# Australian experiments as part of PW18001 'Investigation of skin hardening and splitting disorders in sweetpotato November 2019 to November 2020'.

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To address the question posed by the Sweetpotato Industry, "Do Calcium and Boron influence splitting and skinning in sweetpotato?" we conducted two exploratory glasshouse experiments at the Bundaberg Research facility (BRF).

**Experiment location**
Experiments were established under controlled screen house conditions in 2019 and 2020 at the Bundaberg Research Facility in Queensland; Australia located 24°50'54''S, 152°24'14''E and 14 m above sea level.

**Experimental design**
Aligned with the Louisiana studies, these pot experiments were designed in collaboration with the PRG to investigate any individual effects of boron and calcium on splitting and skinning. Randomised complete block design experiments incorporated three nutrition treatments and four susceptible cultivars with six replicates of 1 plant/plot. The first experiment was planted on December 3, 2019 and the second experiment was established on February 18, 2020. Each experiment contained 144 plants. Two destructive sampling dates were determined at the initial PRG planning meeting; 50 days after planting (DAP), to gather baseline data, prior to the commencement of the differing nutrient treatments and at 140 DAP to replicate commercial harvest.

The cultivars selected for the experiments were Beauregard (known control), Bienville and Bellevue, (highly susceptible to splitting at harvest) and Murasaki (susceptible to skinning (Table 1). Two nodes of virus (pathogen) tested standardised vine tip cuttings (25 to 35cm long) were vertically planted into PVC pots containing washed sand. Pots were placed onto moveable mesh benches. Each bench was allocated 24 pots (one replicate) and benches were rotated weekly to ensure even lighting.

Figure 1: PVC pipes were filled with sand and planted with Pathogen tested planting material.

From day one to day 50 after planting (DAP), the time of the first harvest, all plants were provided with 450 ml of quarter strength of complete Hoagland's nutrient solution three times per week (Table 1). The primary interest of the first harvest and analysis was to obtain baseline data and understand cultivar effect. At 25 DAP the nutrient concentration was doubled to half-strength to prevent nutritional deficiencies, and when nutritional toxicities were observed, the concentration was again reduced to quarter-strength. From day 51 to day 140 (time of the final harvest) each plant was supplied with one of three designated nutrient solutions.

- Hoagland's nutrient solution (complete)
- Hoagland's nutrient solution minus Boron (-B)
- Hoagland's nutrient solution minus Calcium (-Ca)

At this time the amount of nutrient solution administered to each plant was increased to 600 ml, three times per week.

Table 1: Experimental design, cultivars and nutrient treatments.

| Cultivar | Reason for inclusion | Treatments *Day 1 to day 50* | Sampling time | Treatments *Day 51 to day 140* | Sampling time |
|---|---|---|---|---|---|
| Beauregard | Control | Complete | Day 50 | Complete minus Calcium minus Boron | Day 140 |
| Bellevue | Prone to Splitting | Complete | Day 50 | Complete minus Calcium minus Boron | Day 140 |
| Bienville | Prone to Splitting | Complete | Day 50 | Complete minus Calcium minus Boron | Day 140 |
| Murasaki | Prone to Skinning | Complete | Day 50 | Complete minus Calcium minus Boron | Day 140 |

Reverse Osmosis (RO) water was used in all nutrient solutions to eliminate effects of any volatile chemicals such as chlorine in the local water supply. When required the RO water was also used for direct watering of plants. The sand and nutrient solutions were sent for nutrient analysis. The temperature and relative humidity in the screen house were monitored with a Lascar USB Relative Humidity and Temperature Data Logger. Standard practices were followed to control pests and diseases, with a scheduled preventative insecticide spray program and yellow sticky traps used to attract flying insects especially, whiteflies and aphids.

Figure 2: Experiment 1 prior to harvest.

**Harvest and assessment**

At 50 and 140 DAP, the plants were harvested to determine canopy weights and root morphology traits. The vines were cut at soil level and weighed to determine the plant canopy weight. The root system of each plant was washed to remove sand and left to air-dry to ease the root assessment process. Storage roots were assessed for splitting, and the whole root system including stem photographed and weighed. Plants were visually assessed, and data captured included individual root weight, root length, root diameter, fibrous root weight per plant, total number of roots per plant and number of roots per node. Two average-size storage roots from each replicate were randomly selected for skin adhesiveness or time to peeling assessment. All storage roots were later sent to the sweetpotato research team at the Gatton Research Facility for periderm thickness assessment and image analysis to quantify skinning.

***Data analysis***

Due to the balanced nature of the trial design Data were analysed using analysis of variance (ANOVA) in Genstat 19th edition. Treatment means and differences between cultivars were deemed significant at the 0.05 level. Pairwise comparisons were performed using the 95% least significant difference (LSD) on significant effects. Means with a letter in common are not significantly different.

The variables analysed are; total number of roots per plant, total number of nodes per plant, mean root weight, mean root length and mean root diameter, time to peeling and periderm thickness. Any nodes, which produce no roots, are considered missing values for root weight, length and diameter.

Figure 8: Beauregard, Murasaki, Bellevue and Bienville roots after water pressure testing at 50 DAP.

**Experiment 1**

*Root morphological attributes*

There were no significant differences between nutrient treatments for root weight, root length, root diameter and roots per plant across all four varieties (Table 2). The minus boron treatment had the highest root weight and root length compared with the minus calcium and control treatments for Beauregard and Bienville. Whilst the minus calcium treatment had the largest root diameter for Beauregard, the minus boron treatment had the largest root diameter for Bienville and Murasaki. The control treatment had the highest number of roots per plant for Beauregard, Bellevue and Murasaki compared with the minus boron and calcium treatments. Individual variety analysis showed significant differences in nutrient treatments for Beauregard's root length ($P < 0.047$) and Bienville's root weight ($P < 0.004$). The minus boron treatment had significantly higher root length and root weight for both varieties, respectively.

*Time to skinning/peeling*

At 50 DAP: All plants received the same nutritional treatment and there were no significant differences between cultivars. At 140 DAP, there were significant variations in nutrient treatments for Beauregard, Bellevue and Bienville for time to skinning (Table 2, Figure 1). The Beauregard and Bienville control treatment took a significantly longer time to peel compared with the minus calcium and minus boron treatments. In Bellevue and Murasaki, the minus boron treatment took longer time to peel compared with the control and minus calcium treatments, though this was not significant. Individual variety analysis showed no significant differences in nutrient treatments for Bienville ($P > 0.05$).

*Periderm thickness*

At 50 DAP: All plants received the same nutritional treatment and there were no significant differences between cultivars. At 140 DAP, significant variations between nutrient treatments were noted for periderm thickness in Beauregard and Bellevue ($P < 0.05$) (Table 2, Figure 2). The Beauregard minus calcium treatment had significantly thicker periderms compared with the minus boron treatments, though neither were significantly different to the control. Bellevue roots were significantly thicker in the minus calcium and minus boron treatments than the control. Murasaki mean periderm thickness was significantly higher in roots receiving the control treatment. No significant differences between treatments were observed for Bienville ($P > 0.05$)

Table 2: Summary of nutrient treatment effects on various sweetpotato cultivars root morphological attributes in experiment 1 at 140 DAP. Treatments followed by the same letter(s) are not significantly different.

| **Experiment 1** | | **Root morphological attributes** | | | | | |
|---|---|---|---|---|---|---|---|
| Cultivar | Treatment | Root weight (g) | Root length (cm) | Root diameter (mm) | No of Roots per plant | Time to peeling (Sec) | Periderm width (µm) |
| Beauregard | Complete | 41 | 7 | 30 | 5 | 3c | 345bc |
| | -Ca | 51 | 7 | 34 | 5 | 2a | 385c |
| | -B | 55 | 8 | 32 | 5 | 2ab | 328b |
| Bellevue | Complete | 40 | 8 | 29 | 8 | 2ab | 234a |
| | -Ca | 43 | 7 | 28 | 7 | 2a | 316b |
| | -B | 41 | 7 | 28 | 7 | 2ab | 234a |
| Bienville | Complete | 28 | 4 | 21 | 3 | 5d | 246a |
| | -Ca | 31 | 5 | 21 | 3 | 2abc | 236a |
| | -B | 56 | 5 | 28 | 2 | 3bc | 258a |
| Murasaki | Complete | 38 | 6 | 21 | 5 | 2abc | 327a |
| | -Ca | 31 | 5 | 22 | 3 | 2abc | 251a |
| | -B | 31 | 6 | 24 | 4 | 3abc | 217a |
| *P*-value | | 0.129 (ns) | 0.447 (ns) | 0.582 (ns) | 0.156 (ns) | 0.007 | 0.008 |
| *Interaction* | | 0.145 (ns) | 0.895 (ns) | 0.924 (ns) | 0.590 (ns) | 0.027 | <0.001 |

ns = not significant ($P > 0.05$); complete = complete Hoagland solution; -Ca = minus calcium; -B = minus boron

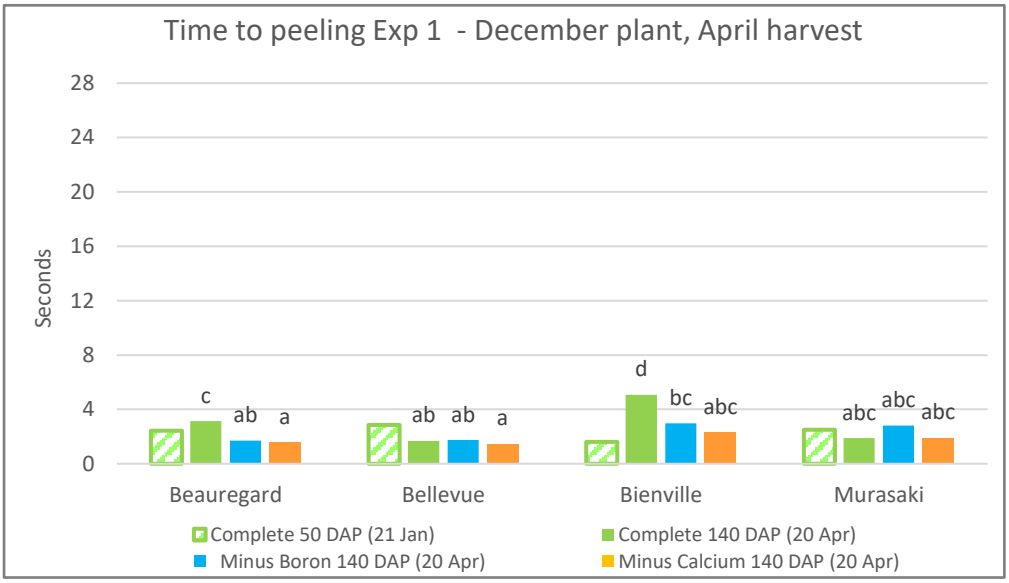


Figure 3: Experiment 1 mean time to peeling for various cultivars under different nutrient treatments ($P = 0.027$). Treatments followed by the same letter(s) are not significantly different.

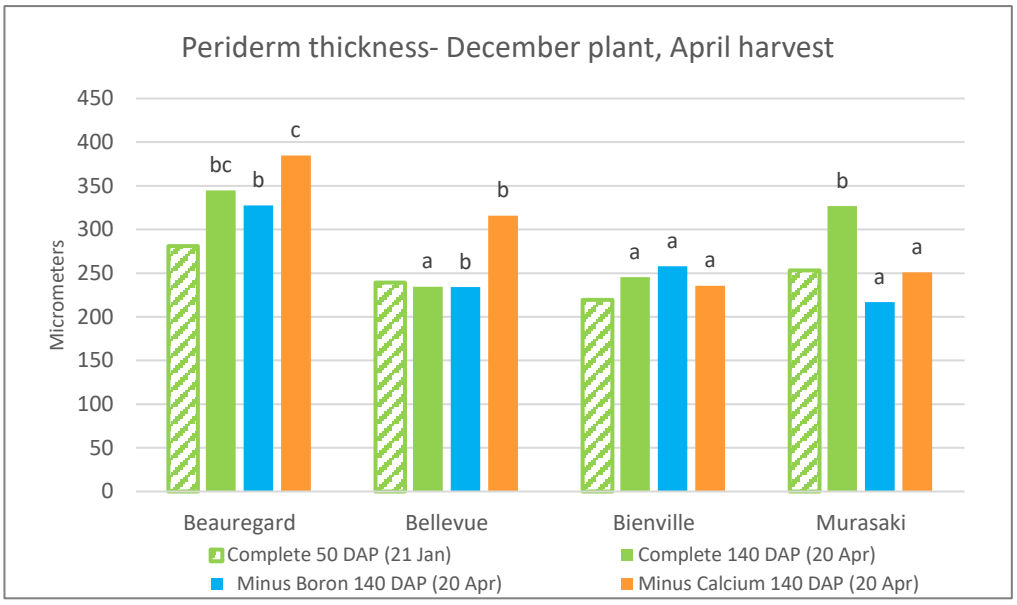


Figure 4: Experiment 1 mean periderm width for different cultivars under different nutrient treatments at 140 DAP ($P < 0.001$). Treatments followed by the same letter(s) are not significantly different.

**Experiment 2 at 140 DAP**

*Root morphological attributes*

Nutrient treatments were not significantly different across all varieties for root length, root diameter and roots per plant (Table 2). Significant differences in nutrient treatments across all varieties occurred in root weight ($P < 0.05$). In Beauregard, the minus calcium and control treatments had bigger roots than the minus boron treatment. In Bellevue, the minus boron and control treatments had higher root weight than the minus calcium treatment. For Bienville, the minus boron and minus calcium treatments had significantly higher root weights (52g each) than the control (16g), with a three-fold increase. Whilst in Murasaki, the minus boron treatment had significantly higher root weight than the minus calcium and control treatments.

*Time to skinning/peeling*

At 50 DAP, All plants received the same nutritional treatment, so the only differences are related to cultivars. At 140 DAP, significant variations in treatments for time to peeling/skinning were noted in Bellevue and Bienville where the minus boron and minus calcium treatments took significantly longer time to peel compared with their respective nutrient treatments (Table 2). In Beauregard and Murasaki, there were no significant statistical differences between nutrient treatments, however, the minus calcium treatment took longer time to peel than the minus boron and control treatments. Individual variety analysis showed significant differences in nutrient treatments for Bellevue ($P = 0.009$), with the minus boron treatment taking 13 seconds to peel compared with the 6 seconds for the minus calcium and control treatments.

*Periderm thickness*

At 50 DAP, All plants received the same nutritional treatment so the only differences are related to cultivars. Bellevue roots measured significantly thicker periderms than Murasaki roots which were also significantly thicker than both Beauregard and Bienville roots. At 140 DAP, nutrient treatments were not significantly different across all varieties for periderm thickness (Table 2). The control had thicker periderms in Beauregard and Murasaki whilst the minus boron and minus calcium treatments had thicker periderms in Bellevue and Bienville, respectively. Individual variety analysis showed significant differences in nutrient treatments for Bienville ($P < 0.004$) with the minus boron and minus calcium treatments having significantly thicker periderms than the control.

Table 3: Summary of nutrient treatment effects on various sweetpotato cultivars root morphological attributes in experiment 2 at 140 DAP. Treatments followed by the same letter(s) are not significantly different.

| **Experiment 2** | | **Root morphological attributes** | | | | | |
|---|---|---|---|---|---|---|---|
| Cultivar | Treatment | Root weight (g) | Root length (cm) | Root diameter (mm) | No of Roots per plant | Time to skinning (Sec) | Periderm width (µm) |
| Beauregard | Complete | 36bc | 9 | 30 | 8 | 8ab | 301 |
| | -Ca | 36bc | 8 | 28 | 9 | 9ab | 335 |
| | -B | 30ab | 8 | 24 | 6 | 7ab | 304 |
| Bellevue | Complete | 46bc | 8 | 30 | 7 | 5ab | 353 |
| | -Ca | 33ab | 8 | 25 | 9 | 6ab | 284 |
| | -B | 43bc | 9 | 29 | 7 | 13b | 318 |
| Bienville | Complete | 16a | 6 | 21 | 4 | 5a | 273 |
| | -Ca | 52c | 8 | 29 | 4 | 25c | 329 |
| | -B | 52c | 7 | 31 | 5 | 7ab | 349 |
| Murasaki | Complete | 33ab | 4 | 28 | 6 | 9ab | 332 |
| | -Ca | 29ab | 5 | 29 | 6 | 11ab | 340 |
| | -B | 45bc | 5 | 31 | 4 | 6ab | 292 |
| *P*-value | | 0.096 (ns) | 0.808 (ns) | 0.628 (ns) | 0.162 (ns) | 0.019 | 0.914 (ns) |
| *Interaction* | | 0.002 | 0.773 (ns) | 0.06 (ns) | 0.381 (ns) | 0.002 | 0.1 (ns) |

ns = not significant ($P > 0.05$); complete = complete Hoagland solution; -Ca = minus calcium; -B = minus boron

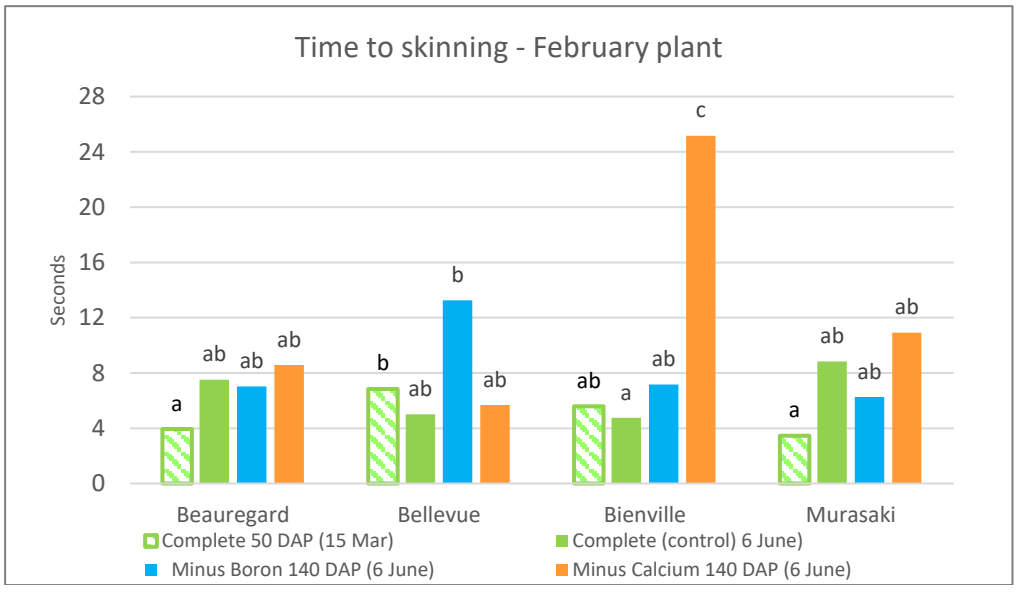


Figure 5: Experiment 2 mean time to peeling for different cultivars under different nutrient treatments at 140 DAP ($P = 0.002$). Treatments followed by the same letter(s) are not significantly different.

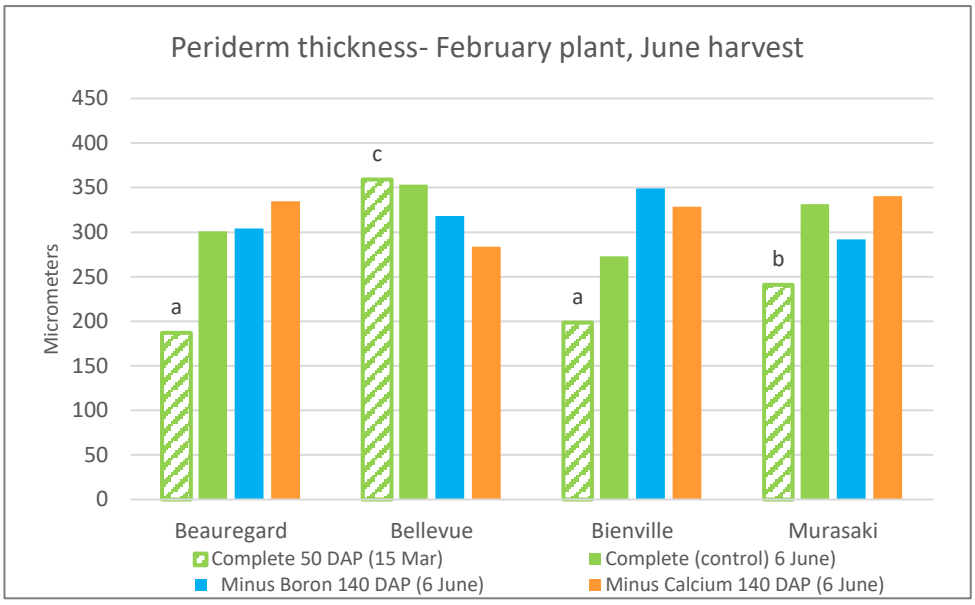


Figure 6: Experiment 2 mean periderm width for different cultivars under different nutrient treatments at 140 DAP ($P < 0.001$). Treatments followed by the same letter(s) are not significantly different.

**Gene-environment interaction (GxE)**

Planting dates for trials 1 and 2 had no significant effect on the nutrient treatments performance ($P > 0.05$). However, a significant interaction between planting date and cultivar was noted for root weight ($P = 0.044$), root length ($P = 0.004$), root diameter ($P = 0.01$), total nodes per plant ($P = 0.004$) and periderm width ($P < 0.001$). Beauregard had a significantly higher average root weight in trial 1 (49 g) than trial 2 (31 g). No significant differences in root weight were observed for Bellevue, Bienville and Murasaki. A similar trend was observed for root diameter. Bienville had significantly longer roots in trial 2 than trial 1 with an average of 7 cm and 5 cm, respectively. The average periderm thickness was larger in trial 2 than trial 1 for all nutrient treatments for Bienville and Murasaki. Bellevue had a larger periderm thickness in trial 2 than trial 1 for the complete and minus boron nutrient treatments whilst Beauregard had a larger periderm width for trial 1 than trial 2 for all nutrient treatments.

**Results summary**

No splitting was observed in either experiment. Preliminary findings indicate that there are significant cultivar differences with reference to root weight, root length, root diameter, total nodes and roots per plant, periderm thickness and time to peeling. Nutrient treatment application had a significant effect on time to peeling, with the minus calcium treatment having a longer time to peeling than the complete Hoagland and minus boron treatments. Interestingly, the minus calcium treatment also had the largest average periderm width, which is hypothesised to influence skin hardening. The lack of boron or calcium had no significant effect on root weight, root length, root diameter and total nodes and roots per plant. Periderm width values were generally higher in all cultivars in experiment two conducted during cooler weather. A gene-environmental analysis showed that trial planting date had no significant effect on nutrient treatments performance albeit cultivar response variations were evident for root morphological attributes assessed.

**Project team resources developed during the project:**

- Protocol for Hoagland's nutrient preparation for sweetpotatoes
- Standard safe preparation procedure for the Hoagland nutrient preparation
- Risk assessment for Hoagland nutrient preparation
- Protocol for removing roots from sand culture and capturing root development data
- Protocol for hydraulic periderm quantification exercise
- Standard safe operating procedure for hydraulic periderm quantification exercise
- Standard safe operating procedure for removing roots from sand culture and capturing root development data