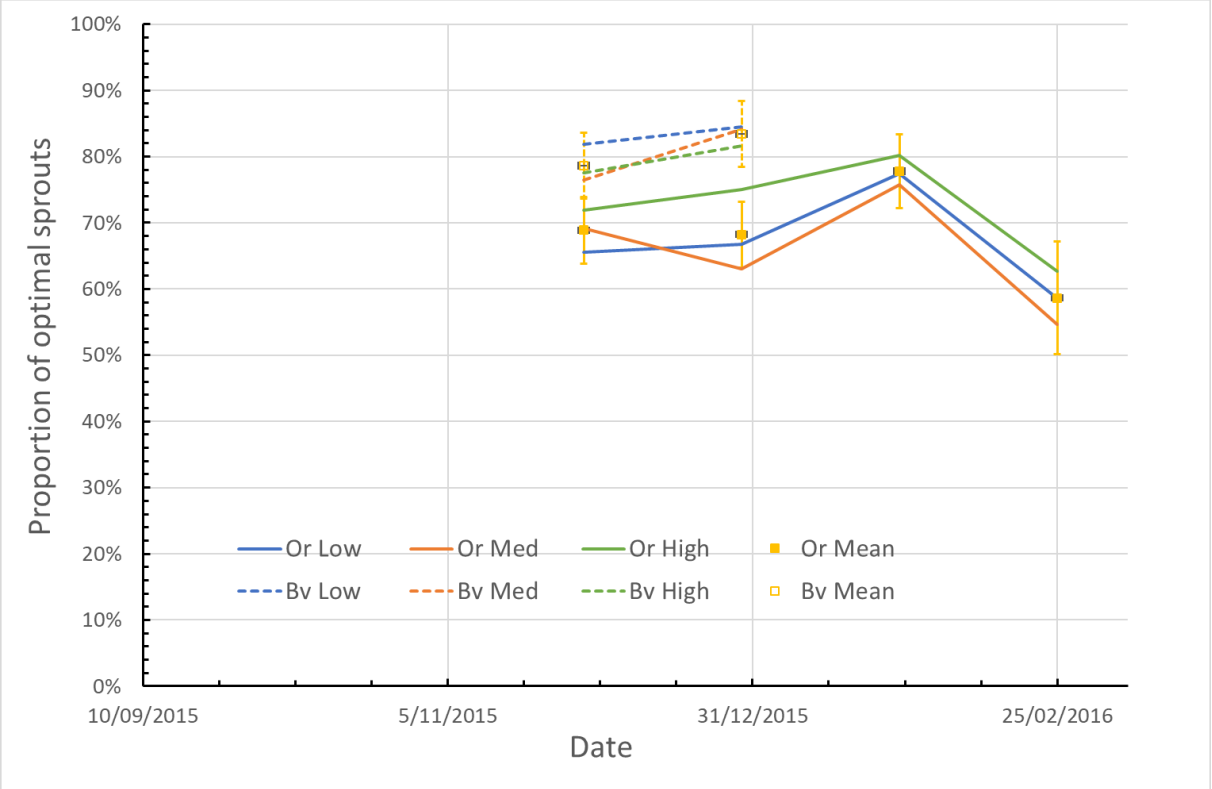


Fig. 19. The proportion of premium sprouts at BRG affected by cultivar, but not nitrogen nutrition treatments – bars show the 95% confidence intervals for the respective Cut means for *Orleans* (Or) and *Bellevue* (Bv).

Across all the cuts at both sites, 60-90% of *Orleans* and *Beauregard* premium sprouts were more than 4 mm in diameter at the cut end. In contrast, a much lower proportion of *Bellevue* sprouts were this thick; the percentage was only more than 60% when there were few optimal sprouts.

All cultivars consistently produced 3-4 nodes in the distal 15 cm at both sites.

Soil nitrogen levels

There was no consistent relationship between fertiliser treatment and measured soil nitrate levels at either site (Tables 4, 5).

Table 4. Soil nitrate levels in GRF plant beds not clearly linked to nitrogen fertiliser strategy.

Soil Nitrate (ppm)	09/11/15	02/12/15	16/12/15	11/01/16	19/02/16
Low	7	15	6	6	21
Medium	7	10	8	6	23
High	8	17	23	8	16

Table 5. Soil nitrate levels in BRG plant beds not clearly linked to nitrogen fertiliser strategy.

Soil Nitrate (ppm)	1/12/15	30/12/15	28/01/16	25/02/15
Low	13	36	20	17
Medium	12	34	15	14
High	13	38	14	35

Discussion

Bedding root supply

The PT bedding roots supplied for the GRF experiment were high quality, consistent lots, demonstrating close attention to size and quality specifications. The pre-sprouting treatments were successful in this instance and did not appear to exacerbate any breakdown issues in the bedding roots, either in storage, or subsequent field plantings.

Nitrogen strategy

The lack of any obvious or consistent impact of changing nitrogen application rates on plant bed performance was interesting. At GRF for example, the High N treatment received 250 kg/ha more nitrogen than the Low N treatment, yet we did not pick up any significant differences in sprout productivity, sprout quality, plant bed breakdown, or even in soil nitrate levels. This lack of effect was consistent across all cultivars, at both sites.

The initial soil fertility was high at both experiments, so the background nitrogen supply may have been sufficient to generate good nitrogen levels for sprout production. Potentially, the bedding roots themselves may have supplied a level of nitrogen to the sprouts for an extended period of time, both through initial direct transfer, and at later stages, even if they were breaking down, from microbial mineralisation.

Biomass removal calculations suggest harvesting sprouts from high productivity beds is removing 4-10 times more nitrogen than is being replaced in general side-dressings. The lack of any N response in this situation is intriguing, indicating the situation is more complex than anticipated. The current conclusion is that there is no reason to shift from current industry practice, although perhaps the rates of side-dressing should be increased in low fertility soils as a risk management strategy, as there appears no issue with over-fertilising.

Future work may also focus on developing critical plant tissue tests for sprouts, as well as their morphological characteristics, as a guide to fertiliser requirements. It is unlikely that soil testing will provide much useful information for decision making for side-dressing requirements, if adequate basal fertiliser has been applied at bed installation. It is very unlikely that growers can over-fertilise their plant beds.

Cultivar performance and the timing of harvest

At GRF, *Bellevue* produced more sprouts than *Beauregard* at three of the five cuts. This was unusual, as *Bellevue* is generally not considered a strong plant bed performer. Perhaps in this instance the heat pre-treatment optimised sprout production for this cultivar. *Orleans* is a very strong sprout producer, as shown at the BRG site, generating nearly 300 sprouts/m^2 at peak performance. There was some evidence of plant bed breakdown with *Bellevue* at BRG, however it did not change between cuts, indicating it was not catastrophic, and the total productivity was acceptable for this cultivar. By Cut 4 at BRG, *Orleans* was suffering productivity issues, with both the quantity and quality of sprouts declining. By this stage, the plant bed was 6 months old, so such a decline is understandable.

Generally, *Orleans* is a high-quality sprout producer, generating, reliable, thick and robust planting material. At the BRG site, perhaps the very high productivity of *Orleans* was causing competition between the sprouts, and the plant beds could have been left to grow for a few more days before harvesting, to ensure a premium length sprout.

All three cultivars generated sufficient nodal densities for premium sprouts in these experiments.

The results of early cutting at GRF clearly demonstrated the productivity and quality penalty associated with prematurely harvesting plant beds Cutting early resulted in fewer sprouts, and a lower proportion making premium length. It also demonstrated that *Bellevue* may be slower to produce sprout length than the other two cultivars.

Given adequate nutrition levels, the number of days to reach optimal cutting is primarily a function of weather (temperature and solar radiation), however it is also dependent on cultivar characteristics, as well as sprout densities and induced inter-sprout competition. Growers will need to factor these variables into their judgements when developing their planting schedules and correlated plant bed requirements.

Appendix 5

Assessing the potential impact of excessive irrigation on productivity and breakdown rates in *Bellevue* plant beds

Introduction

During the latter years of VG13004, it was apparent that the commonly grown Gold cultivar *Bellevue* was the most susceptible to premature breakdown in plant beds. The project team and a few sweetpotato growers recognised that there appeared to be both physiological and disease aspects to this breakdown. However, the actual initial causes of premature breakdown were hard to elucidate, and particularly in highly variable field situations. Management options to alleviate this issue are an important grower priority.

Plate 16c. *Bellevue* breakdown in plant beds.

A model for breakdown in sweetpotato cultivar *Bellevue*

In field and controlled environment observations, we have observed *Bellevue* bedding roots deteriorating due to both physiological and pathological processes (see Appendix 7 for examples).

In long term storage, even in reasonable temperature conditions, the roots get lighter, and in some cases become extremely hollowed out. This is likely just respiration driving use of internal storages of sugars and starches, although this has not been determined. Higher temperatures drive more rapid respiration.

One hypothesis is that at a certain respiration rate, the build-up of respiratory metabolites causes initial death of internal tissues. This then starts a negative feedback loop, where the decaying tissue releases more toxic compounds, until the root starts to rapidly breakdown. This decaying tissue is also a very suitable environment for pathogenic organisms, particularly fungi such *as Fusarium spp.* and bacteria such as *Erwinia spp.*

Conditions that slow or prevent the root exchanging oxygen for metabolite gases (such as carbon dioxide or ethylene) at a sufficient rate would exacerbate any physiological breakdown process. According to this hypothesis, physiological breakdown would be greater with:

- Higher temperatures, driving increased respiration rate.
- Storage with other roots that are breaking down, thus reducing oxygen and increasing the concentrations of toxic metabolites.
- Larger bedding roots, with a smaller per gram surface area for gas exchange.
- In the field, conditions that slow gas exchange, such as deep burial, waterlogging, soil compaction.

We have certainly observed those conditions driving breakdown in storage, and in field planting beds.

Although we have not had the opportunity for a comprehensive pathological study, we know that we are picking up *Fusarium* and *Erwinia spp.* in breaking down bedding roots. Many pathogenic organisms are endemic in most of our horticultural soils. It may be that very high loads of these organisms can cause substantial breakdown, even when the conditions for physiological breakdown are not present. However, our current view is that generally we require at least one of the above conditions to be in play, to drive breakdown.

Although beyond the scope of this project, *Bellevue* may be inherently less resistant or tolerant of the pathogens causing breakdown, compared to other cultivars.

The purpose of this final series of project experiments was to examine the role of irrigation management in driving productivity and breakdown in *Bellevue* plant beds, and, whether over-watering is a substantial risk factor.

Methodology

We established detailed experimental sites on two growers' properties in Bundaberg, investigating impacts of irrigation management on productivity and longevity of *Bellevue* plant beds. One experiment commenced in September 2017, with the other installed in mid-November 2017, to provide contrasting environmental and seasonal influences.

Grower 1

This site had no commercial sweetpotato crop for at least 4 years. It was a red kraznozem soil, which was fumigated with metham-sodium eight weeks before installing the plant beds. Plant beds were formed 20 cm high and 100 cm wide. A high grade organic fertiliser (based on chicken manure) was applied at a rate of 1000 g/m^2. The collaborating grower installed around 17 kg/m^2 of graded *Bellevue* PT bedding roots on top of the organic fertiliser, and then covered the roots with 5 cm of soil. The surface of the plant bed was then additionally fertilised with a further 50 g/m^2 of Amgrow slow release fertiliser.

Plate 17. Installation of *Bellevue* into plant beds.

The plant bed was installed on 4 August 2017 and irrigated with 48 mm of irrigation the following day. A solid-set irrigation system, delivering approximately 12 mm/hr of irrigation, with a uniformity of around 80%, was used for this plant bed.

We designated 25 m of plant bed as our experimental area, dividing it evenly into 15 plots, with the central 1 m of each plot as the datum area. The experimental design was a randomised complete block, comprising three treatments replicated five times. The three irrigation treatments were:

4. **Grower** – grower practice as per the rest of his plant bed, with no additional irrigation, apart from that applied by the grower.
5. **Moist**, - with an additional 10-15 mm of irrigation per week, targeted at keeping plots moist, i.e. less than 25 kPa soil water potential.
6. **Wet** - with an additional 20-30 mm of irrigation per week, targeted at keeping plots wet, i.e. generally in the 5-15 kPa soil water potential range.

The additional irrigation treatments were applied by watering can once or twice a week. Care was taken to avoid runoff, although at times Rachael Langenbaker had to reduce the amount of water applied, because of runoff. In periods of heavy rainfall, no additional irrigation treatments were applied. The first additional irrigation treatments commenced on 20 September 2017, and continued through until 26 February 2018, when the formal experimentation ceased.

Plate 18. Installation of Chameleon sensors.

On 9 September 2017, Ms Langenbaker installed Chameleon soil moisture and temperature sensors in each of the three treatment plots in Blocks 1, 3 and 5. In each plot, two moisture sensors were buried at 7 cm deep, level with the upper surfaces of the bedding roots. A third moisture sensor was buried at 20 cm. close to the level of the original soil surface. A temperature sensor was buried at the same depth as the shallow moisture sensors. The Chameleon sensors logged every two hours and recorded the data for later downloading.

Plate 19. Chameleon sensors and plot layout.

Grower 2

This site was regularly used for commercial sweetpotato cropping or plant bed installations for several years prior. It was also a red kraznozem soil, which was fumigated with metham-sodium several weeks before installing the plant beds. Plant beds were formed 30 cm high and 60 cm wide. The collaborating grower installed around 19 kg/m^2 of graded *Bellevue* PT bedding roots and then covered the roots with 7 cm of soil. This soil was levelled and slightly compressed mechanically with a pallet. No fertiliser was applied to the plant beds at installation.

The plant bed was installed on 3 November 2017 after having been moistened with 18 mm of irrigation four days before. A solid-set irrigation system, delivering approximately 12 mm/hr of irrigation, with a uniformity of around 70%, was used for this plant bed.

The experimental layout, design and treatments were exactly the same as with Grower 1, although each plot was 2 m long, providing an additional buffer between datum areas.

At this site, the first additional irrigation treatments commenced on 1 December 2017, and continued through until 26 February 2018, when the formal experimentation ceased. Ms Langenbaker installed the Chameleon sensors using the same processes as at Grower 1 on 6 November 2017, only 3 days after bed installation.

General site management

At both experimental sites, the collaborating grower managed nutrition, weeding and pests along with their normal plant beds. Ms Langenbaker would advise the grower if she saw any issues arising from her regular field inspections (usually once, and often twice a week). Each grower has a history of high performance plant beds, so we were very confident they were managed in line with their concept of best practice.

Experimental data collection.

Before the bedding roots were installed, Ms Langenbaker took several 20 kg subsamples from the commercial lots to assess their grade characteristics. For each sample, she measured the length, maximum diameter and weight of each root in the sample.

After plant bed installation, Ms Langenbaker took photos of each plot every few weeks, to be used for visual assessment of any obvious differences in sprout growth or plant bed breakdown between treatments. Ms Langenbaker regularly liaised with the growers and organised to be on-site each time they were going to harvest sprouts from the experimental area.

When the sprouts were being cut for commercial planting, RL would liaise with the cutting crews, and get them to place the sprouts they were keeping from the plot datum areas into a separate crate for each plot. She counted and weighed the sprouts from the 1 m^2 datum, and then randomly select 20 sprouts as representative of that plot.

Plate 20. Collaborative experimental sprout harvesting.

Plate 22. Measuring sprout width.

Plate 21. Measuring sprout length and nodal density.

For each of the 20 spouts in the sample, she measured their total length, their width, and counted the number of nodes in the 15 cm interval closest to the cut end.

By April 2018, there had been 7 cuts by Grower 1, and 5 cuts by Grower 2.

Results

Analysis of bedding roots

As discussed, we assessed bedding root grades at both sites, before the beds were installed.

See Appendix 3 p60 for interpretation of the box/whisker plots. The analysis of the root size distributions for the two Growers (G1 samples A-E, G2 samples A-C) demonstrated relatively tightly graded commercial lines, well within the medium size category (Fig. 20). In each sample, there were only a few size outliers. Almost all roots were within 3-8 cm in diameter, with 50% between 4-7 cm.

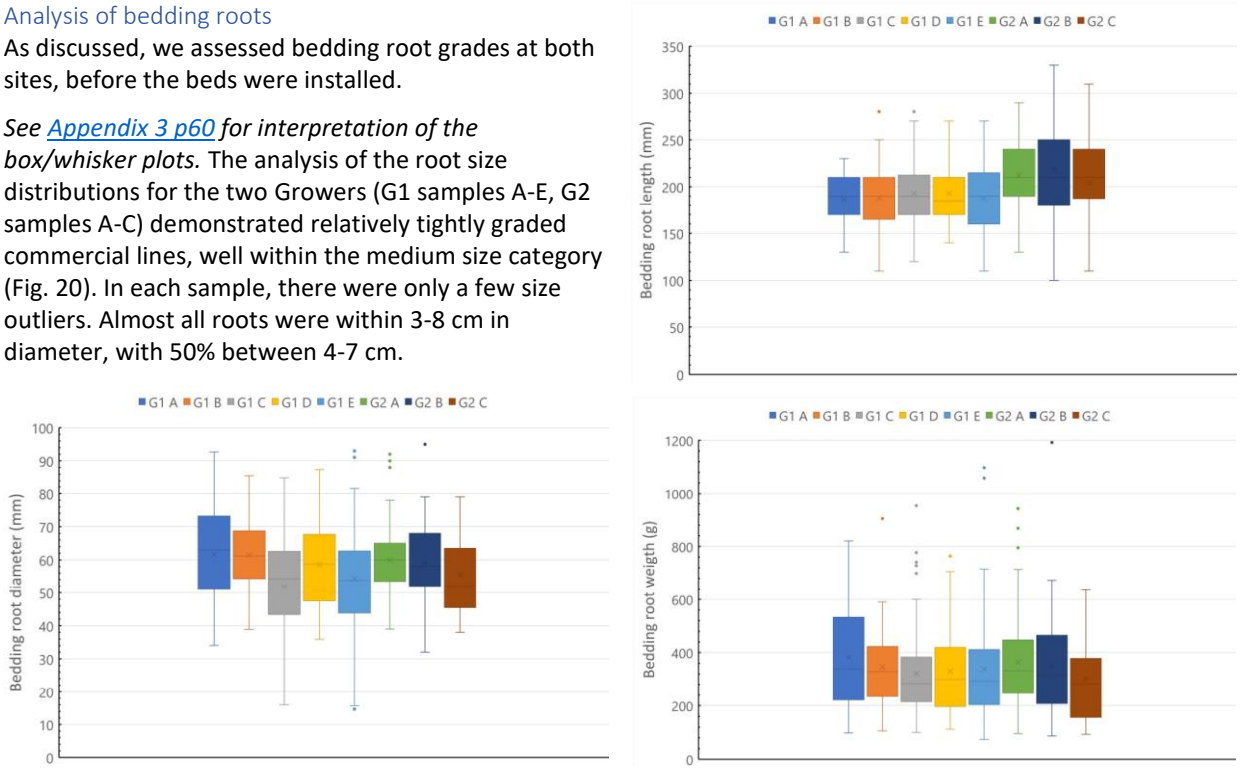


Fig 20. Length (top right), diameter (left) and weight (right) of individual bedding roots from the 2017/18 plant bed irrigation experiments, for sites Grower 1 and Grower 2.

Treatment implementation

At Grower 1, the irrigation treatments commenced 7 weeks after the beds were installed (Fig. 21), when sprouts had started to emerge. With Grower 2, Ms Langenbaker implemented the treatments 5 weeks after bed installation (Fig. 21). Sprouts had started to emerge earlier at this site, because of the warmer weather. The total weekly amount by a treatment is the value shown by the top boundary of its colour. Where a weekly total does not have a treatment colour, it only received rain during that week.

Both sites experienced very heavy rain in October and December 2017, and January and March 2018. This rain dominated water applications during those months.

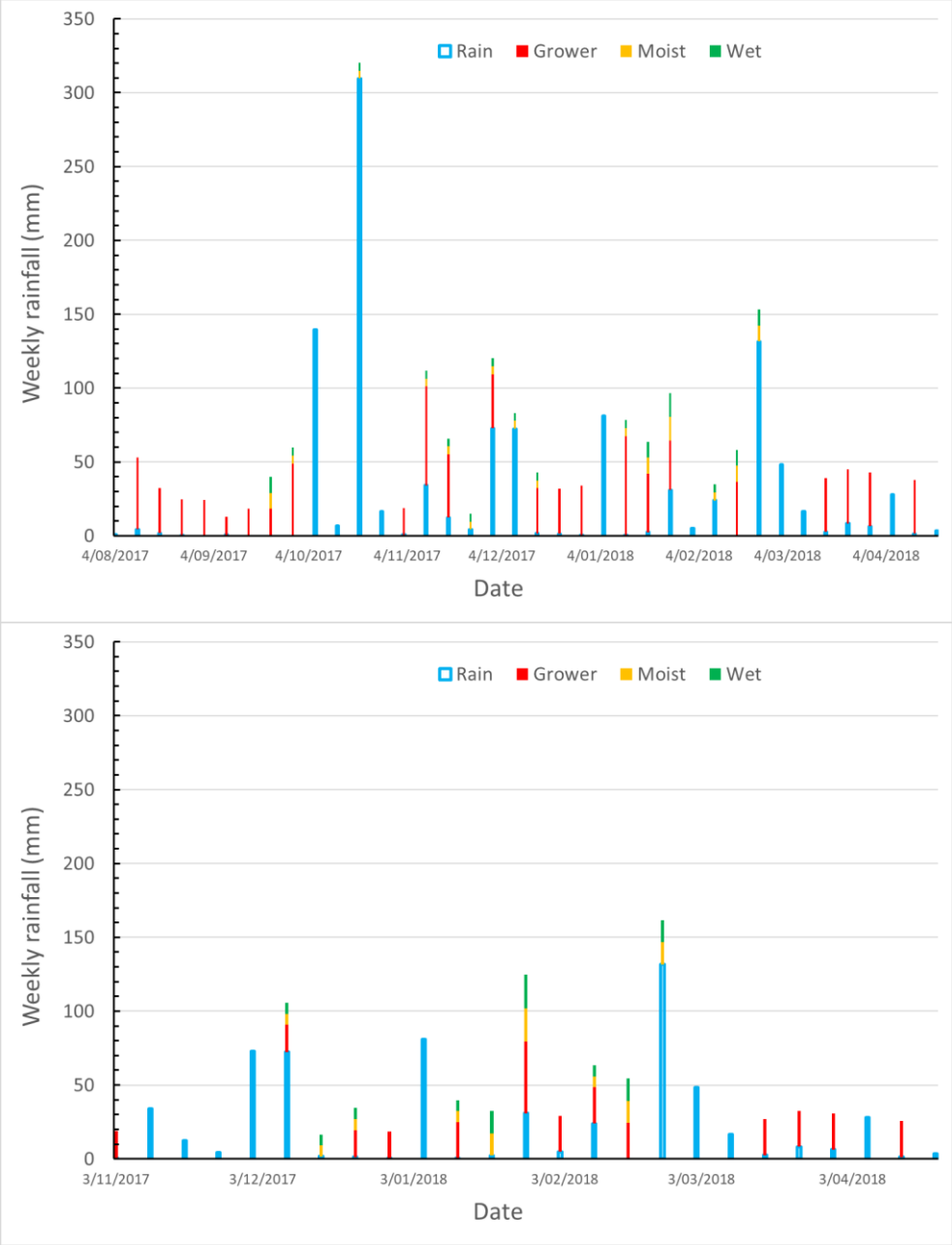


Fig. 21. Weekly water received by Grower 1 (top) and Grower 2 (bottom) plant beds from installation to 20 April 2018.

We had to reduce the amounts of irrigation we applied to the additional treatments, as particularly with the Wet treatment, we were unable to add more water without it running off. We did not have the resources to be able to irrigate twice a week, which may have improved our irrigation capacity. Certainly, rain and grower irrigation dominated the total amounts of water applied, especially with Grower 1

Table 6. Total water received(mm) by Grower 1 plant beds from installation to 20 April 2018.

Source	Grower	Moist	Wet
Rain	1069	1069	1069
Grower irrigation	808	808	808
Additional irrigation	0	113	227
Total	1877	1990	2104

Table 7. Total water received (mm) by Grower 2 plant beds from installation to 20 April 2018.

Source	Grower	Moist	Wet
Rain	589	589	589
Grower irrigation	317	317	317
Additional irrigation	0	105	210
Total	906	1011	1116

Soil water conditions

The additional irrigation did drive differences in the soil water potential, and therefore the 'wetness' perceived by the bedding roots and other rhizosphere biology. Soil water sensor data (examples Fig. 22) showed that as we added more additional irrigation, there were certainly more extended periods of wet conditions, and fewer dry periods. The other interesting observation from the soil moisture data, and backed up by the amounts of irrigation applied, was that the Grower 2 plant beds were substantially drier than the Grower 1 beds.

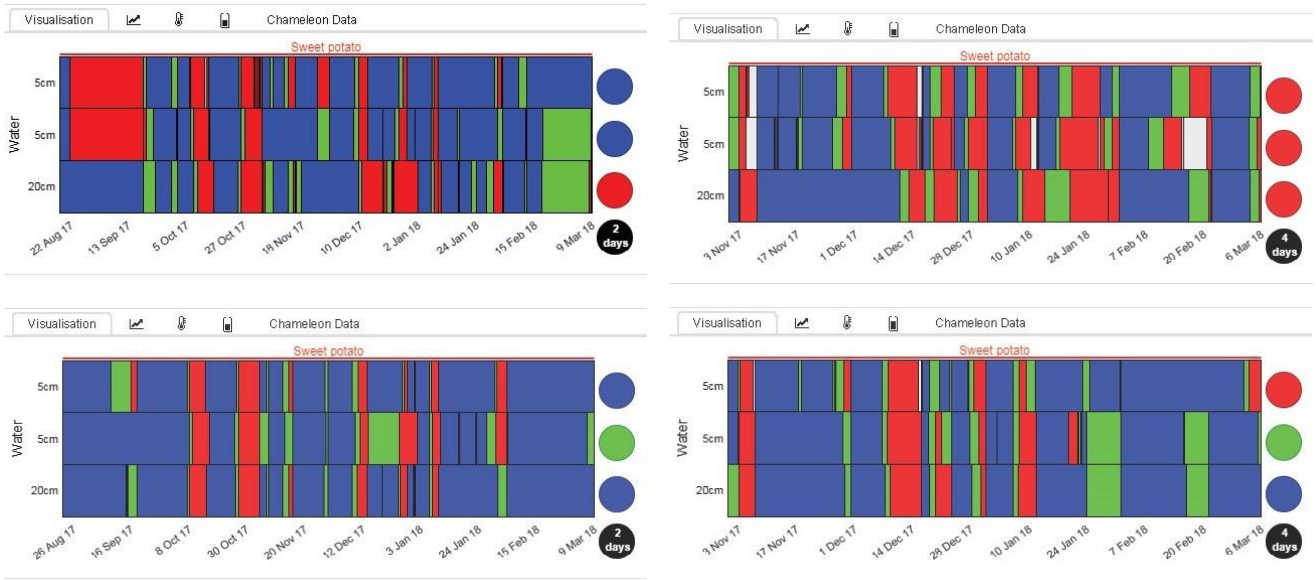


Fig. 22. Seasonal Chameleon soil moisture data for shallow (5 cm) and deeper (20 cm) zones in plant beds. Colours show periods of dry (red), moist (green) and wet (blue) soil water status for Grower 1 (left) and Grower 2 (right) experiments, with the upper and lower charts representing Grower and Wet treatments respectively.

When we calculated the total amount of time during the experimental period the beds spent in either dry, moist or wet conditions (legend in Fig. 23), we can see the differences due to grower irrigation practice, as well as our additional irrigation treatments.

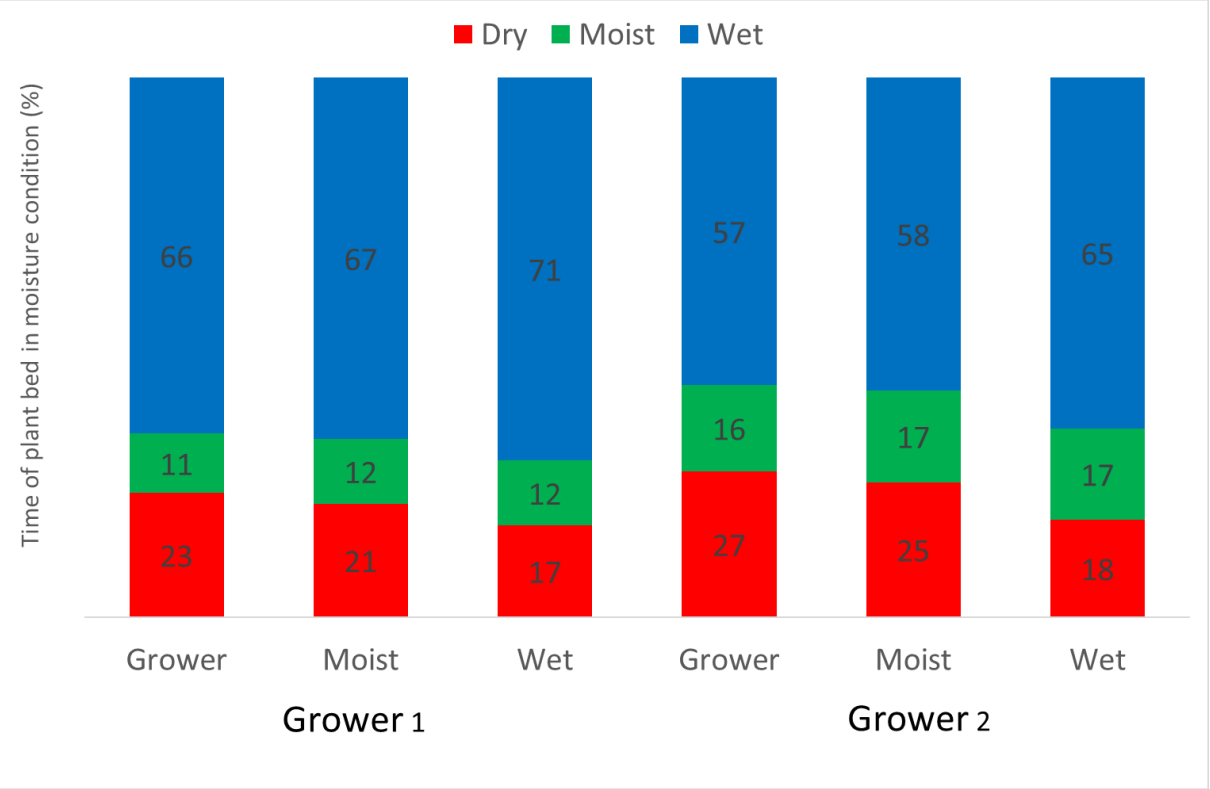


Fig. 23. Grower 2 plant beds experienced more dry conditions that Grower 1, while additional irrigation also drove wetter soil.

Soil temperatures

In reviewing the soil temperature data, we can see that both grower irrigation and our additional watering influenced temperatures within the plant beds (Fig. 24).

At the Grower 1 site, by late August maximum temperatures were around 27°C, although minimums of 16°C were still sufficiently low to restrict sprouting, particularly of *Bellevue*. Interestingly, around early to mid-October, the driest (Grower-irrigation) treatment was regularly 4-5 degrees hotter than the other treatments that received additional irrigation, peaking for several days at 35°C. This effect persisted until the massive 300 mm rain in mid-October 2017, after which there was no segregation of the irrigation treatments, in terms of soil temperatures.

In contrast, temperatures at the slightly drier, Grower 2 site, fluctuated over a greater temperature range for most of the season, and peaked at much higher temperatures as well. It is unclear whether this was a result of land aspect, drier soil conditions, or the narrower, taller plant beds exposing the soil mass to greater solar incidence over a smaller thermal mass. It was also very notable at this second site that the least irrigated treatment certainly consistently recorded the hottest temperatures, throughout the growing period. For several weeks, those plots were reaching maxima of well over 40°C.

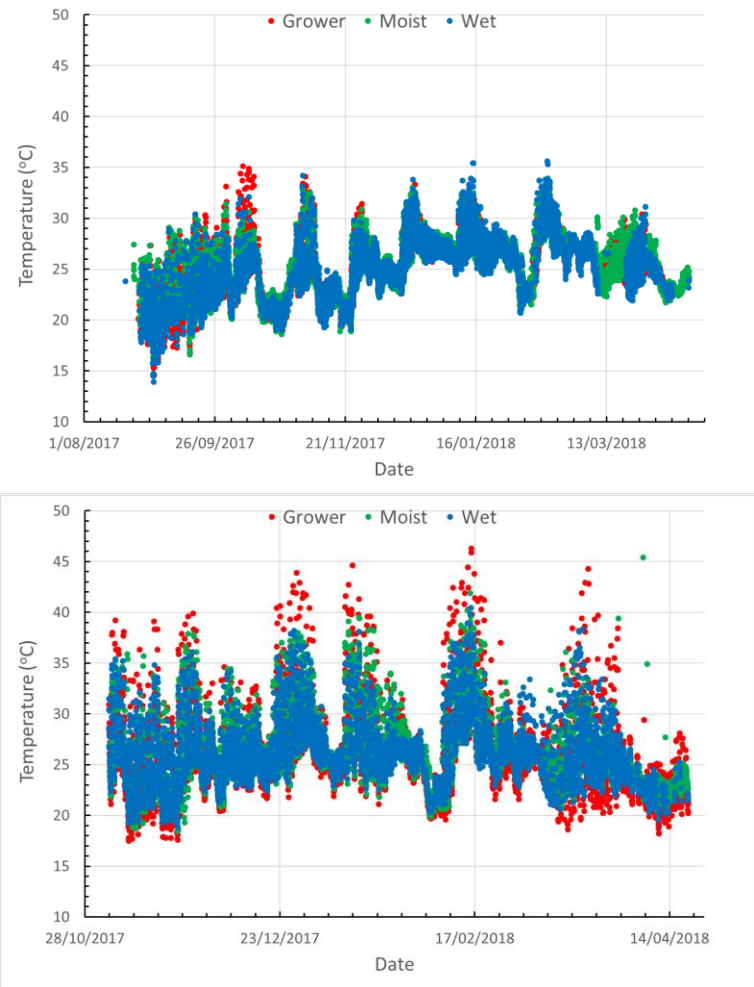


Fig. 24. Grower 2 plant beds (bottom) experienced higher soil temperatures than Grower 1 (top), while additional irrigation reduced maximum temperatures at both sites.

Plant bed productivity

It took nearly 9 weeks from bed installation for the Grower 1 plant bed to produce sufficient sprouts to justify cutting for commercial planting. Cuts 2-4 were only around 24 days apart, with productivity rapidly improving to a peak of 300 sprouts/m^2 in early January 2018 (Fig. 25). Grower 2 plant bed came into production 7 weeks after installation, and also peaked in productivity in early January, although this was only its second cut. The next three cuts at both sites showed deteriorating productivity; only minor reductions from January to February 2018, but more substantial in the ensuing cuts. By the mid-April cuts, productivity at both sites was down to around 130 sprouts/m^2.

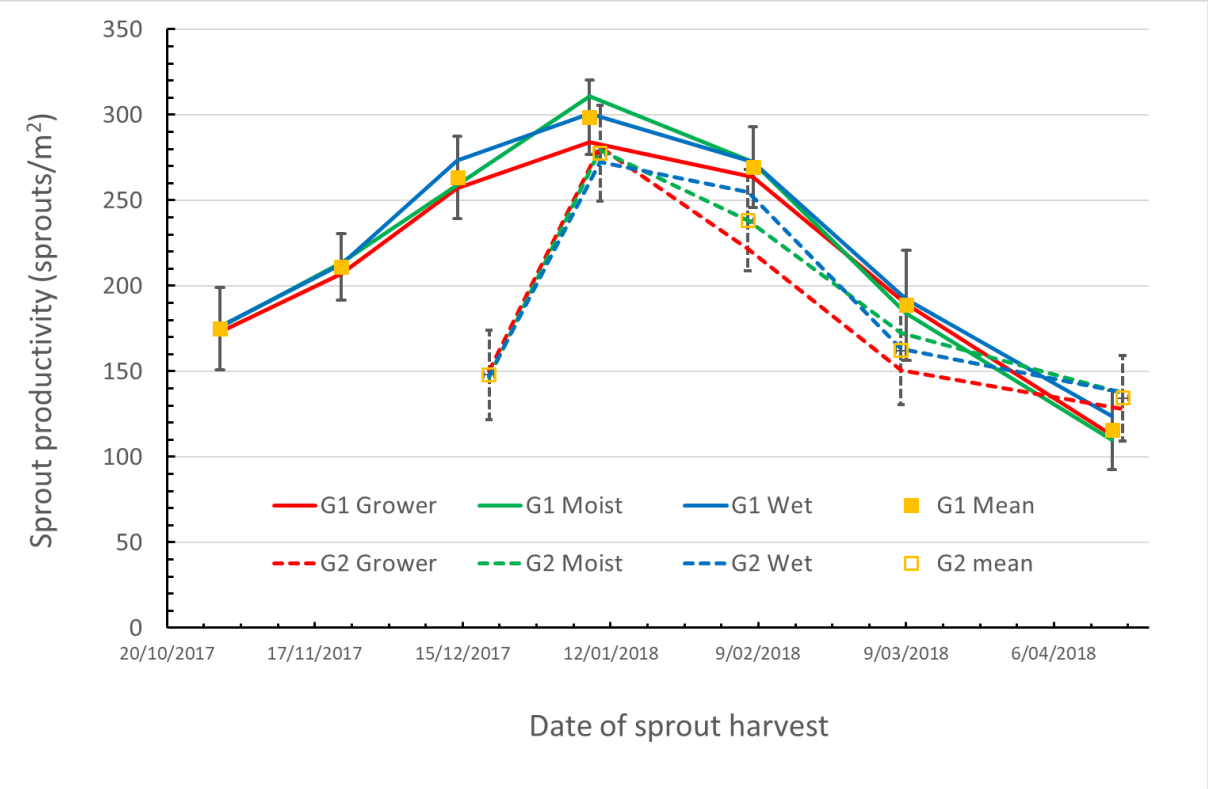


Fig. 25. Sprout productivity peaked at an exceptional level, then declined in late summer and autumn. Irrigation had no impact on sprout productivity – bars show the 95% confidence intervals for the respective Cut means for Grower 1 and Grower 2.

There was no impact of additional irrigation on sprout productivity, at any cut at either site (Fig. 25). It should be noted that the driest irrigation treatment was consistently the least productive, however this difference was not significant. There was a consistent trend for the Grower 1 plant beds to be slightly more productive than the other site at each cutting occasion. This former site also maintained its productivity above the benchmark 250 sprouts/m^2 for several cuts, before falling away.

Sprout quality

Mean sprout lengths were consistently around 34-38 cm for Grower 1, apart from being slightly lower at Cuts 2 and 3, probably because the grower required the plant material early for his planting schedule (Fig. 26). Grower 2 started with a mean sprout length of around 36 cm, increasing to 45 cm by Cut 3, and keeping at that length for the next two cuts. There was no impact of irrigation treatment on the lengths of sprouts.

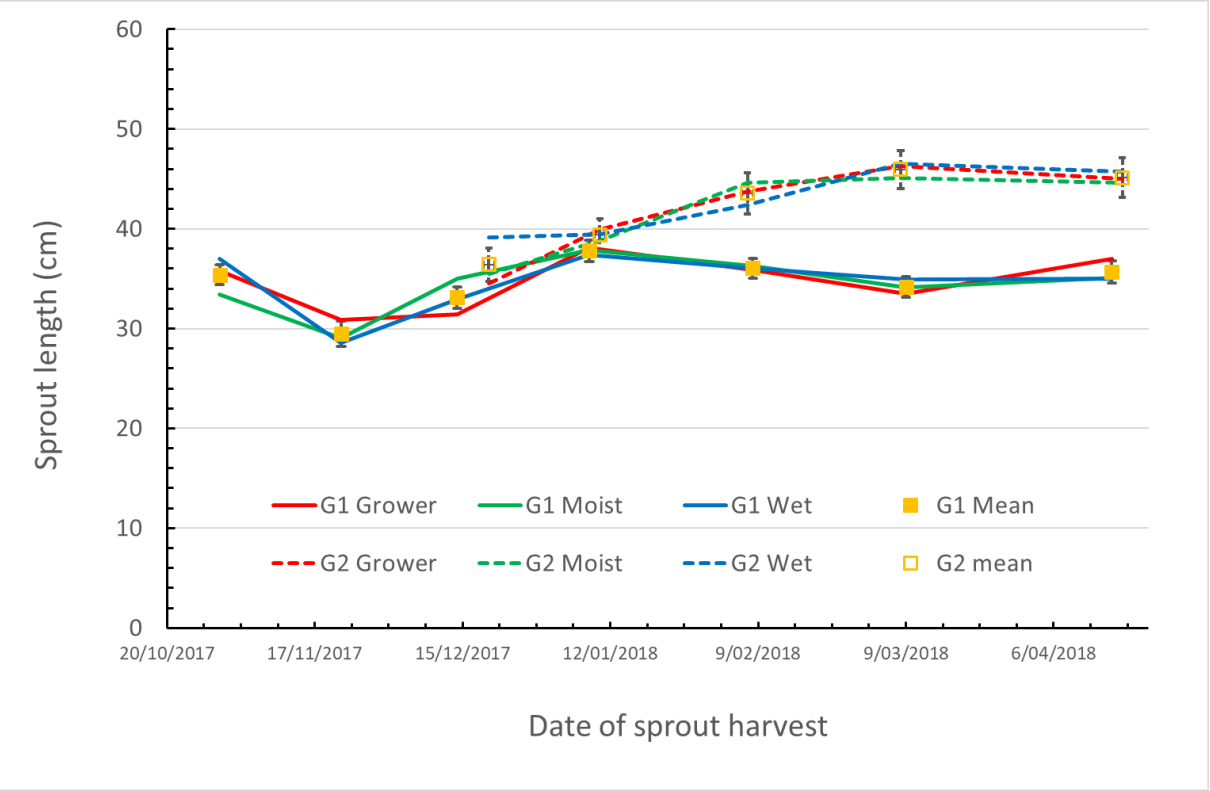


Fig. 26. Sprout length was unaffected by irrigation treatment, however Grower 2 harvested consistently longer sprouts at later cuts - bars show the 95% confidence intervals for the respective Cut means for Grower 1 and Grower 2.

The range of sprout lengths across all the cuts for Grower 1 and Grower 2 (Fig. 27), show the former had a tighter specification, with a maximum length of 50 cm. In contrast, Grower 2 had a substantial proportion of sprouts more than 50 cm long, particularly from Cut 3 onwards. Their maximum length was 60 cm.

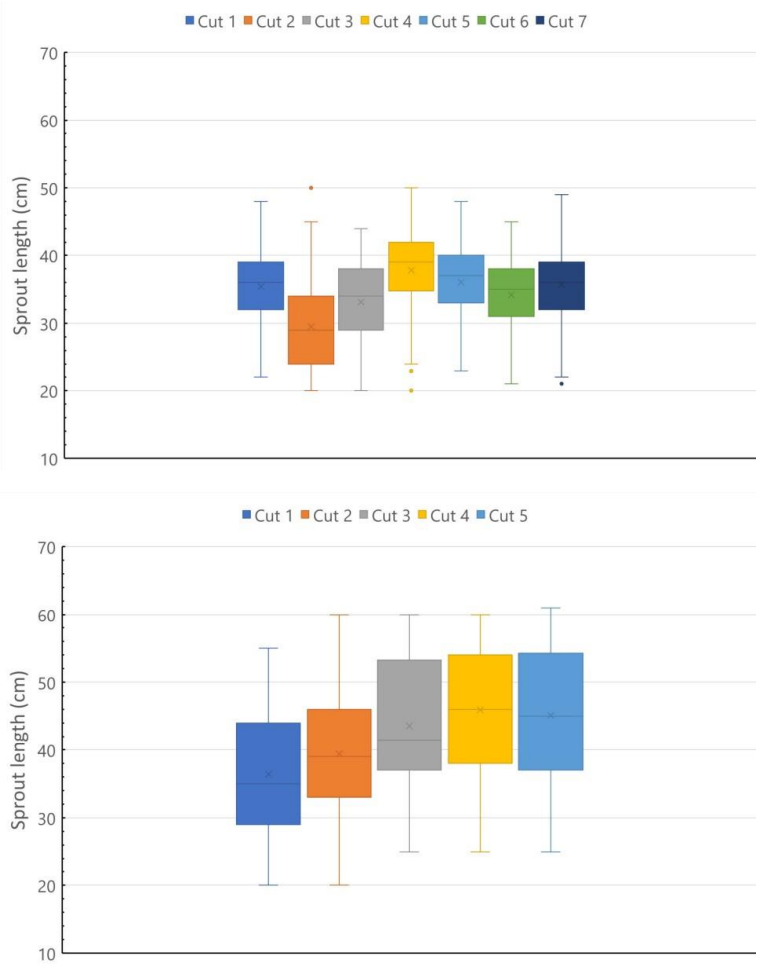


Fig. 27. Grower 2 (bottom) cut a greater range of sprout lengths and longer maximum sprout length than Grower 1 (top).

The average width of *Bellevue* at both sites was around 3-3.5 mm, reasonable for this cultivar. As with other characteristics, there was no influence of irrigation on this trait (Fig. 28).

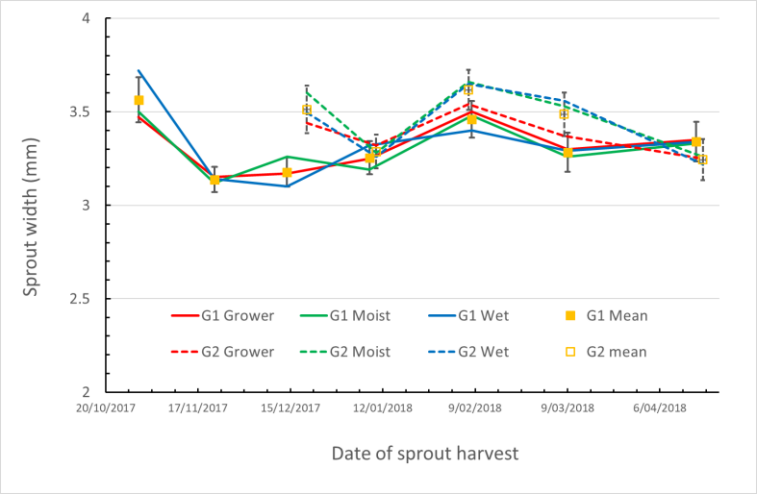


Fig. 28. Sprout width unaffected by irrigation treatment.

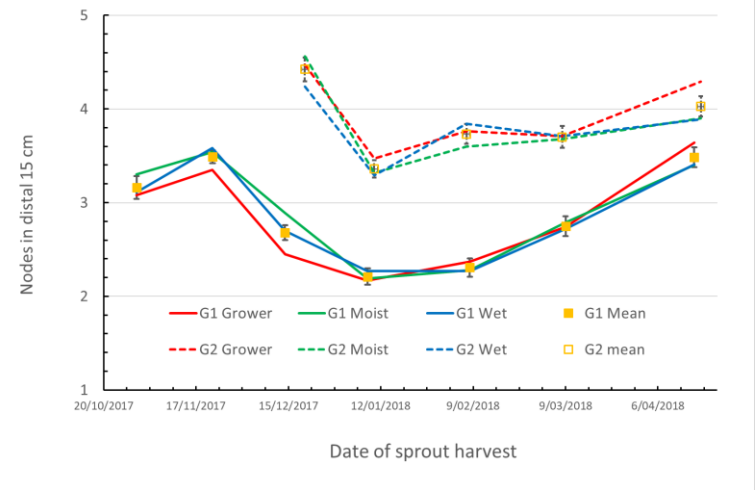


Fig. 29. Sprout nodal density was unaffected by irrigation treatment, while Grower 2 sprouts consistently had greater nodal density than grower 1.

For Grower 1, the mean number of nodes in the distal 15 cm of the sprouts started off at around 3.5, and then fell to just more than 2 during peak production, and then climbed back to 3.5 as fewer sprouts were produced in the later cuts (Fig. 29). Sprouts from the Grower 2 site consistently had higher sprout densities, peaking at 4.4 at the first cut, falling to 3.4 at the most productive Cut 2, before increasing again with sequential cuts. Until the last cut in April 2018, the Grower 2 site generally had at least one more node per 15 cm than Grower 1.

Overall sprout quality at both sites was impressive. Around 90% of the sprouts at both sites were premium lengths (>28 cm), except for the Grower 1 Cuts 2 (60%) and 3 (80%), and Grower 2 Cut 1 (80%), which were probably a few days early, due to commercial planting requirements.

Breakdown

Reductions in productivity from mid-January 2018 were associated with rotting bedding roots. Other bedding roots were still intact. A proportion of sprouts were still producing based on independent root systems, after the underlying bedding roots had disappeared. By March-April 2018, some of these independent sprouts were also dying from disease. Although diagnostics have not been completed on materials collected from these sites, *Fusarium* and *Erwinia* were both symptomatically evident in the breakdown areas. Breakdown started relatively isolated, but patches were starting to become more widespread and coalescing by mid-March at both sites.

From photographic evidence, field observations and productivity assessments, there was no association between irrigation treatments and breakdown extent at either site.

Discussion

The grade analysis of the *Bellevue* commercial lots supplied to both growers confirmed showed that specifications for this cultivar had been tightened to exclude large sweetpotatoes, and were generally within the small-medium range of 3-8 cm. This was a deliberate move by the supplier to reduce potential risks of premature breakdown. It should be recalled that smaller roots are more expensive to produce (lower yields per ha), and growers also require fewer bins of smaller roots to produce the same number of sprouts. So, all things being equal, producing smaller roots, and grading out large roots is a cost currently born by the supplier.

Whilst it was difficult to supply the levels of additional irrigation initially targeted in the experiment, we still managed to create wet plant bed conditions for the designated treatments for much of the growing period. Further, heavy rain in several months did create the potential for waterlogging on many occasions. In hindsight, more water addition during the pre-sprout emergence phase may have been a useful test as well.

The soil temperature results were interesting, suggesting there may be some benefit for light surface watering to reduce soil temperatures during peak heat periods. Temperatures of 35-45^{o}C would certainly be sufficient to drive increased respiration and subsequent bedding root deterioration. For growers that insist on installing new beds in peak summer, it may be worthwhile to reduce temperatures by slightly deeper soil covering and cooling with frequent, light watering.

Productivity from both plant beds, (up until mid-January 2018) was excellent, and for several cuts was well above the early project benchmark of 230-250 sprouts/m^2. The decline in both plant beds post-January was not obviously due to any one factor. Certainly, there was a major rain event, however there were bigger events in October and December 2017. There were hot temperatures, however it was also similarly hot before the New Year. At this stage, the best guess is an accumulation of deterioration factors, including simple ageing of roots, making them susceptible to pathogenic attack. Perhaps at a certain point, one more adverse rain or heat weather event was sufficient to catalyse point of no return deterioration for individual roots. This breakdown may adversely impact on neighbouring roots, or act as reservoirs for infection of independent sprout root systems.

The capacity of both growers' plant beds to cope with extreme weather events was very encouraging. The fact that there was no impact on productivity or breakdown from adding additional irrigation suggests it was possible to minimise the influence of excessive water during the sprout production period by ensuring good bed height and drainage. These well-structured plant beds would be more resilient to sub-optimal irrigation practices, as well as less uniform irrigation distributions.

This experiment did not address water requirements at bed installation. Although we did not have sensors installed for the first few weeks after installation, Grower 1 did irrigate reasonably regularly, so there was at least some moisture in the bed. Grower 2 did not irrigate early, but certainly started before sprout emergence. Best strategy is probably providing some early moisture, with no requirement to ramp up irrigation until sprout development and canopy expansion is well underway. Management of moisture in situations with plastic covering remains a significant unknown.

We were hoping to use an off-the-shelf app to assess canopy cover on plots, however this was confounded by *Bellevue* foliage being substantially purple, not green! This reduced the capacity to analyse images in a reasonable time frame for this project.

Although these experiments did not elucidate any treatment differences, they did demonstrate that using best current practice, high plant bed performance is possible, even under trying seasonal weather conditions. Rapid sprouting of *Bellevue* was still problematic, taking nearly 9 weeks from an early August installation to first cut (without plastic). This experiment confirmed that high rates of nutrition did not adversely affect plant bed performance and was an effective strategy to ensure good sprout quality.

Treatment with metham sodium did not give long-term management of soil pathogens, as initial diagnostics suggest *Fusarium* and *Erwinia* were apparent in large numbers in breakdown areas.

Appendix 6

Cudgen bedding root size demonstrations 2017/2018

Introduction

During the latter years of Project VG13004, it was apparent that the commonly grown Gold cultivar *Bellevue* was the most susceptible to premature breakdown in plant beds. The project team and a few sweetpotato growers recognised that there appeared to be both physiological and disease aspects to this breakdown. However, the actual initial causes of premature breakdown were hard to elucidate, and particularly in highly variable field situations.

Craig Henderson and Rachael Langenbaker initiated grower demonstrations on two properties in Cudgen, northern New South Wales, to:

- Ascertain whether there was a difference in breakdown rates from commercially-supplied PT bedding roots, if they were sorted into distinct size classes.
- Recheck the hypothesis that there were no major differences in sprout productivity from sorted sizes of commercially-supplied bedding roots.

Although this work was unreplicated, it was on a sufficient scale that we are confident the results reflect commercial reality. We felt it was useful to report what occurred in this project final report.

Methodology

Demonstration 1

On 15/08/2017, Ms Langenbaker and the cooperating grower sorted a 450 kg bin of commercially-supplied *Bellevue* bedding roots into two lots, a small-medium grade (SM), and a medium-large grade (ML).
Ms Langenbaker then randomly collected a 20 kg sample from each lot. For each sample, she measured the length, maximum diameter and weight of each root in the sample.

Plate 23. Small-Medium (left) and Medium-Large (right) *Bellevue* bedding roots for installation in Demonstration 1 plant beds.

The following day, the grower installed 6 m of plant bed for each of the two root lots. He provided a very small amount of irrigation, to slightly moisten the bed. Contrary to practice in previous years, he initially did not use a plastic cover, as he was concerned it would enhance breakdown of this sensitive cultivar. However, by the 10/09/2018, there were still no signs of sprouting, so he covered the plant bed with hooped plastic for two weeks, until late September.

Ms Langenbaker regularly liaised with the grower, whilst both Ms Langenbaker and Mr Henderson visited the site about once a month.

On 09/01/2018, Mr Henderson harvested sprouts from three separate one square metre samples from each of the demonstration plots. For each sample, he counted the total number of useable sprouts (>20 cm in length, nil damage or disease, intact tip). He then took a random 20 sprout subsample from each sample. For each of those sprouts, he measured their total length, their width, and counted the number of nodes in the 15 cm interval closest to the cut end. Note that sprouts longer than 45 cm long were trimmed to 45 cm.

Demonstration 2

On 09/10/2017, Ms Langenbaker and the cooperating grower sorted a 150 kg bin of commercially-supplied *Orleans* bedding roots into two lots, a small-medium grade (SM), and a medium-large grade (ML). Ms Langenbaker then randomly selected a 100 root sample from each lot. For each sample, she measured the length, maximum diameter and weight of each root in the sample.

On the same day, the grower installed 3 m of plant bed of each of the two root lots. He provided a very small amount of irrigation, to slightly moisten the bed. Because it was later in the season, and *Orleans* is a good sprout producer, no plastic covering was required.

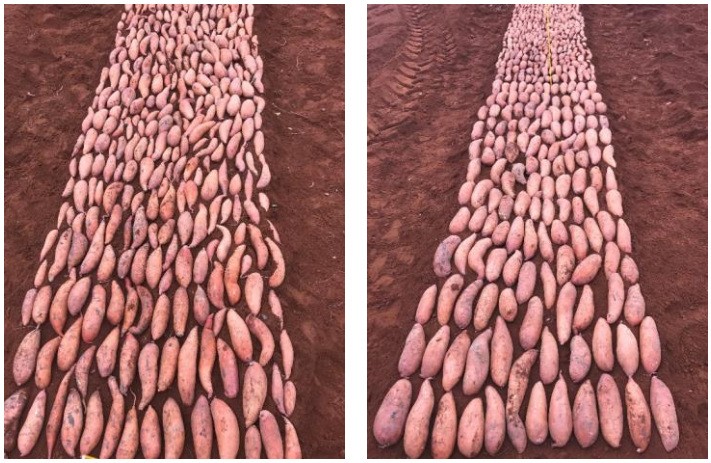


Plate 24. Small-Medium (left) and Medium-Large (right) *Orleans* bedding roots installed in Demonstration 2 plant beds.

Ms Langenbaker regularly liaised with the grower, whilst both Ms Langenbaker and Mr Henderson visited the site about once a month.

On 09/01/2018, Mr Henderson harvested sprouts from the full length of each of the demonstration plots. For each plot, he counted the total number of useable sprouts (>20 cm in length, nil damage or disease, intact tip). He then took a random 50 sprout subsample for each plot. For each of those sprouts, he measured their total length, their width, and counted the number of nodes in the 15 cm interval closest to the cut end. Note that sprouts more than 45 cm long were trimmed to 45 cm.

Plate 25. Covering the *Orleans* plant bed in Demonstration 2.

Results

Bedding root size classes

See Appendix 3 p60 for interpretation of the box/whisker plots. The analysis of the root size distributions in the two classes (SM and ML) demonstrated that the grading was effective. For each of the characteristics of root length, diameter and particularly root weight, we achieved good segregation between the size classes, at both Demonstrations (Figs. 30). Interestingly, the *Orleans* roots in Demonstration 2 were consistently larger than the *Bellevue* roots in Demonstration 1, for both the SM and ML size classes.

Bedding root breakdown and sprout productivity

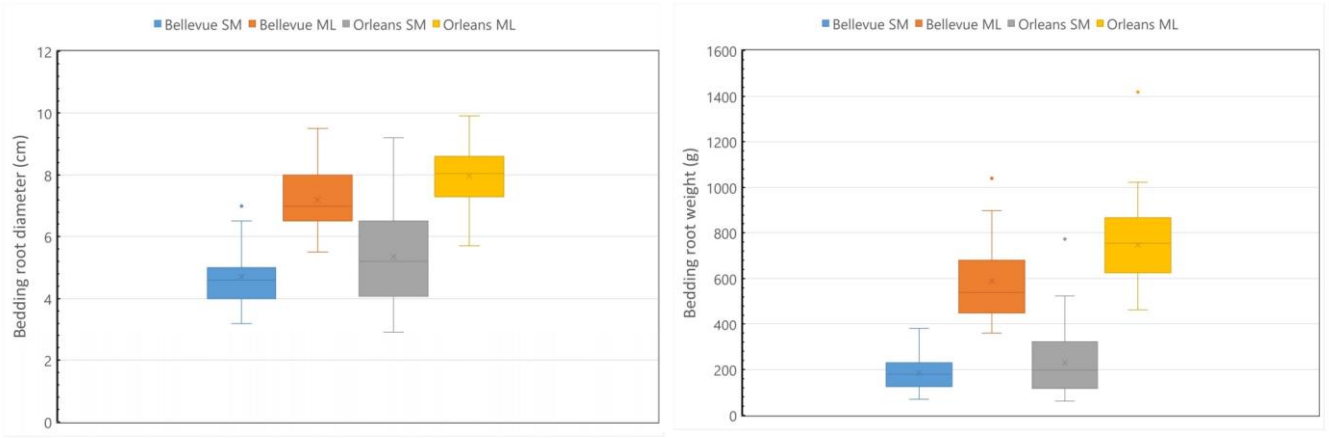


Fig. 30. Length (top right), diameter (left) and weight (right) of individual Small-Medium and Medium-Large bedding roots; cultivars *Bellevue* and *Orleans* installed in Demonstrations 1 and 2.

Neither our project team, nor the collaborating growers, detected any visible differences in sprout production, or amount of bedding root breakdown, in any of the plots, at either Demonstration. Both growers kept their plant beds relatively dry (unpublished sensor data), particularly early in the production period.

Sprout performance and breakdown assessment

The plant beds were on their fourth sprout harvest in early January 2018, when Mr Henderson did the final site assessment. The grower at Demonstration 1 was not going to use the sprouts, as he had finished planting *Bellevue* for that growing season. The Demonstration 2 site was also overdue for sprout cutting by 4-5 days. Therefore, the sprout lengths at both sites were longer than usual. This can be observed by the fact that across all the plots, an average 35% of the sprouts were trimmed to the maximum of 45 cm long.

The sprout performance at both Demonstrations was very good (Table 8). Our benchmark is 250 sprouts m^{-2}, which all plots exceeded. To still be achieving those numbers by the 4th cut is an excellent result. Those high commercial sprout densities, as well as visual observations, indicate there was no commercially significant breakdown in any of the plots.

Table 8. Production of and thickness of sprouts is unaffected by bedding root size in the 4th cut from Demonstration plant beds.

Demonstration	Cultivar	Bedding root size class	Sprout productivity (sprouts m^{-2})	Individual sprout thickness (%>4 mm)
Grower One	*Bellevue*	Small-Medium (SM)	262	30
		Medium-Large (ML)	257	40
Grower Two	*Orleans*	Small-Medium (SM)	271	86
		Medium-Large (ML)	285	76

Looking at sprout quality, only around 35% of the *Bellevue* sprouts were thick, compared to 80% of the *Orleans* cultivar. *Orleans* is known as a very robust and resilient sprout, so this result is not surprising. The size of the bedding roots did not impact on the thickness of the sprouts (Table 8).

The lengths of harvested sprouts were also unaffected by either bedding root size or cultivar, with 85-95% of sprouts being 28 cm or longer (our benchmark of a premium sprout length). The mean length was 35-38 cm across all the beds (Fig. 31).

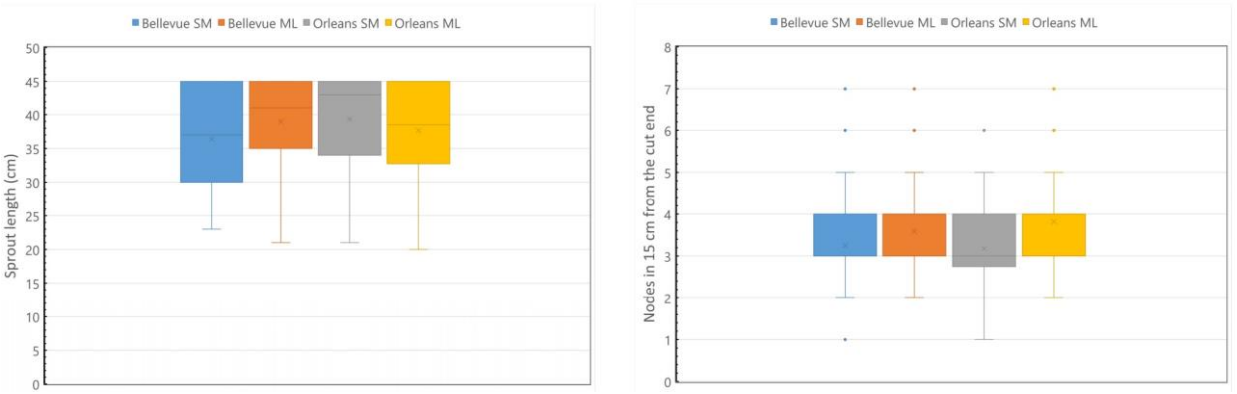


Fig. 31. Sprout length (left) and nodal density (right) of sprouts from cultivars *Bellevue* and *Orleans*, harvested from beds installed in Demonstrations 1 and 2.

Similarly, across all the beds, the sprouts had an average of 3-4 nodes in the 15 cm closest to the cut end, independent of bedding root size or cultivar (Fig. 31). Around 25% had only 2 nodes, and another 25% had 5 nodes, with a few outlier sprouts having closely packed nodes.

Discussion

The performance of these plant beds was excellent, indicating growers have implemented sound agronomic strategies to achieve the benchmark potential of 250 sprouts m^{-2}. To maintain this performance into the 4th cut is also a good result. The grower management of plastic in Demonstration 1 was obviously effective, in that it did not cause premature breakdown of *Bellevue*.

The reliable performance of these plant beds into the 4th cut indicates that the bedding root sizes provided by the supplier are not inherently an inevitable risk factor in causing plant bed breakdown. Obviously, this depends on the plant bed agronomics being optimal as well. The supplier has made a conscious decision not to provide bedding roots beyond around 9 cm in diameter, or around 600 g for the *Bellevue* cultivar in particular. This is reflected in the size grades of the commercial lots in this study.

Orleans is a very strong plant bed performer, providing high numbers of long, thick sprouts, with good node densities. *Bellevue* also has good plant bed productive potential, although it can definitely be slower to establish in initial Spring cuts. It also tends to have thinner sprouts than other cultivars.

Within the grades commercially provided, there appears to be no influence of bedding root size on the production or quality of sprouts from the plant beds. This confirms our result from earlier in the project.

Appendix 7

University of Southern Queensland *Bellevue* breakdown pilot study, January/February 2018

Introduction

During the latter years of project VG13004, it was apparent that the commonly grown Gold cultivar *Bellevue* was the most susceptible to premature breakdown in plant beds. The project team and a few sweetpotato growers recognised that there appeared to be both physiological and disease aspects to this breakdown. However, the actual initial causes of premature breakdown were hard to elucidate, and particularly in highly variable field situations.

Craig Henderson and Dr Bree Wilson (Research Fellow, University of Southern Queensland), initiated a pilot study to:

- Explore the potential for using regulated growth cabinets to monitor breakdown of bedding roots under controlled conditions
- Discover what physiological changes occurred in bedding roots after installation, and what organisms could potentially be involved in root breakdown

Although this work was very preliminary, and the diagnostics of disease organisms is still underway, we felt it was useful to report what occurred in this project final report.

Methodology

We purchased 5 kg of sweetpotatoes from the supermarket, relatively confident they were *Bellevue* cultivar, from their shape and colour. We sorted and selected twelve medium roots of a similar size to use in the study. We measured their length, diameter and weight (Table 9).

Table 9 Uniformity of sweetpotato bedding roots for growth cabinet study

Size attribute	Median	Minimum	Maximum	Standard deviation	Coefficient of variation (%)
Length (mm)	193	160	220	18	9
Diameter (mm)	75	55	80	7	10
Weight (g)	461	370	559	60	13

Plate 26. Selecting experimental bedding roots.

On 16 January 2018, we prepared three plastic tubs to place in the USQ growth chambers. The tubs were drilled to provide drainage holes for excess water and lined with geotextile, to prevent sand falling through the drainage holes. We used unsterilised, coarse, builder's sand as the growing medium.

Tub 1

We mixed 25 kg of dry sand with 25 g of RICHGRO All Purpose Complete Garden Fertiliser (8-1-6-9). We added a 2 cm layer to the bottom of the tub, and then placed 4 randomly selected sweetpotato bedding roots on the sand. We added the remainder of the soil, until the roots were covered with 2 cm of sand above their upper surfaces.

Tub 2

We mixed 38 kg of dry sand with 25 g of RICHGRO All Purpose Complete Garden Fertiliser (8-1-6-9). We added a 6 cm layer to the bottom of the tub, and then placed 4 randomly selected sweetpotato bedding roots on the sand. We added the remainder of the soil, until the roots were covered with 2 cm of sand above their upper surfaces.

Plate 27. Placing bedding roots in Shallow tub.

Tub 3

This was the same as Tub 2, however no fertiliser was added to the sand mix.

We buried Chameleon soil moisture and temperature sensors 5 cm below the sand surface in each of the three tubs. The sensors logged soil moisture and temperature every two hours for the duration of the experiment.

Plate 28. Tubs installed in USQ growth cabinet.

We added sufficient water to each of the tubs to moisten them to field capacity. We installed the tubs in the growth chamber, with the lighter one (Tub 1) on the upper shelf.

We set the growth chambers for 13 hr light at 30^{o}C, and 11 hr dark at 24^{o}C, with the relative humidity constant at 60%.

Dr Wilson checked on the tubs regularly and applied sufficient water to maintain the sand at field capacity, for the duration of the study. She took regular photographs of the sprouts as they emerged.

On the 26 February 2018, we processed Tub 1, by removing the shoots, and then washing out the sand from around the bedding roots. Some of the roots had already broken down, however, we tried to keep these as intact as possible. We photographed the root systems, and the conditions of the remaining bedding roots.

Dr Wilson took samples from healthy and diseased plant tissues, including sprouts, root pieces and bedding root mass (Plates 29-32). She then prepared these materials for plating and diagnostics, using standard surface sterilising and plating media.

Plate 29. Internal sweetpotato bedding root.

Plate 30. Diseased sweetpotato bedding root.

Plate 31. Infected bedding root skin.

Plate 32. Sweetpotato bedding root ooze.

We repeated this process for Tub 2 and Tub 3, on 28 February 2018.

After one week, Dr Wilson did a preliminary assessment on the organisms isolated from the various plant tissues. Further diagnostics on these organisms is ongoing.

Results

There was no obvious difference between the two deep tubs (with or without fertiliser), so for simplicity, the study will simply refer to Shallow and Deep tubs.

According to the moisture sensors, all tubs were maintained in a wet state (5-15 kPa soil suction) for the 43 days of the study.

Growth cabinet temperatures

For the first ten days after installation, we had major issues maintaining the correct temperatures in the growth cabinet. This was primarily due to a previously undetected coolant leak. During the first lighting period, the sand temperature in both Shallow and Deep tubs climbed to around 40°C, and then fluctuated irregularly between 20-30°C for the next seven days. To fix the growth cabinets, they were turned off for a 24 hr period, in which time the minimum temperature fell to 15°C. For the remainder of the study, the temperature actually performed as expected, ranging between 23-30°C (Fig. 32). Note that the temperatures in the Shallow tubs were regularly 2-3°C higher than the Deep tubs.

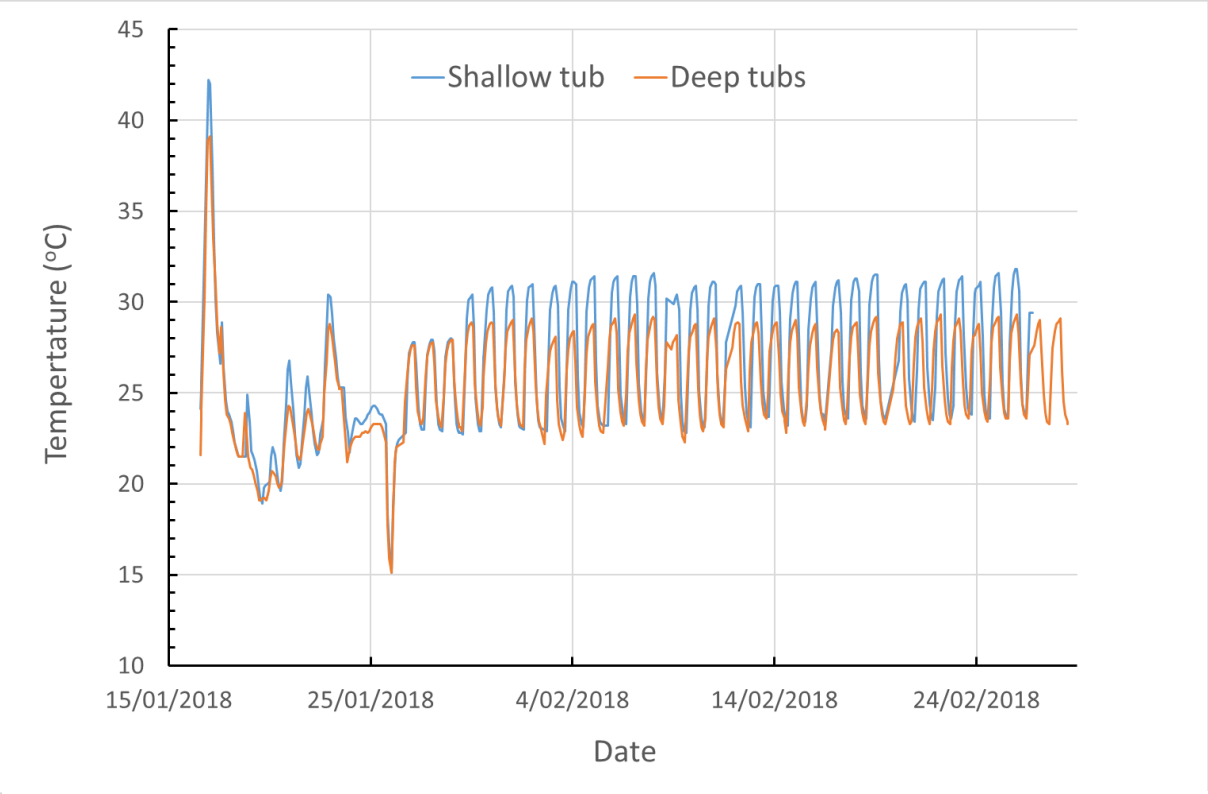


Fig. 32. Soil temperature fluctuations in sweetpotato bedding root tubs.

Sprout emergence and establishment

The first sprouts emerged in both the Shallow and Deep tubs around 23 days after installation (DAI).

Shallow tub

As can be seen in Plates 33-36, only one of the four bedding roots produced sprouts, At 34 DAI, the sprouts from this root were looking reasonably healthy, however by 41 DAI, these sprouts were wilted and obviously dying.

Plate 33. Sprout development in Shallow tub at 23 days after installation.

Plate 34. Sprout development in Shallow tub at 26 days after installation.

Plate 35. Sprout development in Shallow tub at 34 days after installation.

Plate 36. Sprout development in Shallow tub at 41 days after installation.

Deep tubs

In contrast, the Deep tubs produced sprouts from more installed roots, and retained the health of those sprouts through to the final assessment date, 43 DAI.

Plate 37. Sprout development in Deep tub at 23 days after installation.

Plate 38. Sprout development in Deep tub at 26 days after installation.

Plate 39. Sprout development in Deep tub at 34 days after installation.

Plate 40. Sprout development in Deep tub at 43 days after installation.

Plate 41. Healthy sprouts harvested from Deep tub at 43 days after installation.

Bedding root condition 6 weeks after installation

Bedding roots with healthy sprouts

When we looked at the bedding roots supporting healthy sprouts, they had the following characteristics:

- The bedding roots themselves were still firm, and relatively dense (Plates 42, 43).
- They had developed and maintained an established root system directly from the distal end of the bedding root (Plate 44, 45).
- Each of the emerged sprouts had its own, well-developed root system (Plates 45, 46).

Plate 42. Dense, healthy bedding root at six weeks after installation.

Plate 43. Dense, healthy bedding root at six weeks after installation.

Plate 45. Healthy sweetpotato bedding root, supporting good sprout production.

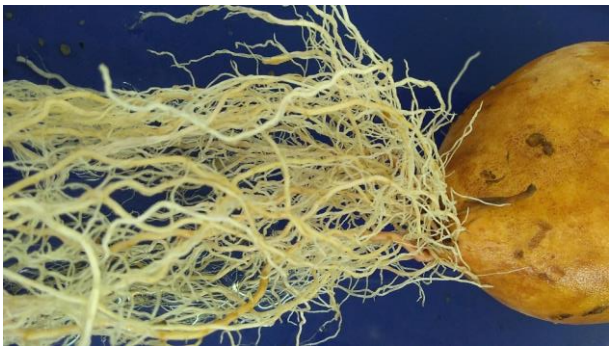


Plate 44. Excellent root production from healthy, sweetpotato bedding root.

Plate 46. Excellent root production from healthy, sweetpotato sprout.

Poor or absent sprouts

When we observed bedding roots from areas without healthy sprout production, we observed various levels of breakdown.

In some instances, we saw evacuation and vacuole development in the distal end of the bedding root (Plates 47, 48). This potentially indicated use of the starch and sugars in respiration, and/or development of roots and sprouts. We also noted some necrosis around those evacuated areas (Plates 49, 50). These changes were often, but not always, associated with browning and necrosis of the proximal end of the root.

Plate 47. Moderate physiological evacuation of bedding root at six weeks after installation.

Plate 48. Severe physiological evacuation of bedding root at six weeks after installation.

Plate 49. Initial internal browning of bedding root at six weeks after installation.

Plate 50. Internal browning of bedding root at six weeks after installation.

Plate 51. Disease entry into bedding root at six weeks after installation.

We also saw browning and necrosis of the internal sweetpotato tissue without evacuation, often associated with a diseased proximal end. This was probably a fungal pathogen; the exact organism is yet to be determined (Plate 51).

In several instances we also encountered complete internal breakdown of the bedding root (Plates 52, 53), associated with bacterial infection. Certainly *Erwinia spp.* were involved, however the diagnostics suggests other bacteria as well. Again, the exact organisms are still being classified.

Plate 52. Bacterial infection contained within shell of sweetpotato bedding root at six weeks after installation.

Plate 53. Bacterial infection pouring from shell of sweetpotato bedding root at six weeks after installation.

Diagnostics

The plating out of the plant materials has demonstrated a range of fungi and bacteria species associated with the breakdown. Because of the range of organisms involved, separating them out, classifying, and then determining their actual pathogenic potential, is beyond the scope of this current project.

Fungi

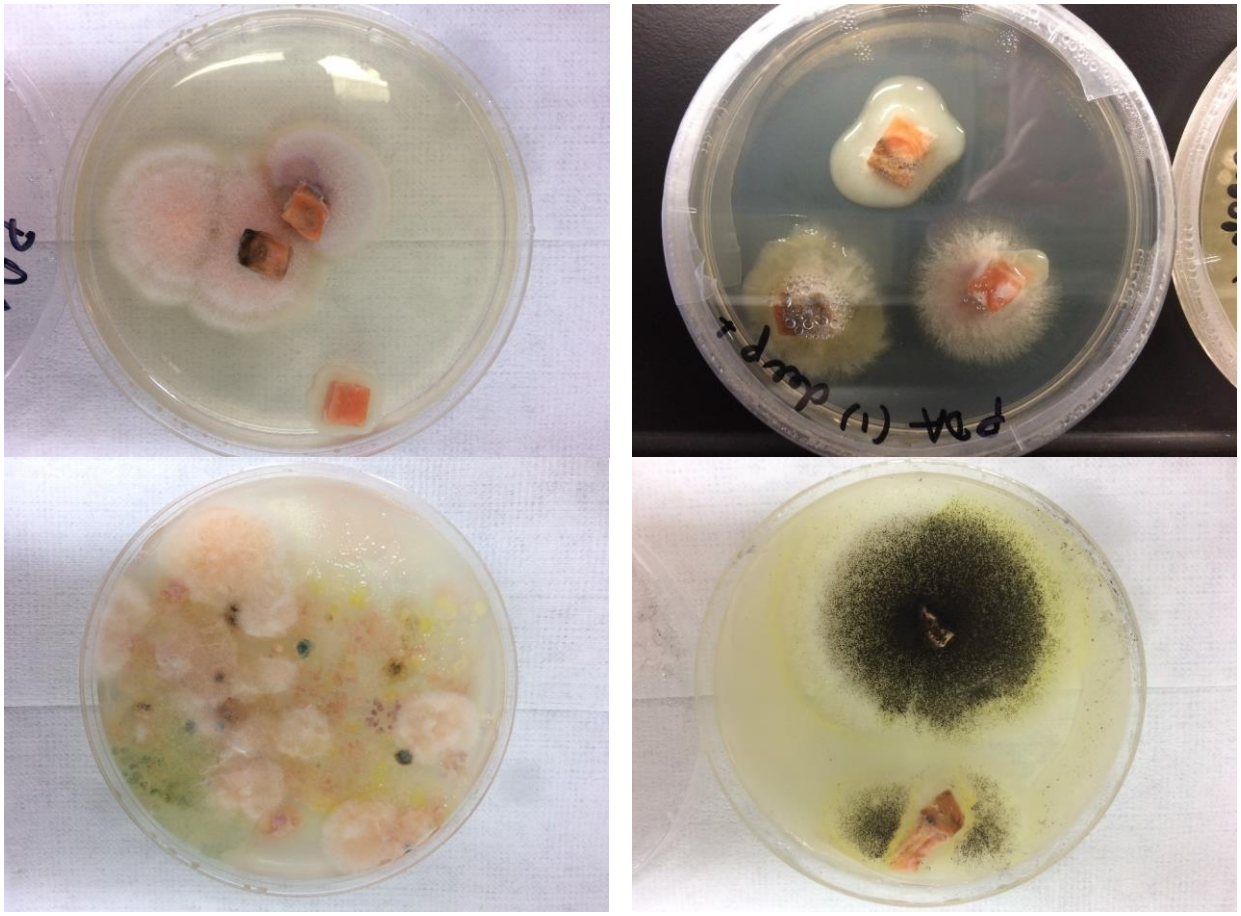


Plate 54. Fungal species extracted from diseased sweetpotato bedding root materials.

Bacteria

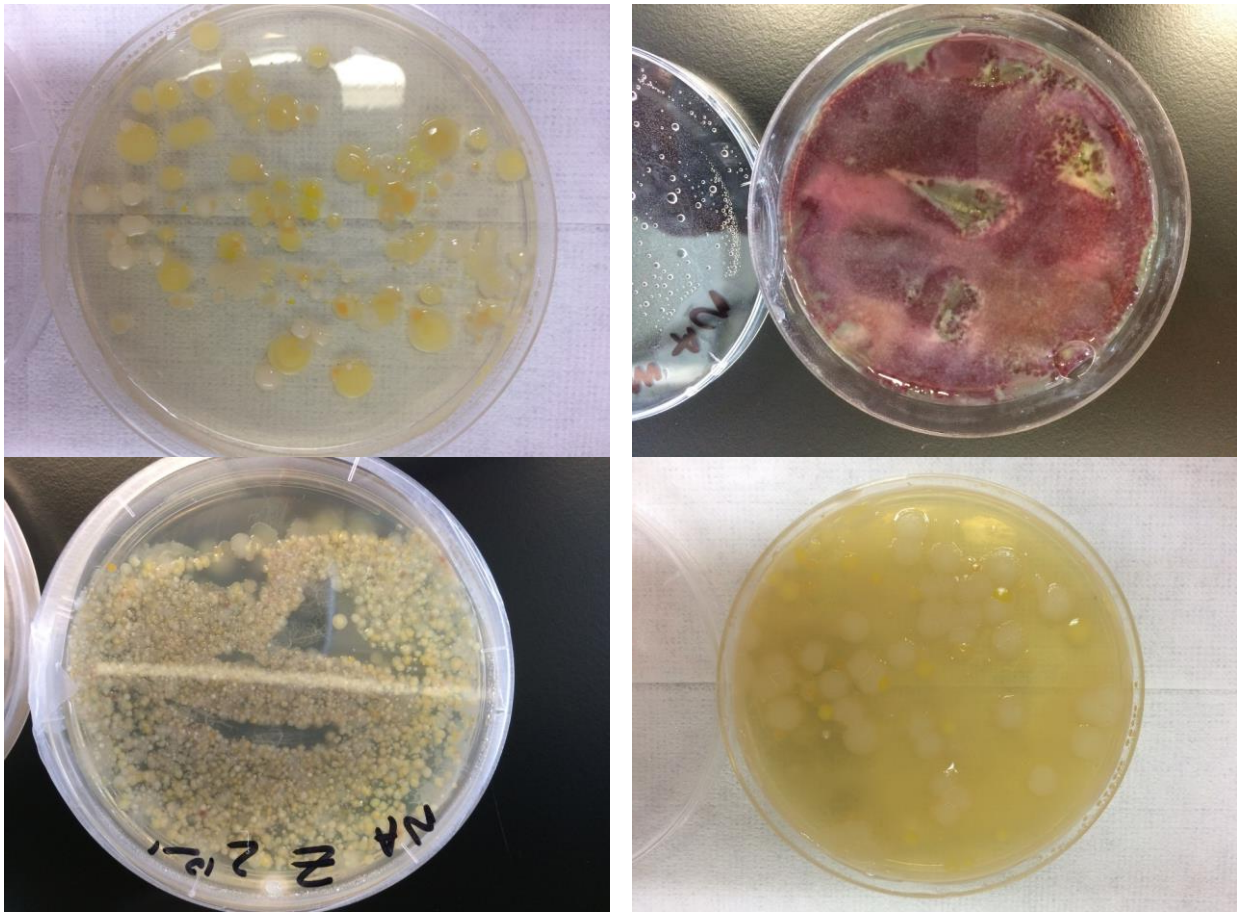


Plate 55. Bacterial species extracted from diseased sweetpotato bedding root materials.

Plate 56. Disease complexes extracted from diseased sweetpotato bedding root materials.

Discussion

Efficacy of growth cabinet

The early issues with the growth cabinet operation outlined the importance of ensuring they are functioning properly, before commencing experimentation. In hindsight, and in discussions with Prof. Villordon from LSU, even our consistent operational temperatures were probably too high, although they do reflect reality for Australian plant bed conditions.

The weight capacity of the growth cabinet shelves limited us to one tub off the floor of the cabinet. For future work, potentially a supportive frame would need to be installed to increase the load capacity of the upper shelf. Particularly as it appears that there is a benefit for having more media below the bedding roots; i.e. the Deep tub arrangement.

For physiological studies, it may be beneficial to go with a lighter, potting mix type media, rather than a pure sand culture. This may allow full use of the upper shelf. Also, for physiological studies, it would be preferred to sterilise both the bedding root surfaces, as well as the growing media, before installation.

The geotextile did prevent sand egress; however, it also formed a penetrable matting for the sweetpotato roots, making extraction of the roots difficult. Rather than a contiguous mat, perhaps small discs of geotextile or a fine sieve material over the drainage holes would be better.

Effective development of first sprouts

Our observations of the extracted bedding roots showed substantial root development from the roots themselves. So, although sweetpotatoes stored ex-soil do develop sprouts (without any obvious root development), bedding roots buried in moist soil do develop substantial root networks. We are not aware of the role of this root development in sprout initiation and development, which would be an interesting future study. In the interim, we are recommending that growers provide a small amount of soil moisture at installation, to encourage this early root development. However, we know that excessive soil moisture and waterlogging can be associated with catastrophic plant bed breakdown, so we continue to recommend good drainage and careful water management early on.

The healthy sprouts also developed very extensive root systems from their buried stems (between the bedding root and the soil surface). Our field studies show that these root systems can support the sprouts, even if the bedding root has disappeared. However, it is important to note that this probably depends on the timing and cause of the bedding root breakdown. If the bedding root breakdown is associated with pathogenic organisms that can also attack the stems and roots, then it is unlikely the independent sprouts will be maintained.

Physiological breakdown of bedding roots

In several bedding roots that appeared otherwise unaffected by diseases, we did notice evacuated areas, and even some necrosis, within their tissues. This observation appears to support our contention that there are physiological processes going on within the buried roots that are breaking down the internal tissues. This is very likely to be depletion of starches and sugars, simply associated with respiration, as well as provision of sugars for root and shoot development. The initial elevated temperatures in the growth cabinet would certainly have supported enhanced respiration and enzymatic breakdown of those internal storages.

It is unclear whether that physiological breakdown in itself is detrimental to sprout production, or whether its negative influence is via enhanced attacks by pathogens.

It is interesting to note that not all roots appeared to suffer the physiological evacuation of tissues. Whether this was related to the age of the bedding roots, or their agronomic treatment and post-harvest storage is unclear. For future experimentation, it would be important to source root lots from a uniform sample of known growth and storage history.

Pathogenic breakdown of bedding roots

In this study, we clearly encountered a wide range of organisms associated with breakdown of the bedding roots. The bacteria were the most destructive organisms, and no sprouts emerged or survived where the bedding roots were subject to bacterial attack.

The fungal organisms tended to be more localised, although the roots with substantial infections looked like they were on the route to rapid deterioration, which may have spread to the sprouts.

Apart from *Erwinia* and a *Rhizopus spp*., the other pathogens have yet to be determined. However, at this time it is unknown to what extent they are causal, or opportunistic pathogens, taking advantage of the weakened state of bedding roots impacted by physiological breakdown.

Management impacts

This was a very early pilot study; however, it does lead to the following interim suggestions for growers in managing premature breakdown of *Bellevue* in plant beds.

- Where possible, use small-medium bedding roots, preferably no more than a few months old, and kept in consistently cool storage conditions (16^{o}C) prior to installation.
- Don't use roots with unhealed wounds or apparent disease.
- If using plastic to heat beds, make sure the plastic structure is well ventilated, and monitor soil temperatures. Ideally keep soil temperatures below 30^{o}C, and perhaps even around 25-26^{o}C. If temperatures are likely to rise above that level, take the plastic off. Similarly for row covers.
- Avoid installing *Bellevue* into plant beds in circumstances likely to experience hot temperatures. Established plant beds can potentially survive, but it's possible new beds are more vulnerable.
- Ensure any irrigations at installation, and before sprouts are established, are even and light.
- Good drainage is essential.
- Avoid installing plant beds in ground with a known history of diseases, particularly bacterial.

Acknowledgements

Although not officially an original member of the VG13004 project team, Dr Wilson's endeavours in this study, and the support of USQ via provision of their facilities, were invaluable. Her diagnostic expertise, and commitment were very welcome and effective. Hopefully this collaboration in sweetpotato research, development and extension can continue for the Australian and international sweetpotato industries for many years.

Appendix 8

Assessing sweetpotato sprout potential productivity

Introduction

Initially in the project, we planted out large numbers of sweetpotato sprouts from our plant bed research experiments in commercial areas in growers' fields, and at Gatton Research Facility. Our intention was to investigate the productivity potential of the experimental sprouts and ascertain if our plant bed treatments impacted. In the initial studies we grew the cuttings through to maturity yield assessments.

Because of environmental conditions, soil variability, and the inherent differences in planting practices and field conditions, we experienced substantial variability in the performance of plots, and plants within plots. More importantly, these evaluations were very time consuming and resource intensive, and apart from the example where we compared short and optimal sprouts, we did not detect any differences in performance resulting from our plant bed treatments. The results were so variable that they were not considered sufficiently useful to include in this final report. The data has been archived and may be revisited for more detailed analysis in potential future projects investigating storage root formation, or even in Mr Henderson's PhD. Such analysis was beyond the scope or resources of this project.

As identified in the mid-term review, and in discussions with project team members and collaborators, we decided we needed to get a more fundamental understanding of the sprout characteristics, as key drivers of storage root initiation and development.

Literature review

The project team supported starting collaborative studies with CQU on physiological processes involved in sweetpotato storage root initiation and physiological development, as well as key factors impacting those processes. As part of that collaboration, student Tham Dong has commenced her PhD *Understanding agronomic factors that affect the development of sweetpotato (Ipomoea batatas (L.) Lam.) storage roots – The role of N fertilisation and organic soil amendments and factors.* Ms Dong has completed the initial literature review, which includes understanding the development of storage roots from sweetpotato cuttings. This review is confidentially available by request from the project team.

The review identifies the characteristics of potential and early developing storage roots, including pentarch or hexarch steles and enlarged apical meristems or adventitious roots (AR) with a pith and centrally located metaxylem element. The initial sign for the development of storage roots was the continued activity of the vascular cambium and anomalous primary and secondary cambia, which in turn leads to formation of thin-walled, starch-storing parenchyma cells. During storage root development, none, or only a small proportion of the cells between the protoxylem points and the central metaxylem become lignified, allowing for further root expansion.

Formation of storage roots appears to start around 21 days after planting, and the numbers and potential shape of storage roots (or at least those likely to become marketable roots) should be clearly identifiable by 50 days after transplanting. In ideal growing conditions, experimenters may be able to make an initial estimate as early as 30 days after transplanting.

Unfortunately, there are very few studies investigating sweetpotato storage root initiation and development in conditions relevant to evaluation of Australian sweetpotato sprouts. Most studies have either been conducted in field conditions, or with glasshouse experiments involving few attempts to standardise optimal temperature, moisture or light conditions. Probably the biggest limitation is the use of vertical planting methods, whereas sweetpotato sprouts in Australia are generally flat planted.

Collaboration with Louisiana State University

Visits to Although the literature review has been useful for understanding fundamental storage root development processes, the respective USA visits and reciprocal visits to Australia with Professor Arthur Villordon have been far more useful in developing approaches to sprout assessment. See Appendices 9, 12-14 for more detail.

Professor Villordon also uses vertical planting, however his work with aeroponic and sand culture assessment of storage root development have been very helpful to our project. Crucial factors for consideration are:

- Consistent, high light levels, to maximise photosynthate accumulation of developing shoots. This requires the use of artificial light sources, rather than relying on natural light, particularly with the shading in most glasshouse structures.
- Use of a standard nutrient solution in either an aeroponic or sand culture system.
- Avoidance of aeration issues, by manipulating solution-spraying intervals (aeroponics) or solution-addition intervals (sand culture).
- Avoidance of compaction/compression issues in growing media, by loose addition of sand, and ensuring large enough pots to not restrict root development.
- Regulation and standardisation of growing temperatures where possible.
- Use of standard control cultivars, of known productivity, for comparison.

Investigations

Aeroponics/hydroponics

The project team has attempted aeroponic and hydroponic growing of sweetpotato sprouts at experiential small scales. Generally, the sprouts were very slow to develop both tops and root systems, and the techniques were certainly not capable of identifying the productivity potential of the sprouts. In hindsight, below optimal light levels were probably the major factor affecting performance, as well as insufficient attention to consistent solution management.

Ms Dong has been collaboratively developing hydroponic systems to undertake her nutrient impact studies as well. At this stage, she has produced some storage roots in her system, however it is still at the stage of developing a suitable protocol, rather than explicitly generating treatments and experimental work.

Rhizotrons

After Craig Henderson's USA visit, the project team conducted a preliminary rhizotron study (Appendix 9), modifying the techniques use by Pro. Villordon. This work was possible with the advent of a new, dedicated sweetpotato hothouse structure at the Bundaberg Research Facility, and provision of some internal facilities, including benches and watering systems.

Field plantings

In the nutrition studies, we planted out the sprouts from our experimental plant beds and assessed storage root initiation and development at 40-60 days after planting. By that stage the initiated storage roots were just starting to bulk. This methodology focussed on early nodal data, rather than waiting until a commercial harvest of mature sweetpotatoes, with the consequent confounding issues of seasonal environmental and agronomic issues discussed previously. However, we still found the performance of sprouts to be highly variable.

Discussion

All the methods described above are very time and resource intensive. None of them are useful as a routine measure for assessing the productivity potential of large numbers of sprouts. The experience in this project, and collaborative discussions with Prof. Villordon demonstrate we still have very limited understanding of the drivers of storage root initiation and early development in sweetpotatoes. Without that understanding, it is very difficult to generate systems that can reliably index the potential storage root production of an individual sprout.

There is potential to do additional aeroponic studies in conjunction with our ongoing ACIAR project, under the supervision of Mike Hughes, DAF North QLD. Mr Hughes has extensive experience in the use of aeroponics in potato planting material multiplication and has done some initial work on multiplying sweetpotato planting materials. His focus has been on top growth, rather than root development.

Ms Dong will continue to explore the topic of storage root initiation and development within her ongoing PhD. And the sweetpotato industry has clearly prioritised collaborating closely with Prof. Villordon in his current and potential studies on storage root development.

The experience suggests future hothouse studies of storage root development in Australia should focus on sand culture in large plots, using flat-planted sweetpotato planting material. This is outlined in more detail in Appendix 9.

Best guide to sprout productivity is:

Sprouts come from a high nutrition, (particularly phosphorus) plant bed, using verified, first-generation, pathogen-tested bedding roots. Plant beds are well managed for virus vector control and rouging of diseased plants. Sprouts are harvested cleanly, stored in a moist, cool environment, and preferably planted within 1-2 days of harvesting.

Acceptable sprouts

No visible damage or disease, an intact and vigorous tip, at least 20 cm long, at least 3 mm diameter at the cut end, and at least 1 node within 15 cm of the cut end.

Premium sprout (increased resilience in difficult planting conditions, yield advantage of around 10%)

No visible damage or disease, an intact and vigorous tip, at least 28 cm long, at least 4 mm diameter at the cut end, and 2-5nodes within 15 cm of the cut end.

Appendix 9

Using rhizotrons for assessing potential productivity of sweetpotato sprouts

Introduction

Initially in the project, we planted out large numbers of sweetpotato sprouts from our plant bed research experiments in commercial areas in growers' fields, and at Gatton Research Facility. Our intention was to investigate the productivity potential of the experimental sprouts and ascertain if our plant bed treatments impacted performance. In the initial studies we grew the cuttings through to maturity yield assessments. In the later studies, we assessed storage root initiation and development at 40-50 days after planting.

Because of environmental conditions, soil variability, and the inherent differences in planting practices and field conditions, we experienced substantial variability in the performance of plots, and plants within plots. More importantly, these evaluations were very time consuming and resource intensive, and apart from one or two examples, we seldom detected any differences in performance between our treatments.

As identified in the mid-term review, and in discussions with project team members and collaborators, we decided we needed to get a more fundamental understanding of the sprout characteristics, as key drivers of storage root initiation and development.

Rachael Langenbaker and Craig Henderson took the opportunity to borrow rhizotrons built for another root investigation project at Gatton Research Facility, to evaluate their usefulness as research tools in understanding early sweetpotato root development. This was a very preliminary study, primarily around developing techniques and initial observations of sweetpotato development in a rhizotron environment. Most of the installation, maintenance and measurement work was undertaken by Ms Langenbaker, with initial guidance and assistance from Mr Henderson, and additional support from Dr Bree Wilson.

Methodology

A total of 12 rhizotron boxes were transported to the DAF Bundaberg Research Facility. Ms Langenbaker made two carrier boxes, each capable of supporting eight rhizotrons. Each rhizotron was 60 cm wide and 60 cm high, with an internal thickness of 4 cm. They comprised an aluminium frame, with clear Perspex sheet screwed to both sides.

We lined the bottom edge (with regular drainage holes) and side frame edges with geotextile. We filled each rhizotron with around 20 kg of washed, coarse river sand.

The rhizotrons were placed in a large metal tray, which we filled with 4 cm of water, hopefully to facilitate bottom watering. This was ineffective, and eventually became algal infested, so we stopped using this method, and just let the rhizotrons freely drain. The Perspex faces were covered with builders' insulation foil, to keep the roots in the dark, and reduce temperature fluctuations.

Plate 57. Rhizotrons in carrying frame.

The rhizotrons were grown inside the BRF sweetpotato hothouse facility. It does not have precise temperature control, and so regularly reached mid 40oC temperatures on hot days. A datalogger recorded the air temperatures during the study.

On 23 January 2018, Ms Langenbaker horizontally planted two sprouts of cv. *Orleans* in each rhizotron, ensuring at least two nodes were buried for each sprout. The plants were regularly watered manually twice a day. After two weeks, Ms Langenbaker installed a drip system to automatically water the plants, with two drippers per rhizotron. The drippers were initially set to irrigate for two minutes every 6 hours, however this was resulting in too much drainage. After two weeks, Ms Langenbaker changed it back to one minute per irrigation, which remained the schedule for the rest of the study.

Once a week, Ms Langenbaker made up a nutrient solution containing 5 g of Aquasol (23:4:14) per 9 L of water. Each rhizotron received 1.6 L of this solution.

Plate 58. Installing rhizotron and plants.

Plate 59. Sweetpotato plants in rhizotron at 29 days after planting.

Plate 60. Sweetpotato roots in rhizotron at 29 days after planting.

We sacrificed one rhizotron during the visit of Prof. Arthur Villordon to BRF. We removed one of the faces and observed the roots on the exposed face. We then gently shook the sand from the rhizotron, exposing the root systems. We had substantial and easily-identifiable storage root development, which we showed to the Bundaberg grower group that night at our presentation event.

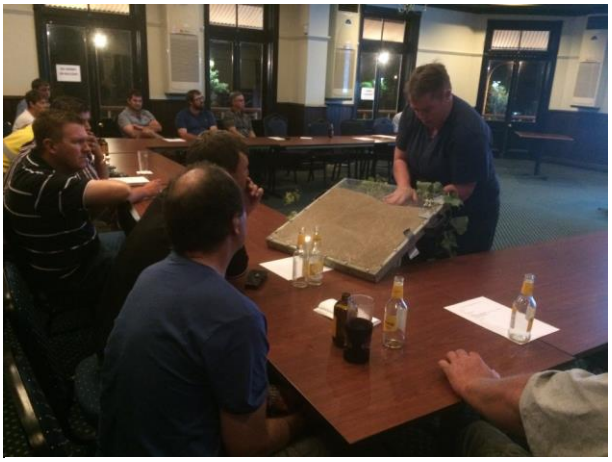


Plate 61. Extracting sweetpotato root systems from a rhizotron during the visit of Prof. Arthur Villordon to Bundaberg in March 2018.

On the 9 April 2018, 76 days after planting, Ms Langenbaker removed all the rhizotrons, cleaned away the sand, and exposed the root systems. She photographed all the root systems for both plants per rhizotron. For each buried node, she counted the number of initiated and developed storage roots and measured their maximum diameter with electronic callipers.

Results

Due to the intensity of work load associated with field experiments, and the logistics of Prof. Villordon's visit to Australia in March 2018, the 'harvesting' of the sweetpotato root systems for the bulk of the rhizotrons was delayed. We initially anticipated harvesting around 50 days after planting. By 76 DAP, the top growth was excessive, and the root systems were certainly constrained by the rhizotron dimensions.

Before removing the sand, we could get some appraisal of the root system, however it was purely chance if we could observe any storage root development against the Perspex. However, once the sand was removed and the root systems extracted, we got very good appreciation of the root systems, and the stages of storage root development.

Plate 62. Storage roots difficult to detect in rhizotron, even at 76 DAP. However, easy to observe once sand is removed.

We observed substantial amounts of variability in the size, shape and position of storage roots across the eleven rhizotrons, even though we intended that all the sprouts and rhizotron growing conditions were relatively uniform. We observed:

- Some plants set only one or two storage roots, others produced 8-10 storage roots.
- Although most storage roots were produced on the first three buried nodes (from the shoot end), many plants also produced storage roots from the callus formed at the sprout cutting point.
- Some storage roots were set shallow, others were set 30-40 cm deep; even on the same plant.
- Storage root shape ranged from short and bulbous, through to long and thin.
- Sometimes a single storage root expanded at both a shallow and deep position.

Plate 63. Variation in storage root productivity between plants.

Plate 64. Storage roots produced from callus at the sprout cut end.

Plate 65. Variable shape and depth of sweetpotato storage roots.

Plate 66. Variable shape and depth of sweetpotato storage roots.

Plate 67. Expansion independently at two depths within a single storage root.

When we looked at storage roots at each node, we only considered those with a maximum diameter greater than 10 mm. We assumed that thinner roots would be unlikely to make size from a commercial production perspective.

Mr Henderson analysed the nodal storage root productivity data, resulting in the following observations. Firstly, there was some variability in the number of nodes buried for each sprout (Fig. 33). Of the 22 plants, seven had two, and ten had three nodes buried. Another four had four buried nodes, while due to some planting issues, the remaining two had six and nine buried nodes respectively.

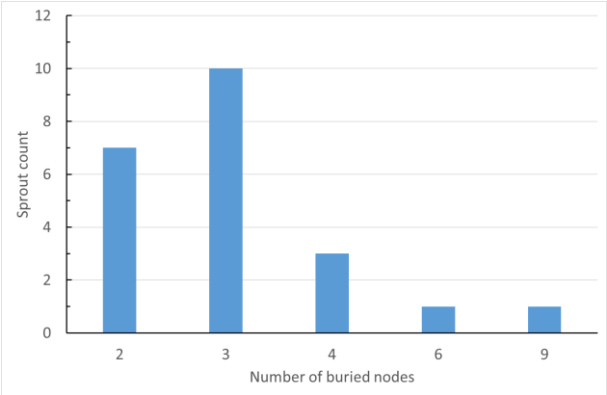


Fig. 33. The number of buried nodes for each plant was mostly 2-3, but sometimes more.

The following points are relevant to understanding the box and whisker plot in Fig. 34. The data only includes counts for plants that had the specific node buried. As an example, for the Node 3 plot (grey), a zero result is only for those plants that had a buried third node. It does not include a zero for those plants that did not have a third node buried.

In understanding the graphic, use the Total storage root for the plant (green) as the example. The lower 'whisker' (value 2), represents the least number of storage roots for any plant. The lower boundary of the box (value 3) indicates that 25% of plants had 2-3 total storage roots. The upper boundary of the box (value 5) shows 50% of the plants had 3-5 storage roots. And the top 'whisker (value 8) shows the maximum number of storage roots per plant, and that 25% of the plants had 5-8 storage roots. The x (value 4.3) marks the mean number of storage roots per plant. A similar interpretation applies for the storage root counts at each of the individual nodes.

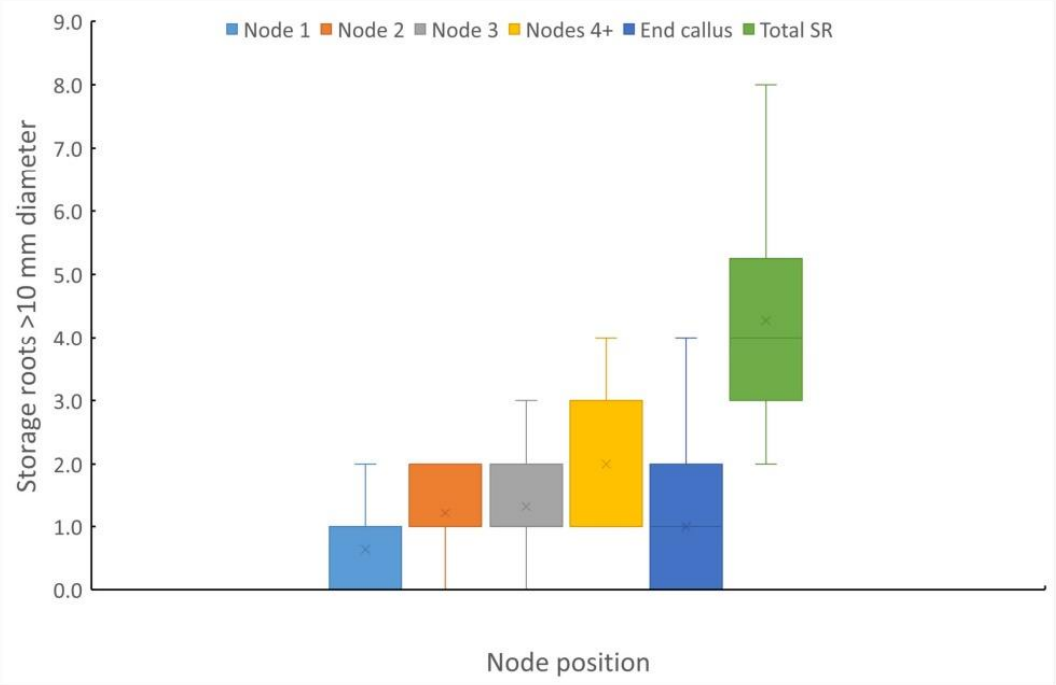


Fig. 34 Distribution of sweetpotato storage roots, and total storage roots, from 22 sprouts grown for 76 days in rhizotrons at Bundaberg Research Facility.

Generally, the first node was less productive than Nodes 2 and 3. Where more than 3 nodes were buried, there was generally one to two additional storage roots produced from this extended area. Fifty percent of plants produced at least one storage root from their cut end callus. There was no relationship between the number of buried nodes, and the number of storage roots developed from the callus.

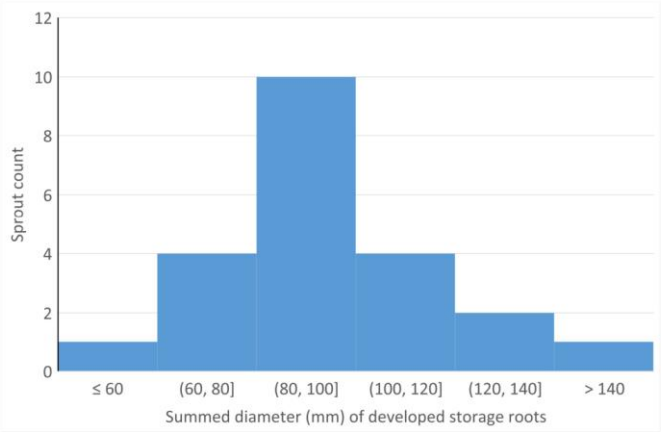


Fig. 35. Distribution of sweetpotato plant productivity estimate, from 22 sprouts grown for 76 days in rhizotrons at Bundaberg Research Facility.

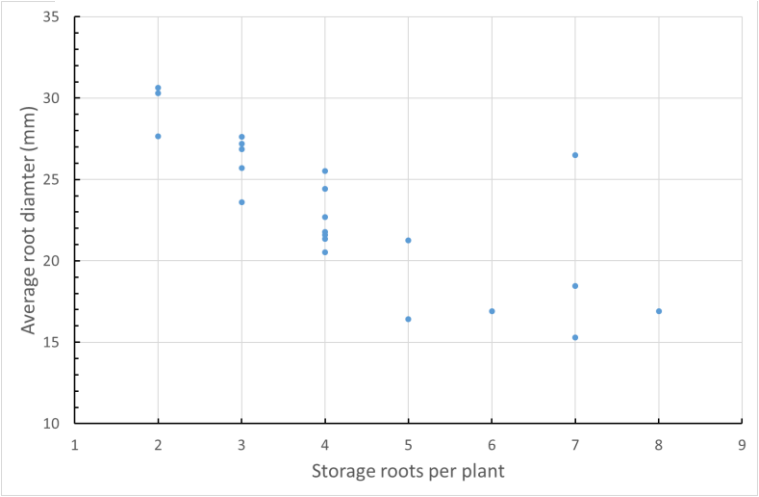


Fig. 36. Average diameter of storage of storage roots at 76 days declines with increasing storage root number per plant, for sweetpotatoes grown in rhizotrons at Bundaberg Research Facility.

We used a technique of summing the diameters of all the storage roots for each plant, to get a sense of the plant's overall productivity. Our thinking was that this would determine whether the plants were compensating for fewer roots, by filling them out more, or whether diameter was independent of the number of roots.

Nearly half of the plants were in a narrow productivity range (80-100 mm summed diameter), with only one plant less than 60 summed mm, and 3 plants more productive than 120 summed mm (Fig. 35).

There was a steady decline in the average diameter of storage roots as the number per plant increased, indicating competition between those storage roots for photosynthate, even at this early developmental stage (Fig. 36).

Discussion

The project team was successfully able to generate storage roots on flat-planted sweetpotato sprouts in rhizotrons at Bundaberg Research Facility. It is likely that similar to other sweetpotato studies in the hothouse, there is insufficient light intensity for optimal production. It is even possible that the sub-optimal light intensity affects storage root initiation, as well as the more obvious impact on the availability of photosynthate to fill the roots. Ideally, more refined temperature control in the hothouse would also be useful to keep conditions more suited to reliable sweetpotato plant growth. This is particularly the case when trying to reproduce conditions across time, for generating absolute indices of sprout performance.

The drip irrigation certainly provided more even watering of the rhizotrons. Because of the width, it would be better to have three stably located drippers per rhizotron, so they were precisely applying water across the whole width of the sand. We did see patterned distributions on some occasions.

The other aspect that probably needed attentions was nutrition. We used a general-purpose liquid fertiliser, however recent research by Prof. Villordon (pers. comm.) suggests the amounts and distribution of nitrogen, and particularly phosphorus, may have a critical role in storage root initiation and early development. In these hothouse growth studies, it is almost certainly critical to pay close attention to early nutrition. We have since accessed the Hoagland's nutrition solutions and quantities used in Prof. Villordon's USA studies, and will use these protocols in any future sand culture driven investigations.

Although we managed to initiate and develop substantial numbers of storage roots in the rhizotrons, we encountered several issues. Firstly, although the visual access was good at presenting a picture of general root system development, the visibility of the actual storage roots was minimal. So, there was little inherent advantage from having the visible Perspex system, as opposed to a standard pot. We were not able to generate a time series understanding of storage root development by visual assessment. Although probably not relevant during the first 6-8 weeks after planting, it is likely that the later development of storage roots was constrained by the narrow dimensions of the rhizotrons. We would not be looking to run an investigation for so long. Similarly, the proximity of the rhizotrons to each other may have constrained top growth, via competition for light during those last few weeks.

Once we extracted the root systems, the variability in storage root initiation and growth was immediately apparent. This was despite us conducting a uniformity study, with no treatments. In hindsight, we could have been more selective in the sprouts we used. We did select sprouts of uniform length and thickness. However, we should also have focussed on using sprouts with very similar nodal spacings and buried similar node numbers. There was a trend for more total storage roots as the number of buried nodes increased.

The positioning and shape of the storage roots was by far the most variable aspect within the study.
Prof. Villordon's most recent work suggests this inconsistent setting and expansion is associated with local nutrient availability, and particularly phosphorus. Thus, the patterns of expansion we observed may indeed reflect uneven nutrient distribution, through application, and then differential availability through water movement, in the sand.

We did identify substantial competition for photosynthate between the storage roots by the time we exposed the systems. This also suggests we need to improve the growing environment for generating photosynthate, as discussed above. It also underlines that these systems are not suitable for long studies, however are more targeted at early storage root development and initial expansion.

We still feel there are benefits from flat planting of cuttings, in terms of understanding the total productivity potential of sprouts (as opposed to investing external influences, such as media nutrition or nematode infestation). Because there was no apparent capacity to see storage roots initiating or growing via the Perspex, standard pots would be suitable. Dimensions of 30 cm long, and at least 40 cm high would be preferable for a single sprout. The width of the pot, (if not round) could be as narrow as 15 cm. although then stability may be an issue.

Using sand culture, an automated drip system is certainly useful, although with a greater volume of soil per plant, it may be less critical, except for weekends or holiday breaks. Other critical factors are sufficient spacing between the pots, and particularly provision of sufficient lighting, i.e. a minimum 400 uM m^{-2} s^{-1} of photosynthetically active radiation.

Any future work on indexing sprout productivity should focus on these aspects of the system, as well as high uniformity of testing material. The principal uniformity characteristics are sprout length, thickness, and node interval.

Appendix 10

Survey of virus incidence and development of diagnostics in the Australian sweetpotato industry

Introduction

Previous work had recorded a range of sweetpotato viruses present in Australia See Virus detection review (Outputs p13). It was unclear how many of these viruses were prevalent in the commercial sweetpotato growing regions, and whether they were impacting on productivity. At the same time, it was also important for the Australian industry to develop the capacity to test for important sweetpotato viruses, in a timely and cost-effective manner.

The virus component of this project undertook to survey the principal sweetpotato growing regions, as well as evaluate current diagnostic procedures, develop standard protocols, and explore the opportunities of new molecular-based technologies.

Method

Sample collection and maintenance

From January 2014 to January 2018, sweetpotato samples from the major Australian sweetpotato growing regions of Bundaberg, Cudgen, Rockhampton and the Atherton Tablelands were collected from seed beds and commercial crops as well as fallow areas (regrowth) and reject root dump sites. Ipomoea weeds were collected from within commercial crops, from headlands and roadsides. Samples were collected year-round as vine cuttings and/or storage roots and transported to Gatton Research Facility (GRF). Samples from gardens in Far North Queensland and Torres Strait were dried over silica gel. Collected material (vine cuttings and/or roots and dried tissue) were allocated an identification number and entered into the sample database with collection details including: grower; region; symptoms and cultivar details. Collected samples (roots and vines) were then potted into 100 mm pots (labelled with the grower's name and the accession number) containing modified pasteurised UC mix as per Dennien *et al.* (2013) for future testing.

Potted plants were placed into a quarantine meshed area within an insect-proof glasshouse. Quarantined plants were sprayed with insecticides and miticides at fortnightly intervals and hand watered as required. After establishment (4-6 weeks), quarantined plants were relocated to an insect-proof quarantine mesh screened bench, within a larger insect proof quarantine meshed igloo, and base-watered using an automatic micro-irrigation system. A strict precautionary spray program incorporating both miticides and insecticides was continued at fortnightly intervals with igloo access strictly limited to prevent inadvertent insect incursions.

Known positive control plants, assembled as part of previous DAF sweetpotato research projects were housed in a dual walled quarantine mesh insect proof igloo at GRF. Over a number of years, (from when the particular control plant was first identified) the control plants have been subjected to regular repeated virus indexing using biological indicator plants, NCM-ELISA and/or conventional PCR. These plants also receive fortnightly preventative insecticide treatments.

Biological indexing

Biological indexing using *I. setosa* is the first step in the Australian sweetpotato pathogen testing (PT) process at GRF. Each sample undergoes the three-month long virus detection process, and is grafted to a more virus sensitive, herbaceous indicator plant. Seeds of *I. setosa* were produced Between January 2014 and January 2018, at GRF in a dedicated insect-proof, quarantine meshed, seed production igloo. Ripened seeds were collected and stored in a refrigerator in an airtight container to which a small net bag of silica gel was added to remove moisture. Currently, there is no published data to suggest that any sweetpotato infecting viruses are seed transmitted either in *I. setosa* or *I. batatas*.

From September 2014, individual *I. setosa* seeds were potted into 100 mm pots filled with new pasteurised modified UC potting mix as above and placed into a quarantine mesh screened area within an insect proof glasshouse. Watering was delivered via an automated system using micro irrigation sprinklers for 1 to 5 minutes per day, depending on ambient glasshouse temperature. Granular fertiliser, Osmocote®, was applied to pots at label direction rates to ensure optimal indicator plant growth as per Dennien *et al.* (2013). Germinated seedlings were graft- ready at full expansion of the second true leaf.

Annually graft inoculation commences in October and concludes in late April, a period when suitable weather conditions occur in south east Queensland. *I. setosa* growth is optimal at 25°C and favours increasing day length and warmer night time temperatures, with symptom expression affected at higher or lower temperatures (Fuentes pers. comm.). Graft inoculation of collected samples to *I. setosa* (Plate 68) commenced in October 2014 and concluded in January 2018. Vine sections incorporating at least 3 nodes were removed from both the proximal (tip) and distal ends of collected plants to be tested.

Plate 68. Left to right: *I. setosa* seedlings, a side veneer graft and grafted plants showing virus symptoms, *S.* Dennien, DAF Qld.

Cuttings were placed into individual, labelled zip lock bags and kept cool either in an insulated box with ice or in a refrigerator. Sweetpotato vine sections (scions) containing at least one node were grafted onto *I.* setosa plants (stocks). One end-cleft graft, and one side-veneer graft, as per (Love *et al.* 1987; Beetham and Mason 1992; Fuentes 2010; Dennien *et al.* 2013) were performed on each *I. setosa* plant. This was replicated twice (two *I. setosa* plants per sweetpotato plant to be tested). Previously tested known positive control plants for a range of viruses; SPFMV; SPCV; SPLCV SPCFV (either single or mixed infections) and Phytoplasma (where infection was suspected) as well as negative pathogen tested control plants were also grafted onto *I. setosa* as above.

After grafting, plants were covered with plastic bags and relocated to the insect-proof glasshouse where they were placed into large, plastic lined shallow trays (Plate 69). These were placed on the glasshouse floor to keep the plastic-covered plants out of direct sunlight. Trays were filled with water to a depth of 10 mm and a foliar fertiliser (Aquasol®) was applied (16 grams per 10 litres of water). Water was checked twice weekly.

Plate 69. Grafted plants covered with plastic bags and set out on benches, S. Dennien, DAF Qld.

At four days post grafting (PG), plastic bags were lifted slightly, depending on ambient glasshouse temperature. All plastic bags were then removed seven days PG. Plastic trellis, 90 cm high was cut into one metre lengths, rolled into a cylinder roughly 150-200 mm wide and secured with cable ties. Cylindrical trellises were then attached to weld-mesh covered nursery benches also with cable ties. Individual grafted plants were then placed into individual 200 mm pot trays, with each replicated pair (for each sample to be virus indexed) arranged side by side adjacent to a single trellis to aid in foliar symptom observation.

Grafted plants were hand watered twice weekly as required depending on ambient glasshouse temperatures over the 28 to 42 day (56 days for Phytoplasma) testing period. Aquasol®, liquid fertiliser was applied with a watering can to the base of plants at fortnightly intervals to ensure optimal growth as per Dennien *et al.* (2013). As *I. setosa* vines grew they were trained onto the plastic trellises. Insecticides were applied during the late afternoon using a fogger (producing fine droplets) to minimise spray damage to indicator plant foliage, to avoid confusion between any potential leaf damage and virus symptoms.

As some virus symptoms can initially be transient, indicator plants were observed twice weekly until 21 days post grafting (PG), then weekly until 42 days PG. Symptom data was recorded weekly and each pair of replicated *I. setosa* plants were photographed. Data recorded included the number of surviving grafts and a range of symptoms (Plate 70) such as mottling, vein-clearing, chlorosis, chlorotic spots, chlorotic flecks, necrosis, leaf cupping/dishing, leaf roll, rugosity, stunting, leaf deformation, leaf balling, flowering and spatial symptom occurrence.

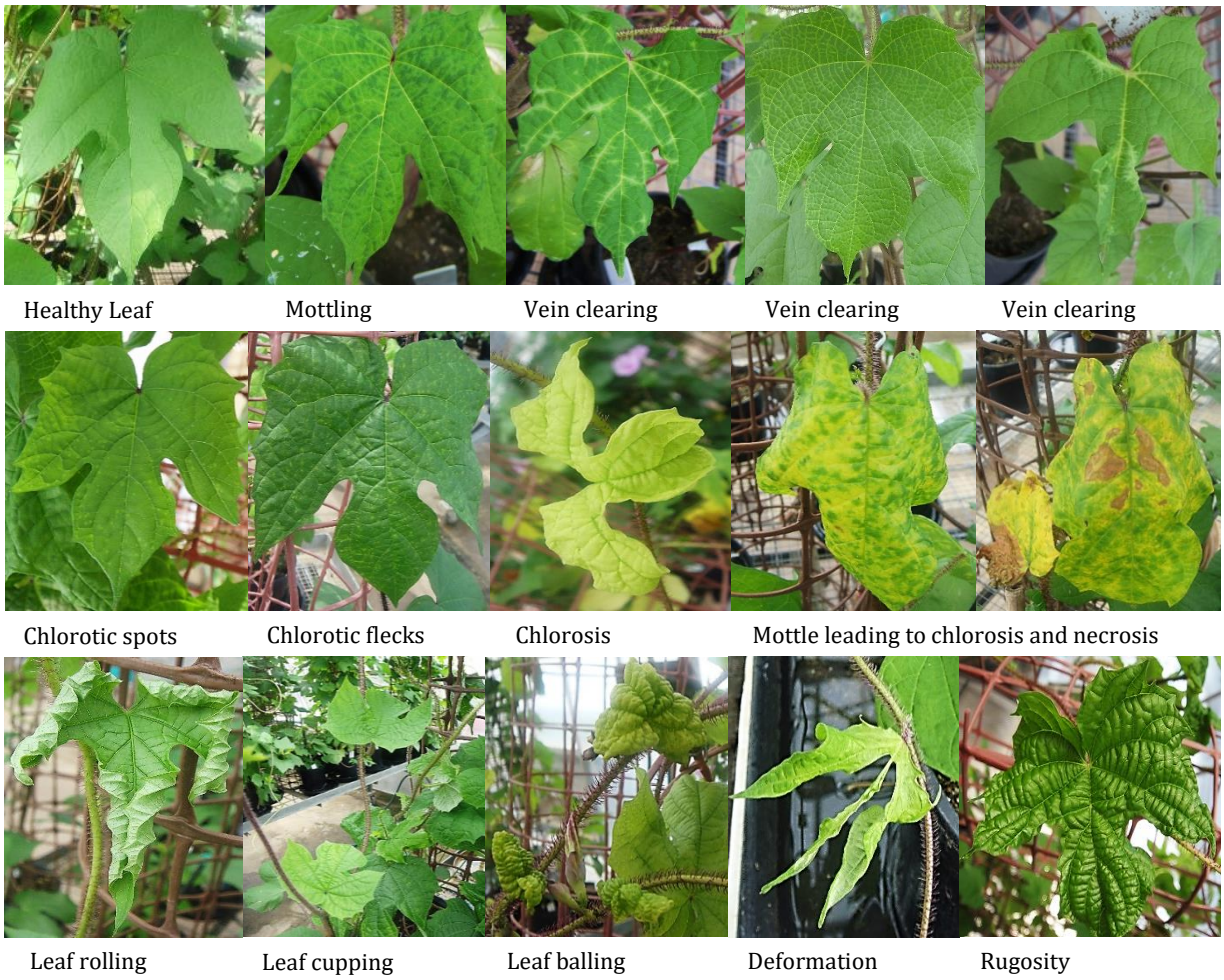


Plate 70. Visual virus symptoms on *I. setosa*, Dennien *et al* (2016).

At the conclusion of biological indexing (around 42 days PG), all plant material including roots was placed into freezer bags, and frozen for several days, before being disposed of via domestic rubbish collection. Soil was stockpiled as waste not to be reused for virus indexing and checked regularly for any emergent *I. setosa* seedlings as I. setosa is classed as a prohibited weed and only permitted in Australia for research purposes. Any seedlings emerging from used soil were immediately destroyed. Pots were cleaned in soapy water, then soaked in a 4% bleach solution and left to air dry. Plastic trellises were sprayed with 70% alcohol and left empty for at least seven days prior to the next round of biological indexing.

Serological indexing - NCM-ELISA

The second step in the virus indexing process is serological indexing using a sweetpotato virus specific antibody test to detect proteins on the virus particle coating. A specific detection antibody conjugated to an enzyme, produces a reaction, resulting in a colour change (Plate 71). Nitrocellulose membrane, enzyme linked immunosorbent assay (NCM-ELISA) is the main serological method currently used to detect viruses in sweetpotato This test is purchased from the International potato centre (CIP) in kit form and contains antibodies for 10 known sweetpotato infecting viruses (C-6, CMV, SPCV, SPCFV, SPCSV, SPFMV, SPLV, SPMMV, SPMSV and SPVG). It is recommended that infected material is first grafted on *I. setosa,* as the virus concentration will be higher in the indicator species and the test will not be affected by inhibitors present in sweetpotato sap (Fuentes pers com.).

Between 14 and 21 days PG, three leaves were removed from one plant out of each replicated pair of grafted *I. setosa* plants. Leaf removal was performed as early as possible in the morning prior to the commencement of photosynthesis. One symptomatic leaf was selected from each region of the plant; top, middle and base to allow for differences in virus distribution within plants. If symptomatic leaves were absent, then asymptomatic leaves were used. The three leaves from each plant were collected into new, labelled zip lock bags and kept cool in transit to the laboratory where the NCM-ELISA assay was performed as per kit instructions. A positive result is indicated by a purple colour change to sample blots.

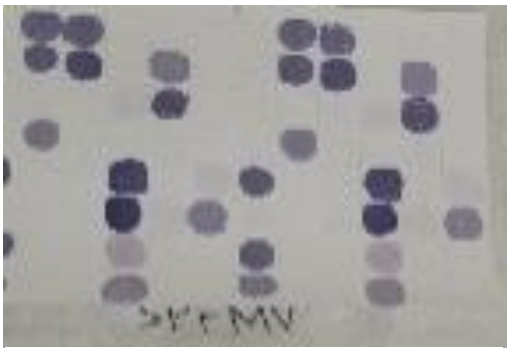


Plate 71. Developed membranes positive samples turn purple, S. Dennien, DAF Qld.

Tissue preservation

As a back up to the live plant samples held at GRF, tissue was obtained from collected sweetpotato plant samples as well as *I. setosa* indexed samples for preservation using 2 methods: RNAlater™ (to preserve RNA) and drying over silica gel (to preserve DNA). Between 14 to 28 days PG, three leaves from each pair of *I. setosa* grafted plants and the respective sweetpotato plants, as well as known positive and negative control plants, were collected as per the above NCM-ELISA protocol.

A total of 6 to 8 leaf discs were cut from the mid-rib sections of the collective three leaves as per above tissue sampling method. Leaf discs were placed into new, labelled 2 ml tubes to which five volumes of RNAlater™ were added making sure that all leaf tissue was totally submerged. Leaf discs in RNAlater™ were kept at 4°C for 24 hours, with RNAlater™ removed the next day as per manufacturer's instructions. Leaf discs (samples) were catalogued, added to the sample database and stored at -20°C for downstream RNA extraction.

At the same time, whole leaves were also collected in labelled paper bags and dried over silica gel within 24-48 hours. Once leaves were fully dried, leaf samples were catalogued, added to the sample database and stored for future extraction in their respective paper bags within food grade vacuum seal bags. A small amount of silica gel was added to the bags to both ensure complete moisture removal and as storage monitoring tool as a colour change would indicate a break in the airtight seal.

Extraction of DNA and RNA from leaf tissue

Between 14-28 days PG, three leaves from each pair of *I. setosa* grafted plants and the respective sweetpotato plants were collected as per the above NCM-ELISA protocol and taken to the laboratory for DNA and RNA extraction. One disc was cut from each of these three leaves from the midrib area using the large end of a disposable 1 ml pipette tip. A new pipette tip was used for each plant sample. Leaf discs (3 to 6 in total) or dried tissue sections (Far North Qld samples) were placed into 2 ml safe-lock tubes and ground with stainless steel beads, using a Bullet Blender Storm™ tissue homogeniser (Next Advance). DNA extraction was performed using either Dneasy™ plant mini kit (Qiagen), or Magjet™ plant genomic DNA kit (Thermo Scientific) as per manufacturer's instructions. RNA extraction was performed using Rneasy™ plant mini kit (Qiagen) or the Isolate II™ RNA plant mini kit (Bioline). A Qubit 2.0™ fluorimeter (Life Technologies) was used to quantify extracted DNA and RNA and samples were catalogued, added to the sample database and stored at -20°C for downstream qPCR.

qPCR and qRT-PCR

All qPCR and qRT-PCR assays were performed on a StepOnePlus™ (Applied Biosystems) real time quantitative PCR system, using optical 96 well plates or 8 tube strips and included both no template controls and known negative sweetpotato plant controls. The following published qPCR protocols were used in the detection of sweetpotato viruses.

SPLCV: To detect Sweetpotato leaf curl virus (SPLCV), published protocols as per Barkley (2011) were used with positive control plants GRF 300 and GRF 3 consistently producing leaf cupping and rolling symptoms in *I. setosa* Dennien *et al.* (2016) and previously testing positive for SPLCV in conventional PCR. Pathogen tested plant GRF 100 was used as a negative control.

SPFMV and SPV2/IVMV: Detection of Sweetpotato feathery mottle virus (SPFMV) along with the closely related Sweetpotato virus 2 (SPV2), syn: Ipomoea vein mosaic virus (IVMV) was performed using published protocols as per Kokkinos and Clark (2006), SPFMV positive control plants GRF 300 and GRF 1, consistently producing vein clearing symptoms in *I. setosa* Dennien *et al.* (2016) and previously testing positive for SPFMV in conventional PCR and NCM-ELISA were used as positive controls with GRF 100 (PT Beauregard) used as a negative control.

SPVG: Detection of Sweetpotato virus G (SPVG) was also performed using published protocols as per Kokkinos and Clark (2006). Samples previously testing positive to SPVG in NCM-ELISA were imported on FTA cards as part of ACIAR project HORT 2014/097 and used as positive controls. Pathogen tested GRF 100 was again used as a negative control.

SPCSV: Published protocols for the detection of Sweetpotato chlorotic stunt virus (SPCSV) as per Kokkinos and Clark (2006) and Fuentes (2012), were used with FTA card samples previously imported as part of ACIAR project HORT 2014/097 with GRF 100 used as a negative control.

Phytoplasma: Phytoplasma *Candidatus aurantifolia* protocols were obtained from DAF Mareeba staff. Positive control plants consistently producing phytoplasma like symptoms (stunting, chlorosis and small leaves), at 56 days PG on *I. setosa*, plants GRF 357, GRF 356 along with GRF 1008 were used as positive controls with GRF 100 used as a negative control.

SPCV: Positive control plants GRF 69 and GRF 85 with a history of producing chlorotic flecks, spots and fine isolated vein clearing symptoms leading to necrosis in *I. setosa* and previously testing positive to Sweetpotato collusive virus (SPCV) in NCM-ELISA, were sent for next generation sequencing. The resultant data identified SPCV sequences within both cultivars that were not distinct from each other or from the only published accession No: HQ694978 from Portugal. Specific SPCV qPCR primers and probes were then developed in conjunction with Amit Sukal (QUT) based on sequence data from the three accessions (in publication). qPCR Protocols are currently undergoing review and efficacy testing prior to publication. Positive control plants GRF 69 (Beni aka) and GRF 85 (Alley's Red) and negative control plant PT Beauregard were used in this assay.

SPCFV: In the absence of any published qPCR protocols to detect the Carlavirus SPCFV this project facilitated the development of a specific qPCR primer/probe set based on published sequence data on identified isolates from Australia, East Timor, Uganda and South Korea (Table 10). SPCFV qPCR primers and probe were designed in conjunction with Dr Amit Sukal (QUT). Plant GRF 323, consistently producing chlorotic flecks, chlorotic spots and isolated vein clearing symptoms in *I. setosa* and multiple positive results for SPCFV in NCM-ELISA, was used as positive control with GRF 100 used as a negative control.

Table 10. SPCFV accessions used in primer design.

ACCESSION	DESCRIPTION	COUNTRY	ISOLATE
KU707475	Sweet potato chlorotic fleck virus isolate AusCan, complete genome (Maina 2016)	Australia	AusCan
AY461421	Sweet potato chlorotic fleck virus, complete genome (NCBI)	Uganda	
KP115606	Sweet potato chlorotic fleck virus isolate SC20, complete genome (NCBI)	South Korea	SC20
KP115607	Sweet potato chlorotic fleck virus isolate UN210, complete genome (NCBI)	South Korea	UN210
KP715159	Sweet potato chlorotic fleck virus isolate HG176, complete genome (NCBI)	South Korea	HG176
KR072674	Sweet potato yellow mottle virus, complete genome (NCBI)	South Korea	Yeongdeok
KU720565	Sweet potato chlorotic fleck virus isolate Tm37, complete genome (NCBI)	East Timor	Tm37
NC006550	Sweet potato chlorotic fleck virus, complete genome (NCBI)	Uganda	

Next Generation sequencing

Twelve sweetpotato cultivars were sent for Next Generation Sequencing (NGS) (Table 11). Cultivars were chosen based on symptom expression on *I. setosa* as well as both negative and positive results in NCM-ELISA testing. Dried leaves from *I. batatas* were sent to Amit Sukal (QUT) for DNA extraction, rolling circle amplification (RCA) and RCA purification. The purified RCA products were sent to Macrogen, Korea where NGS libraries were prepared using the Nextera™ XT sample preparation kit (Illumina) and sequenced on an Illumina platform.

NGS data analysis was performed by Amit Sukal using Geneious® v11.0.2 (http://www.geneious.com).

Table 11. GRF DAF Sweetpotato accessions sent for NGS.

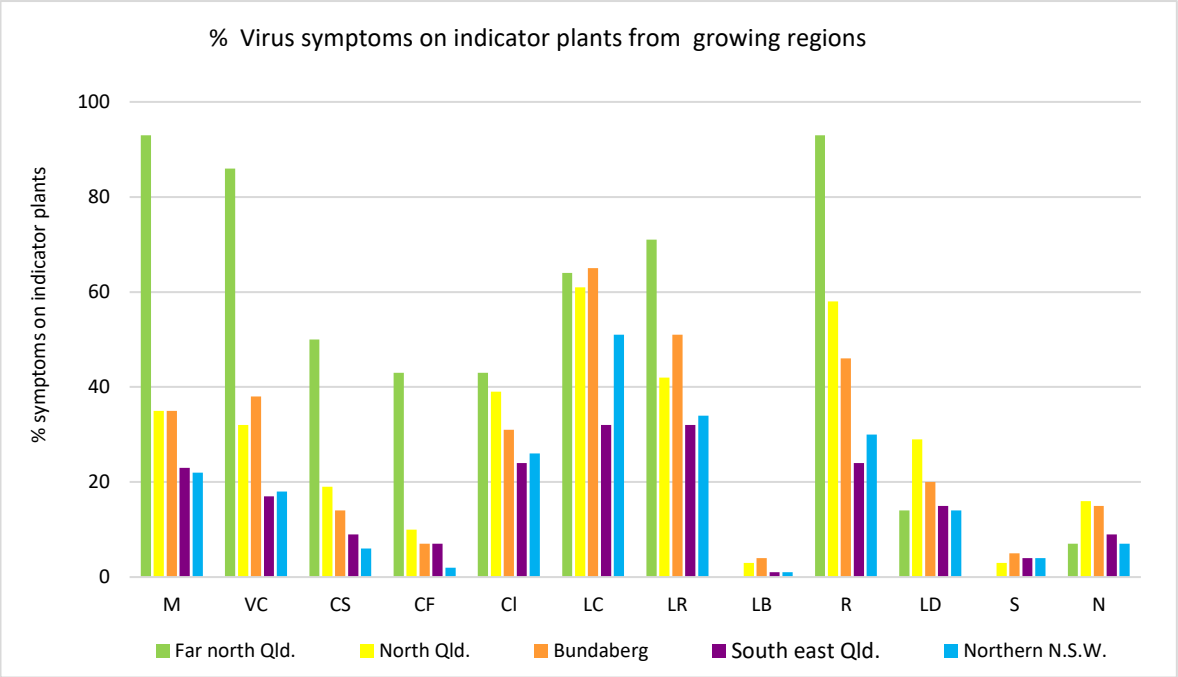
SAMPLE ID	GRF ACCESSION NO	ORIGIN
SPO1	GRF 001	Nth QLD
SPO2	GRF 069	Imp Japan 2001
SPO3	GRF 085	Nth QLD
SPO4	GRF 341	IMP USA 2007
SPO5	GRF 014	NSW
SPO6	GRF 340	IMP USA 2007
SPO7	GRF 300	Nth QLD/ IMP USA 1980s
SPO8	GRF 330	Rockhampton
SPO9	GRF 328	Bundaberg
SPO10	GRF 353	PNG 1980s
SPO11	GRF 303	PNG 1980s
SPO12	GRF 315	Imp Japan 2001

Results

Over 670 plant samples were collected with more than 950 samples (sweetpotato plants, *Ipomoea* weeds and alternate *Ipomoea* indicator plants) virus indexed from March 2014 to January 2018. Plant samples for virus detection were collected from commercial crops, plant beds, headlands, road sides, reject root dump sites and regrowth (fallow fields) throughout the Atherton Tablelands, Rockhampton, Bundaberg, Esk, Lockyer Valley, Central Qld, Northern Qld, Far northern Qld and Northern NSW regions.

Samples collected /region

Virus indexed samples from commercial farming systems and non-commercial settings were grouped into regions (Figure 37). Samples from Central Qld (two growers) were added to the Bundaberg samples; commercial samples from the Atherton Tablelands and Bowen were classified as North Qld samples; samples from Esk, the Lockyer Valley and Redland Bay were assigned to the South east Qld region; samples originating from home gardens in Atherton, Cooktown, Hopevale, Weipa and Torres Strait were designated as being of Far North Qld origin. Sweetpotato plants from this region were non-commercially grown and mostly found in home or market gardens, purchased from local markets or found on road sides and dump sites.



M=Mottle, VC= Vein clearing, CS = Chlorotic Spot, CF = Chlorotic Fleck, Cl = Chlorosis (interveinal Chlorosis), LC = Leaf cupping, LR = Leaf roll, R = rugosity, LD= Leaf Deformation, S= Stunting, N= Necrosis.

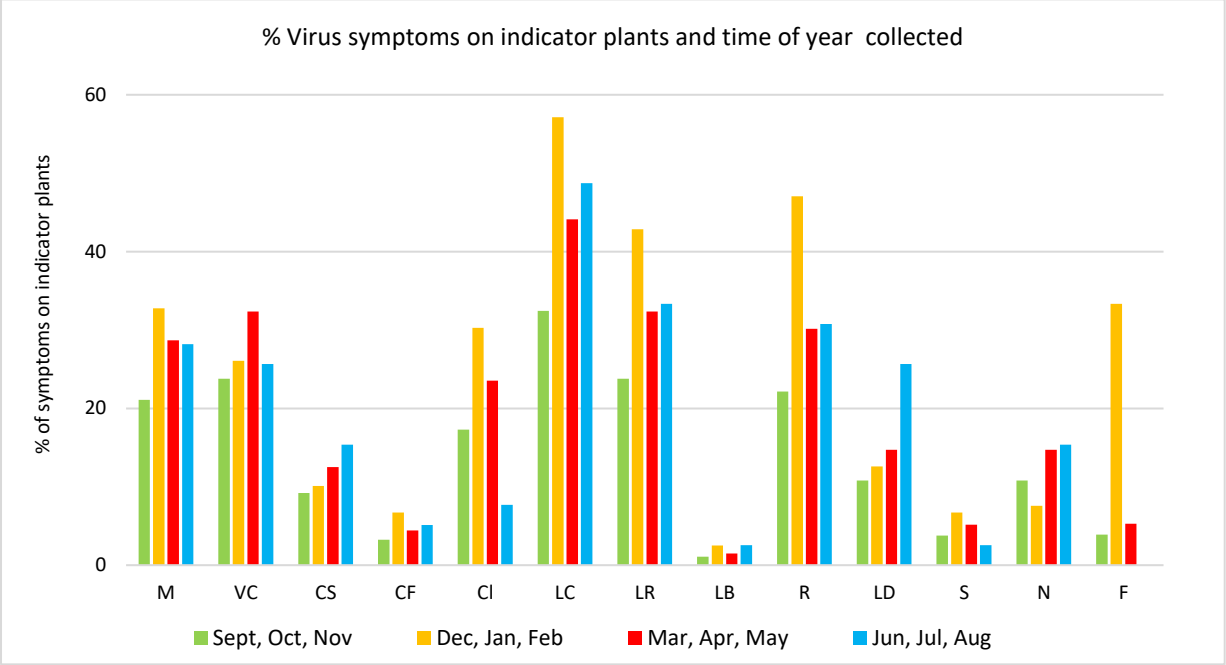
Fig. 37. Percentage of virus symptoms on indicator plants from growing regions.

Samples from Far North Qld had the highest percentage of symptom expression on indicator plants with 93% exhibiting mottling and rugosity, 86% producing vein clearing, 71% exhibiting leaf roll, 52% developing chlorotic spots and 43% having chlorotic fleck and chlorosis. There was an absence of stunting and flowering and 7% necrosis. Samples from commercial crops in North Qld were mostly affected by leaf curl (61%) and leaf roll (42%), while 58% exhibited rugosity, 39% chlorosis, 35% developed mottle and 32% produced vein clearing.

Plants from the Bundaberg region mostly displayed leaf curl and leaf roll, 61% and 51% respectively. Rugosity was also seen in 46% of graft inoculated plants, with vein clearing, mottle and chlorosis appearing in 38%, 35% and 31% of plants respectively. South east QLD indexed samples had the lowest amount of leaf cupping and leaf roll symptoms both at 32% with only 24% developing rugosity and chlorosis. We found 23% produced mottling and vein clearing. Of the Northern NSW samples, 51% exhibited leaf cupping but only 34% developed leaf roll, with 30% or roughly one third of plants exhibiting rugosity. In our samples from that area, 26% had some form of chlorosis, 22% developed mottle and 18% displayed chlorotic spots, but chlorotic fleck was only seen in 2% of plants.

Samples collected /time of year - commercial crops

Graft inoculated samples were assembled according to the time of year that they were collected (Figure 38). The year was divided into seasons for example: Season 1 (September, October, November) to coincide with the commencement of the majority of planting; Season 2 (December, January and February), aligned with the main summer planting period and February marked the conclusion of commercial plantings in Northern NSW); Season 3 (March, April, May), Autumn, winter plantings in some areas and cessation of active plant bed production; Season 4, (June, July, August), winter months. Non-commercial grower samples from Far North Qld were excluded from the assessment on virus incidence by time of year.



M=Mottle, VC= Vein clearing, CS = Chlorotic Spot, CF = Chlorotic Fleck, Cl = Chlorosis (interveinal Chlorosis), LC = Leaf cupping, LR = Leaf roll, R = rugosity, LD= Leaf Deformation, S= Stunting, N= Necrosis.

Fig. 38. Percentage of virus symptoms on indicator plants by time of year.

A similar number of plants were sampled throughout each season, with the exception of Season 4 where a smaller number of plants were collected. Plants collected in Season 1 (spring) had the lowest percentage of most symptoms on indicator plants, the highest being 32% leaf cupping. 24% of grafted plants developed leaf roll, 22% vein clearing, 21% rugosity and mottle and 17% chlorosis.

Season 2 saw an increase in symptoms with 57% of plants having cupped leaves, 47% rugosity, 33% mottle 43% leaf roll, 33% mottle and flowering and 26% vein clearing. The highest incidence of vein clearing was in Season 3, (autumn), with 44% of plant affected. Symptoms from autumn also included leaf cupping, 32% showed leaf rolling, and vein clearing, 30% rugosity, 29% had mottling, 24% chlorosis and 15% exhibited leaf deformation and necrosis. Graft inoculated plants samples from Season 4 (winter) developed leaf curl symptoms (49%), and leaf roll (33%), rugosity 31%, mottle 28% and same amount of vein clearing (26%) as in mid-summer.

Virus detection

SPFMV was the most commonly detected virus in collected plants (Plate 72) tested using NCM-ELISA. NCM-ELISA also detected SPFMV, SPCFV and SPCV from one sweetpotato sample (home garden) in Far North Qld. Another plant at the same site was infected with SPCV alone. This virus was also detected in two plant samples from Cooktown and one commercial grower sample from north Qld. The most common virus detected by herbaceous indexing (Plate 73) was SPLCV (no NCM-ELISA antisera).

Plate 72. SPFMV symptoms seen in the field on sweetpotato (*I. batatas*) plants and *I. plebia*. DAF Q, 2014 and 2017.

Plate 73. Left to right: *I. setosa* graft inoculated with Grower sample GRF 929 showing leaf roll and leaf cupping on sweetpotato. *Ipomoea* weed sample GRF 2206 (Gold coast airport) and Far North Qld sample GRF 2194 producing isolated vein clearing and rugosity on *I. setosa.* Weipa sweetpotato sample displaying SPFMV symptoms.

Ipomoea species were imported or obtained from local nurseries and 36 species were planted into the quarantine meshed tunnel at GRF for seed multiplication. Initial work identified germination issues for many species, as well as the inability to procure the required number of seeds. A pilot study was conducted to investigate possible rapid and reliable low-cost scarification methods to break seed dormancy. Nicking the seed coat and soaking for several hours proved to be the most successful and cost-effective method of pre-seed treatment of *Ipomoea* species.

Details on Ipomoea species investigated are listed within the *Ipomoea* as alternate indicator report (Outputs p13). As part of the small honours research project, seeds of six *Ipomoea* species were propagated and graft inoculated with SPFMV infected sweetpotato plants in replicated experiments. Results are detailed in the thesis document, Alternate indicator plants for sweetpotato virus detection. In addition to this work, Australian native *Ipomoea* species *I. plebia*, *I. pes caprae* and *I. muelleri* were inoculated with SPFMV, SPLCV and SPCV (Plate x) to assess their susceptibility to some the most commonly detected sweetpotato viruses in Australia. Native desert species *I. costata* did not produce any viable seeds so was excluded from testing. Symptoms associated with SPFMV infection were often observed on *I. plebia* within commercial crops.

I. pes caprae remained asymptomatic (Plate 74) when inoculated with all three viruses, indicating that the commonly known beach convolvulus is either tolerant or possibly resistant to the above sweetpotato infecting viruses. *I. plebia* produced cupping, vein clearing, rugosity and leaf distortion, *I. muelleri* produced vein clearing, rugosity and cupping, indicating both of these species are susceptible to infection by sweetpotato viruses.

Plate 74. Left to right: *I. pes caprae*, *I. plebia* showing vein clearing and rugosity, *I. plebia* produced vein clearing and leaf distortion, and *I. muelleri* exhibiting vein clearing, cupping and rugosity.

Primers and fluorescent probes (Table 12) suitable for use in qPCR were obtained to detect sweetpotato infecting viruses reported from Australia as well as exotic viruses posing a threat to the Australian industry. Published primer/ probe assays were purchased and protocols followed as per Kokkinos and Clarke (2006), Fuentes (2012) and Barkley (2011). Universal *Candidatus aurantifolia* (Phytoplasma) qPCR protocols were obtained from DAF Q Mareeba. New primer/probe assays were designed to detect SPCFV and SPCV. Antisera to detect ten sweetpotato infecting viruses was purchased from CIP.

Table 12. Primers and antisera sourced as part of this project.

ORIGIN	VIRUS	NCM-ELISA (CIP)	QPCR, QRT-PCR	SOURCE
Endemic	SPFMV	✓	✓	Kokkinos and Clark (2006) Fuentes (2012)
	SPV2		✓	Kokkinos and Clark (2006)
	SPVC		✓	Fuentes (2012)
	SPCFV	✓	✓	This work - VG 13004
	SPCV	✓	✓	This work - VG 13004
	SPLCV		✓	Barkley (2011)
	Phytoplasma		✓	DAF QLD, Mareeba
Exotic	SPCSV	✓	✓	Fuentes (2012), NCPN (n.d.)
	SPVG	✓	✓	Kokkinos and Clark (2006)

Plants (including known positive controls) returning positive results in NCM- ELISA for SPFMV and displaying Potyvirus symptoms (mottle, vein clearing, chlorotic spots and chlorotic flecks) on *I. setosa* were tested by qPCR. A one step SPFMV qRT-PCR was performed and testing produced a positive result 78% of the time. qPCR assays for strains (RC, C, O and EA) were postponed until future optimisation of SPFMV universal test could be completed. Plants testing positive for SPCFV in NCM- ELISA (including positive control plant GRF 323) and displaying symptoms associated with Carlavirus infection (mottle, vein clearing, chlorotic spots and chlorotic flecks) were positive 67% of the time in qPCR using newly developed primers.

New qPCR primers to detect the Cavemovirus SPCV produced 65% positive results when tested. Assay samples included positive control plants GRF 69 and GRF 85 previously tested by NCM-ELISA and *I. setosa* (chlorotic flecks, chlorotic spots and necrosis). Published primers used to detect the exotic virus SPVG were run using imported samples. All samples previously produced positive results for SPVG in NCM- ELISA in the country of origin as well as symptoms on *I. setosa* and 100% of samples were positive in qPCR. Known positive control plants previously tested using PCR and *I. setosa* and grower samples producing leaf curl and rolling symptoms on *I. setosa* returned positive results 68% of the time in qPCR.

Positive controls were obtained for SPCSV and these were tested using published primers and protocols by qPCR. All results in were negative with no amplification occurring. Likewise, published SPV2 primers and protocols also produced negative results and no amplification of samples. Known positive controls and grower samples producing positive results on indicator plants (small leaves and stunting at 49+ days PG) were tested by qPCR for phytoplasma with 100% giving positive results.

Several *Ipomoea* weeds collected around packing sheds and roadsides in commercial cropping areas tested positive in qPCR for SPLCV and SPFMV.

Table 13. Comparison of virus detection methods.

Current methods		Indicator plant symptoms													New method
	Virus	M	VC	CS	CF	Cl	LC	LR	LB	R	LD	S	N	F	qPCR % positive
	SPFMV	x	x	x	x										78
NCM-ELISA	SPCFV	x	x	x	x										67
	SPCV	x	x	x	x										65
	SPVG*						n/a								100
Indicator plant	SPLCV					x	x	x		x					68
only	Phytoplasma											x			100

* Exotic virus, W. Maso data, PNG. Blank cell indicates no symptoms.

All samples of *I. setosa* grafted with positive SPFMV control plant, GRF 300, produced a positive result for SPFMV in qRT-PCR using both fresh and preserved leaf tissue. RNA was extracted from dried leaf tissue stored for four years as well as from samples preserved in RNAlater™ for two years and from fresh tissue. However, all samples extracted directly from *I. batatas* (sweetpotato) tissue were negative.

DNA extractions from both fresh and dried tissue samples of *I. setosa* grafted with positive SPLCV control plants produced positive results for SPLCV in qPCR as did samples extracted directly from *I. batatas* (sweetpotato).

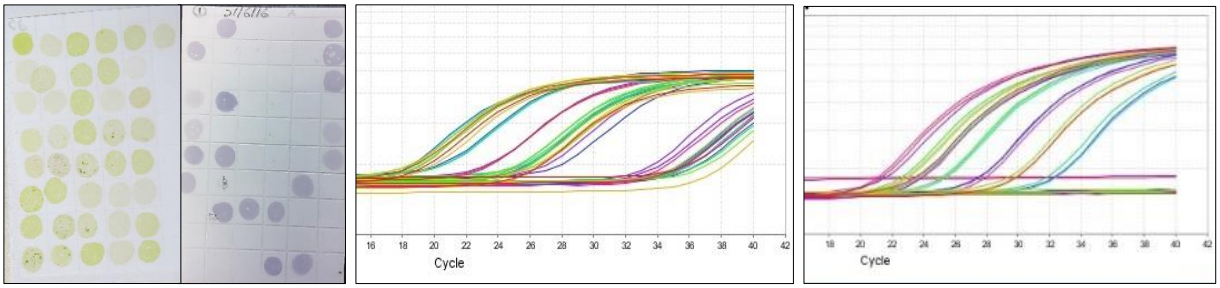


Fig. 39 Left to right: Plant samples blotted to membranes and developed NCM-ELISA test (purple colour denotes positive result). SPLCV qPCR amplification plot (Cudgen grower samples) and Phytoplasma qPCR amplification plot, S. Dennien DAF QLD.

Per sample costs of each of the virus detection methods used at GRF were calculated (Table 14). qRT-PCR was the lowest per sample cost, followed by biological indexing and qPCR. NCM-ELISA was the most expensive at $39 per sample. However, the cost of the NCM-ELISA includes the cost of biological indexing, as it is a requirement that NCM-ELISA tests are run on *I. setosa* samples. Overall qPCR and qRT-PCR are the most affordable and time saving diagnostic technologies.

Table 14. Cost of virus detection methods.

Virus indexing costs per sample		
Indexing method	Cost	Days to complete
Biological indexing	$31.24	49
NCM-ELISA*	$39.04	51
qRT-PCR	$21.94	1
qPCR	$29.43	1
Total per sample	$129.45	

*Includes biological indexing

Initial results sequencing

Data analysis of sequencing results is ongoing, however, initial RCA results, genome assembly and sequence analysis has revealed possible infections of multiple strains of Sweet potato leaf curl virus (genus Begomovirus) and Sweet potato collusive virus (genus Cavemovirus). Complete or partial genomes of some of the virus isolates have been assembled (Table 15), however, further NGS analysis and assembly is ongoing.

Table 15. Initial sequencing results.

GRF Accession No.	Origin	Cultivar	SPLCV	SPCV
GRF 001	Nth QLD	93-93-Q24	+	-
GRF 069	Imp Japan 2001	Beni Aka	+	+ Complete genome
GRF 085	Nth QLD	Alleys Red	+	+ Partial genome
GRF 341	IMP USA 2007	Regal	+	-
GRF 014	NSW	Whitestar Australia	-	-
GRF 340	IMP USA 2007	Excel	+	-
GRF 300	Nth QLD/ IMP USA 1980s	Lester's Original Beauregard	+	-
GRF 330	Rockhampton	Rocky Gold	-	-
GRF 328	Bundaberg	Rusty's Red	+	-
GRF 353	PNG 1980s	L46	+	-
GRF 303	PNG 1980s	L49	+	-
GRF 315	Imp Japan 2001	Beni Kokei	-	-

Discussion

Discussion regions

Leaf curl, leaf roll and chlorosis indicating SPLCV infection (Dennien *et al.* 2016) were the most common symptoms recorded overall closely followed by rugosity. Far North Qld and Bundaberg inoculated plants displayed the highest percentages, with south east Qld plants exhibiting the lowest. The second most prevalent symptoms were the suite of symptoms often associated with Potyvirus (including SPFMV) and or Carlavirus (SPCFV) infection, (mottle, vein clearing, chlorotic spots and chlorotic fleck). South east Qld plants displayed the lowest percentage of symptoms overall, closely followed by Northern NSW, where sweetpotato is intensively cropped within a relatively small area but use of PT planting material is widespread.

It is surmised that the high incidence of a range of symptoms produced on indicator plants inoculated with samples from Far North QLD is a result of the lack exposure to PT planting material. The vegetative nature of sweetpotato propagation, and the use of virus infected planting material facilitates a build-up of viral pathogens over time. Moyer and Salazar (1989) suggest that viruses are present in most non- PT material wherever sweetpotatoes are grown.

Discussion seasons

Symptoms on indicator plants associated with leaf curl were also highest for each season, followed by rugosity and Potyvirus symptoms. The incidence of chlorotic spots increased from Season 1 through to Season 4. The lowest occurrence of symptoms was from plants collected in Season 1 at the onset of planting when seedbeds were recently installed, and planting had commenced. Virus symptoms increased over summer in line with more favourable climatic conditions for vector population increases, and as planting beds aged.

The highest percentage of most symptoms occurred in mid-summer, with climatic conditions suitable to vector population increases and therefore more exposure to insect vectors. This was followed by autumn when plant beds been in ground for long time, symptoms decreasing with the exception of vein clearing which was detected at the highest percentage in autumn. Symptoms on plants collected during winter decreased further possibly as vector populations lowered.

Virus detection

SPLCV was the most commonly detected virus on herbaceous indicators and SPFMV was the most commonly detected virus in NCM-ELISA tests. SPCV was detected in five samples and SPCFV in one sample. The positive SPCFV plant was also positive for SPFMV and SPCV. SPCV and SPCFV positive samples were collected in the Northern and Far Northern regions of the state and all but one was grown in a non-commercial system.

Several *Ipomoea* species were shown to be susceptible to graft transmitted sweetpotato viruses and Morning glory weeds collected from commercial areas tested positive to SPFMV and SPLCV, indicating that that they provide a virus reservoir within commercial cropping regions.

qPCR assays produced accurate results comparable to existing diagnostics for the detection of the exotic SPVG virus and the endemic Phytoplasma. Results for other endemic sweetpotato viruses such as SPFMV, SPCFV, SPCV, SPLCV and V2 using qPCR and qRT-PCR suggest that more work is needed to optimise assays to improve accuracy. Whilst every attempt was made to sample plants concurrently and to use the same individual symptomatic leaves in NCM-ELISA and qPCR/qRT-PCR assays, this was not always practical. It is possible that movement of virus particles within indicator plants between sampling times, unknown distribution of virus particles within plants and sampling of different leaves as well as low titres (common to some sweetpotato viruses) all contribute to differing accuracy levels between diagnostic tests. Sweetpotato plants are mostly asymptomatic, but although indicator plants produce foliage symptoms readily, symptoms are often confined to a limited number of leaves which need to be utilised for DNA and RNA extraction, NCM-ELISA and tissue preservation, hence asymptomatic leaves from virus infected plants are sometimes used.

Initial qRT-PCR assays for SPV2 and the exotic SPCSV unfortunately did not produce any amplification and as such were null and void. This could be due to several factors such as non-viable controls, quality of primers and experimental error. New positive controls for qRT-PCR assays for SPV2 and the exotic SPCSV would need to be obtained and assays retested.

Tissue from sweetpotato plants and indicator plants yielded similar results in qPCR for SPLCV. However, sweetpotato plant tissue yielded negative results in initial SPFMV qRT-PCR assays, whilst tissue from indicator plants (*I. setosa*) yielded positive results from both dried and fresh tissue. This could be influenced by inhibitors in sweetpotato sap, the area of the plant that was sampled (differing distribution of virus particles within plants), the time of year that plants were sampled (stress may affect virus particle distribution and titre), and whether plants were symptomatic or asymptomatic (affecting titres).

Initial results sequencing

Further analysis and assembly of sequencing mega data is ongoing, and identification of specific strains present in country could facilitate the design of specific primers for accurate detection of viruses reported from Australia and the production of specific SPLCV antisera to be added to the NCM-ELISA suite of tests.

There are no antisera as yet developed for SPLCV in NCM-ELISA, but negotiations are underway to initiate development, based on strains identified in next gen sequencing.

Summary

The endemic nature of sweetpotato viruses and varying tolerance levels of cultivars complicates virus detection. In addition, the lack of visual symptoms, low titres, mixed infections, diverse strains and uneven distribution of virus particles within sweetpotato plants further confounds the issue. The presence of inhibitors in sweetpotato sap also interferes with serological and conventional PCR detection (Fuentes pers com. 2007). To further hinder accurate virus isolation and identification, the presence of SPFMV thought to be endemic, in sweetpotato growing countries often masks the presence of other viruses, especially other Potyviruses, (Valverde *et al.* 2007). Nutritional deficiencies also cause symptoms sometimes mistaken for those caused by virus infection. Although infected sweetpotato plants are mostly asymptomatic, abiotic stresses can induce occasional symptom expression.

When present, virus symptoms are often difficult to distinguish, and expression often varies with virus strain, host cultivar, age of plants, abiotic factors, multiple infections and synergistic reactions. Symptoms of SPLCV are rarely seen in the field, as evidenced by detection in grower samples that were asymptomatic on collection. Symptoms can be seen in G2 planting material and where plants are co-infected with SPFMV and SPLCV.

For this reason, the current Australian PT system relies on a combination of complimentary diagnostic techniques, Phyto-diagnostics, NCM-ELISA and the development of qPCR. Phyto-diagnostics can detect the presence of virus infection including new or “novel” viruses using biological indicator plants. As indicator plants are highly sensitive to most sweetpotato infecting viruses, leaf symptoms produced are more apparent than those observed on sweetpotato plants. This sensitivity of indicator plants to virus infections also facilitates rapid increases in virus titres, enhancing downstream diagnostics such as NCM-ELISA and PCR. Phyto-diagnostic indexing as part of the Australian PT protocol is conducted by experienced, skilled staff at Gatton Research Facility (GRF) as some virus symptoms can be transient (Potyviruses), difficult to discern at lower titres (SPLCV) and mixed infections influence symptom expression. The lack of symptoms on some rounds indicates the need for repetition as recommended in Dennien *et al.* (2013). However, this is time consuming and requires considerable glasshouse space.

The (NCM- ELISA (CIP) test is able to detect 10 known sweetpotato infecting viruses, (C-6, CMV, SPCaLV [now called SPCV], SPCFV, SPCSV, SPFMV, SPLV, SPMMV, SPMSV and SPVG) and whilst accurate takes a day and a half to complete and requires sap from graft inoculated indicator plants. These virus detection technologies offer high levels of repeatability and reproducibility. qPCR is cost effective and labour saving and has the potential to speed up routine virus diagnostic capability and is a valuable addition to the suite of complimentary methods used in sweetpotato virus detection at GRF. Further work needs to be done on optimisation of primers and protocols to optimise accuracy and efficiency. Boonham *et a*l. (2013) suggests that qPCR will become the most widely used diagnostic technology in testing laboratories around the world. NGS technologies are rapidly becoming more affordable and are a promising platform for developing future portable devices for plant pathogen detection devices. While there is a need to adopt new molecular technologies, when evaluated during this study, varying levels of accuracy were observed, thus the inclusion of herbaceous indicators remains essential to the Australian sweetpotato virus testing process, due to its continuous and wide-ranging ability to detect all sweetpotato viruses.

Appendix 11

Engagement of VG13004 project team with international scientists around IHC2014

During the IHC2014, project team members presented an oral paper and two posters and were co-authors on another oral paper presentation (see Outputs). These were well attended and received, even in the last hour of the last day of the Congress!

The highlight for our sweetpotato project team (even those who did not specifically attend the IHC) was two weeks of engagement with sweetpotato scientists from the USA, and particularly Louisiana and North Carolina State Universities (listed below). On the Tuesday of the IHC, members of the project team, both scientists and growers, visited Cudgen with Don Labonte, Arthur Villordon, Theresa Arnold, David Picha and Craig Yencho. On the following Monday through Wednesday, members of the project team, both scientists and growers, toured the Bundaberg sweetpotato district with Don Labonte, Arthur Villordon, Theresa Arnold and Jonathan Schultheis.

Plate 75. LSU and DAF sweetpotato researchers meeting with ASPG leaders and Bundaberg growers.

Plate 76. LSU and DAF sweetpotato researchers meeting with ASPG leaders and Cudgen growers.

At both Cudgen and Bundaberg, apart from the numerous individual farm visits, we also had formal grower shed and night meetings. More than 100 growers and industry personnel attended the presentation events in Cudgen and Bundaberg. Both our sweetpotato team and the USA visitors all presented various sweetpotato RDE findings to these large groups of sweetpotato growers and industry people. The industry got lots of interesting ideas on cultivars, nutrient management and general agronomy. We all received valuable feedback on our programs and identified future research needs and opportunities.

On the Saturday post-conference, DAF staff and ASPG R&D Committee members were visited at Gatton Research Station by Don Labonte, Arthur Villordon and Theresa Arnold, and they reviewed our current sweetpotato virus testing facilities and operations. Queensland sources nearly all its current and emerging cultivars from the Louisiana S.U. breeding program, so it was invaluable to build the interaction with Don Labonte, gaining his expertise on niche characteristics of his cultivars, as well as increasing his familiarity with the requirements of the Australian industry. Arthur Villordon is the foremost world authority on sweetpotato physiology and agronomic interactions, and we continue to probe his knowledge as we develop our sweetpotato RDE. Arthur is currently supervising Craig Henderson's PhD on sweetpotato agronomy at Central Queensland University. Jonathan Schultheis provided some useful ideas about the North Carolina sweetpotato agronomy program, with particular insights with relevance to our recently started plant bed management project.

Louisiana State University

Professor Arthur Villordon – world's leading sweetpotato physiologist

Professor Don Labonte – breeder of newest sweetpotato cultivars emerging in Queensland

Professor David Picha – sweetpotato post-harvest specialist

Theresa Arnold – sweetpotato agronomist and PhD student

North Carolina State University

Dr Jonathan Schultheis – sweetpotato agronomist and extension specialist

Professor Craig Yencho – sweetpotato breeder of major USA cultivars

Pacific region

Our sweetpotato project team also had the opportunity to interact with colleagues from the Pacific region, particularly Fiji, Vanuatu and New Zealand (see below). The scientists from SPC have a very important role in virus diagnostics and germplasm maintenance, particularly given that they now hold the reserve collections of sweetpotato germplasm for cultivars previously held in Australia. (In Australia, we now only hold germplasm of commercially relevant cultivars, apart from a few specimens that Sandra Dennien keeps for ongoing virus detection work). Sandra made the most of the time to firm up links with these scientists, as well as explore opportunities for coordinating virus diagnostic and germplasm movement protocols around the region.

Dr Mary Taylor – sweetpotato tissue culture specialist from Secretariat for the Pacific Commission (SPC) in Fiji

Valerie Tuia – sweetpotato germplasm specialist from Secretariat for the Pacific Commission (SPC) in Fiji

Amit Sukal - sweetpotato virus diagnostics specialist from Secretariat for the Pacific Commission (SPC) in Fiji

Professor Vincent Labot - sweetpotato tissue culture and germplasm specialist from Vanuatu

Dr Bruce Searle – sweetpotato agronomist, New Zealand Crop and Food

Andre de Bruin – sweetpotato industry leader, New Zealand Vegetables

Dr Susan Miyasaka – sweetpotato agronomist, University of Hawaii

Relevant outcomes for Australian sweetpotato industry

Great interactive discussions on managing plant beds, including use of row covers to promote earlier sprout emergence, importance of pre-sprouting to maximise plant bed performance of some cultivars, and how to properly ventilate plant beds under row covers.

DAF now able to effectively and rapidly diagnose Begomovirus diseases in Australian sweetpotatoes using new Real-Time PCR equipment provided by ASPG. Expertise shared with SPC scientists means this capacity is now growing exponentially, building both biosecurity and PT planting material abilities in Australia and our pacific neighbours.

Plate 77. Sandra Dennien discussing sweetpotato development with Professor Arthur Villordon from LSU.

Australian growers and researchers are now directly informed of exciting new developments in understanding sweetpotato physiology and nutrient management. Examples are sensitivity of some cultivars to ammonium fertilisers, and the importance of where fertilisers are in the sweetpotato rootzone, to trigger the best size and shape in sweetpotato roots. This is information that hasn't even been published in the scientific literature yet, and our growers will be able to implement these ideas in the current growing season!

Sweetpotato industry is now directly able to question USA experts on cultivar characteristics and future cultivars that may soon be available in Australia. Industry is also able to let breeders know what characteristics of cultivars will be important for Australia-bound germplasm.

Strong relationships are now enhanced between ASPG, DAF and USA scientists, to really build RDE capacity.

Appendix 12

Sandra Dennien and Rachael Langenbaker visit to USA sweetpotato industry, August 2015

Sandra Dennien and Rachael Langenbaker (the latter fully funded by ASPG outside Project VG13004) visited several USA sweetpotato districts in Louisiana, Alabama and California, 16-29 August 2015. In Louisiana/Alabama they were hosted by eminent Louisiana sweetpotato scientists, Chris Clarke, Don Labonte, Mike Cannon and Arthur Villordon, each a world authority in their respective disciplines. In California they were accompanied by Scott Stoddard. A range of sweetpotato researchers, institutions and growing areas were visited and large scale sweetpotato production was observed in different environments throughout Louisiana, Alabama and California.

Virus issues

Ms Dennien's focus was to capture some of the knowledge and experience of Dr Chris Clarke from Louisiana State University, the foremost world authority on sweetpotato viruses, before he retires. Ms Dennien gained specific understanding of new sweetpotato virus research, including new sweetpotato virus diagnostic protocols, and exchanged virus management strategies and technologies being developed in this specialised field.

Plate 78. Ms Dennien discussing sweetpotato viruses with Dr Chris Clarke.

In USA they have:

Potyviruses: SPFMV Sweetpotato feathery mottle virus, SPVG sweetpotato virus 2, SPV2 sweetpotato virus G and SPVC sweetpotato virus C. We have not found sweetpotato virus C or sweetpotato virus G in Australia so far, but we have found it in PNG and the Solomon Islands. In USA they are still seeing a reduction in yield in Generation 2 and Generation 3 planting material; potentially due to SPVG and SPFMV/SPV2/SPVC.

Geminivirus: SPLCV (not common). It may not be the same strain as we have in Australia; potentially why they believe they can use G2/G3 material? That attitude may also be because in their growing areas, they have less overall virus/whitefly pressure compared to Australia

Other observations from Dr Clarke's work include:

Most virus impact occurs in sweetpotato 3-7 weeks after planting, when plants are growing vigorously, and viruses are therefore multiplying rapidly.

There is a high presence of *Ipomoea* weeds everywhere in Louisiana and Alabama. As a consequence, it is nearly impossible to keep plants clean in the field. At their primary multiplication site, Dr Clarke walks around propagation fields fortnightly to check for virus infection.

Plate 79. Tissue culture laboratory at Louisiana State University, Baton Rouge.

Louisiana State University are looking at providing a service to growers for a fee - testing bedding roots to provide a decision support tool, along with yield data, to assess how on-farm bedding roots have deteriorated.

Concern that most of the evaluation on effects of viruses on yield has been done with cv. *Beauregard*, however now there are many cultivars with no data on yield effect of viruses.

Dr Clarke has developed a new multiplex PCR assay showing excellent results.

The USA PT sweetpotato scheme is being recalibrated into a national network. However, it is still predominantly providing G2 or G3 bedding roots to growers, who only use that material for around one third of their planting. They use kept roots from the previous commercial harvest for the rest of their plantings. It reflects how fortunate the Australian industry is to have access to sufficient G1 bedding roots to plant the bulk of their commercial crops, if they so wish.

Dr Clarke and Ms Dennien had many discussions on the intricacies of different virus analytical methods, the results of which will be used to improve diagnostic methods in Australia.

Additional study tour findings

Water is a major limiting factor in Louisiana and California, with many sweetpotato farms not irrigated. This results in lots of missed establishment of plants within rows, as well as regularly poor initial growth and storage root set. Additionally, key nutrients are poorly available in dry soils. Paradoxically, flooding is also a major sweetpotato production problem in Louisiana and Alabama.

Plate 81. Missing plants caused by drought in unirrigated sweetpotatoes.

Plate 80. Drought symptoms in Californian drip irrigated sweetpotatoes.

High levels of nutrients are used in Louisiana and California; around 120 units of N, 150 units of P and 150 units of K. Part of this is probably due to the reality of poor uptake, due to no or inefficient irrigation. The USA scientists suggested growers seem to be slightly reducing the amount of nitrogen they use in the last few years.

Sweetpotato growers in the USA predominantly rely on herbicides for managing weeds at planting and post-emergence. Ms Langenbaker and Ms Dennien both observed damage to sweetpotatoes from use of metolachlor and halosulfuron-methyl in commercial crops. No herbicides appeared appropriate for Australian growers chasing good crop performance.

Arthur Villordon's nutrient work was very interesting, reinforcing the role of nutrients as signals for storage root production and quality, as well as their macro effects. His particular emphasis was the roles of N and P in storage roots shape, and Ca and B on root structures. Of particular interest to project VG13004 is Prof. Villordon's aeroponic system for observing early storage root formation and development. This technique may be very useful for understanding sprout potential from planting beds, reducing the need for lengthy and resource-consuming commercial plant out experiments.

Plate 82. Herbicide damage in sweetpotatoes.

Plate 83. Professor Arthur Villordon (left) and his colleagues demonstrate aeroponic experimentation with sweetpotatoes at a LSU Research Station field day.

The visiting tour observed how Don Labonte develops new sweetpotato cultivars, which are currently the basis for the Australian industry, including all the new introductions in recent years. Apart from reviewing the breeding facility, the team also saw the cultivars performing in field evaluations and commercial crops. One comment from the group in discussing *Orleans* and *06-52* was that the former was probably more suited for cool season production, with the latter mainly to be grown during summer, to avoid splitting issues. It was obvious that the new cultivars require different production strategies to current cultivars.

Plate 84. Dr Don Labonte discusses sweetpotato cultivars and breeding at a LSU Research Station field day.

Plate 85. Polycross sweetpotato breeding lines at Louisiana State University.

In summary, knowledge gained from this visit and the collaboration now established with LSU sweetpotato researchers, has greatly improved the capacity for the Australian Sweetpotato industry to detect current sweetpotato viruses as well as potential new virus risks. There is also an increased capacity to respond to biosecurity threats, through improved diagnostic techniques and knowledge of virus management, particularly considering new viruses recently detected in Papua New Guinea.

Plate 86. Californian sweetpotato production is broadacre, and highly mechanised (left, and produces a high-quality product through careful handling.

Excellent collaboration between world authorities in sweetpotato virus detection and management, crop production, nutrition, new cultivars and plant bed management was established through engagement with experts at Louisiana, and California research institutions. A meeting was also held to discuss potential arrangements to mutually conduct future collaborative research in Australia and the USA.

Industry extension

Ms Dennien and Ms Langenbaker are giving presentations on their tour to the sweetpotato industry at their AGM in Cudgen on 29 October 2015. Following the presentations, there will be an open forum with the growers, to determine what aspects they felt were most useful, and these will be highlighted in the report to be placed on the ASPG website in the coming weeks. The tour presentation and forum will be repeated in a planned meeting in Bundaberg in December 2015.

These excellent presentations are available from Mr Henderson.

Acknowledgments

Ms Dennien and Ms Langenbaker acknowledged the wonderful hospitality of all the people they met on this visit, and their generous allocation of time and resources to making it such a worthwhile exercise. The LSU scientists Chris Clark, Don Labonte and Arthur Villordon were very welcoming and generous with sharing their knowledge, as well as their time, and even their homes. The leadership and staff at Chase Research Station, took time out from their major field day organisation and presentation to include the Australian visiting team in their activities.

Scott Stoddard in California was also very forthcoming and supportive of the team's visit to the USA West Coast. A very special thanks to all the growers and industry support people, who took time out of their busy days to show the Australians around, and share knowledge of their respective operations and industries.

Ms Dennien and Ms Langenbaker would especially like to thank the Directors of Aus. Sweetpotato Seeds, Eric Coleman and his wife Kristie, for their very tolerant and supportive tour hosting for this USA visit. Without their knowledge and contacts, it would not have been anywhere near as useful and informative.

Finally, Ms Langenbaker would particularly like to thank ASPG for financially supporting her participation on this tour. She found the trip extremely worthwhile, and hopefully can return to Australia with innovative ideas, and a better appreciation of international sweetpotato science and commercial operation.

Appendix 13

Craig Henderson visit to Louisiana sweetpotato industry, November 2017

Introduction

Craig Henderson visited Louisiana sweetpotato research facilities and farms between 4-18 November 2017. The tour was a new project activity, negotiated in response to the VG13004 MTR. Mr Henderson spent most time at the Louisiana State University (LSU) Sweet Potato Research Station at Chase, Louisiana, in collaboration with Professor Arthur Villordon, one of the world's premier sweetpotato physiology scientists.

Plate 87. LSU Sweetpotato Research Station, Chase, Louisiana, USA.

Plate 88. Professor Arthur Villordon, Sweetpotato Physiologist, LSU

Production of Pathogen Tested (PT) roots

The research station is the provider of Foundation PT Roots to the Louisiana (and other states, such as Alabama, Mississippi and California) sweetpotato industry, multiplying tissue culture materials from LSU Baton Rouge. This system is analogous to the Australian situation, although the Louisiana situation is obviously government and institution supported, as opposed to the fully-commercialised Australian system. Interestingly, a biosecurity issue, with sweetpotato weevil (*Cylas formicarius*) being found in stored sweetpotatoes at Chase, meant the Research Station was quarantined for a week. In addition, all the harvested and stored sweetpotatoes had to be shipped to weevil infected areas, rather than sold to usual planting material customers and multipliers. This may have substantial impact on both the budgets for the research facility, as well as availability of PT planting material for Louisiana growers in weevil free zones in the northern half of the state. A learning for Australia is the threat of biosecurity issues to the PT chain, as well as risks associated with sourcing materials from one location.

Plate 89. Chase PT sweetpotato storage.

Plate 90. Harvesting PT Foundation Roots at, Chase, Louisiana, USA.

Plate 91. Investigations of P status on sweetpotato storage root development.

Late in the visit, Mr Henderson assisted harvesting Foundation PT Roots from fields released from quarantine. An observation was that yields were relatively low, reflecting the situation in Louisiana where both weather conditions at crop establishment, and perhaps lack of facilities and focus on irrigation management during that key phase, mean yield potentials are sacrificed at the outset.

Glasshouse studies of sweetpotato roots

The key focus of the visit was viewing the glasshouse studies of Prof. Villordon into sprout and storage root development. Currently he is undertaking investigations into the impact of N and P nutrition on storage roots, and these are the experiments Mr Henderson viewed and assisted with during his visit. He also collaborated with Prof. Villordon initiating a pot study investigating the impact of sprout defoliation, and hence photosynthate provision, on development of sweetpotato storage roots.

Plate 92. Collaborative pot experiment on slip defoliation impacts on storage root development.

Sweetpotato root research using aeroponics

Prof. Villordon has an excellent aeroponic system for studying the early development of sweetpotato roots from cuttings. It currently uses a vertical planting set-up, with two nodes in the aeroponic environment. The key aspects of the system are:

- High light environment
- Standard nutrient solution
- Timed operation of the spraying solution, so the roots stay moist, but are also given some time for effective gas exchange

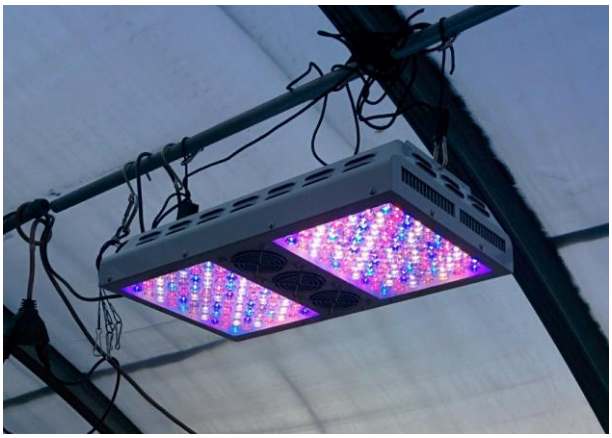


Plate 93. High lighting critical for successful glasshouse sweetpotato studies.

Plate 94. Installation of sweetpotato sprouts in aeroponic system.

Plate 95. Early aeroponic root growth.

Plate 96. Early aeroponic root growth. Note the early lateral root development.

The roots grow very rapidly and reach the underlying solution within 10-14 days of transplanting. Prof. Villordon is identifying different root growth patterns under variable nutrient management, within a few days of application.

Plate 97. Aeroponic shoots.

Plate 98. Aeroponic shoots (low P).

Plate 99. Aeroponic roots developed.

Plate 100. Aeroponic roots developed (low P).

Plate 101. Aeroponic roots final.

Plate 102. Aeroponic roots final (low P).

Aeroponics in the commercial PT program

Plate 103. Foundation PT plant multiplication hothouses at Chase Research Station.

Plate 105. Commercial aeroponic units for plant multiplication.

Plate 107. Aeroponic roots develop well and support rapid shoot multiplication.

The Research Station has also just started using commercial aeroponic systems for early multiplication of tissue culture plantlets from their Baton Rouge facility. The early multiplication is undertaken using aeroponics, with further multiplication enhanced in specialised media beds.

Plate 104. Foundation PT plant multiplication using aeroponics and media beds.

Each aeroponic tub can support 180 plantlets direct from tissue culture and has a very rapid multiplication rate. The equipment is readily available, due to large scale hydroponic industries established for other edible, ornamental and medicinal crops.

Plate 106. Dedicated staff monitor and maintain production.

Plate 108. Aeroponic roots suffering after less than 30 hours when solution had run dry.

Plate 109. Layout of sand culture pot experiment.

Plate 110. Demonstration of nutrient impacts on early storage root initiation and development.

This aeroponic system appears to be working well, although absence of staff availability (and hence attention) during the biosecurity issue demonstrated vulnerability, with some root death during hot days after solution levels dropped.

Sweetpotato root research using sand culture

Prof. Villordon also uses sand media in 15 cm diameter by 30 cm deep pots, to examine sweetpotato storage root development. The same principles apply to encouraging effective root development as in the aeroponic studies. Within 45 days, storage roots are clearly identifiable in those experiments, with substantial swelling having taken place. Prof. Villordon has identified that soil compaction and constraint by pot size do affect storage root development and swelling. This means that with flat planting, much larger pots will be required in the Australian studies.

Prof. Villordon suggests that he can detect storage roots likely to develop into marketable roots by 30 days, however he generally runs his experiments for 50 days, to be certain of effects.

Plate 111. Freshly exposed root system.

Both the aeroponic and sand culture systems require very intensive management and attention to detail. The aeroponic system is vulnerable to disruption of even a few hours, particularly before the roots reach the underlying nutrient solution. Prof. Villordon or his team monitor their experiments at least once a day, and have automated systems to manage water timings, lights and temperature control.

Field experimentation

Mr Henderson assisted Prof. Villordon and his team harvest a nutrient management field experiment, identifying impacts of nutrient addition and distribution on yields and quality of sweetpotato roots. Field yields were generally lower than in Australia, probably for reasons previously discussed with their foundation root program, although Prof. Villordon does try and use Australian intensive practices when resources (staff and equipment) make it possible.

Plate 112. Sweetpotato nutrition experiment.

Plate 113. Harvesting the sweetpotato nutrition experiment.

Mr Henderson also assisted Research Station Leader Dr Tara Smith and her team assess an experiment identifying best practices for sweetpotato weevil management. This was an excellent opportunity to get a feel for their pest management issues, as well as a further sense of their commercial grading specifications.

Plate 114. Assessing roots harvested from a weevil management experiment.

Sweetpotato breeding

Mr Henderson was shown the polycross LSU breeding plots, where promising parents are naturally cross pollinated to generate new breeding materials. In this system, only the female parent is identified. Seedlings are multiplied, and then run through screening processes for soil disease resistance in infected fields. Resistant plants are then further multiplied and evaluated over several years for quality and productivity.

Plate 115 Sweetpotato polycross breeding lines (frost affected).

Plate 116. Preparing breeding line evaluation site for digging.

Mr Henderson assisted the Plant Breeder, Dr Don Labonte, as well as Prof. Villordon and the research team, assess an evaluation experiment for a full day during his visit. He observed low selection pressure on weevil and *Rhizopus* resistant lines, with greater culling of lines as they get closer to release as a named cultivar. The evaluation environment is relatively harsh, with often only one or two nodes buried, and suspected tough conditions at planting. A particular focus this year has been purple cultivars, as well as red-skinned, gold flesh cultivars. The latter are difficult to market in Australia, due to resistance from wholesalers, citing confusion with red-skinned, white-flesh cultivars.

Provision of PT mother lines and plants

Mr Henderson visited LSU in Baton Rouge with Prof. Villordon, principally to meet with Prof. Chris Clark, the leading world sweetpotato pathologist. Mr Henderson saw their diagnostic equipment and processes, which has previously been reviewed by project team member Sandra Dennien. He also was introduced to their tissue culture facility, which provides all the mother plant material for the LSU cultivars currently in production. These include gold cultivars *Beauregard, Orleans, Bellevue* and *Evangeline*, which dominate Australian sweetpotato production. Red-skinned cultivars *Bayou Belle* (high yielding processing cultivar) and *Burgundy*, as well as white *Bonita* and red *Murasaki* round out their commercial lines. They also hold the stock for all the breeding material.

Interestingly, the Louisiana team only use meristem therapy to remove viruses from their lines, and not the additional thermotherapy as used in Australia. It appears they rely on volume of operation, as well as advanced meristematic excision skill, to remove viruses from their planting material.

Louisiana sweetpotato industry

Mr Henderson was fortunate to tour four sweetpotato growers with renowned sweetpotato consultant Mike Cannon. Noted was the degree of mechanisation of their plant multiplication and production systems. They rely on volume of production and treat sweetpotatoes more as a broadacre crop. Yields are about 30-50% of those achieved in Australia. Of relevance to the Australian situation was machinery for harvesting sprouts, although it is potentially damaging to the longevity of plant beds (not important in the USA system).

Plate 117. Mr Henderson (left) and Mike Cannon (right) inspect Louisiana sweetpotatoes with grower Vendy Kinnaird.

Plate 118. Large scale machinery typical of Louisiana sweetpotato production.

One grower was having serious issues with skinning of *Orleans* during pack-out. These sweetpotatoes were coming out of storage, and our USA colleagues felt they had not be cured sufficiently before storage. Australian sweetpotatoes are not cured before packing, however perhaps we have more focus on skin-setting through topping and drying before harvest.

The final grower was a major corporate supplier to national supermarkets. They had an outstanding, highly mechanised packing line. USA size grading standards are smaller than in Australia, particularly their premium grade. Sweetpotatoes graded as large premiums in Australia would be classified as Jumbo (processing) lines in the USA.

Plate 119. Large Louisiana packing shed.

Although small sweetpotatoes in the USA were traditionally sold into canning products (sweet, dessert styles), this market is declining. It is becoming increasingly important to focus on premium lines making up the bulk of the harvested sweetpotatoes. Fortunately, this grower had negotiated many different pre-pack lines for different sweetpotato sizes and grades, and the company was thus able to use almost all their harvested product.

Mr Henderson had several hours discussion with their farm manager, who was very interested in the Australian planting system. In conjunction with Prof. Villordon, they are very keen to evaluate several hectares next season, to assess its application in their growing environment.

Louisiana- Australia information exchange

Mr Henderson presented a seminar on the Australian sweetpotato industry to research station staff, growers, and an online audience at LSU Baton Rouge. With the talk going for 40 minutes, there was obviously much interest, as questions and discussion continued for another 80 minutes after the end of the presentation. Several growers were keen to follow up with Prof. Villordon on application of aspects of the Australian production system in their enterprises.

Acknowledgements

Mr Henderson acknowledged the wonderful hospitality of all the people he met on this visit, and their generous allocation of time and resources to making it such a worthwhile exercise. The leadership and staff at Chase Research Station, Dr Tara Smith, Theresa Arnold, Cole Gregorie and Jimmy Ronsonet were very welcoming, and always had a friendly word every day. Their LSU colleagues Dr Chris Clark and Dr Don Labonte at Baton Rouge were similarly kind on the day I visited there, and when I was involved with harvesting their breeding evaluation experiment.

Mike Cannon and the growers we visited, Ken Thornhill, Vendy Kinnaird, Ricky and Jeanette Ables and Todd O'Neal were exceptionally supportive and interested in talking about their farming practices, as well as what we were doing in Australia. Mike Cannon has an amazing knowledge of sweetpotatoes, and its place in American culture. The day touring with him was an excellent opportunity to observe first-hand the successes and challenges of the USA industry.

Prof. Arthur Villordon was the most kind, sincere and generous host of Mr Henderson's visit. He spent the best part of 10 days sharing his knowledge, experiences and practical skills, as well as providing Mr Henderson with numerous learning opportunities through practical involvement in research and extension activities.
Prof. Villordon, his wife Theresa and son Michael also shared their home with Mr Henderson for the bulk of his stay. This was an absolute gift, and a wonderful opportunity to experience life in an extremely interesting place and culture.

Appendix 14

Arthur Villordon visit to Australian sweetpotato industry, March 2018

Introduction

Professor Arthur Villordon from Louisiana State University is a world renowned sweetpotato physiologist. More details on him and his work can be found in Appendix 13. Following the mid-term review, additional project funds were provided to ASPG for him to visit Australia in March 2018. There were five aims for his visit.

- To check-in with the Australian industry and see what practices have changed since his last visit, as well as get an understanding of current issues. To also offer his perspective on those issues and practices, particularly in relation to his knowledge of the USA cultivars, as well as specific sweetpotato applied physiology
- To present his most recent research and ideas, particularly around sweetpotato storage root initiation and development, to Australian industry, marketers, scientists, and research support organisations.
- To observe the sweetpotato agronomy research being undertaken in Australia, and offer advice and support, as well as get a sense of what is sweetpotato science is going on.
- To get an understanding of the value chain for the Australian industry.
- To discuss the organisation, funding and collaborative opportunities with ASPG, research providers, and research funders

Prof. Villordon visited Australia 11-24 March 2018. His visit was organised by the ASPG Executive, and he was accompanied for his whole trip by their R&D Project Manager (voluntary) and sole Australian supplier of PT bedding roots, Mr Eric Coleman.

At the time of writing this report, due to his heavy early season workload, Prof. Villordon was still compiling his trip report. This summary provides information on the VG13004 components of his visit, as far as the project team is aware.

Industry review

Prof. Villordon visited farms and growers in Cudgen, Bundaberg, Rockhampton, and the Atherton Tablelands. In the Cudgen and Bundaberg sections, he was accompanied by VG13004 project team members, Craig Henderson, Sandra Dennien, Rachael Langenbaker, and collaborator Dr Bree Wilson (USQ). In Cudgen, they visited the farms of Sam Tully; Doug, Steve and Jim Paddon; Stuart Kennedy; and Matthew and Henry Prichard. In Bundaberg, they visited the farms of Russell Mortimer, Darren Zunker, Peter Greensill, David Holt, Troy Prichard and Mal Beutel. Arthur also solely visited specialist sprout producer and agricultural consultant Mr Russell McCrystal for an afternoon, observing and reviewing his operation. ASPG President Mr Rodney Wolfenden accompanied the group during their Bundaberg grower visits.

In Rockhampton, Arthur visited Mr Coleman and his wife Kristie's pathogen tested bedding root production facility at Gracemere, as well as Mr Wolfenden's farm at Rossmoya. The final leg of Prof. Villordon's grower visits was in on the Atherton Tableland, where he was accompanied by DAF scientist and sweetpotato specialist Mr Mike Hughes. They visited the farms of Don Murray, Richard Grima, Bruno Cuda, Simon Godfrey and Pompey Pezzalatto / Daryl Tindel.

In each location, Prof. Villordon observed the growers' production chain, from plant bed, through commercial fields, to their packing operations. He re-familiarised himself with Australia's intensive production systems, and our focus on high yields and productivity. In talking with the growers and accompanying scientists, there was lots of interest around the different cultivars from the USA, their advantages and pitfalls, and their different management requirements. He provided many handy tips for growers on some specific management issues, such as temperatures for plant beds, optimal fertiliser practices, and disease susceptibility. There were particularly lengthy discussions with most growers around the drivers for storage root initiation, and development of good root shape. The focus was on nutrient availability and distribution (particularly phosphorus), and soil temperature profiles early in the season. He also provided insights into how USA growers and scientists manage the sprout initiation vs breakdown experiences with *Bellevue*.

Having those discussions in the field, looking at real commercial crops, was an excellent opportunity for growers and scientists alike. In turn, it also gave Prof. Villordon a very clear overview of the agronomic and other issues facing Australian growers.

Plate 120. LSU Professor Arthur Villordon visiting Australian sweetpotato growers in Cudgen (top), Bundaberg (bottom), North Queensland (centre-left), and the well-known Australian industry 'wall of sweetpotatoes (centre-right).

Industry presentations

Along with the VG13004 team, Prof. Villordon gave an extended presentation of his recent sweetpotato agronomy research to packed grower meetings in Cudgen, Bundaberg and Kairi (see Outputs). Most Australian sweetpotato businesses attended one of those presentations. His various talks, commonly themed *Fifty shades of phosphorus: a sweetpotato story*, are available from Mr Henderson.

His presentations covered the following topics:

- USA sweetpotato production, location, production systems, markets.
- The USA breeding program, strategies, targets, and review of the LSU cultivars currently grown in Australia.
- The importance of soil phosphorus in sweetpotato root development, storage root initiation and expansion.
- How phosphorus and sucrose content of sprouts affects storage root initiation.
- Optimal sprout characteristics (stem thickness, foliage, nodal distribution) for peak field performance.

At all the meetings, growers were highly attentive, engaged and asked many questions at the end.

Plate 121. Prof. Villordon presented to most of the Australian sweetpotato industry during his visits to the principal growing districts.

Reviewing sweetpotato science

On 15/03/2018, Prof. Villordon toured the Bundaberg Research Facility, and specifically the sweetpotato hothouse. He reviewed the nematode investigations being conducted by Dr Jady Li and her team from Central Queensland University (CQU), as well the sweetpotato hydroponic systems for investigating storage root initiation and early development. The latter is being undertaken as a PhD study by Ms Tham Dong (also from CQU). He also observed the small aeroponic system and rhizotron study (see Appendix 9) managed by Ms Langenbaker.

Prof. Villordon had previously communicated with Ms Dong to assist her with her interpretation of microscopic root sections and point out the various important components of a developing sweetpotato storage root. His main comments on the work in the hothouse facility were:

- The imperatives of good supplemental lighting to ensure effective sweetpotato growth. It needs to be at least 400 uM m^{-2} s^{-1} of photosynthetically active radiation.
- Having effective diameter and depth of pots for growing sweetpotatoes. Even in studies where vertical sprout planting is acceptable, the pots still need to be at least 15 cm in diameter, and 40 cm deep, to not adversely compromise root development.
- Effective temperature management to maintain the plants in a non-restrictive growing range is very desirable.
- Ensure very uniform sprout selection, with good initial nutrient and foliage status, at least two uniformly distributes nodes for burial, and a uniform thickness and quality. Variable sprouts mean variable storage root potentials.
- Consistent and optimal provision of nutrients in aeroponics, hydroponics, or sand culture systems. He provided the base recipe he uses for all his studies. Ms Langenbaker now has those solutions at the Bundaberg Research Facility.

Plate 122. Prof. Villordon reviewed Queensland sweetpotato science activities. Sandra Dennien indexing viruses (top left), Rachael Langenbaker developing aeroponics (top right), Jady Li investigating nematode management (bottom left) and Tham Dong examining sweetpotato storage root initiation (bottom right).

Prof. Villordon also visited the field experiment where Mr Henderson and Ms Langenbaker were evaluating the impact of irrigation strategy on productivity and breakdown of *Bellevue* plant beds (Appendix 5). He provided further discussion around his ideas about what drove the *Bellevue* cultivar to initiate sprouts, and his experiences with *Bellevue* breakdown.

At lunchtime, Prof. Villordon gave a more detailed scientific form of his industry presentation to the participating VG13004 project team, as well as additional scientists from CQU, DAF researchers and two crop consultants.

While in North Queensland, Prof. Villordon visited the Mareeba laboratories and discussed sweetpotato aeroponics with Mr Hughes.

Plate 123. Rachael Langenbaker explains the VG13004 experiment investigating impacts of excessive irrigation on *Bellevue* plant beds.

Other activities.

In the last two days of his visit, Prof. Villordon accompanied Mr Coleman to Sydney, where he visited the wholesale markets and met with several large produce merchants. He also attended a meeting at Hort Innovation, and met with the Marketing, Industry Development and Research Management Teams. He gave his presentation to that audience as well.

Acknowledgements

The VG13004 project team acknowledges the generous investment of time for Prof. Villordon to come to Australia and participate in his visit. They also wish to thank all the growers, collaborators, industry staff, and institutional people who were involved in either organising, or hosting various aspects of the tour. A particular thanks to Mr Coleman for accompanying Prof. Villordon throughout his visit, and to grower Matthew Prichard for also being heavily involved in both organisation and hosting.

Appendix 15

VG13004 project mid-term review

Methodology

As approved by Hort Innovation in October 2015, ASPG contracted Professor Calum Wilson from University of Tasmania as the independent professional for the formal mid-term review (MTR). ASPG and the research team negotiated with Prof. Wilson to coordinate a suitable itinerary. As a precursor to the review, the research team provided Prof. Wilson with the following documentation:

- Hort Innovation review guidelines, report structures, review terms of reference and VG13004 Project Contract.
- Planting bed management and virus management SWOT analyses developed during the Project stop-go milestone review in October 2014.
- Project work plans from September 2014 through March 2016
- ASPG Research Priorities 2011-2016 and Industry profile
- Project milestone reports MS102, MS103, MS104; Plant bed management and virus management literature reviews; Plant bed management and virus management grower guides; extension articles and 10 grower presentations.

Review schedule

On Monday 15/02/16, Mr Henderson and Ms Dennien picked up Prof. Wilson from Brisbane airport and drove to Cudgen NSW. The group visited growers Matthew Prichard, Henry Prichard, Doug Paddon and Jim Paddon, inspecting plant beds and commercial crops. Over 3 hours, the group discussed sweetpotato production issues, specific plant bed and virus management issues, project conduct, effectiveness and areas for change or improvement. They also visited experimental areas where plant beds and commercial crops were monitored for viruses and sprout performance, as well as planting-out sites from plant bed experiments. *This process can be taken as read for all other industry visits discussed in the context of this review.* The research team and Prof. Wilson then drove to Gatton Research Facility. Ms Dennien presented the GRF diagnostic facilities, including hothouses for maintaining sample, control and indicator plants, grafting areas, indexing areas, and the sweetpotato diagnostic laboratory. Mr Henderson showed Prof. Wilson the current plant bed management experiment, investigating nutritional requirements of differing cultivars. The group had specific discussions about methodologies, and potential improvements. The group overnighted in Gatton.

On Tuesday 16/02/15, Prof. Wilson, Mr Henderson and Ms Dennien visited a large sweetpotato farm at Esk, holding discussions with growers Michael, Aaron and Nick Jess. After approximately one hour, the review team drove through to Bundaberg, discussing sweetpotato and project issues on the 4 hour journey. In the afternoon, the group was joined by sweetpotato planting material supplier and ASPG Project Account Manager Eric Coleman, project team member Rachael Langenbaker, as well as large grower 'Crow' Prichard. This expanded group visited growers Darren and Linda Zunker, who have been extensively involved in on-farm sweetpotato research, and are collaborating in plant bed monitoring, as well as a companion study to the GRF nutrition investigation. The final grower visit for the day was to Russell Mortimer, again discussing sweetpotato and project issues. That night in Bundaberg, the people mentioned above attended an informal dinner, which was expanded to include several other sweetpotato growers and their families.

On Wednesday morning 17/02/16, Prof. Wilson, and the scientific team reviewed the Bundaberg Research Facility hothouses and laboratories, as well as Ms Langenbaker's preliminary work on using aeroponics to assess sprout potential. The formal review meeting took place for 3 hours in the BRF meeting room, attended by Prof. Wilson, Mr Henderson, Ms Dennien, Ms Langenbaker, Mr Coleman, as well as ASPG Secretary John Maltby, and grower Dean Akers. The group went through the project terms of reference and discussed project methodology, strengths and weaknesses, industry engagement, and potential future improvements. As a final activity, the group visited grower Troy Prichard, reviewing his large-scale plant beds and commercial practices. Prof. Wilson departed by air at around 2 pm.

The project team and participating growers were positive about the review, both from a participation and project improvement perspective. The research scientists appreciated Prof. Wilson's commentary and innovative ideas. The project team received Prof. Wilson's report in early April 2016, and circulated it to ASPG Executive, as well as project team members for their consideration. The MTR document is available on request to Hort Innovation, or Mr Henderson.

Outcome

The project team discussed the MTR recommendations in October 2016 and submitted a response with proposed actions and budgets to Hort Innovation in November 2016. Communications continued, until a final Project Variation Request, with upgraded Project Schedule and Project Budget was completed in early March 2017. The total budget increase was $88,960, around 9% of the overall Hort Innovation contribution to VG13004.

The following extra project activities were proposed and are described in the future tense, as in the original project variation request:

Additional project outputs include new qPCR primers and NextGen sequences for sweetpotato viruses, a literature review of sweetpotato shoot productivity research, and a report on R&D collaboration opportunities between Australian and USA sweetpotato physiology researchers and the Australian sweetpotato industry.

All project changes are intended to place in the final 12 months of the project. As recommended in the MTR, planned sampling for viruses to be limited to three farms in each growing region, to enable increased focus on development of diagnostic protocols.

The project team will attempt to develop methods of undertaking qPCR on sweetpotatoes, rather than requiring grafting onto *I. setosa* where possible.

Specific diagnostic developments to be undertaken are:

qPCR detection of SPLCV

New one step protocol (CIP) for RT-qPCR (RNA viruses) under assessment for –

- SPFMV (O, C, RC and EA strains);
- SPV2 (IVMV);
- SPCSV (EA and WA strains)
- SPVG

As recommended by the MTR, the project will also investigate the development of qPCR primers for sweetpotato phytoplasma and SPCV, increasing industry detection capacity, as well as team skills. They will also arrange for NextGen sequencing through a commercial laboratory of 4 current samples displaying unusual diagnostic symptoms.

The MTR recommended Sandra Dennien visit another virus diagnostic laboratory in Australia, to compare their diagnostic techniques with those undertaken in this current project. This visit will be undertaken and reported on by October 2017.

Craig Henderson will undertake an initial literature review of potential methodologies for efficiently indexing sweetpotato planting material productivity potential by October 2017. After this review, the project team (principally Rachael Langenbaker) will conduct an initial practical assessment of the most promising method identified in the review. This research will be reported by project completion.

As recommended by the MTR, the project team is mentoring two new University of Queensland students doing honours work in 2017. One student is studying alternative indexing plants to I. setosa. The other is investigating the impacts of nitrogen fertiliser type on plant bed performance and longevity. The project will support these studies through team engagement and instruction, and in return achieved enhanced research capacity.

To enhance development of the sweetpotato industry's international links, Mr Henderson will travel to the USA for 14 days in August/September 2017, to visit sweetpotato agronomists, and particularly Professor Arthur Villordon, a world renowned sweetpotato agronomist. Of interest are his methodologies for doing glasshouse work with early sweetpotato root development, which would be very relevant to indexing sweetpotato sprout/shoot potential. The outcomes of this visit will be reported to industry, as well as incorporated in the remaining RDE activities within this project, and for development of future work.

The project will also support Prof. Villordon to visit Australia in February/March 2018. The principle objectives are to review the agronomy aspects of the project, to enhance the quality of the final extension activities and project reporting. He will take part in the interstate tour of sweetpotato regions, to present his latest research findings, as well as participate in ongoing industry development and capacity for international collaboration.

Signing off the variation contract was finalised by Hort Innovation in mid-May 2017, leaving only 10 months for completion of all the additional activities.