FINAL REPORT 2015/004

Impact of stool architecture on ratooning ability

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ABSTRACT

Crop productivity is very much dependent on ratoon performance with at least 75% of the crop consisting of ratoons of varying age. There are efforts to improve crop management techniques to maintain ratoon yields, but there are significant gaps in our knowledge of traits that contribute to ratoonability and which could provide the basis for selection of improved varieties. The aim of this project was to investigate stool architecture traits that may contribute to ratoon performance in the context of a highly mechanised industry. Under the guidance of an industry consultative panel, we developed methods to compare genotypes, including measures of the number and position of sprouted buds in the stool. These methods were applied to analysis of pot-grown plants (with optimisation of pot size), commercial field samples, a range of *Saccharum* progenitor species, and a field trial of 32 genotypes encompassing a 50-year timeline of released varieties. The results showed significant genotype differences in all measured traits including bud position. Trait changes were observed in the 1st ratoon compared to the plant crop, but importantly, there was no indication that modern varieties are significantly different from older varieties. Industry yield data was tested for correlations with the trait measurements. Continuation of the field trial will provide valuable insights into changes in stool structure that can be used to understand ratoon performance.

EXECUTIVE SUMMARY

Crop productivity is very much dependent on ratoon performance with at least 75% of the crop consisting of ratoons of varying age. After harvest, the stool remains in the ground and new buds germinate on the remaining underground nodes to produce the stalks for the subsequent year's crop. There is evidence that ratoon yields are declining, so that growers incur the substantial costs of replanting more frequently. The causes of yield decline are complex. There is evidence for genetic variation for ratoonability, and the declining productivity is also influenced by environmental factors, such as rainfall or diseases, and by management practices including fallow crops and harvester operation. The genetic potential of varieties for ratooning cannot be achieved unless management practices are addressed and this is a focus for ongoing research. There are also significant gaps in our knowledge of traits that contribute to ratoonability and which could provide the basis for selection of improved varieties. The aim of this project was to investigate stool architecture traits that may contribute to ratoon performance in the context of a highly mechanised industry.

We developed methods to analyse stool architecture traits using pot and field-grown plants. The new methods include a formula to express the number and position of sprouted buds in the stool. We also measured morphological traits that contribute to yield, including rate of stalk emergence, stalk flexibility, stalk diameter, stalk height, stalk weight, number of millable stalks, stool area, and number of suckers. We used these methods to compare the structures of the progenitors of modern sugarcane varieties (including *Saccharum spontaneum* and *S. officinarum* accessions) and to characterise a purpose-designed field trial of 32 genotypes spanning 50 years of variety release.

Significant genotype variation was found in all measured traits, including bud position and number in the stool, and branching complexity of the stool structure. Although large initial stalk populations did not translate to large millable stalk numbers, they contributed to more complex underground stool structures containing live buds. There was some evidence that a larger stool structure contributed to better ratoon yields. Stalk strength was only partially explained by stalk diameter, suggesting that biochemical or anatomical differences may play a role.

Comparisons across genotypes showed no indication of a systematic change to stool structure in modern varieties compared to older varieties. The stools of the *Saccharum* progenitor species were similar to those of the hybrid cultivars, suggesting that the improvements to ratoonability brought by *S. spontaneum* introgression were not due to radical changes in stool structure.

When the features of the 1st ratoon crop were compared to the plant crop, a number of changes in stool structure were observed. In general, the area of the stools and the number of millable stalks increased, but the stalk diameters decreased. The complexity of the stool structures increased, with additional hierarchies of branching and an increase in the ratios of sprouted buds to millable stalks. While most varieties showed similar trends, we identified some varieties that behave differently in the transition from plant to 1st ratoon and these varieties may be important to focus on in the future. Although we analysed correlations between industry yield data and the trait information, the approach was limited by the lack of genotype diversity in the yield data sets; this highlights the value of the project's field trial which can provide valuable data on a wider set of genotypes.

The research was guided by an industry consultative panel including members of the SRA breeding team, and has been communicated at ASSCT conferences and through discussions with individual researchers. In the short term, the goal is to refine the methods for trait measurement and to continue monitoring of the trial into subsequent ratoon cycles, as this is typically where ratoon yield declines become apparent. In the longer term, biochemical or genetic markers could be a cost-

effective high-throughput method but this would require further trials of heritability and genetic correlations across populations. Ultimately, the results may contribute to a change in the way that plant breeders select for ratoon yields, resulting in more productive varieties, with increased ratoon yields and less frequent plough-out and replant, resulting in net profit gains.

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1.1. Stalk architecture and bud outgrowth in grasses

A characteristic feature of stalk anatomy in the grasses (family Poaceae) is the repeating phytomers consisting of nodes with associated leaves separated by internodes. Axillary meristems, or buds, located in the axils of the leaves, have the potential to give rise to new shoot branches or tillers. The growth of axillary buds is under genetic and hormonal control and may also be influenced by environmental factors such as light intensity (McSteen 2009). The control of axillary bud outgrowth results in the different growth habits found amongst the grasses. For example, in maize, a single stalk predominates and the buds are suppressed, while in wheat and rice, the buds produce multiple tillers (McSteen 2009). In some perennial species such as switchgrass and Bermuda grass, buds produced on underground nodes develop into underground shoots termed rhizomes. Rhizomes contain buds which can give rise to new tillers and since they grow horizontally, they contribute to the lateral spread of the plant. Rhizomes also have an important role in storage of nutrients, particularly in temperate grasses that die back over winter. Growth in the following season is dependent on the withdrawal and storage of water, carbohydrates and nitrogen into the rhizome (Wayman et al. 2013). Rhizome traits in grasses have also been shown to influence performance under drought stress (Zhou et al. 2014).

In sugarcane, the axillary buds are the basis of crop production. When stalks or billets are planted for establishment of sugarcane crops, axillary buds located at the nodes each give rise to a single stalk. The growth of the shoots is sustained initially by the sugars stored within the billet until the new root system is established and photosynthesis in the above-ground portion of the shoot commences (O'Neill et al. 2012). Secondary stalks (tillers) then arise from buds on the underground internodes of the primary stalk. These may give rise to third order tillers and so on, generating a complex branched structure which is termed a stool (Moore 1987), composed of underground internodes (rhizomes) and associated roots.

The stool remains in the ground after harvest. Stored resources in the stool permit the germination of new buds from the remaining underground nodes to produce the stalks for the subsequent ratoon crop (Moore 1987). Shoots emerge more quickly from ratoon crops than from plant crops (Keating et al. 1999). This is probably due to the existing root system and the presence of sub-surface buds. The point of development of shoots tends to move upwards in the stool as ratoon cycles proceed (Whiteing and Kingston 2008).

1.2. Diversity of stool structures in sugarcane

Although *Saccharum officinarum* is not strongly rhizomatous, *S. spontaneum* produces rhizomes prolifically, leading to its application in the past as an introduced species for stabilising river banks (Bonnett et al. 2008). Studies in other grasses such as sorghum have shown that the genetic regions associated with rhizome production are also associated with ratooning ability (Jang et al. 2006). It is likely that the ratooning ability of modern hybrid sugarcane cultivars is derived from the introduction of *S. spontaneum* genes related to rhizome production and tillering (Matsuoka and Garcia 2011). Introgression of a wider range of *S. spontaneum* genotypes has been proposed as a solution for improving ratooning ability as well as introducing other valuable traits such as disease resistance.

Our knowledge of the below-ground characteristics of sugarcane has been hampered by the size of the plant and the difficulty of removing the soil. As a consequence, stool architecture has only been examined in a limited number of sugarcane varieties. However, variation between genotypes in growth habit has been described. For example, there is variation in the number of stalks arising from a stool and also in the angle of tiller emergence, resulting in stalks that may be erect or recumbent to various degrees (Moore 1987). It is known that many morphological traits in sugarcane are highly heritable, although they are quantitative traits, likely to be controlled by many genes (Aitken et al. 2008).

In many cases, knowledge from other grass crops can provide the basis for investigating key aspects of sugarcane biology, for example, in root development or control of flowering. The insights that can be gained from other crops about ratooning ability are limited, since sugarcane has until recently been the only large-scale perennial grass crop. As interest in biofuel crops has grown (see http://www.newenergyfarms.com/About.php for example), other perennial grasses such as switchgrass and *Miscanthus* have become the focus of study.

1.3. Economic importance of ratoon yields

Crop productivity is very much dependent on ratoon performance with at least 75% of the crop consisting of ratoons of varying age. The yield (TCH) of a ratoon crop is typically lower than that of a plant crop and continues to decline in subsequent ratoons. A grower must balance the declining value of ratoon crops against the costs of replanting, which are substantial (Schroeder et al. 2009a).

In the Australian industry, three ratoon cycles following the plant crop have typically been achieved before the old stools are ploughed out and a new crop is replanted (Schroeder et al. 2009b), however there is evidence from mill data that the number of profitable ratoons has fallen from three to two in some regions.

The causes of yield decline are complex. In addition to the inherent genetic potential of a variety for generating new tillers, the declining productivity across subsequent ratoon crops is also influenced by environmental factors, such as rainfall or diseases, and by management practices including fallow crops and harvester operation. Historically, 10 or more productive ratoon cycles could be achieved in a sugarcane crop but this was only viable when stalks were harvested by hand. In regions where hand-harvesting is being replaced by mechanical harvesting, a decline in the number of ratoon cycles has occurred (Ramburan et al. 2013). The decline has been attributed to physical damage to the stool and soil compaction by the heavy machinery (Garside and Bell 2006; Ramburan et al. 2013). A study of harvester efficiency showed damage to the stool which can allow pathogen entry (Whiteing and Kingston 2008). Harvester damage can prevent bud germination and allow pathogen entry; increasingly likely in later ratoons as the position of bud recruitment from the stool tends to move closer to the soil surface (Garside and Bell 2006, Whiteing and Kingston 2008).

A study in South Africa assessed the relative effects of cultivar, environment and management on ratoon yields. Significant cultivar effects were found for cane yield (TCH), CCS and TSH. This is consistent with other evidence that there is substantial genetic variation for ratoonability (Piperidis 2017). However, the cultivar effects were frequently superseded by environmental and management effects. For example, environment (trial location) accounted for 83% of the variation in TCH, and irrigation, fertiliser and trashing all had significant effects (Ramburan et al. 2013). It is clear that the genetic potential of varieties for ratooning cannot be achieved unless management practices are addressed. There are also significant gaps in our knowledge of traits that contribute to ratoonability and which could provide the basis for selection of improved varieties.

1.4. Breeding and selection for ratooning ability

Since assessment of multiple ratoon cycles during breeding can considerably lengthen the time taken for selection and release of new varieties, researchers have attempted to identify key plant growth parameters that predict ratooning performance. Genetic correlations have suggested that sugar yield of the plant cane is an adequate predictor of the performance of the ratoons, at least in the early stages of selection (Jackson 1992).

Particular morphological traits have been shown to be associated with ratoon performance. In a study of six older Australian varieties, the authors noted that tiller angle was an important factor in ratoon yields, since low angles lead to stool tipping and gaps in ratoons (Chapman et al. 1992a, b, c). In the breeding program, some of these traits are selected as part of the assessment of habit and general appearance. Seedlings showing an extreme tendency towards recumbent stalks would be eliminated from the program. A study of modern Indian varieties showed that early emergence and vigorous growth of tillers in ratoons was particularly advantageous (Gomathi et al. 2013). The position of emergence of new tillers was also important; sucker shoots recruited from deep in the stool played an important role in stool longevity, even though suckering is generally regarded as a detrimental trait (Whiteing and Kingston 2008). It has been suggested that older varieties had better ratooning ability compared to modern varieties. A systematic evaluation of stool architecture traits in current and past varieties is important to establish the extent of diversity and whether any changes have occurred over time.

Ratooning has previously been addressed as part of the SRA core breeding program, where assessment of ratoon yields is an important factor in variety selection. In order to broaden the genetic base of commercial varieties and bring in new traits, there has been a considerable investment in introgression of wild germplasm over the last 10 years. Novel *S. spontaneum* and *Erianthus arundinaceous* genotypes have been introduced, with good results especially in seedling families derived from *S. spontaneum* (Piperidis 2017).

Reliable methods and trait knowledge for characterizing ratooning ability are currently lacking. It is important that a repeatable methodology be established for analysing stool architecture so that it can be used to screen varieties in selection trials and to dissect the influences of genetic, environmental and management factors. This research will also provide the opportunity to describe the ideotype of a good ratooning variety in the context of current management practices (i.e. a highly mechanized industry).

2. PROJECT OBJECTIVES

The objectives defined in the project proposal were:

(i) Form an industry consultative panel to scope essential criteria for analysis, guide selection of varieties and review results

(ii) Develop a pot trial system that models ratoon regrowth of tillers to allow analysis of stool morphology traits

(iii) Establish methods and define parameters for measurement of key morphological traits. The traits that will be examined include (i) developmental traits (e.g. time of tiller emergence, rate of growth, persistence), (ii) morphological traits (e.g. number of buds, position in stool, angle of emergence), and (iii) biochemical traits (e.g. sucrose content of stool internodes).

(iv) Test the hypothesis that stool architecture has changed in modern varieties by characterising a range of commercial varieties released over a 50 year period.

(v) By consultation with the industry panel identify appropriate trials for sampling which have been rated for ratooning ability (stalk number, stalk weight, CCS). Collect field samples and analyse to test correlation between morphological traits or combinations of traits and ratooning capacity.

3. OUTPUTS, OUTCOMES AND IMPLICATIONS

3.1. Outputs

The major outputs delivered by the project are:

(i) Knowledge of stool architecture trait variation.

- New methods and key parameters for assessing sugarcane stool architecture traits in plants grown in controlled environments and in the field were developed
- Application of these methods demonstrated that there is genetic variation for stool architecture traits amongst current commercial lines

(ii) Identification of key morphological traits linked to ratooning performance.

- Analysis of stool architecture traits in modern varieties compared to older varieties showed that there has been no consistent change over a 50-year timeline of released varieties.
- Analysis of field trial data showed changes in stool structure with subsequent ratoon cycles and identified genetic variation within these trends.
- Industry ratoon yield datasets were examined but genetic variation was too low to enable effective correlations with stool architecture trait data.

The outputs are relevant to the breeders and the research community, as the improved knowledge and methodology can contribute to selection methods and assessment of program performance. The breeding program is actively testing examining new methods for selection of complex traits, including ratooning. The project results have been communicated to the breeding team through the consultative panel and other meetings.

Further development of two aspects of this work would increase its utility:

- The parameters that have been used to assess stool architecture to date are still labour intensive and require mature plants to be grown. Identification of biochemical markers that are linked to particular stool attributes would speed up selection, particularly if they could be applied to immature plants. In the longer term, genetic markers could be a cost-effective high-throughput method but this would require further trials of heritability and genetic correlations across populations.
- Only two seasons of field trial data have been collected to date (plant crop and 1st ratoon). Monitoring the changes that were identified through further ratoon cycles when ratoon yield decline typically becomes more pronounced (2nd and later ratoons) would strengthen these trends, particularly focussing on varieties that behave differently.

3.2. Outcomes and Implications

The immediate outcome of this project is that more rigorous methods for comparing between genotypes for stool architecture traits are now available for researchers to use. The longer term outcome for this research area is a change in the way that plant breeders select for ratoon yields, resulting in more productive varieties. The ultimate beneficiaries of better selection methods are the growers and millers, with increased ratoon yields and less frequent plough-out and replant, resulting in net profit gains.

To achieve this, the project outputs will be used by plant breeders to develop indirect methods of selection for ratoon yield. Currently, yields in plant crop and early ratoons are assumed to be adequate predictors of continuing ratoon yields. This avoids the time and cost of directly assessing ratoon yields by growing variety selection trials for additional cycles. The methodology for testing stool architecture traits developed in this project and correlations between particular traits and productivity can be used to screen varieties in selection trials. For a new indirect method, new morphological or biochemical markers linked to ratoon performance would be tested for heritability and for the feasibility of using these markers in the selection index. In the short term the selection criteria could be applied to late-stage selection in the FAT trials. In the longer term, with further testing of genetic correlations across populations, the new morphological or biochemical markers could become part of a genomic selection system. The outcome would be a step-change in the efficiency of the breeding program.

4. INDUSTRY COMMUNICATION AND ENGAGEMENT

4.1. Industry engagement during course of project

(i) An ASSCT paper was published and presented by Dr Donna Glassop at the 2017 conference:

Glassop D, Pollock D, Perroux JM and Rae AL. (2017) Variation in stool architecture and bud sprouting; morphological traits that may contribute to ratooning. Proceedings of the Australian Society of Sugar Cane Technologists 39.

(ii) A poster was presented at the 2017 ASSCT conference:

Glassop D, Perroux, J and Rae A. (2017) Sugars for ratoon growth: sugars within the underground stool structure to germinate ratoon growth. Proceedings of the Australian Society of Sugar Cane Technologists 39.

(iii) Consultative panel meetings

The role of the consultative panel was to review progress of the project, guide future directions and assist with pathways for adoption of new practices and information. Feedback from the panel was incorporated into the project plans. In order to capture a broad range of views and expertise for the project, we sought involvement from researchers, breeders, grower group representatives, and the SRA RFU program manager. The members of the panel were:

- Dr George Piperidis – SRA Sugarcane Breeder based in Mackay
- Dr Roy Parfitt – SRA Sugarcane Breeder based in Bundaberg
- Dr Graham Bonnett – Research Director, Integrated Agricultural Systems, CSIRO Agriculture
- Mr Peter Rose – Sugarcane grower at Harwood NSW and also responsible for Harvest Best Practice trials

- Dr Harjeet Khanna – Program Manager, SRA Research Funding Unit
- Project Team – Dr Donna Glassop, Mr Jai Perroux and Dr Anne Rae

Panel meetings were held annually at the CSIRO laboratory in St Lucia with videoconferencing available for panel members outside Brisbane. Meetings were held on Friday 16 October 2015, Thursday 4 August 2016 and Wednesday 26 July 2017.

(iv) SRA Ratooning workshop

The project data on methods for analysis and initial comparisons from the first year's experiments were communicated as a slide presentation to the participants at the SRA Ratooning Workshop on 3 August 2016 and the team participated in the round table discussions following the presentations.

(v) Meetings with other industry personnel

Sunshine Sugar: planting methods were examined and project goals discussed during a field trip hosted by Malcolm Warren at Condong Mill, Sunshine Sugar, NSW in August 2015.

MSF Sugar: Stools for destructive harvesting, from three varieties and across different crop cycles (2R – 4R), were supplied by MSF Sugar (Maryborough) in 2016. Mr Barry Callow and Mr Tony Coutts-Smith were able to assist with identifying field grown samples relevant to the project goals. These samples were used to assess bud viability (via germination or staining), classification of stalks and available sugars within the below ground internodes.

Wet Tropics Sugar Industry Partnership, Herbert River Canegrowers: experiments conducted by Don Pollock on the germination of below ground buds in three varieties were reanalysed and presented in the ASSCT paper. Results from Don's research supported findings from the project.

(vi) Meetings with researchers:

Dr Barry Croft (SRA Pathologist) was consulted about potential disease effects on ratooning and the best approach for screening to rule out confounding effects. Several meetings were organised with Dr Jo Stringer (SRA Biometrician) to discuss industry ratoon performance data sets and best approaches for testing correlations with project data. Stalk strength results were discussed with Dr Geoff Kent (QUT). Dr Frikkie Botha (SRA Executive Manager, Technology) was consulted to discuss the concept of bud dormancy, bud sprouting and resources available to support bud sprouting.

4.2. Industry communication messages

Key communication points are as follows:

- We have developed new methods for assessing and comparing the stool architecture features of field grown plants, including plants from commercial fields and a field trials of 32 genotypes that span 50 years of variety release
- The features assessed were rate and number of stalk emergence in the plant crop, stalk flexibility, stalk diameter, stalk height, stalk weight, number of millable stalks, brix, stool area, number of suckers and stalk positional classification in the stool.
- There was significant variation in all of these features, however there were no significant trends towards particular features in our varieties over time.
- Between plant and 1st ratoon crop, there were significant changes in stool structure and some varieties performed differently.
- *Spontaneum* genotypes and backcrosses did not have fundamentally different stool structures, except that underground parts of the stalks were longer.

5. METHODOLOGY

5.1. Selection of varieties

One of the major objectives of the project was to characterise a range of commercial varieties released over a 50 year period to test the hypothesis that stool architecture has changed in modern varieties. Varieties selected for experiments were chosen after assessing data from SRA variety notes, and advice from consultative panel members and Dr Felicity Atkin at SRA. The list of varieties is shown in Table 1. Treated single eye setts were supplied as planting material by SRA Meringa. Setts were planted into trays containing soil/sand mix and germinated in the Controlled Environment Facility (CSIRO, St Lucia) with conditions set to 12 h light, 32 °C, 60% humidity and 12 h dark, 24 °C, 75% humidity, and watered by hand daily. Single plants were transplanted to 500 mL pots prior to planting in the field trial at Gatton or use in the pot trials.

Table 1. Sugarcane varieties used in pot and field trials. * Information from QCANESelect^{TM}

	Variety	Year first planted	Parents*
1	Co290		
2	Comus		*S.officinarum* (Noble cane)
3	Empire		unknown
4	KQ228$^{(b}$	1998	QN80-3425 x CP74-2005
5	MQ239$^{(b}$	1993	Q96 x MQ77-340
6	NCo310		Co421 x Co312
7	Pindar	1937	
8	POJ2878		
9	Q77		
10	Q96	1959	Q63 x Q68
11	Q113		
12	Q117	1963	
13	Q124	1969	NCo310 x QN54-7096
14	Q138	1975	QN58-829 x QN66-2008
15	Q151	1981	Q96 x QC66-807
16	Q167$^{(b}$	1977	QN58-829 x QN66-2008
17	Q190$^{(b}$	1986	Q107 x H56-752
18	Q200$^{(b}$	1989	QN63-1700 x QN66-2008
19	Q208$^{(b}$	1987	Q135 x QN61-1232
20	Q231$^{(b}$	1995	QN85-1647 x QS80-7441
21	Q232$^{(b}$	1994	QN80-3425 x QS72-732
22	Q234$^{(b}$	1988	Q107 x QN66-2008
23	Q242$^{(b}$	1997	Q170 x Q150
24	Q249$^{(b}$	2002	QC83-625 x QC90-289
25	Q252$^{(b}$	2000	Q208$^{(b}$ x Q96
26	Q256$^{(b}$	2002	N21 x Q135
27	QB01-10005		Introgression, *S.spont*, F1, POJ2878 + Mandalay
28	QBYC05-20853		Introgression, Phil Jackson, BC1, *S.spont* cross in China
29	QC91-580		
30	QN04-121		
31	SRA1$^{(b}$	2005	QN86-2139 x QC90-289
32	SRA5$^{(b}$ - QN04-668		H72-8597 x QN89-109
Guard	Q240$^{(b}$		

5.2. Definitions and terms used.

Swollen or sprouted (sprouted including mature stalks) buds at nodes with emerged roots were considered to be sprouted and were measured. Classification of underground branching pattern was done in accordance with Barber (1919) and republished by Moore (1987). Classification is based on the primary stalk arising from the billet being classified as ‘a’, secondary stalks that branch from the ‘a’ stalk are classified as ‘b’ stalks and numbered 1 onwards from the lowest occurring stalk (i.e. b1 occurs lower on stalk ‘a’ than b2), stalks arising from the ‘b’ stalks are classified as ‘c’ stalks, etc.

Node position 1 is at the base of the stalk, unless stated otherwise, and was identified as the first ring of roots on the stalk.

5.3. Field trial at Gatton

As this project was designed to test a larger number of varieties, the use of a field trial at the CSIRO research station at Gatton, provided an environment similar to commercial fields. Varieties were randomly assigned to plots, with 3 replicate blocks for each variety.

Planting methods were examined during a field trip hosted by Malcolm Warren at Condong Mill, Sunshine Sugar, NSW. Because of the potential overlap created by billet planting, the stools produced are very complex and may be composed of tillers from multiple buds on adjacent billets. For the purposes of the project, planting of single-eye setts was used, as this facilitates the analysis of individual stools. To ensure good establishment, setts were pre-germinated, as described above, and then transplanted into the field site.

The Gatton field was laid out with 1.8 m row spacing and plants within rows were spaced at 50 cm. For each variety, three replicates each consisting of eight plants in a row were located randomly in the field plan. Each set of eight plants was surrounded by guard cane rows to ensure that the competition from neighbouring cane was uniform and that the varieties were exposed to the same inter-row root competition. The whole plot was surrounded by two rows of guard cane to limit edge effects from light and wind. The field was irrigated as required to ensure plants were not water stressed. Nitrophoska Special fertiliser was applied during field preparation for the plant crop and after harvesting at a rate of 300 kg ha^{-1} (N 36 %, P 15 %, K 42 %; Incitec Pivot Fertilizers, Victoria Australia). Measurements were taken on 8 stalks from each replicate (Table 2).

Table 2. Measurement taken at each harvest and details of measurements.

Measurement	2016	2017	Details of measurements
Rate of stalk emergence	✓	✓	The number of stalks on each plant was counted every 2 – 4 weeks until a maximum of 30 stalks were present. Plants were then assessed to note when tiller die back occurred.
Stalk diameter	✓	✓	Stalk diameter 1 m from the base of the plant.
Stalk height	✓	✓	Height from the base of the plant to the TVD.
Stalk weight	✓	✓	Combined weight of 8 stalks.
Brix	✓	✓	Juice was collected from 8 full length stalks and brix measured with a refractometer.

Stalk flexibility	✓		Relative stalk flexibility was measured in the internodes using an Imada Digital Force Gauge (details below).
Number of millable stalks	✓	✓	Total number of mature stalks per plant.
Area of stool	✓	✓	The area encompassed by each stool at ground level.
Number of suckers	✓	✓	Total number of suckers, number of mature suckers (~1 m) and immature suckers (~ 30 cm).
Ratoon stunting disease	✓		Xylem sap was collected from each replicate and analysed by SRA according to industry standards.
Stalk classification	✓	✓	One plant from each rep was removed to assess the stalk classification.

Stalk flexibility: Basal regions of stalk were cut and rested across the block of the apparatus so that the internode was centered under the pressure point (Fig. 1). In order to accommodate the central alignment of the internode to the point of force, the height from the base of the stalk to the point of force ranged from 180 to 510 mm. Increasing pressure was then applied to the stalk until either: (i) the stalk bent to the maximum distance of 10 cm allowed by the instrument design; (ii) the force reached the maximum instrument capacity of 1000 newtons without the stalk bending to the maximum degree; or (iii) the stalk broke, leaving the rind on the upper side still intact.

Figure 1. The Imada texture analysis meter and motorised tower.

5.4. Soil testing

Soil samples were collected from random areas of the field, in accordance with sampling procedures provided by the SRA Tully Soil Assay Laboratory, for testing by SRA following harvesting in 2016 and 2017. A soil corer was used to obtain 15 samples to a depth of 25 cm from in row and row edge. All in-row samples were pooled together and similarly with the row edge. A sample of 250 g from each pooled sample was sent to the Tully Soil Assay Laboratory along with an ice block to keep the samples cool. The samples were tested for Pachymetra and nematode (fungal and bacterial) pathogens. Results indicated that the Gatton field is within the Low Hazard range.

5.5. Stool assessment in pot-grown plants

Another major objective of the project was to develop a pot trial system that models ratoon regrowth of tillers. This allowed us to develop methods and define parameters for measurement of key morphological traits that may underpin ratoon performance. This experiment used a range of current and near-to-release varieties, selecting a broad range of phenotypes. Eight varieties were selected for the pot trials: Q234$^{\Phi}$, Q208$^{\Phi}$, Comus and Empire in the CEF (five replicates of each); and 96, Q138, Q113 and QBYC05-20853 in the glasshouse (six replicates of each). These varieties were selected for their varied architecture and ratoon rating (based on SRA Variety Guides). Searles Peat 80 Plus Premium Potting mix was used to fill 52 L pots to the ¾ mark, the plantlet node was placed on top of the soil and then approximately 10 cm of hydroponic beads layered around the plant. Temperature and humidity settings for the CEF were as indicated above. The glasshouse at Queensland Crop Development Facility – Redlands Research Facility maintained temperatures between 23 and 28 ˚C. Plants were watered several times a day to maintain a humid environment in the hydroponic beads.

After 110 days growth, the plant stools were harvested and underground buds assessed. The stool was removed from the pot and roots trimmed from the stalks to reveal the buds (Fig. 2). Data were recorded on the sprouted buds including number of sprouted buds, length of sprouted bud and relative position on the stalk.

Figure 2. Sugarcane stool of variety Q234$^{\Phi}$ grown in the pot trial, pre- and post-root removal.

5.6. Assessment of stools from commercial fields

5.6.1. Bud sprouting experiments

These experiments were conducted in the 1990's by Don Pollock and the statistical analysis and results were completed by Donna Glassop during the present project. Stools of mature cane were collected from the Burdekin SRA research station (Brandon) and surrounding farms. Varieties include Q124, Q117 and Q96 (three replicates of each). Each mature stalk was dissected into single eye setts, with approximately 25 mm of internode either side of the bud. The setts were planted in the James Cook University glasshouses, in moistened washed river sand covered with shade cloth. The glasshouses maintained temperature between 20 and 26 ^{o}C. The length of swollen and sprouted buds were measured 51, 47 and 42 days after set up for Q124, Q117 and Q96, respectively.

5.6.2. Classification of stalks, bud viability and bud sprouting

Access to commercial sugarcane farms in Maryborough was kindly granted by Maryborough Sugar Factory (MSF) Sugar Limited (Mitr Phol Sugar Corp Ltd) to supply stool/clump samples for analysis. Three varieties were collected: Q238$^{\Phi}$, Q208$^{\Phi}$ and Q242$^{\Phi}$, at various crop cycle ages, including plant crops (planted in 2015), 1R – 1st ratoon crops (planted in 2014), 2R – 2nd ratoon crops (planted in 2013), 3R – 3rd ratoon (planted in 2012) and 4R – 4th ratoon (planted in 2011). MSF Sugar Limited have adopted the SRA Farming system developed by industry researchers, including controlled traffic, green cane trash blanketing (GCTB), legume rotations and minimum tillage. Crops are fully irrigated. Stools were excavated within a week of harvesting.

Stools for bud sprouting experiments were carefully washed to remove soil and broken into individual stalks which were planted into large containers holding 30 L Searles Peat 80 Plus Premium Potting mix in the CEF. A soil moisture level of approximately 30% was maintained. The extent of sprouting was recorded after 30 days and un-sprouted buds assessed for damage and viability by staining in 1% tetrazolium solution overnight. Pink stained buds were scored as viable and buds without pink colour were scored as non-viable.

Stools for stalk classification were carefully washed and roots removed to reveal the stool architecture. Within most of the clumps assessed there were two to four billets contributing to the clump. Classification of ‘a’ stalks could be discerned from the attachment to the billet. Along with stalk classification the number of mature stalks harvested each year was noted when possible.

5.7. Appropriate pot size to achieve field equivalent sugarcane growth.

Plants grown in pots in the CEF and glasshouses for the stool assessment experiment had significantly fewer stalks than the equivalent plants grown in the field at Gatton. After discussions with the consultative panel and Dr Phillip Jackson (CSIRO) we determined that one of main reasons for this difference is the exposure to natural light. While field grown plants may be optimal, there are occasions where a pot is desirable (e.g. for a rhizomatous *S. spontaneum* or applying treatments to the root system). A second limitation on pot based experiments may be the size of the pot. To this end, an experiment was designed to test three different size pots; 35 L, 52 L and 70 L, all with 50 cm saucers (Table 3). Pots were filled with garden mix from Brooks Quarries (Gatton) and supplemented with Osmocote controlled-release all-purpose fertiliser (applied as per directions, Scotts Australia Pty Ltd, NSW). Varieties included in the experiment were Q234$^{\Phi}$, Q208$^{\Phi}$ and Empire.

Plants were germinated (16/3/16), transplanted into the large pots (12/5/16) and harvested (8/9/17; ~16 months growth). Plants were initially slow to grow and this was most likely due to being planted at the end of autumn with the onset of cold weather. Plants were watered to capacity once per day.

Table 3. Pot dimensions, volume and colour.

Diameter (cm)	Height (cm)	Volume (L)	Colour
30	50	35	White
30	100	70	White
50	38	52	Black

Pots were blocked and varieties blocked within the pot layout (Fig. 3). Steel trench mesh was positioned approximately 1 m above the top of the pots to hold stalks and prevent the pots from toppling over, especially the 1 m tall pots (Fig. 4). There was a space of approximately 2 m between groups of different pot sizes.

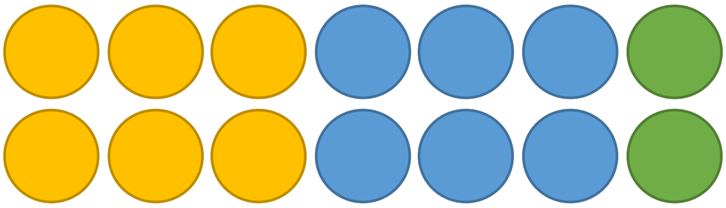

Figure 3. Layout of varieties within each pot size group. Q208$^{\Phi}$, yellow; Empire, blue; Q113, green.

Figure 4. Pots set up outdoors at Gatton showing the three sizes being tested: 70 L to the left, 35 L in the centre and 52 L to the right.

5.8. Differences in stool architecture amongst Saccharum species.

Following the preliminary pot experiments and discussion with the consultative panel, a pot experiment was established to compare the stool structure in progenitor species and wild *Saccharum* species with modern hybrids.

A range of genotypes were obtained from SRA Meringa (Table 4). The *S. spontaneum* lines were selected with advice from Dr Karen Aitken to ensure that a diverse range of origins and genetic backgrounds were included. Nodes were planted in trays in the controlled environment facility, sprouted plants were transplanted to 75 L pots located at Gatton in November 2016. Fertiliser and a wetting agent was applied as per the manufacturer's instructions (Osmocote All Purpose Fertiliser and Scotts Everydrop Premium Soil Wetter; Scotts Australia Pty Ltd, NSW). Plants were set up to grow in natural light and while they were also exposed to rainfall, the pots were irrigated once per day to ensure no drought stress occurred.

Table 4. List of genotypes in pot trial at Gatton for comparative analysis of stool structures.

Name	Genotype
Mandalay	*S. spontaneum*
Coimbatore	*S. spontaneum*
SES196	*S. spontaneum*
NG51-2	*S. spontaneum*
Okinawa	*S. spontaneum*
U469-4	*S. spontaneum*
Badilla	*S. officinarum*
NCo310	Hybrid
Q208⏀	Hybrid
Comus	*S. officinarum*
QB01-10005	*S. spontaneum* backcross
QBYC05-20853	*S. spontaneum* backcross

5.9. Correlations between stool architecture traits and industry ratoon yield data

One of the project objectives was to test for possible correlations between the plant traits measured and the ratoon yields observed in the field. The field trial at Gatton can provide information on yields in a single location and season. However to gain an overall view of ratoon performance across a broad set of locations and seasons, we accessed industry yield data for this analysis. The methodology and available data sets were discussed with Dr Jo Stringer at SRA to ensure consistent approaches.

Based on Jo's experience (project 2014/054), we used historical datasets from QCANESelect™ from selected mill regions. We defined the ratoon yield index as the rate of decrease in TCH from 1R to 3R crops. Since season-to-season variation in weather can greatly influence yields, we calculated the yield index for each year based on the 1R, 2R and 3R crops grown across the region within that year, ensuring that all crop classes had been exposed to the same growth conditions for that year. The following criteria were applied to selection of datasets:

- Data sets that contained yield for P to 3R - 5R crops were used to produce graphs and trend lines for yield. Various combinations of data were assessed including P – 3R, P – 5R, 1R – 3 R and 1R – 5R and the same values normalized to P or 1 R.
- Regions with different environments and good data were selected: South Johnstone, Tully, Proserpine and Plane Creek.

The approach used for analysis was as follows:

- Datasets selected according to criteria above.
- Derived slope of trend line (defined as ratoon yield index) for yields over 1R-3R for each variety and year. Statistical analysis showed that for each variety, there was no significant difference ($P<0.05$) between regions or years assessed.
- As there was no statistical difference, the slope of the trend line was averaged across all regions and years, excluding slopes with a positive value (as this is not the norm).

- The value for ratoon yield index for each variety was added to the trait data collected from the P crop in the field trial at Gatton and a correlation analysis was performed.

6. RESULTS AND DISCUSSION

6.1. Determination of measurement methods and key parameters for assessing stool architecture traits

In this section, the analysis of stool structure using pot-grown plants is discussed. These results have been published in Glassop et al. (2017).

(i) Positional hierarchy of stalks in the stool

In order to assess variation in bud sprouting, eight varieties were grown in pots in the CEF and glasshouse. As all plants were germinated from single eye setts there was only one ‘a’ stalk per pot regardless of genotype. However there were differences in the number of ‘b’ and ‘c’ stalks produced (Table 5). Empire and Q208⁽ᴾ⁾ produced the largest number of ‘b’ stalks while Q96 produced none except for one replicate which had a single (b) stalk. Only a small number of plants produced ‘c’ stalks, though these were not present in all replicates.

Table 5. Number of above-ground stalks per branch position classification for the eight sugarcane varieties grown in pots (mean, with ± SE in brackets where applicable). Tukey’s LSD (<0.05) was applied to the ‘b’ stalks. Values with the same letter are not significantly different from each other

Cultivar	‘a’ stalks	‘b’ stalks			‘c’ stalks
Comus	1	1.6	(0.24)	b	
Empire	1	3.0	(0.32)	c	
Q208⁽ᴾ⁾	1	3.8	(0.37)	c	1
Q234⁽ᴾ⁾	1	1.4	(0.24)	ab	
Q113	1	1.2	(0.22)	ab	
Q138	1	1.3	(0.22)	ab	
Q96	1	0.2		a	
QBYC05-20853	1	1.5	(0.22)	b	1

(ii) Number of sprouted buds relative to the depth of the node in the stool.

Roots were removed to reveal the below ground buds of the stools (Fig. 2). Although only a small number of stalks were visible above ground at this stage, a much larger number of sprouted buds were observed below ground (refer to Fig. 2). The total number of sprouted buds (including both emergent stalks and below-ground sprouted buds) showed significant variation (Fig. 5). The average number of sprouted buds was higher in varieties with more emergent stalks. This suggests that the ability for a variety to produce stalks translates to a larger number of buds available for sprouting.

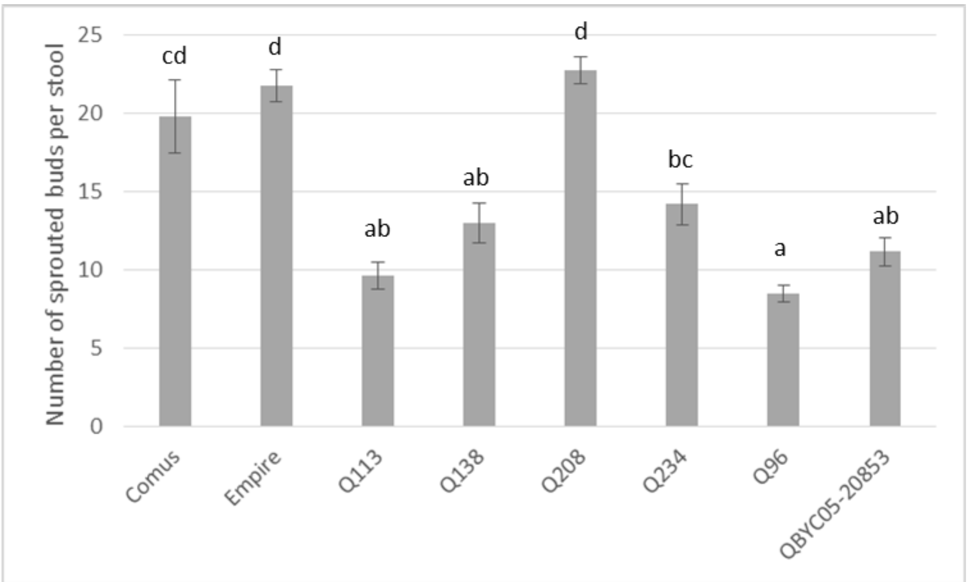

Figure 5. Number of sprouted above and below-ground buds within the stool of the eight varieties (mean ± SE). Tukey's LSD (<0.05) was applied; values with the same letter are not significantly different from each other.

The extent of sprouting of buds from nodes at various positions along the stalks (a, b and c stalks collectively) is detailed in Table 6. For this experiment, each stalk within a stool was treated as an individual regardless of its positional classification. Three varieties, Empire, Q113 and QBYC05-2083, had no sprouting of the lowest visible bud compared with the other five cultivars (Table 6, values highlighted in yellow). Comus had the largest number of sprouted buds along the stalk while QBYC05-20853 had the fewest (Table 6). The main source of mature stalks arose from buds located between positions two and six (Table 6, values highlighted in green). Uniform to all cultivars was the germination of mature stalks occurring at node 3 (data not shown). Assessing the sprouting that occurred only on the 'a' stalk revealed significant differences in the pattern of germination between the varieties (Fig. 6). As revealed in the assessment of all stalks, bud position 3 was uniformly sprouted across all of the varieties. Q208⏀ showed a relatively even sprouting from bud positions 2 to 6, with evenly sized bars for those bud positions (Fig. 6). Q234⏀ preferentially sprouted buds lower in the stool profile, followed by Empire, Q113, Q138 and QBYC05-20853, with more than 80% of the buds sprouting from buds positions 2 to 4. Conversely, Q96 and Comus showed a propensity to recruit from higher in the stool, bud positions 5 and higher, with approximately 55% and 38% sprouting, respectively.

Table 6. Number of sprouted buds at each position along the stalk for the eight varieties (mean ± SE). There were statistical significant differences between the varieties and bud position P<0.01. Yellow shaded cells indicate no germination in the lowest position for those varieties. Green shaded cells indicate uniform sprouting in these node positions for all varieties.

Node pos.	Comus	Empire	Q113	Q138	Q208⏀	Q234⏀	Q96	QBYC05-20853
1	0.6 (0.4)	0.0	0.0	0.2 (0.17)	0.2 (0.2)	1.4 (0.5)	0.5 (0.2)	0.0
2	2.0 (0.6)	3.8 (0.4)	1.5 (0.3)	2.3 (0.2)	4.4 (0.4)	2.2 (0.2)	1.0	2.7 (0.4)
3	2.4 (0.4)	4.0 (0.3)	1.7 (0.4)	2.2 (0.3)	4.4 (0.6)	2.2 (0.2)	1.2 (0.2)	2.7 (0.2)
4	2.6 (0.2)	3.6 (0.2)	2.0 (0.3)	2.2 (0.3)	4.4 (0.2)	2.2 (0.2)	1.2 (0.2)	2.7 (0.2)

5	2.4 (0.2)	3.4 (0.2)	1.3 (0.2)	1.8 (0.3)	3.8 (0.4)	2.0 (0.3)	1.0	2.0 (0.3)
6	2.4 (0.2)	2.8 (0.4)	1.3 (0.2)	1.3 (0.2)	3.2 (0.4)	1.4 (0.4)	1.0	1.0
7	2.0 (0.3)	2.0 (0.3)	1.0	1.2 (0.2)	1.0 (0.3)	1.2 (0.2)	1.0	1.0
8	1.6 (0.2)	0.8 (0.2)	1.0	1.0	0.8 (0.3)	1.0	1.0	
9	1.2 (0.2)	1.0	1.0	0.8 (0.2)	0.5 (0.5)	1.0	1.0	
10	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
11	1.0	1.0						
12	1.0							
13	1.0							

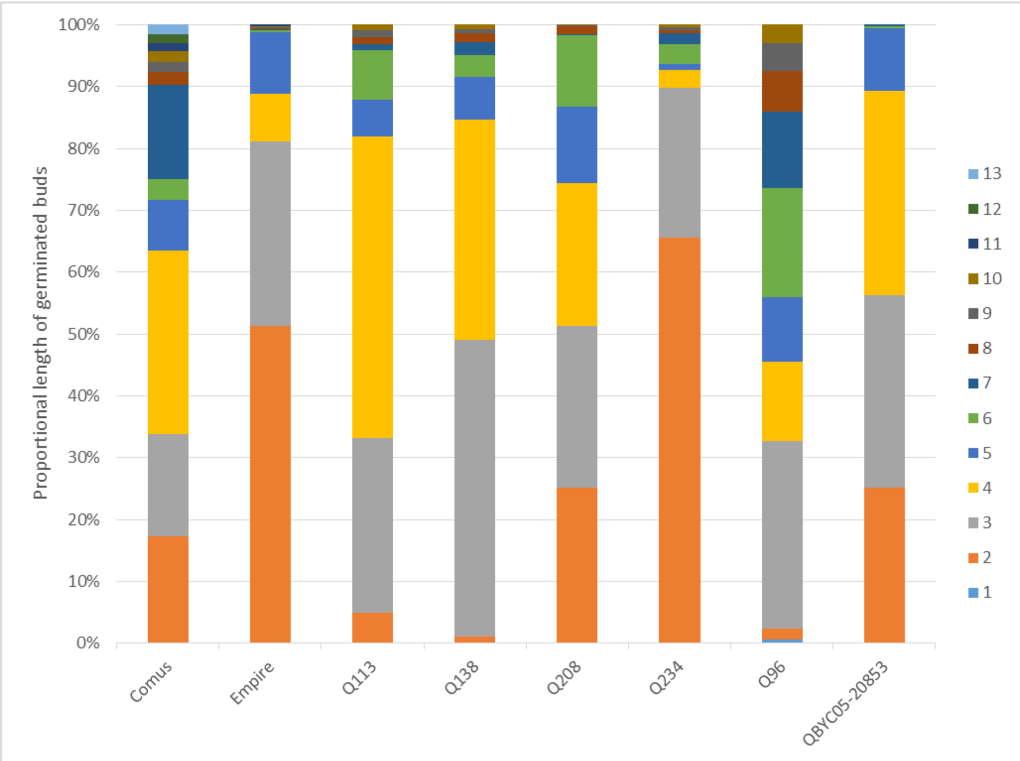

Figure 6. Cumulative length of sprouted buds from nodes on 'a' stalks as a proportion of the total length displayed relative to node position. Bud positions have been allocated different colours, with bud 1 located at the base of the 'a' stalk and furthest from the soil surface and numbering continuing up the stalk.

The examination of branching within pot-grown varieties allowed for the successful development of methods to examine sprouting, order of sprouting and effect of relative position on the stool. Between the eight varieties examined there was a significant difference in the degree of branching and the location of the buds from which mature branches arose. Correlated with the number of above-ground stalks is the number of below-ground buds which can be recruited.

It is logical to see how the stalk classifications would change with increasing crop age as shown in Table 7. While stalk classification may not directly influence the crop's propensity for a high yielding ratoon growth, a variety with recruitable buds lower in the stool profile that is capable of producing a larger stool with a range of different classified stalks may contribute to ratoonability. Further research to investigate differences in stalk classification patterns between good and poor ratooning varieties may be useful.

Table 7. An example of stalk classifications that may be found with progressive age of a sugarcane crop.

	'a'	'b'	'c'	'd'	'e'	'f'	'g'
Plant	✓	✓	✓				
1R		✓	✓	✓			
2R			✓	✓	✓		
3R				✓	✓	✓	
4R					✓	✓	✓

Preferential sprouting from certain bud positions may influence the survivability of a sugarcane clump and help to maintain an acceptable yield. Ferraris and Chapman (1991) claimed that the first shoots to emerge following harvest arose from buds that were higher in the stool and soil profile, within 5 cm of the soil surface; however there was a higher degree of sprouting overall from the lower buds, which may also contribute to the final number of mature stalks. A similar degree of lower bud sprouting was seen in six of the eight cultivars examined in the pots in our experiments. Varieties Q96 and Comus were noted to recruit buds from higher in the stool structure (Fig. 6), compared with the other varieties. Potential consequences of recruiting higher in the stool are that, as the crop ages, the region of recruitment is removed with harvesting, resulting in ratoon growth initiating lower in the stool or reduced ratoon growth (Whiteing and Kingston 2008). The recruitment of buds in field grown Q96 was also assessed and sprouting was seen in the top three buds; unfortunately buds lower in the profile were not assessed (Fig. 7, discussed further in the following section).

This research has developed methods for the analysis of below-ground buds in the stool with respect to ability to sprout and relative position on the stool. The results have shown a significant variation among genotypes and will be used in conjunction with bud/soil profile, stalk angles and density to further examine correlations with ratoonability.

In summary, methods have been established for analysis of the following parameters: number of sprouted buds, length of sprouted bud, order of sprouting (1^o, 2^o, 3^o) and relative position on the stool. These parameters were able to show differences between genotypes. The results from analysis of the pot-grown plants have shown that:

- Total number of sprouted buds varies according to genotype
- The number of sprouted buds is related to the number of above-ground stalks
- Differences in secondary and tertiary bud numbers account for the variation
- The position of sprouted buds that are recruited as above-ground tillers varies between varieties
-

6.2. Analysis of stool structure in plants grown in commercial fields

The stool structure in field-grown plants has been examined in separate studies over a number of years. In experiments conducted in the 1990s, field grown plants were excavated and nodes individually cut before being replanted and tested for sprouting ability. In this work, 3 to 5 below-ground nodes closest to the soil surface were assessed and nodes were numbered from the top down, with node 1 being closest to soil surface and node 5 furthest from the soil surface. All nodes examined were capable of sprouting, with nodes lower in the stool profile germinating earlier or growing at a faster rate (Fig. 7).

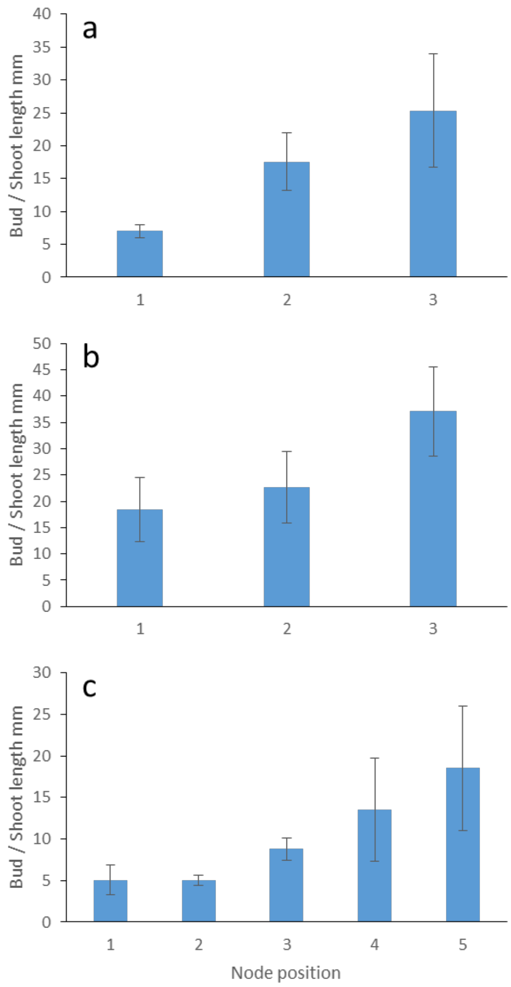

Figure 7. Average length of sprouted buds from below ground nodes of (a) Q117, (b) Q96 and (c) Q124 plants grown in the field. Node 1 is the first below ground node and Node 5 is the furthest from the soil surface (closest to the base of the stalk). Averages are across all mature tillers within 3 stool replicates. Q117 n = 23, Q96 n = 16, Q124 n = 20.

While these initial investigations have focussed on bud position it may be of more importance to note the distance from the soil surface. One reason to consider changing the definition of bud position within the stool is that stalk growth is individual. For example, one stalk may produce long below-ground internodes while others produce short internodes, possibly related to presence of obstructions in the soil. The location of the bud relative to the soil surface can also expose the bud to slightly different environments that may influence its germination. Buds located closer to the soil surface would experience a greater variation in soil temperature and moisture, compared with those further from the surface. While the top three buds of Q117, Q96 and Q124 sprouted, it was the lowest buds that either sprouted first or had faster growth (Fig. 7).

In experiments in 2016, clumps of sugarcane were excavated from MSF commercial fields and roots were removed to examine the extent and variation of stalk production. Comparisons were made between crop classes for a single variety, and between multiple varieties in a single crop class. Stools from various crop cycles of cultivar Q208$^{\Phi}$ were examined for the number of stalks harvested in 2016; there was no significant difference between crop cycles in stalk number (Fig. 8b). Within the clumps the stalks were classified according to the order of branching that occurs with increasing crop cycle. With increasing stool age, increasing branch order was observed, with ‘h’ stalks the maximum (Fig. 8a).

A small number of stools of Q200$^{\Phi}$ from 9R, 10R and 13R were obtained from Mr Vince Russo and Mr Lawrence Di Bella (2016, Herbert region). Although it was difficult to discriminate the order of branching, these stools had no more than 5 levels of stalk classification, suggesting that larger structures break apart over time to re-form “new” stools.

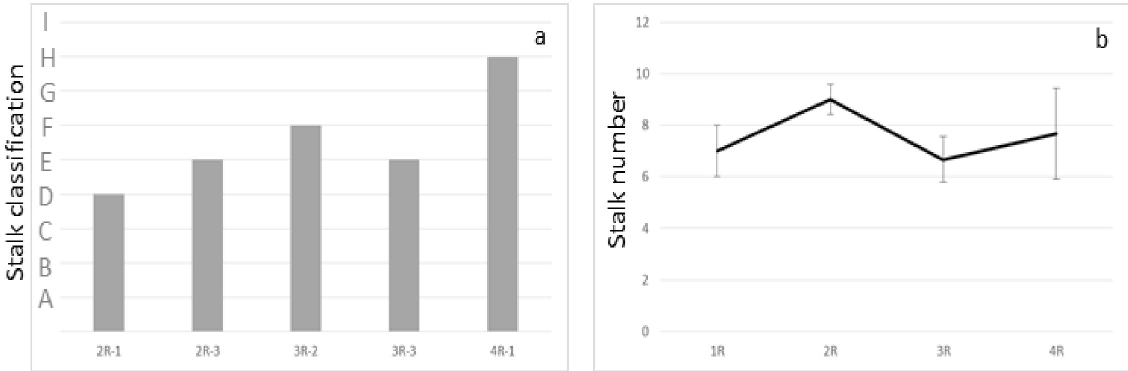

Figure 8. Branching order observed in stools of commercial field-grown Q208$^{\Phi}$ over increasing crop cycles. (a) Classification of stalks within the stools. (b) Number of mature stalks harvested in a single season (2016), including samples from various crop cycles from 1R (1st ratoon) to 4R (4th ratoon).

Stalk branch order was also compared between three different varieties from the MSF site, all at the first ratoon (Fig. 9). All varieties produced stalks to the same branching order, with ‘d’ stalks noted in all clumps. The factors influencing the separation of the cultivars were the larger number of ‘b’ and ‘c’ stalks produced by Q242$^{\Phi}$, with Q238$^{\Phi}$ producing the least. This was not linked to the number of billets within the clump, as the number of sprouted billets ranged from two to five. One of the Q208$^{\Phi}$ clumps contained the highest number of sprouted billets at five.

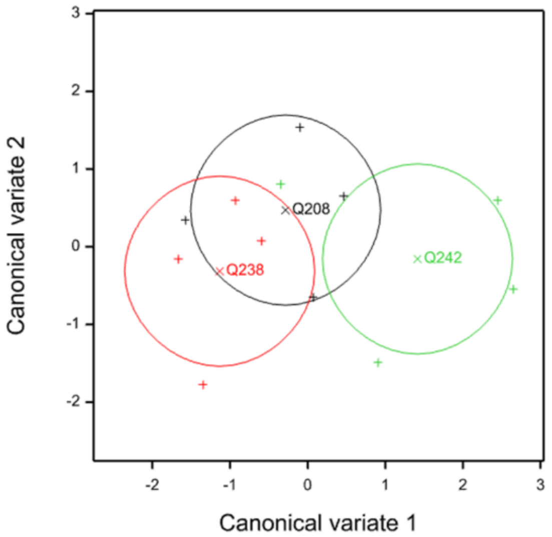

Figure 9. Canonical plot of variation in stalk order amongst stools of commercial field grown Q238$^{\phi}$, Q208$^{\phi}$ and Q242$^{\phi}$. All cultivars were 1st ratoon, planted in 2014. Samples were excavated within a week of harvesting in 2016. Stalks within the clump were classified and the total number of stalks at each classification for each year of harvest (2015 and 2016) was used for statistical analysis.

During the first few months of growth, the sugarcane plant produces more tillers than will become mature harvestable stalks. What was unknown was how far along the tiller does the die back effect extend; i.e. does the die back extend to the junction with the progenitor stalk or do the underground parts of the tiller remain viable. Examining the stools from the field grown varieties revealed structures that we believe were the remnants of the younger tillers. These bulb-like structures had a rounded top resulting from the degradation of the above-ground parts of the stalk, not a breakage or cut (Fig. 10). We referred to these structures as Base of Tillers (BOTs). BOTs of various sizes have been observed ranging from 1–4 cm and they may contain viable buds that can sprout.

Figure 10. Stalk section of Q238$^{\phi}$ 2nd ratoon stool. Arrows indicate BOTs originating on a broken secondary stalk.

Following on from the assessment of the stool branching, questions regarding the number of viable underground buds were raised. Re-planting of below ground buds from field grown clumps resulted in greater than 30 shoots per clump after 30 days growth (results not shown). Buds that did not sprout were assessed for viability by staining with tetrazolium, and were determined to be non-viable. Buds that did not sprout were not localised to any particular bud position along the stalk. Hence all viable nodes were capable of sprouting when separated from the stool and replanted under the optimal growth conditions. Interestingly, on one stool from a 1R crop, a bud from the ‘a’ stalk was observed to sprout after replanting, indicating that this bud had remained viable for two years. The concentration of carbohydrates were also examined within the stool. Two samples were taken below and above a point approximately 8 cm from the base of the initial billet. Sugar concentrations were equivalent to those measured in the base of mature stalks and were not significantly different among the three cultivars examined or position in the stool (Glassop *et al.*, 2017 ASSCT Poster). We concluded from this that sugar concentrations were not limiting bud outgrowth from the stool nodes.

When testing field grown plants from Q238$^{\Phi}$, Q208$^{\Phi}$ and Q242$^{\Phi}$ for the viability of buds within the stools, all buds that were not damaged were able to sprout. Regardless of the genotype, there were sufficient viable buds within the stool, arising from mature stalks and BOTs, that can be recruited for the next crop and there are sufficient carbohydrates to initiate sprouting. The differences in position of tiller recruitment may be under genetic control.

In summary, the methods for comparing stool architecture that were developed using pot-grown plants were found to be applicable to field-grown plants. The results confirmed that:

- The number and position of sprouted buds varies according to genotype.
- Multiple “spare” buds are present in the stool structure including buds on bulb-like structures that may represent the base of tillers that have died back.
- All viable nodes are capable of sprouting when separated from the stool.
- Carbohydrate supply is probably not the factor limiting bud outgrowth.

6.3. Influence of pot size on stool structure

Stool architecture traits in plants of three varieties grown in three pot sizes were compared with parameters measured in field-grown plants. There was a significant reduction in stalk number between the field and pot grown plants (Fig. 11). For example, the stalk number in plants grown in the 52 L pot compared to the field grown plants was lower by 23, 45 and 48% (Empire, Q113 and Q208$^{\Phi}$, respectively). The 35 and 70 L pots had reductions of 46 – 56 % across the different varieties. Despite the reduction in stalk number the ranking of the varieties was the same for all pot sizes compared to the field except the 52 L pots where Empire produced slightly more stalks than Q113 (Fig. 11).

Similar results were measured for the stalk height for Empire and Q208$^{\Phi}$, although variety Q113 was not significantly different between the pot sizes and the field (Fig. 11). Stalk height was reduced by 13 to 24% from the field grown plants, across all of the pot sizes for varieties Empire and Q208$^{\Phi}$. Once again, the rankings of the varieties were maintained across all pot sizes and field grown plants.

Stalk diameter was significantly lower in pot grown plants compared to field grown plants for all three varieties (Fig. 12). Interestingly, stalk diameter was higher in the 52 L pots than the other pot sizes for Q208$^{\Phi}$. For variety Q113 the 52 L pot produced stalk diameters larger than the 70 L pot but not significantly different from the 35 L pot (Fig. 12). The ranking of the varieties at each pot size altered between the varieties Q113 and Q208^{A}, though there was no significant difference between these varieties.

The combination of stalk height and stalk diameter influence the stalk weight of the plant. As with each of the other measured attributes, the stalk weight was significantly lower in the pot grown plants compared to the field grown plants (Fig. 12), except for Q113 in 52 L pots, which was similar to the field grown plants despite a 14% reduction. The other pot sizes saw a reduction in stalk weight of 21 to 46% compared to field grown plants, across all varieties. As seen with the stalk diameter the rankings at each pot size altered due to differences in Q113 and Q208$^{\Phi}$, though again at those points there was no significant difference between the varieties.

The extra root depth available in the 70 L pots did not improve plant growth and in some cases these plants were the least like field grown plants. While pots have not replicated field growth, the 52 L (50 x 38 cm) pot resulted in plants that trended to be better than the 35 L and 70 L pots. The observation that ranks were generally maintained for each parameter means that useful comparative results can be obtained from pot trials.

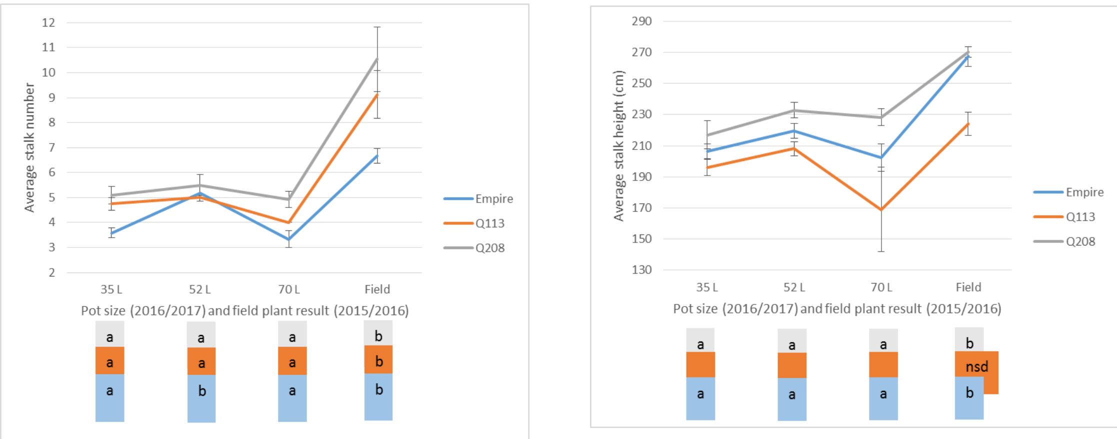

Figure 11. Average stalk number and height, left and right respectively. N = 6 for Q208$^{\Phi}$ and Empire, n = 2 for Q113, error bars are standard errors. Least significant difference letters were determined using Fisher's protected LSD for *P<0.05*. LSD letters can only be compared for individual varieties.

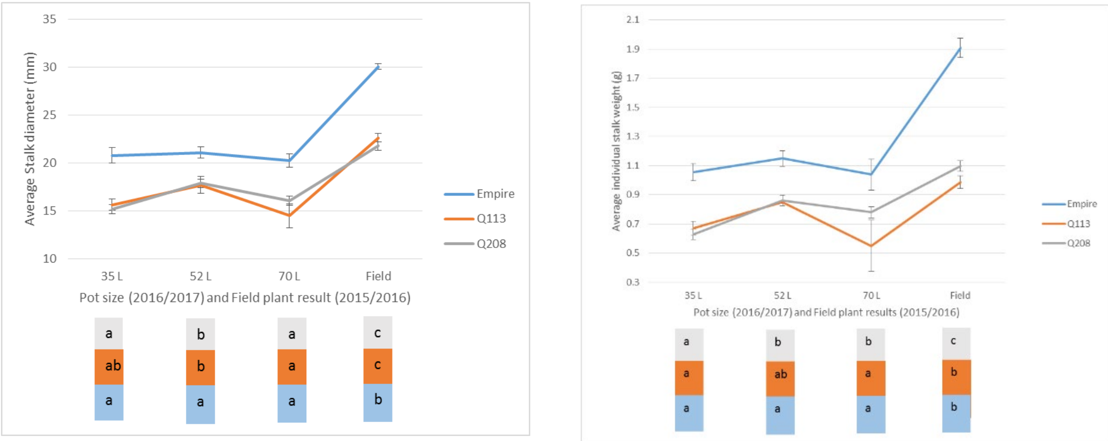

Figure 12. Average stalk diameter and weight, left and right respectively. N = 6 for Q208$^{\phi}$ and Empire, n = 2 for Q113, error bars are standard errors. Least significant difference letters were determined using Fisher's protected LSD for *P<0.05*. LSD letters can only be compared for individual varieties.

In summary, these experiments showed that:

- The 52 L pots were optimal.
- Pot experiments can be a useful model for field growth as variety rankings are maintained.

6.4. Differences in stool architecture amongst Saccharum species.

An experiment to grow a series of wild sugarcane relatives in order to compare the stool structures with hybrid varieties was set up in pots grown outdoors. A classification system used for bamboos was applied to the stools, as the bamboo family includes species with a number of different types of rhizome (Reference: https://www.guaduabamboo.com/identification/bamboo-identification-guide). The key features are:

- Length between culms and diameter of rhizome: Short and thick, or long and slender.
- Rhizome habit: Specialized as props for culm, running over ground, or running underground.
- Occurrence of buds on rhizome neck: Present, or absent.
- Position of roots: At the nodal line only, or at random.

Using these features, bamboo species are classified into types:

(i) Sympodial (also known as pachymorph or clumping bamboos): determinate structure where the growing point either terminates in an inflorescence or dies each year and growth is continued by new lateral branches. Axillary buds on the stem base develop directly into shoots which then grow into new stems. In the next year, axillary buds on the base of the new stems develop into new shoots and repeat the cycle. These rhizome systems can become very highly branched but cannot extend over large distances.

Depending on the length of the "necks" of the new shoots, sympodial systems may be classified as "clumping", with short distances between culms, or as "scattered", with longer distances between culms. The long necks of the sympodial-scattered types are regarded as pseudorhizomes and can extend 50-100 cm.

(ii) Monopodial (also known as leptomorph, or running bamboos) – indeterminate structures with a persistent growing point that produces many lateral organs progressively. In this type, thin rhizomes or “runners” extend horizontally underground for long distances. There is a bud at each node on the rhizome which can grow into either a new rhizome or a new shoot. These rhizome systems can produce complex branched structures that extend over large distances.

Examination of the cleaned stools from various Saccharum species suggested that all stools were of the sympodial type. New growth was continued by lateral branches and there was no evidence for continuously growing “runners”. The differences between genotypes were in the length of the “necks” of the laterals, which in some cases, grew horizontally underground for 10-20 cm before producing a culm. In the hybrids and *S. officinarum*, the necks were short, giving rise to a single clump of stalks, while in *S. spontaneum*, the necks were longer, giving rise to multiple clumps of stalks separated by underground sections of stalk. In summary, although is it generally accepted that crosses with *S spontaneum* increased the ratoonability of *S. officinarum* (Matsuoka and Garcia 2011), our results suggest that this may not be due to fundamental changes in stool architecture.

6.5. Comparison of stool architecture traits amongst 32 varieties grown in the field trial over two seasons

As described in the Methods, 32 genotypes were grown in a field trial and analysed for a range of plant and stool architecture traits during the early growth of the plant crop, at the time of harvest of the plant crop and at the time of harvest of the 1st ratoon crop. The following sections describe the results of these analyses, test for genetic diversity and correlations between traits, and examine how the traits changes between one harvest and the next.

6.5.1. Rate of stalk emergence in the plant crop

The rate of stalk emergence varied between varieties. Fig. 13 shows a subset of varieties illustrating this variation. Within this set, there was statistically significant difference in rate of stalk emergence between variety Q151 and Q77. These measurements highlight differences in variety vigour during establishment and progressing to canopy closure. In temperate cereals, early vigour is a significant trait linked to yield. Rapid growth increases photosynthetic capacity and early canopy closure limits water loss and weed competition. The trait has not received much attention in sugarcane, but is potentially valuable in plant establishment and in productivity of ratoon crops. These results confirm that genetic variation in early vigour exists is sugarcane.

Some varieties produced very large numbers of shoots (>30 per plant). As Fig. 13 shows, some varieties also reached a plateau of less than 30 shoots. Plants with larger numbers of shoots will have a correspondingly greater amount of below-ground biomass. It is well documented that as the canopy closes, the number of new tillers reduces and some young tillers will die back (McSteen 2009). The number of mature stalks at harvest is expected to average 10-12. Die back of shoots was observed in the plants in the field trial. Although some above-ground tillers die back, the underground sections of these tillers may still represent a resource of buds and nutrients that can fuel the next season's growth.

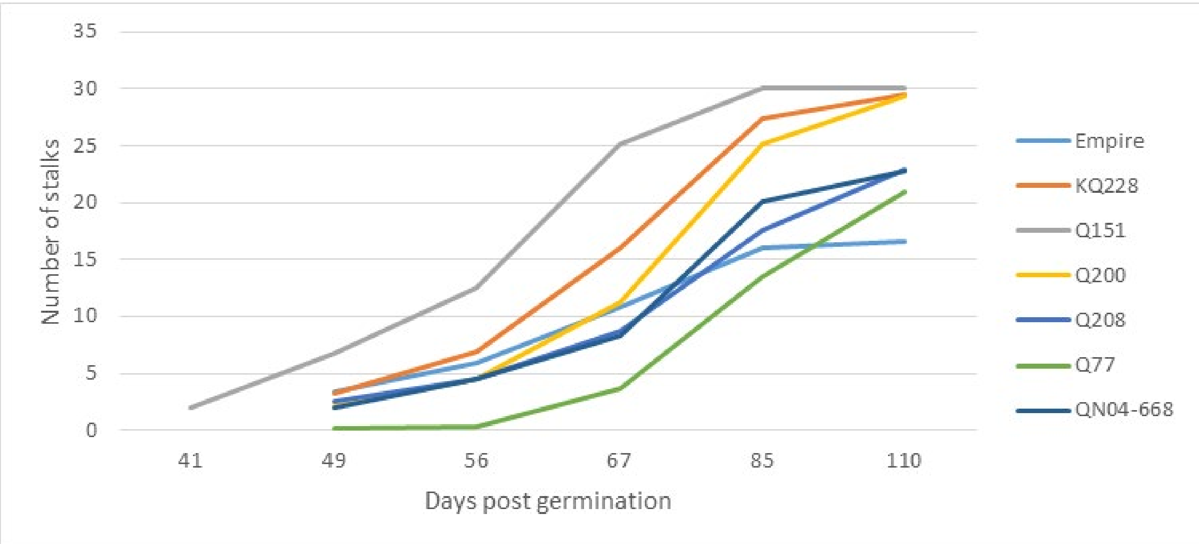

Figure 13. Average rate of stalk emergence in seven sugarcane varieties planted in the field trial at Gatton. N = 24 per variety.

6.5.2. Stalk flexibility measurements in the plant crop

Relative stalk flexibility was measured in the internodes using an Imada Digital Force Gauge . Increasing pressure was then applied to the stalk until either: (i) the stalk bent to the maximum distance of 10 cm allowed by the instrument design; (ii) the force reached the maximum instrument capacity of 1000 newtons without the stalk bending to the maximum degree; or (iii) the stalk broke, leaving the rind on the upper side still intact. In practice, the majority of stalks were able to bend. Only a few individual stalks were able to withstand the maximum 1000 newtons of pressure without bending to the maximum degree. Some of the stalks that bent with the force did not have any observable splits, while in some stalks the rind split into ribbons along the length of the internode and the stalk continued to bend without breaking (Fig. 14A). Breakage occurred in 21 of the 32 varieties (Fig. 14B), but not in every stalk within a genotype. Breaking occurred most frequently in Comus, Q117 and POJ2878.

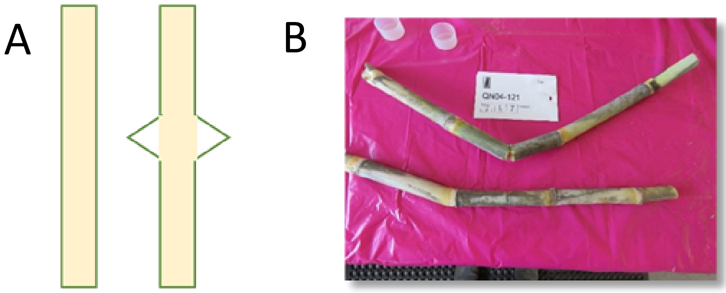

Figure 14. Images of stalk strength measurement. A, schematic top view of stalk prior to and following applied force, demonstrating the rind splitting along the length of the internode and away from the vascular pith. B, example of a snapped stalk after applying force, compared to a stalk prior to applying force.

For each variety, 24 stalks were analysed (8 stalks from each of three replicate plots). The results are displayed in a box and whisker plot that shows the medians of the set of 24 values and the distribution of values in four quartiles (Fig. 15). Fig. 16 shows the same results plotted against seedling date, revealing that there has been no unconscious bias in variety selection affecting this trait. The average forces observed ranged from 216 to 664 N. Stalk strength did not appear to be related to the age of a genotype as there was no evidence for a change in flexibility amongst modern varieties compared to older varieties. The average force required to bend each variety was correlated with the stalk diameter (Fig. 17). When all varieties are considered, a positive trend line yields a slope of 21.07 (R^2 = 0.3). If varieties Comus, SRA1$^{\Phi}$ and Pindar are removed from the analysis the slope increases to 34.1 (R^2 = 0.651). Both trend lines indicate that for a small increase in stalk diameter there is a larger increase in stalk strength. To date there has been no assessment to determine if there are differences in the internal stalk anatomy or cell wall biochemistry that may explain this phenomenon.

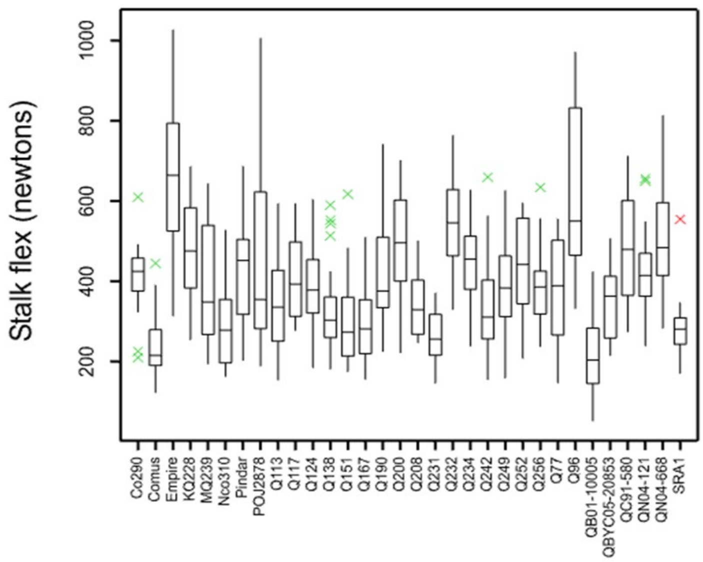

Figure 15. A box and whisker plot of stalk strength for each variety. Crosses indicate outliers (n = 27)

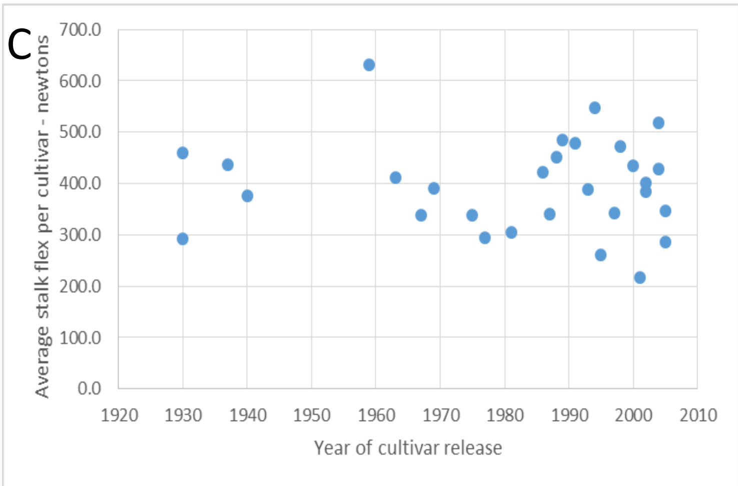

Figure 16. The average stalk strength for each variety plotted against the seedling date for that variety. (n = 27).

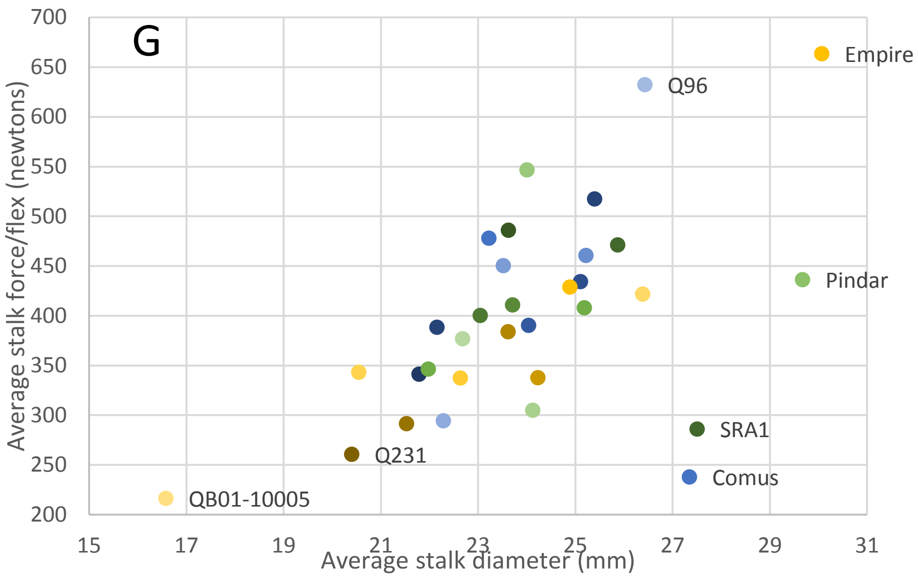

Figure 17. Relationship between the amounts of force applied and stalk diameter.

It is known that damage to the stool during harvest can result in reduced ratoon yields (Hurney et al. 2005; Kroes and Harris 1996). On the sugarcane harvester, the rollers (knockdown and finned feed rollers) push the stalks away from the harvester at a height of 400 to 1035 mm above ground, depending on the roller type and adjustable setting (Kroes and Harris 1996). The angle of the base of the stalk to the top of the stalk pushed over by the rollers can result in the stalks breaking/snapping before the cutters come through. Damage to stools include shallow to deep splits, shattering (resulting in jagged ends) and snapping (Hurney et al. 2005; Kroes and Harris 1994; Kroes and Harris 1996). Assessment of stools in the Tully, Burdekin and Mackay regions showed as much as 60 to 75% damage following harvesting, including approximately 40% seriously damaged. While physical damage plays a role in ratoon yield, it is the resulting infection that has a greater influence on ratoon growth; reducing stalk number by as much as 40% (Hurney et al. 2005). With increased damage, regardless of damage type, the percentage of the stools affected correlates with ratoon yields (Hurney et al. 2005). Plant varieties that can bend or snap cleanly without shattering or splitting may be less susceptible to infection. Another factor is the potential for the stool to tip or be pulled out of the ground if stalks do not snap. While soil environment and root structure will also affect a variety's propensity to tip or be pulled out, clean snapping may be an advantage.

Several observations have been reported on the knockdown and cutter damage to stalks. The force applied to the internodes of three varieties with varying fibre content (10.3 to 11.3%) at a height of 250 mm resulted in the stalks bending and creating splits along the length of the internode (Kroes and Harris 1996). While Kroes and Harris (1996) did not present variety-specific results, a typical force graph indicated that a maximum force of approximately 375 N was achieved and no breakages at the internode were reported. When force was applied to the node, breakages were observed with a typical force of approximately 525 N.

Our study found similar force measurements to those of Kroes and Harris (1996) but the range of values was increased by the inclusion of 32 genotypes. We confirmed the observations of Kroes and Harris that in some stalks the rind split into ribbons along the length of the internode. In the stalks that bent, despite having no visible damage to the rind there would have been some damage of the pith to accommodate the bending.

Stalk strength has been measured in other crops, including maize and canola, to study the propensity for lodging. In a study of canola stems by Grundas and Skubisz (2008), the force required to bend the stalk relative to the stalk diameter was used to compare cultivars. In sugarcane, we found that increased stalk diameter lead to increased stalk strength overall, however, this was clearly not the only factor. For a given stalk diameter, a range of stalk strengths could be found amongst the genotypes, suggesting that there may be other structural differences.

Stalk strength and diameter may be an important indicator of a genotype's ability to withstand the force of the knockdown rollers. This result needs to be further interrogated to include fibre measurements and potentially the strength/integrity of the rind (rind puncture strength), in future harvests.

6.5.3. Other measurements in the plant crop, highlighting genotypic differences between varieties

There were genotypic differences for all of the parameters measured, including stalk diameter, stalk height, stalk weight, number of millable stalks, stalk flex, brix, stool area, number of suckers and stalk positional classification in the stool.

The results from the 2016 harvest of the plant crop were analysed to test for correlations between the measured phenotypes across all of the varieties. Five correlations with coefficients above 0.5 were identified between the traits: stalk flex, stalk diameter, the area of the stool, the number of millable stalks at harvest, stalk length and weight of 8 stalks. When these results were presented at the consultative panel meeting in July 2017, discussion suggested that the correlations be repeated using only the genotypes that had been released as commercial varieties. Consequently 5 varieties were removed from the analysis, including Comus (a noble cane), two varieties arising from an introgression population (QB01-10005 and QBYC05-20853) and two other varieties (QC91-580 and QN04-121). The correlation tests were repeated, resulting in predominantly small increases in the correlation coefficients (Table 8). Two other correlations became significant when only assessing the commercial varieties; these were between the number of C stalks, B:C stalk ratios and number of millable stalks. Within this group of 8 correlated traits, several are used within the breeding program to assess variety potential including number of millable stalks, stalk diameter and length, and stalk weight.

Table 8. Correlation coefficients for traits assessed from the 2016 plant crop harvest including all varieties or including only commercially released varieties. Values highlighted in grey or blue indicate where the correlations strengthened when examining released varieties only, in the positive or negative directions, respectively.

All varieties	Commercial varieties	Phenotype vs.	Phenotype
0.5072	0.5502	Flex	stalk diameter
0.5451	0.5738	Area of stool	No. millable stalks
0.6162	0.6139	Weight of 8 stalks	stalk length
0.6237	0.5592	Flex	weight of 8 stalks
0.791	0.8612	Weight of 8 stalks	stalk diameter

0.497	0.5272	C stalks	No. millable stalks
-0.4416	-0.474	B:C ratio	C stalks

6.5.4. Correlations between measured parameters for plant and first ratoon harvests.

As well as testing correlations between the traits measured, it is of particular interest to note when varieties have not responded in the same manner; which may identify phenotypes linked to differences in ratoonability. Two way ANOVA was conducted for each phenotype to assess significant differences between varieties, years and variety by year. These calculations were conducted only on the commercial varieties, as the other varieties (Comus, QB01-10005, QBYC05-20853, QC91-580, and QN04-121) have measurements that skew the results.

(i) Brix. There was a significant decrease in brix when comparing the 2017 harvest to 2016 for five varieties. Overall there was a trend for reduced brix in 2017 compared to the 2016 measurements, with only three varieties showing an increase, though not significant at $p<0.05$ (Fig. 18, Appendix 2 Table 1). The harvest in 2017 was conducted one month earlier than in 2016, which may explain the reduced brix overall. Xiao *et al.* (2017) reported an increase of 1.5% brix over the last month of growth from mixed juice samples collected throughout the crushing season. If a 1.5% increase was added to those 2017 values that were significantly reduced, this would restore most values to the 2016 level, except for SRA1[Φ] and Comus, which would remain significantly lower. The reason for the reduction in these varieties was not obvious.

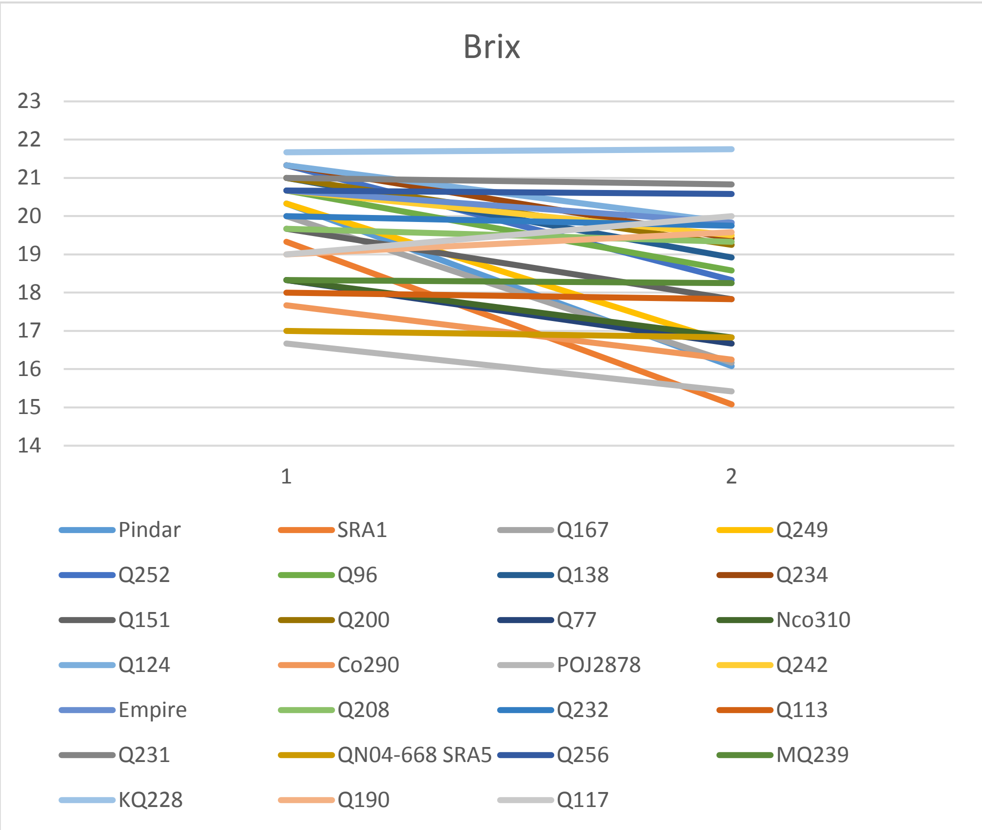

Figure 18. Average brix as °Bx for commercial varieties for each year of growth. The x-axis labels are 1 and 2 representing 2016 and 2017, respectively.

(ii) Suckers and Degree of Lodging. The total number of suckers in all genotypes was reduced in 2017 compared to the 2016 harvest, with 23 varieties showing a significant level of reduction at P>0.05. The difference in sucker number has been attributed to the heavy lodging of the field trial in 2016 following a severe storm, compared to a relatively upright field in 2017. The degree of lodging was assessed in both years using ratings as follows: 1 = upright cane; 2 = cane within 45° of upright; 3 = cane greater than 45° from upright; 4 = lying on the ground. As previously stated, the field in 2017 was more upright overall although this difference was only significant for 3 varieties (Q124, Q231⏀ and Q77). Five varieties were fully lodged (rating 4 in all replicates) in both years (Co290, Q151, Q96, QC91-580 and QN04-668/SRA5⏀) and the remaining varieties were not significantly different between the two years but all trending for being more upright. The flex measurements from the 2016 harvest were examined to test whether any stalk strength characteristics had an influence on lodging, there was no correlation between the flex measurement and the propensity to lodge.

(iii) Millable stalk number and stool area. Stalk number increased for 28 of the 32 varieties examined, with significant increases for 12 varieties at *P<0.05* (Fig. 19). A reduction in stalk number was observed for four varieties, although it was not significantly reduced (SRA1⏀, Q256⏀, Q124, QBYC05-20853). Logically, an increase in stalk number may result in an increased stool area and this was found to be the case, as the area of stools in 2017 was larger than that recorded in 2016 for all varieties (excluding Comus) (Fig. 20). There was a statistically significant increase in stool area for 27 of the varieties. When the slope of the line was examined there was a large range in the values, indicating that not all varieties responded in a similar manner (Appendix 2 Table 2).

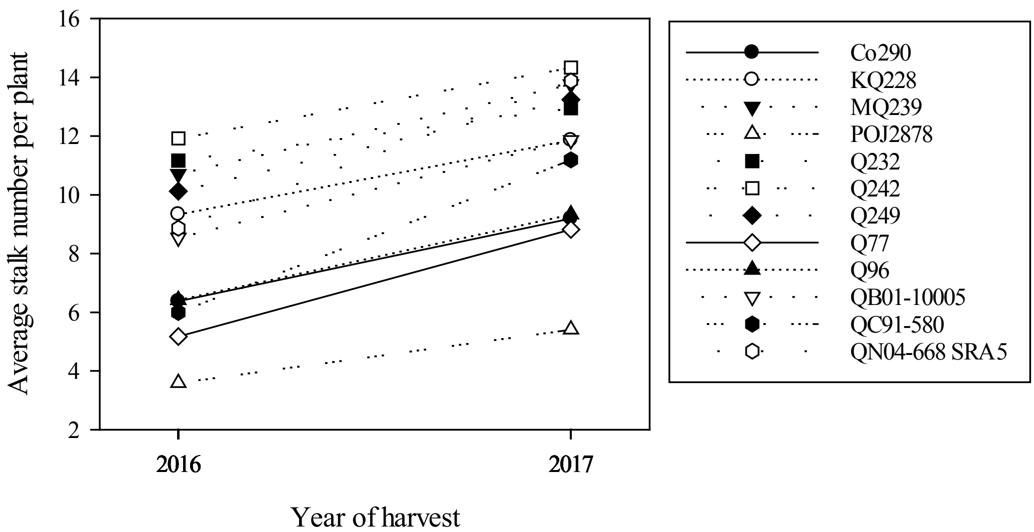

Figure 19. Mean millable stalk number per plant for the twelve varieties that were showed a significant increase from 2016 to 2017. Comus, (an *S.officinarum*), has been excluded from this set.

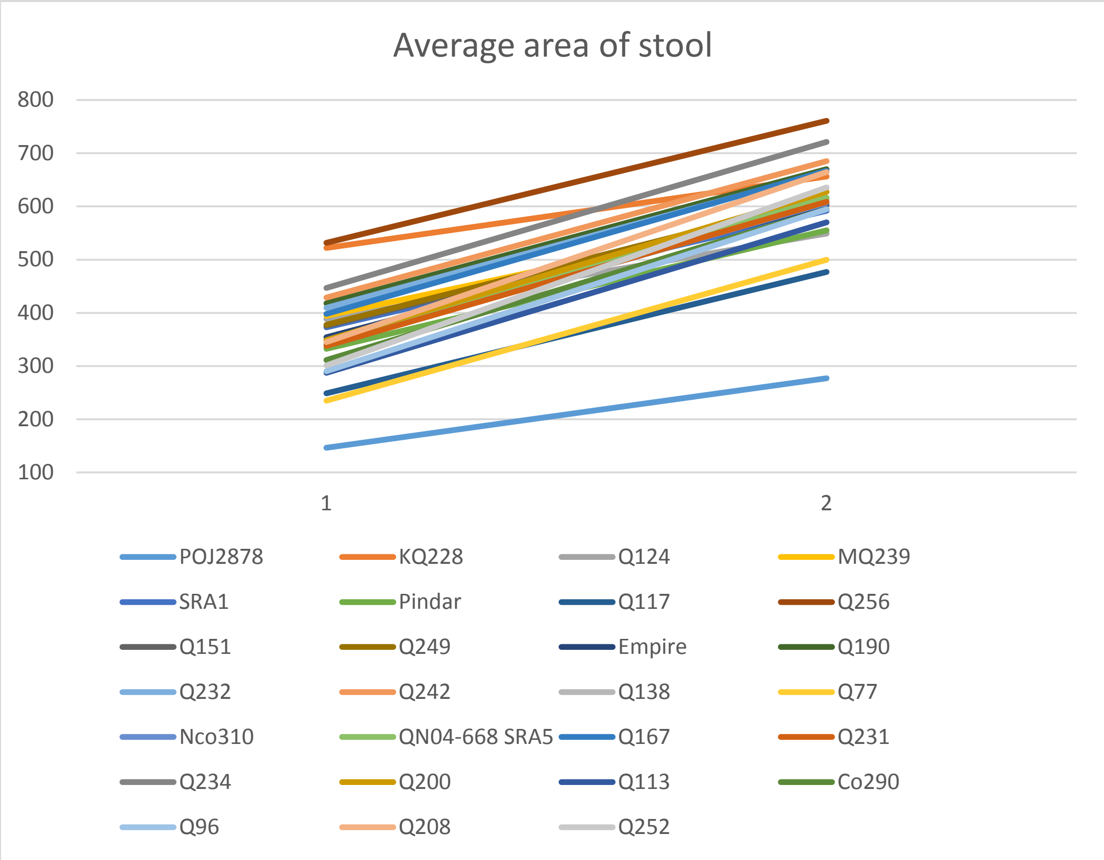

Figure 20. Average area of stool for commercial varieties for each year of growth in cm^2. The x-axis labels are 1 and 2 representing 2016 and 2017, respectively.

For all but four varieties (Q113, Q124, Q190$^{\phi}$, Q242$^{\phi}$) the number of sprouted buds and stalks increased in 2017 compared to 2016 (Fig. 21). Q242$^{\phi}$ was the only variety to not produce any B stalks in the 2017 growth, while all of the other varieties still had viable buds on the A stalk that were recruited in the second year of growth. There were 16 varieties that had a large number of sprouted buds but significantly fewer mature stalks. Depending on the size of the sprouted bud, these may not have been cut during the harvest and would provide an immediate flush of new growth. Varieties without these pre-sprouted buds would require quick activation to sprout buds and produce new growth. This trait may be linked to vigour, assisting a plant to survive stresses that occur early after harvest; three of the four varieties known as good ratooners fall into this category (Q138, Q208$^{\phi}$, Q77; not Q113; Fig. 21). Millable stalk number increased for all but three varieties (Q256$^{\phi}$, SRA1$^{\phi}$, Q124) and there was a fold increase range from 7.5 – 56 for the other varieties (Fig. 22, Appendix 2 Table 3). Increased stalk number has been associated with decreasing yield (Zhou *et al.* 2012; Milligan *et al.* 1990 a,b); while this seems contradictory the coupled trait resulting in reduced yield is reduced stalk diameter.

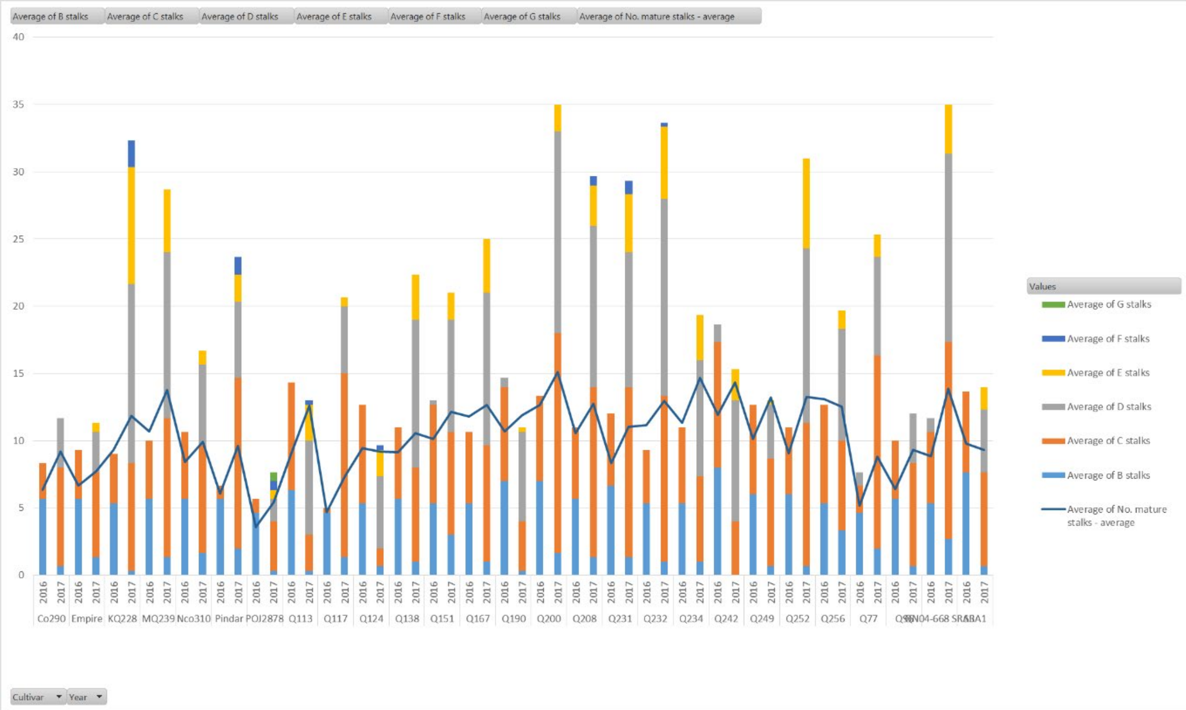

Figure 21. **Average number of stalks, coloured according to their stool position classification observed for each variety and both years. The line indicates the average number of mature stalks counted for that variety and both years.**

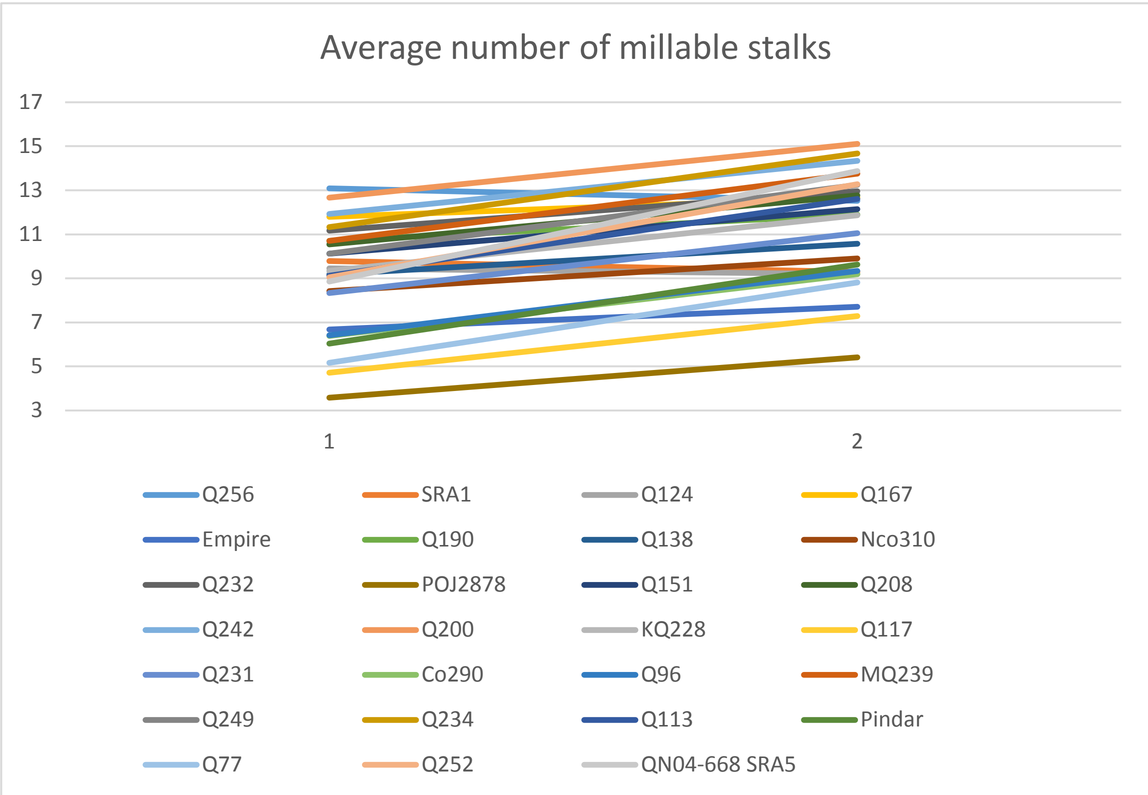

Figure 22. Average number of millable stalks. The x-axis labels are 1 and 2 representing 2016 and 2017, respectively.

(iv) Stalk height, diameter and weight

Stalk height was significantly increased in the 2017 harvest compared to the 2016 harvest for 25 of the 32 varieties grown (commercial varieties detailed in Fig. 23, Appendix 2 Table 4). In these varieties, the stalk height was increased from 12.6 – 32.6 %. Amongst the other seven varieties, five showed a trend towards increased stalk height and two showed a slight decrease in stalk height (Appendix 2 Table 4).

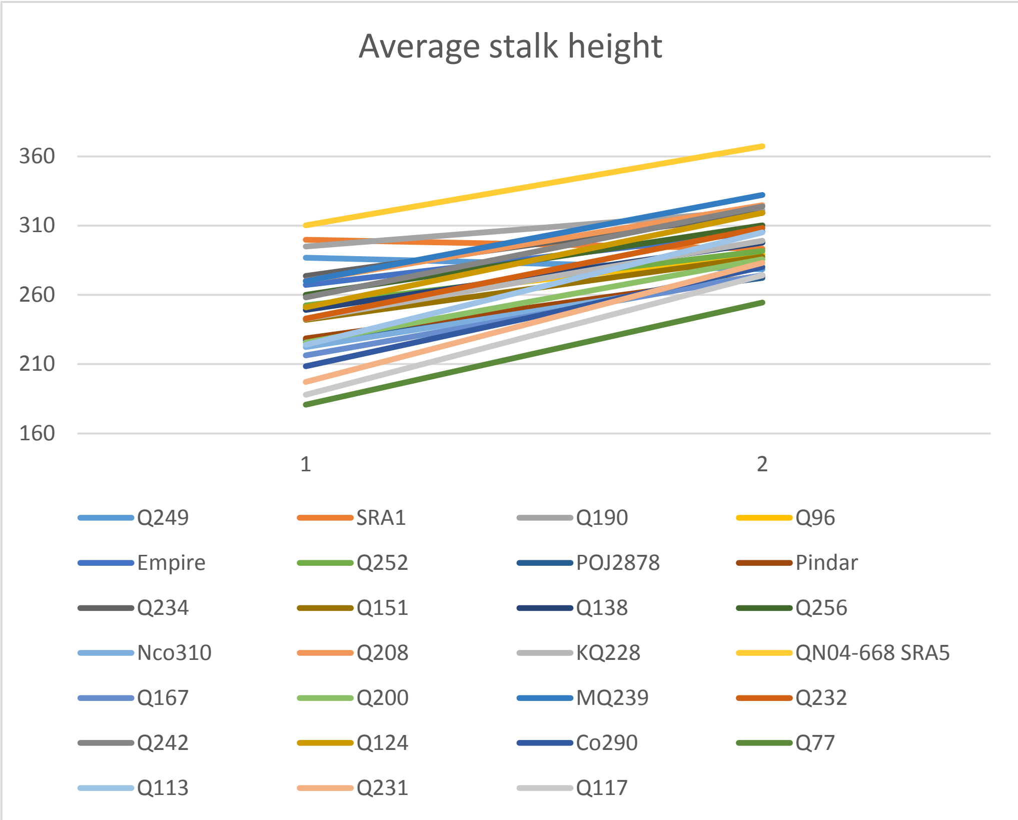

Figure 23. Average stalk height, cm (n = 27). The x-axis labels are 1 and 2 representing 2016 and 2017, respectively.

With such a significant increase in stalk height, it may follow that there is an increase in stalk weight which would in turn affect harvest weights, together with the increased stalk number per plant. Within the 32 varieties analysed for mean weight of eight stalks, 25 and 7 varieties showed increased or decreased stalk weight respectively, although the differences were only significant for 13 varieties (12 varieties showing a significant increase and 1 variety (Q249) showing a significant decrease). The range of significant increases was 16 – 42% (commercial varieties detailed in Fig. 24 and Appendix 2 Table 5).

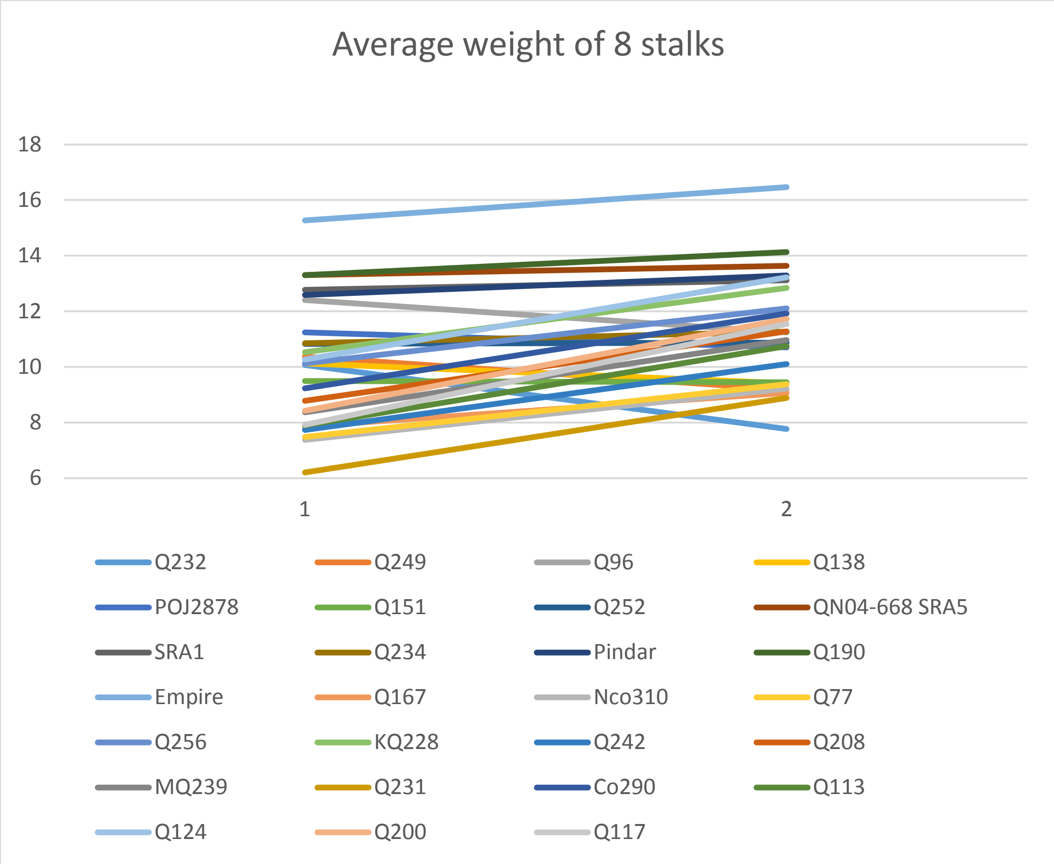

Figure 24. Average weight of 8 stalks for the commercial varieties (kg, n = 3). The x-axis labels are 1 and 2 representing 2016 and 2017, respectively.

Interestingly, it has been reported that reduced ratoon yields were associated with increased stalk number but decreased stalk diameter. All varieties in the trial showed a decrease in stalk diameter when comparing the 2017 to the 2016 harvest (commercial varieties detailed in Fig. 25 and Appendix 2 Table 6). Thirteen varieties showed a significant reduction in stalk diameter, ranging from 7 – 20% reduction (Fig. 26). Despite the decreased stalk diameters there was an increase in stalk weight, which can be attributed to the increased stalk height, effectively negated the loss of yield due to decreased stalk diameter.

Importantly the decrease in ratoon yield is usually reported in the 2nd ratoon harvest, with increases in yield often occurring between the plant and 1st ratoon crops. The 2nd ratoon harvest in 2018 will also be added to this database of variety measurements and allow the continued assessment of varieties, their stool architecture and ratoonability.

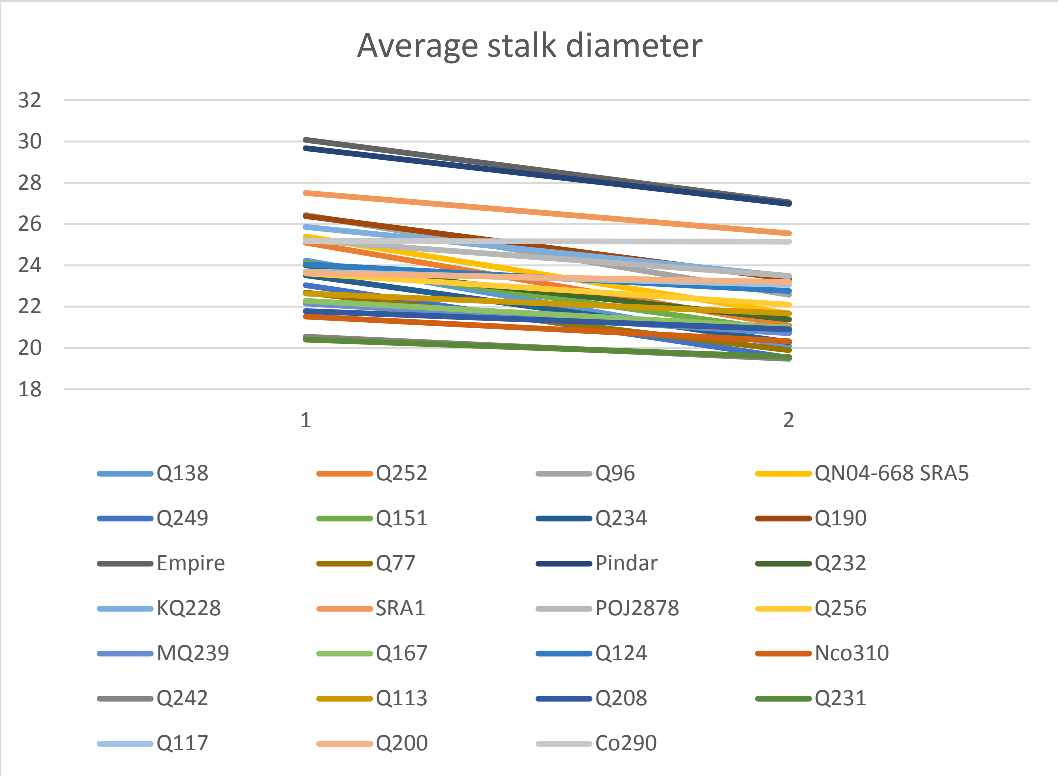

Figure 25. Average stalk diameter (mm, n = 27). The x-axis labels are 1 and 2 representing 2016 and 2017, respectively.

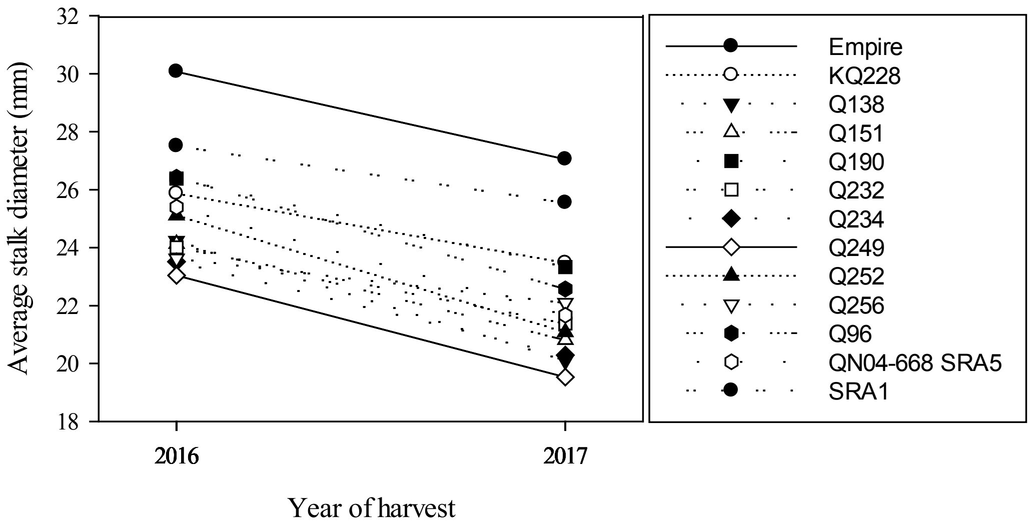

Figure 26. Mean stalk diameter per plant for the 13 varieties that showed significant decreases from 2016 to 2017. Comus (an *S.officinarum*) has been excluded from this set.

(v) Range of stalk classification

In the plant crop, the stalk classification ranged from 2-3, i.e. all varieties had B stalks and some had greater than B stalks. In the first ratoon, the range of stalks generated in the second year of growth was from 2.3 – 5; suggesting that there was more growth in the second year (commercial varieties detailed in Fig. 27, Appendix 2 Table 7). For comparison, 2R stools from commercial fields had 4 – 5 stalk classifications (MSF field results above).

This result is supported by observations of the increased stalk number in the ratoon crop. Varieties Q242[⫛] and Q190[⫛] were the only varieties that had less growth or no greater growth in 2017 than 2016. Those varieties that have a larger range of stalks may also have a larger number of buds available for ratoon growth, as noted previously.

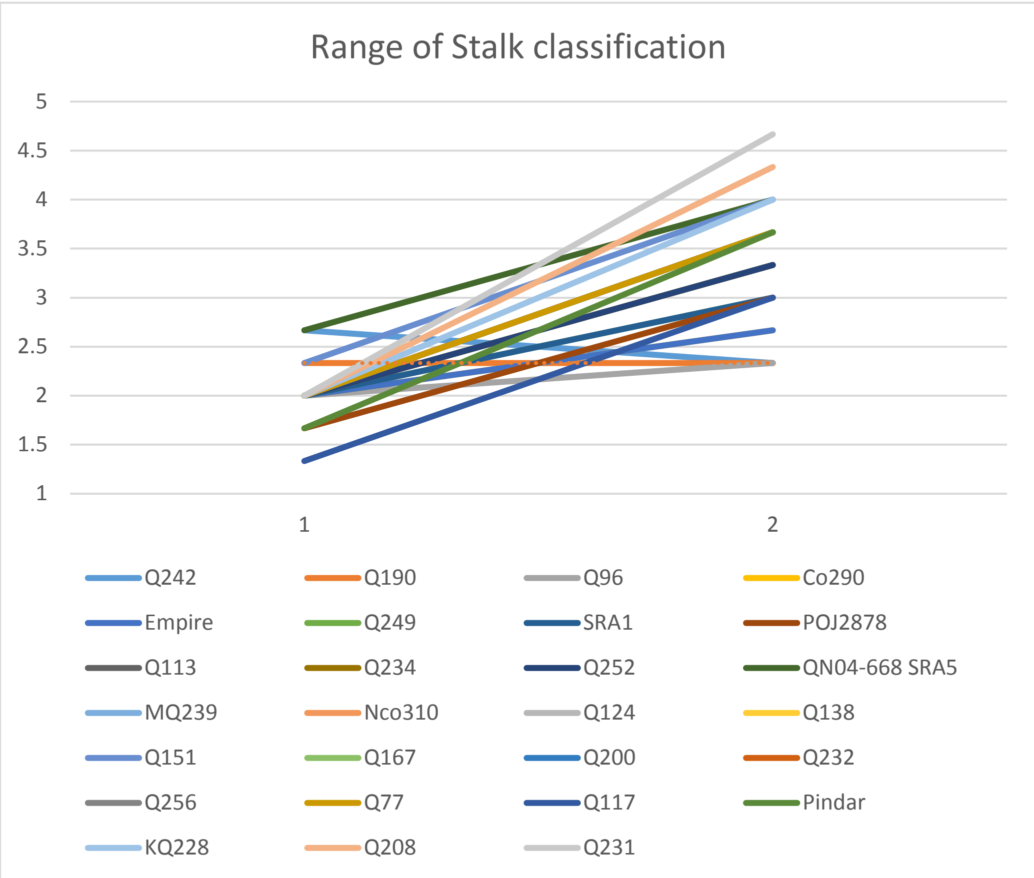

Figure 27. Average range of stalk classifications. The x-axis labels are 1 and 2 representing 2016 and 2017, respectively.

(vi) Correlations between traits that were observed with combined plant crop and 1st ratoon measurements.

When the 2016 and 2017 results were combined a number of phenotypic measurements correlated (Table 9). There were other correlations, not listed as these were obvious links, e.g. number of C stalks correlated with the number of B stalks, which is logical because a C stalk cannot exist without a B stalk. Some of the listed correlations are expected, e.g. area of stool and number of millable stalks (0.6232). With increasing numbers of stalks, the size of the stool would also increase. Similarly the correlation between stalk classification range and area of stool (0.6302) is logical. A couple of the correlations are due to different growth in each year, e.g. stalk height and number of suckers (-0.5073) which is due to the lodging in 2016, where more suckers were produced and stalk height affected compared to 2017 where there was no lodging and therefore reduced sucker numbers and taller stalks. The main correlation that has been reported previously to be associated with ratoon growth is between stalk diameter and weight. That correlation is maintained in these results where an increase in stalk diameter results in an increase in stalk weight (0.5721). These correlations and deviations from correlations will be monitored in future harvests.

Table 9. Correlations between measurements, with 2016 and 2017 results combined.

Phenotype	versus	Phenotype	Correlation
Stalk length		No. millable stalks	0.5443
Area of stool		No. millable stalks	0.6232
Stalk height		Total no. suckers	-0.5073
Stalk height		Area of stool	0.6302
Classification range		Area of stool	0.5932
Stalk diameter		Weight of 8 stalks	0.5721
Stalk height		Weight of 8 stalks	0.5374
Classification range		Stalk height	0.4717

6.6. Correlations between stool architecture traits and industry ratoon yield data

The final objective of the porjecct was to test correlations between architecture traits and ratoon yields. Further work, as detailed in the materials and methods, has been done to find industry ratoon yield values that can be used with the Gatton field trial results. Ratoon yield data was obtained from QCANESelect^{TM} for the South Johnstone, Tully, Plane Creek and Proserpine regions (Fig. 28). As described in the methods the TCH was graphed for each variety, in each year. Statistical analysis for each variety showed that there was no significant difference between years and regions; consequently the slope of yield decline was averaged to give a single value for each variety (Table 10).

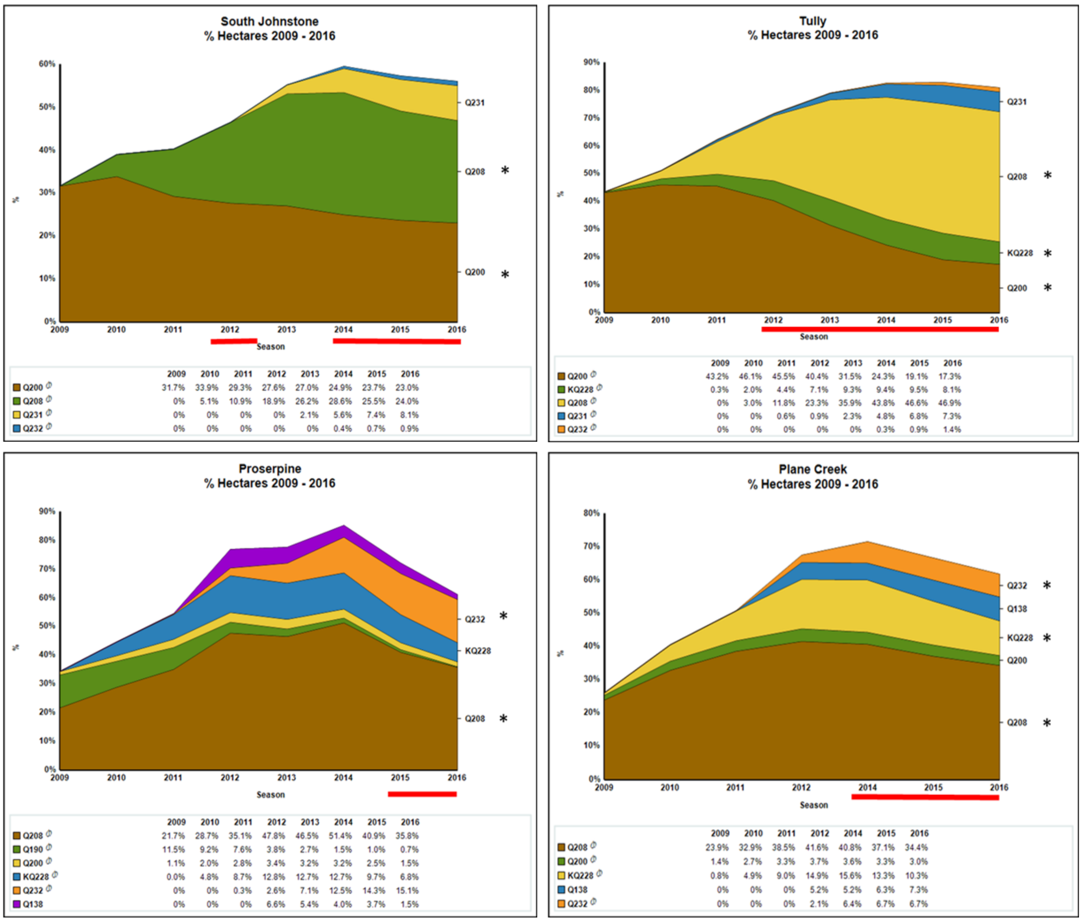

Figure 28. Sets of yield data (TCH) for the four regions selected for initial analysis, showing varieties (asterisks) and seasons (red lines) that meet the selection criteria. Each colour sector represents a different variety and shows the proportion of the region growing that variety for each year.

Table 10. Average ratoon yield index calculated for each variety across all regions and years in the dataset.

Variety	Ratoon yield index across 1R-3R
Q200	-3.5383
Q208	-4.021
Q232	-3.543
KQ228	-4.348

The ratoon yield index was added to the 2016 field measurements to test for any correlations between the parameters measured in the field trial for the four varieties. Principal component analysis resulted in the varieties separating (Fig. 29). The main phenotypes influencing the separation of PC-1 were the number of C stalks (-0.198), ratoon yield index (-0.158), stalk diameter (0.324) and number of tillers at 36 days growth (0.343). The main phenotypes influencing the separation of PC-2 were the length of the stalks (-0.417), ratoon yield index (0.334) and number of C stalks and C classified BOTs (0.341).

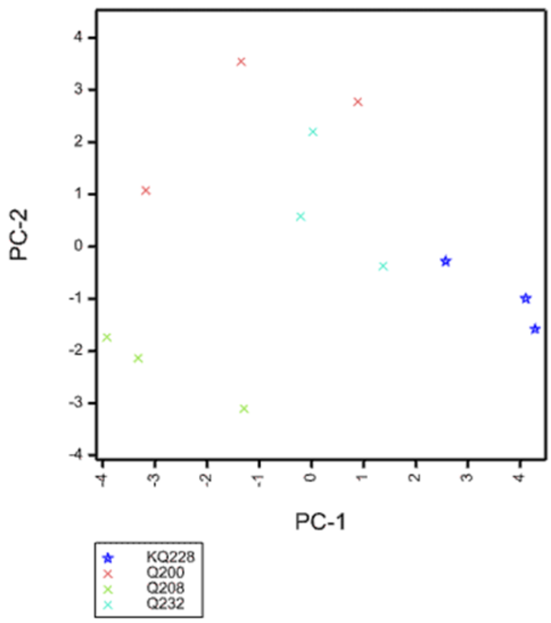

Figure 29. Principal component analysis of trait data and industry ratoon yield index. Each variety has three data points on the graph, representing the three replicates from the trial at Gatton. This analysis only utilised results from the four varieties with a calculated ratoon yield index value. Percentage variations for component 1(PC-1) and 2 (PC-2) were 36.6 and 20.6% respectively.

In order to further examine the relationship between the traits that influences the separation of the varieties in the PCA, a pair-wise correlation analysis between each trait was performed (data not shown).

Several positive and negative correlations with the ratoon yield index were observed:

No. mature stalks at harvest	0.6195
Total number of suckers	-0.6211
No. of tillers at 36 days	0.6195
No. C stalks and C-BOTs	0.6133

The observation that the number of C stalks + C-BOTs correlate with ratoon yields supports the hypothesis that a more complex underground structure contributes to better ratoon potential through a greater availability of buds. This needs to be tested further with a larger data set, including industry yield results and the traits measured in the upcoming harvest of the 2R plants in the Gatton trial.

The major constraint with this approach was the lack of significant difference between the varieties so that any correlations may not be statistically sound. The ratoon yield index values were re-assessed between P – 3R, P – 5R, 1R – 3R, 1R – 5R and repeated with values normalised to the P crop. ANOVA was used to identify ratoon yield index values that were significantly different between varieties. Significant differences were only identified in the Proserpine region and this is due to limited data information on two of the included varieties.

The data to calculate ratoon yield index was not available for a wide range of varieties and did not include all of the varieties grown in the Gatton Field Trial. Data sets were also limited with respect to crop class. The data that is available reflects the recent commercially grown varieties, and these are present in large quantities over multiple years because they likely perform well in TCH and ratooning. In order to perform this analysis effectively, sets of yield data for poorly ratooning varieties would also need to be available: clearly this is not compatible with commercial production systems. These results emphasise the value of the Gatton Field Trial as it will provide comprehensive information on a wider set of varieties, which is not available from other data sources, and will enable better statistical analysis of stool architecture traits in relation to ratoon yields.

Summarising the results from the field trial to date, 32 genotypes of commercial sugarcane, progenitor species, late-stage breeding lines have been analysed for a range of stool architecture traits during establishment of the plant crop, at harvest of the plant crop and at harvest of the 1st ratoon.

- All traits varied according to genotype. Overall trends in many traits (e.g. stalk number, stool area, stalk weight) were observed from P to 1R, but some varieties behaved differently and these varieties may be key to an understanding of ratoonability.
- Shoot emergence rates and maximum number varied. Large initial stalk populations did not translate to large millable stalk numbers but do contribute to more complex underground stool structures containing live buds. There was some evidence that a larger stool structure contributed to better ratoon yields.
- Stalk strength was only partially explained by stalk diameter, suggesting that biochemical or anatomical differences may play a role.
- Attempts to use industry yield data to test correlations with variety trait data were hampered by the lack of genotype diversity in the yield data sets.

7. CONCLUSIONS

Materials used for the project included pot grown plants, plants sourced from commercial fields and a purpose-designed field trial of 32 genotypes that span 50 years of variety release. Using the pot-grown plants, we first established a method to classify stalks where a single plant can be represented as an equation based on the hierarchy (as 'a' stalks, 'b' stalks, 'c' stalks etc.) and number of emergent buds in the stool structure. This equation can then be used to identify similarities or differences between varieties. For example, contrasting varieties could be described as a + 2b + 1c or a + 5b + 10c.

As predicted, varietal differences in the number of sprouted buds and the position of bud recruitment were identified. In general, buds lower in the structure sprouted first, but some varieties had a propensity to sprout buds higher in the stool, which may leave these varieties with fewer viable buds for recruitment in the following ratoon crop following harvesting. Buds remained viable over many seasons, and sprouting is unlikely to be limited by resource availability as adequate concentrations of water and sucrose were present; this suggests that bud release is under genetic control.

Methods for growing plants in pots while achieving comparable stool structures were tested. Glasshouse growth conditions did not replicate tillering patterns, but large pots grown outdoors were a better model. The 52 L pot size was found to be optimal.

We also assessed the stool structures in a range of Saccharum species, including several *S. spontaneum* genotypes. These plants had stool structures that were essential similar to the modern hybrid cultivars, except that the underground portions of the internodes were longer; they did not appear to have the contrasting "runner" type of rhizome. Although introgression of *S. spontaneum*, improves ratooning, this does not appear to be due to any fundamental change in rhizome structure.

Plants grown in the field trial were assessed for a number of traits in the plant crop (2016 harvest) and 1st ratoon crop (2017 harvest) including: rate and number of stalk emergence in the plant crop, stalk flexibility, stalk diameter, stalk height, stalk weight, number of millable stalks, brix, stool area, number of suckers and stalk positional classification in the stool using the method described above. There was significant variation in all of these traits, however importantly, there were no significant trends towards particular features in our varieties over time. The implication are that selection for yield has not resulted in significant changes to the stool architecture.

There was genotypic variation in the rate of stalk emergence and the final number of emerged stalks in the plant crop. All varieties produced a larger number of stalks than their final millable stalk population and the structures remaining underground after stalk dieback appear to contain viable buds which may contribute to growth in following seasons. In the ratoon crop the plants reached their maximum stalk emergence number faster and the differences in rate of production were less pronounced.

We tested relative stalk strength as a feature that may increase the likelihood of damage to the stool during harvesting. Measurements identified varieties that were able to bend with the applied force and others that responded by snapping with some tearing of the rind. There was an overall positive correlation between the stalk diameter and strength, but some varieties were outside this correlation, suggesting that there are other factors involved.

Key findings from the field trial when comparing the traits in the 1st ratoon plants to the plant crop were:

- The number of mature (millable) stalks increased for 28 of the 32 varieties tested.
- The area of the stool increased, as might be expected for a larger stalk population.
- The complexity of the stool structures increased, with additional hierarchies of branching and an increase in the ratios of sprouted buds to millable stalks.
- Stalk height increased, although this was clearly linked to environmental conditions as there was heavy lodging following a storm in 2016.
- Stalk diameter decreased for all varieties, significantly for 14 varieties.
- Stalk weight trended to increase, although this is linked to the stalk height.

While there were trends across all varieties, we identified some varieties that behave differently when comparing ratoon traits to plant crop traits. These varieties will be closely monitored in future work as they may be key to discerning phenotypes related to ratoonability.

We attempted to test for correlations between industry yield data and the traits measured in the field trial, but this approach was limited by the lack of availability of yield data for a wider set of varieties with contrasting phenotypes. This highlights the importance of the purpose-designed field trials, which provides a strong foundation of measurements that can be expanded with future harvests. The traits measurements in the 2nd ratoon will be particularly important as this has been widely cited as the crop cycle where variety ratooning abilities diverge.

8. RECOMMENDATIONS FOR FURTHER RD&A

As described above, the results from the field trial plant and 1st ratoon are a valuable data set for a wide set of genotypes that is not available elsewhere. The immediate need is to continue this trial into subsequent ratoon cycles, as this is typically where ratoon yield declines become apparent. Monitoring the changes that were identified through further ratoon cycles would strengthen these trends, particularly focussing on varieties that behave differently.

The results show that bud recruitment and sprouting varies between genotypes but this was not fully explored within this project. Remaining questions include the activity of the “base-of-tiller” structures resulting from tiller dieback and the fate of buds that sprout near harvest and may be recruited early in the next ratoon. These vigour-related traits may explain contrasting ratoon performances but are difficult to study systematically as they necessitate a destructive sampling of the stool.

Stalk strength measurements highlighted varieties that behaved outside the norm. Although increased stalk diameter lead to increased stalk strength overall, this was clearly not the only factor, as for a given stalk diameter, a range of stalk strengths could be found amongst the genotypes. This relates to concurrent research into fibre traits in sugarcane stalks and the impact on millability of stalks. Better methods for measuring stalk flexibility and strength would improve comparisons between genotypes and enable a genetic study of the contributing factors. A study of the biochemical and anatomical features that correlate with stalk strength would also be revealing.

Finally, the parameters that have been used to assess stool architecture to date are still labour intensive and require mature plants to be grown. Identification of biochemical markers that are linked to particular stool attributes would speed up selection, particularly if they could be applied to immature plants. In the longer term, genetic markers could be a cost-effective high-throughput method but this would require further trials of heritability and genetic correlations across populations.

9. PUBLICATIONS

Glassop D, Pollock D, Perroux JM and Rae AL. (2017) Variation in stool architecture and bud sprouting; morphological traits that may contribute to ratooning. Proceedings of the Australian Society of Sugar Cane Technologists 39.

Glassop D, Perroux, J and Rae A. (2017) Sugars for ratoon growth: sugars within the underground stool structure to germinate ratoon growth. Proceedings of the Australian Society of Sugar Cane Technologists 39. (Poster)

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12. APPENDIX

12.1. Appendix 1. METADATA DISCLOSURE

Table 11 Metadata disclosure 1

Data	Trait data from plant trials
Stored Location	CSIRO secure database
Access	Restricted
Contact	Dr Anne Rae, Anne.Rae@csiro.au Dr Donna Glassop, Donna.Glassop@csiro.au

12.2. Appendix 2. Data Tables

This Appendix contains the tables of data that are described and summarised by Figures in the main section of the report.

Appendix 2 Table 1. Average brix as °Bx for commercial varieties for each year of growth. The data has been sorted on the value for the slope of the line formed between the 2016 and 2017 measurements for each variety.

Variety	2016	2017	slope of line
Pindar	20.33	16.08	-4.25
SRA1ᶲ	19.33	15.08	-4.25
Q167ᶲ	20	16.17	-3.83
Q249ᶲ	20.33	16.67	-3.66
Q252ᶲ	21.33	18.33	-3
Q96	20.67	18.58	-2.09
Q138	21	18.92	-2.08
Q234ᶲ	21.33	19.42	-1.91
Q151	19.67	17.83	-1.84
Q200ᶲ	21	19.25	-1.75
Q77	18.33	16.67	-1.66
Nco310	18.33	16.83	-1.5
Q124	21.33	19.83	-1.5
Co290	17.67	16.25	-1.42
POJ2878	16.67	15.42	-1.25
Q242ᶲ	20.67	19.5	-1.17
Empire	20.67	19.83	-0.84
Q208ᶲ	19.67	19.33	-0.34
Q232ᶲ	20	19.75	-0.25
Q113	18	17.83	-0.17
Q231ᶲ	21	20.83	-0.17
QN04-668 SRA5ᶲ	17	16.83	-0.17
Q256ᶲ	20.67	20.58	-0.09
MQ239ᶲ	18.33	18.25	-0.08
KQ228ᶲ	21.67	21.75	0.08
Q190ᶲ	19	19.58	0.58
Q117	19	20	1

Appendix 2 Table 2. Average area of stool for commercial varieties for each year of growth in cm^2. The data has been sorted on the value for the slope of the line formed between the 2016 and 2017 measurements for each variety.

Variety	2016	2017	slope of line
POJ2878	146.7	277.2	130.5
KQ228$^{\phi}$	521.9	656.5	134.6
Q124	388.3	548.9	160.6
MQ239$^{\phi}$	394.7	594.8	200.1
SRA1$^{\phi}$	372.9	592	219.1
Pindar	332.6	555.4	222.8
Q117	249	477	228
Q256$^{\phi}$	531.8	761	229.2
Q151	375.8	608.9	233.1
Q249$^{\phi}$	377.4	615.3	237.9
Empire	353.9	603.1	249.2
Q190$^{\phi}$	418.2	670.1	251.9
Q232$^{\phi}$	409.3	664.9	255.6
Q242$^{\phi}$	429	685.4	256.4
Q138	348.2	607	258.8
Q77	235.2	499.6	264.4
Nco310	340.4	607	266.6
QN04-668 SRA5$^{\phi}$	348.5	616.8	268.3
Q167$^{\phi}$	397.8	666.8	269
Q231$^{\phi}$	337.6	608.8	271.2
Q234$^{\phi}$	446.8	721.3	274.5
Q200$^{\phi}$	348.9	627.5	278.6
Q113	287.3	570.3	283
Co290	311.4	594.8	283.4
Q96	290.1	594.8	304.7
Q208$^{\phi}$	344.9	664.9	320
Q252$^{\phi}$	301.4	635.9	334.5

Appendix 2 Table 3. Average millable stalk number of commercial varieties (n = 27). The data has been sorted on the value for the slope of the line formed between the 2016 and 2017 measurements for each variety.

Variety	2016	2017	slope of line
Q256[ɸ]	13.08	12.52	-0.56
SRA1[ɸ]	9.79	9.3	-0.49
Q124	9.46	9.19	-0.27
Q167[ɸ]	11.79	12.67	0.88
Empire	6.67	7.71	1.04
Q190[ɸ]	10.71	11.9	1.19
Q138	9.17	10.57	1.4
Nco310	8.42	9.9	1.48
Q232[ɸ]	11.17	12.95	1.78
POJ2878	3.58	5.41	1.83
Q151	10.12	12.14	2.02
Q208[ɸ]	10.54	12.76	2.22
Q242[ɸ]	11.92	14.33	2.41
Q200[ɸ]	12.67	15.1	2.43
KQ228[ɸ]	9.33	11.86	2.53
Q117	4.71	7.29	2.58
Q231[ɸ]	8.33	11.05	2.72
Co290	6.38	9.19	2.81
Q96	6.42	9.33	2.91
MQ239[ɸ]	10.71	13.76	3.05
Q249[ɸ]	10.12	13.24	3.12
Q234[ɸ]	11.33	14.67	3.34
Q113	9.12	12.61	3.49
Pindar	6.04	9.63	3.59
Q77	5.17	8.82	3.65
Q252[ɸ]	9.04	13.27	4.23
QN04-668 SRA5[ɸ]	8.86	13.87	5.01

Appendix 2 Table 4. Average stalk height of commercial varieties, cm (n = 27). The data has been sorted on the value for the slope of the line formed between the 2016 and 2017 measurements for each variety.

Variety	2016	2017	slope of line
Q249$^{\phi}$	286.8	278.4	-8.4
SRA1$^{\phi}$	299.7	293.2	-6.5
Q190$^{\phi}$	295	321	26
Q96	251.2	289.3	38.1
Empire	267.3	305.9	38.6
Q252$^{\phi}$	252.3	291.7	39.4
POJ2878	227.1	272.2	45.1
Pindar	228.6	273.8	45.2
Q234$^{\phi}$	273.6	319.1	45.5
Q151	241.9	287.6	45.7
Q138	249.1	297.7	48.6
Q256$^{\phi}$	260	310.3	50.3
Nco310	222.3	274.2	51.9
Q208$^{\phi}$	270.1	324.7	54.6
KQ228$^{\phi}$	242.8	299.1	56.3
QN04-668 SRA5$^{\phi}$	310.2	367.2	57
Q167$^{\phi}$	216.3	274.2	57.9
Q200$^{\phi}$	225.6	285.7	60.1
MQ239$^{\phi}$	270	332.1	62.1
Q232$^{\phi}$	242.9	308.3	65.4
Q242$^{\phi}$	258.2	323.8	65.6
Q124	250.9	319.2	68.3
Co290	208.4	280.1	71.7
Q77	180.7	254.4	73.7
Q113	223.9	305.1	81.2
Q231$^{\phi}$	197.2	283.1	85.9
Q117	187.9	274.2	86.3

Appendix 2 Table 5. Average weight of 8 stalks (kg, n = 3). The data has been sorted on the value for the slope of the line formed between the 2016 and 2017 measurements for each variety.

Cultivar	2016	2017	slope of line
Q232[PBR]	10.07	7.77	-2.3
Q249[PBR]	10.37	9.18	-1.19
Q96	12.41	11.24	-1.17
Q138	10.13	9.41	-0.72
POJ2878	11.24	10.7	-0.54
Q151	9.5	9.45	-0.05
Q252[PBR]	10.83	10.88	0.05
QN04-668 SRA5[PBR]	13.3	13.64	0.34
SRA1[PBR]	12.77	13.12	0.35
Q234[PBR]	10.85	11.26	0.41
Pindar	12.59	13.29	0.7
Q190[PBR]	13.3	14.13	0.83
Empire	15.27	16.46	1.19
Q167[PBR]	7.86	9.07	1.21
Nco310	7.38	9.22	1.84
Q77	7.48	9.38	1.9
Q256[PBR]	10.13	12.11	1.98
KQ228[PBR]	10.53	12.84	2.31
Q242[PBR]	7.73	10.1	2.37
Q208[PBR]	8.78	11.28	2.5
MQ239[PBR]	8.37	10.97	2.6
Q231[PBR]	6.2	8.88	2.68
Co290	9.23	11.92	2.69
Q113	7.9	10.75	2.85
Q124	10.27	13.22	2.95
Q200[PBR]	8.42	11.73	3.31
Q117	7.92	11.54	3.62

Appendix 2 Table 6. Average stalk diameter, mm (n = 27). The data has been sorted on the value for the slope of the line formed between the 2016 and 2017 measurements for each variety.

Variety	2016	2017	slope of line
Q138	24.23	20.095	-4.135
Q252Ⓟ	25.108	21.086	-4.022
Q96	26.428	22.571	-3.857
QN04-668 SRA5Ⓟ	25.394	21.667	-3.727
Q249Ⓟ	23.041	19.524	-3.517
Q151	24.126	20.81	-3.316
Q234Ⓟ	23.515	20.286	-3.229
Q190Ⓟ	26.385	23.333	-3.052
Empire	30.07	27.048	-3.022
Q77	22.68	19.886	-2.794
Pindar	29.675	26.971	-2.704
Q232Ⓟ	24.008	21.381	-2.627
KQ228Ⓟ	25.868	23.476	-2.392
SRA1Ⓟ	27.505	25.548	-1.957
POJ2878	25.222	23.492	-1.73
Q256Ⓟ	23.62	22.095	-1.525
MQ239Ⓟ	22.154	20.714	-1.44
Q167Ⓟ	22.286	21	-1.286
Q124	24.04	22.762	-1.278
Nco310	21.527	20.333	-1.194
Q242Ⓟ	20.549	19.476	-1.073
Q113	22.639	21.675	-0.964
Q208Ⓟ	21.788	20.905	-0.883
Q231Ⓟ	20.401	19.571	-0.83
Q117	23.71	23.048	-0.662
Q200Ⓟ	23.626	23.19	-0.436
Co290	25.183	25.143	-0.04

Appendix 2 Table 7. Average range of stalk classifications.

Variety	2016	2017	slope of line
Q242Ⓟ	2.667	2.333	-0.334
Q190Ⓟ	2.333	2.333	0
Q96	2	2.333	0.333
Co290	2	2.667	0.667
Empire	2	2.667	0.667
Q249Ⓟ	2	3	1
SRA1Ⓟ	2	3	1
POJ2878	1.667	3	1.333
Q113	2	3.333	1.333
Q234Ⓟ	2	3.333	1.333
Q252Ⓟ	2	3.333	1.333
QN04-668 SRA5Ⓟ	2.667	4	1.333
MQ239Ⓟ	2	3.667	1.667
Nco310	2	3.667	1.667
Q124	2	3.667	1.667
Q138	2	3.667	1.667
Q151	2.333	4	1.667
Q167Ⓟ	2	3.667	1.667
Q200Ⓟ	2	3.667	1.667
Q232Ⓟ	2	3.667	1.667
Q256Ⓟ	2	3.667	1.667
Q77	2	3.667	1.667
Q117	1.333	3	1.667
Pindar	1.667	3.667	2
KQ228Ⓟ	2	4	2
Q208Ⓟ	2	4.333	2.333
Q231Ⓟ	2	4.667	2.667