Environmental and varietal factors predisposing to suckering in sugarcane in the wet tropics

Thesis submitted by

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ABSTRACT

A recent review of the trends in productivity of sugarcane grown in the wet tropics of Australia revealed a decline in sugar content at the mill. Many factors were implicated in this decline. Sugarcane suckers are shoots that appear when the original stalks produced by the crop are more or less mature. Suckers are harvested along with the mature stalks in crops that are mechanically harvested. The low sugar content of suckers, due to their immaturity, causes dilution of the sugar content of the harvested material. Suckers also increase the amount of extraneous matter in the harvested material, this results in further dilution of the sugar content. Farmers are paid on a formula which is biased towards high sugar content. The additional yield, as a result of sucker growth, does not outweigh the loss due to the lower sugar content of the crop. This results in a loss of profitability. Little was known about suckering in sugarcane. The few observations that exist in the literature are mostly speculative. That meant that there was a need to better describe suckering and to establish what environmental factors cause it.

Sugarcane suckers of three cultivars were found to have different morphology to normal stalks of similar age. Suckers had greater maximum breadth of the leaf lamina, longer leaf sheaths, produced their leaves at a greater height above ground and had thicker internodes. When allowed to grow, the buds produced on a sucker did not posses this altered morphology, which indicted that the change in morphology was transient. Gene expression in the apex of sucker stalks was also found to be different to that of normal stalks, which provides further evidence for the differences between the stalk types and

could potentially provide some evidence as to why these differences occur. Some evidence was found for the translocation of sucrose from the mature parent stalk to a young developing sucker. This matter needs to be investigated further as mature stalks may lose substantial amounts of sucrose to sucker stalks even before dilution occurs at the mill. This negative impact of suckering on productivity has yet to be considered by the industry. The presence of a mature parent stalk was also found to have an effect on sucker morphology. In the absence of a mature stalk, sucker morphology changed to being more similar to that of a normal stalk. This too provides evidence for the translocation of substances from the mature stalk to the sucker.

The availability of nitrogen and moisture was shown to increase suckering. A significant interaction effect was also found between these two factors. The availability of light beneath the crop canopy was also shown to have an effect on suckering in some experiments but for the most part the results were inconclusive. Further investigation is required in order to establish the role of light in suckering. The data generated from this study has many implications for crop agronomy and plant breeding. Farmers could potentially reduce suckering by careful management of nitrogen fertilisation. The work has also highlighted a need to understand the link between trash blanketing and suckering. The breakdown of a trash blanket may provide nitrogen to the plant at the time that suckers are being produced. In order to reduce suckering plant breeders may need to alter the weighting of some traits in the breeding program. Many of these traits relate to the ability of the crop to remain erect under wet and windy conditions. Managed environment selection trials may also need to be considered. The required environmental conditions for such a trial have been defined. These trials would provide data on the genetic differences in suckering propensity in years when these differences

would not normally be expressed. While much remains to be done, this work has laid the groundwork for starting to manage the problem of suckering in sugarcane.

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Sugarcane suckers growing in a crop in the Mulgrave district near Cairns, Australia. Photograph provided by Dr. Nils Berding.

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DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

5/01/03

Barry Salter

Part A: Introduction and Literature Review

Chapter 1. Introduction

Sugarcane production is a major agricultural industry in north eastern Australia. Competitive market pressures require continuing productivity improvements to sustain profitability. Therefore, a decline in the sugar content of sugarcane at the mill in far-north Queensland, Australia is a major concern to those in the industry. This was highlighted by an allocation of $(AUS) 13.5 million in 1998 to research and development funding to primarily boost sugar content levels.

A review of the productivity trends in sugarcane in the wet tropics of Australia was conducted by Leslie and Wilson (1996). They analysed the productivity in six mill areas in far-north Queensland for the period 1960 – 1995. They reported a fairly consistent decline in commercial cane sugar (CCS), which is a measure of sugar content. However, this decline was offset wholly or in part by an increase in the tonnes of cane per hectare (TCH) harvested. A mean gain across mill areas of 15% in TCH was offset by a mean loss of 13% CCS. This resulted in a fairly static amount of sugar being produced per unit area over the period studied. However, farmers are paid on a formula that is biased towards high CCS, and therefore the loss of CCS has resulted in lower on-farm profitability. The trend in CCS decline has continued past the period taken in by Leslie and Wilson's review, with the Mulgrave mill recording seasonal averages for CCS of 11.81 and 10.92 for 1997 and 1998 respectively. These two years have the lowest CCS on record (Crook *et al.* 1999). The mill's worst results before 1997 and 1998 were also recorded in recent times, 1993 and 1995 (Figure 1.1).

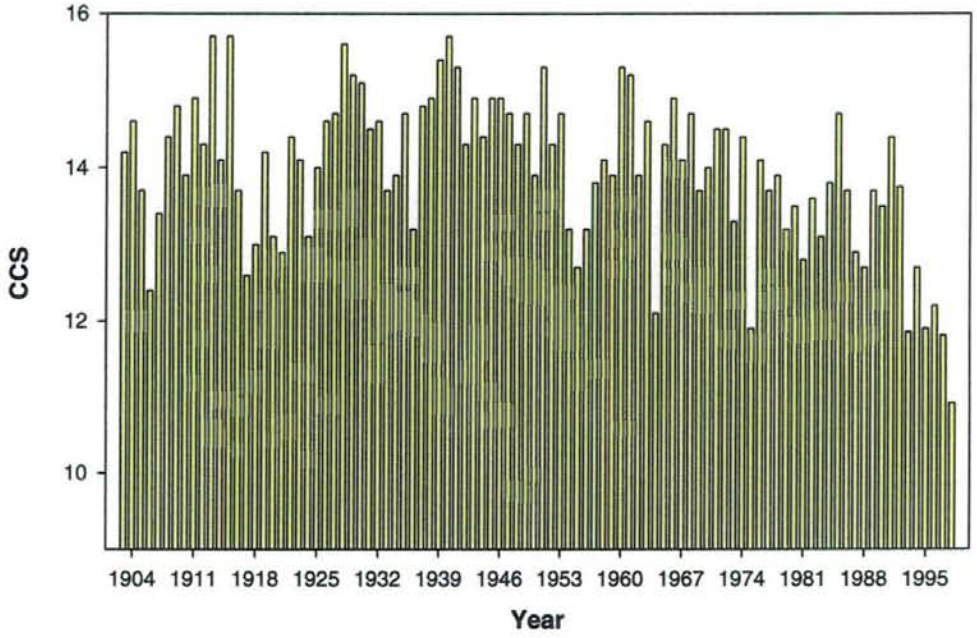

Figure 1.1 Yearly average CCS for the Mulgrave district (1903-1998). Redrawn from Pope (1997). Data for 1997 and 1998 taken from Crook *et al.* (1999).

Further work by Lawes *et al.* (2000) has shown that while CCS measured at the mill was declining, CCS of sound stalks (living undamaged stalks cut at ground level with leaf material and immature stem removed) remained constant. This indicates that the CCS of sound cane stalks has not changed over time, and therefore implicates factors associated with the processing (harvesting, transport and milling) of sugarcane causing the decline in CCS that has been reported.

Numerous factors have been implicated as causes of CCS decline. The change from hand harvesting to mechanical harvesting, the change from harvesting burnt cane to harvesting green cane, the introduction of green cane trash blankets (GCTBs), crop lodging and suckering (Leslie and Wilson 1996).

Sugarcane suckers are shoots that appear late in the growing season, when other tillers (main stalks) have already produced a substantial amount of millable cane (Borden 1948) (Figure 1.2). Suckers are harvested along with mature stalks. In one-year crops, such as those in the wet tropics of Australia, suckers are low in sugar content due to the short period of growth before they are harvested.

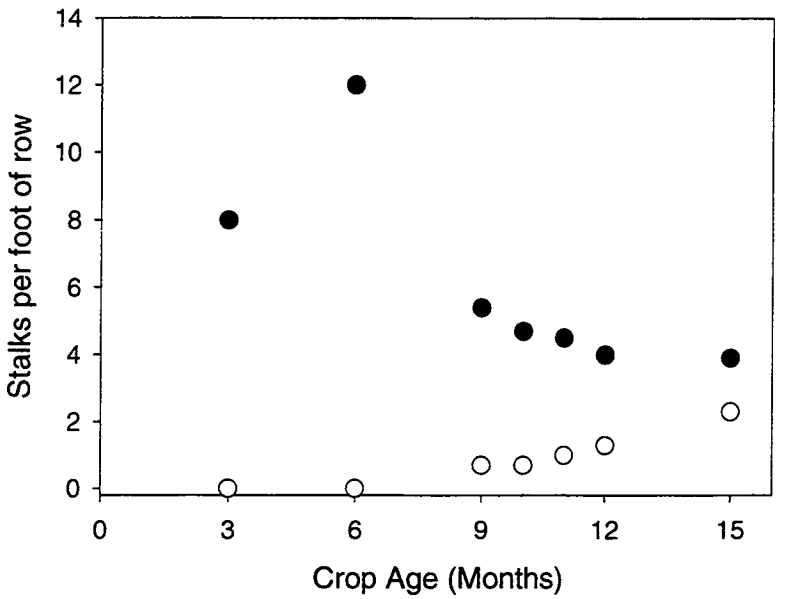

Figure 1.2 Number of suckers (○) and main stalks (●) present in a crop of cultivar 32-8560 planted on 8 November 1944 in Hawaii (redrawn from Borden 1948).

Ivin and Doyle (1989) analysed four cane varieties and found that suckers had an average CCS of 1.3, compared with 14.7 for mature stalks. Data from the Mulgrave mill area for four varieties in 1996 indicated that the CCS of suckers ranged between 3.25 and 7.78 at harvest (Pope 1997). The difference between the measured CCS content of suckers in these two studies may have been due to different sucker age.

A consequence of mechanical harvesting is that low CCS stalk materials, derived from suckers, are crushed with mature stalks. Therefore, sucker stalks lower the overall CCS of the processed crop by diluting the sucrose content of the mature stalks. Hurney and Berding (2000) have shown that CCS could decrease by one unit for every 10% by weight of suckers included in the harvested sample. Suckers also increase extraneous matter (EM) by adding tops (immature internodes and green leaves) and trash (dead leaf) to the harvest. EM reduces CCS due to its low sucrose, and high fibre content (Clarke *et al.* 1988; Ivin and Doyle 1989). Suckers may also draw upon the sucrose in the mature stalk to aid their own growth. However, this is only alluded to in early references (Hes 1954; Barnes 1974; Clements 1980), and is yet to be shown experimentally. This too could result in a reduction in CCS, as the sucrose would be used in the growth of the sucker and would therefore not be recovered at the mill.

Suckering in sugarcane is not a well-described phenomenon in the scientific literature. Suckers appear to have different morphology to other stalks (van Dillewijn 1952; Hes 1954; Barnes 1974), but no data are presented or cited to show what these differences are. It is also not known whether any differences in morphology between suckers and other stalks are similar for different cultivars. The environmental stimuli that cause suckering have not been identified. However, there is some speculation that light, nitrogen and moisture availability are all involved.

The paucity of knowledge on suckering in sugarcane, combined with its apparent importance in reduced CCS realised at the mill, meant that there was a need to better define the phenomenon, as well as determine what environmental signals were causing

it. This was a precursor to trying to reduce the impact of suckering on the profitability of sugarcane growing. To this end, the general aims of the investigation were to:

1. Describe the morphology of suckers
2. Determine the inter-stalk relationship between main-stalks and suckers
3. Determine environmental stimuli for suckering in sugarcane and establish whether responses differed between cultivars

The morphological studies were required as there was some confusion in the literature between the term sucker and tiller, with many studies referring to them as being one and the same. Describing sucker morphology aids in their definition and identification.

The inter-stalk relationship between sucker and main-stalk may be important, as some authors have suggested that suckers derive nutritional support from the main-stalk for their growth. If this does occur, main-stalks with suckers attached could have lower sugar content. This would mean that dilution at the mill is not the only negative effect of suckers on CCS.

Identification of the environmental stimuli for suckering in sugarcane could provide valuable information that can be used in order to reduce their occurrence in sugarcane crops in the future. This knowledge may be applied via plant breeding programs and agronomy to reduce the effects of suckering

In Chapter 2 the literature about suckering in sugarcane and other closely associated traits are reviewed in order to establish a starting point for the experimental chapters

that follow. A brief description of the origins of sugarcane, the Australian sugar industry and some of the agricultural practises used in the industry has been included.

Chapter 2. Literature review

2.1 Sugarcane, its origin and agriculture

2.1.1 Origin of commercial sugarcane

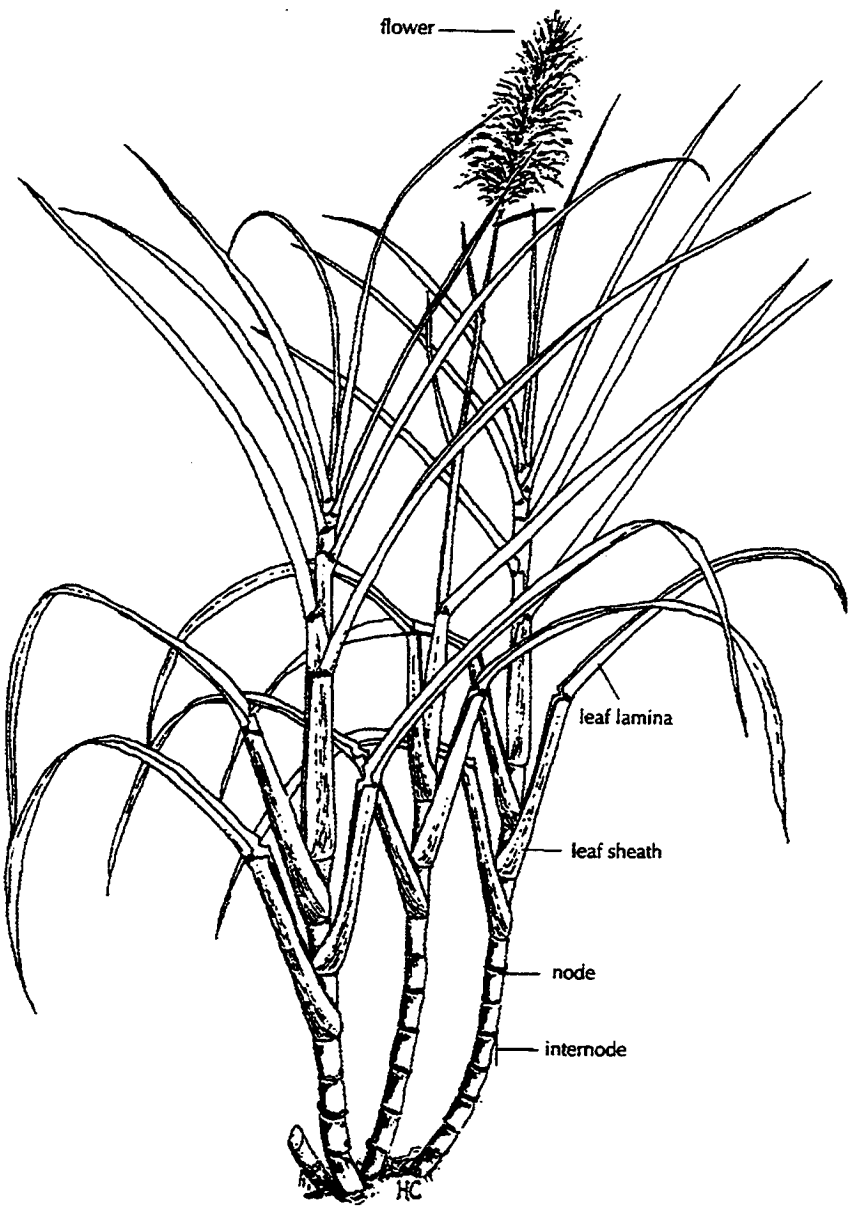

Figure 2.1 The sugarcane plant (drawn by H. Chaillet in Soopramanien 2000)

Sugarcane (*Saccharum spp.*) is a tropical grass that is grown as a commercial crop in many regions/countries around the world that have tropical and/or sub-tropical climates. Commercial cultivars differ from region to region, and are the product of many years of plant breeding. Some of the morphological features of the sugarcane plant and stalk are represented in Figures 2.1 and 2.2.

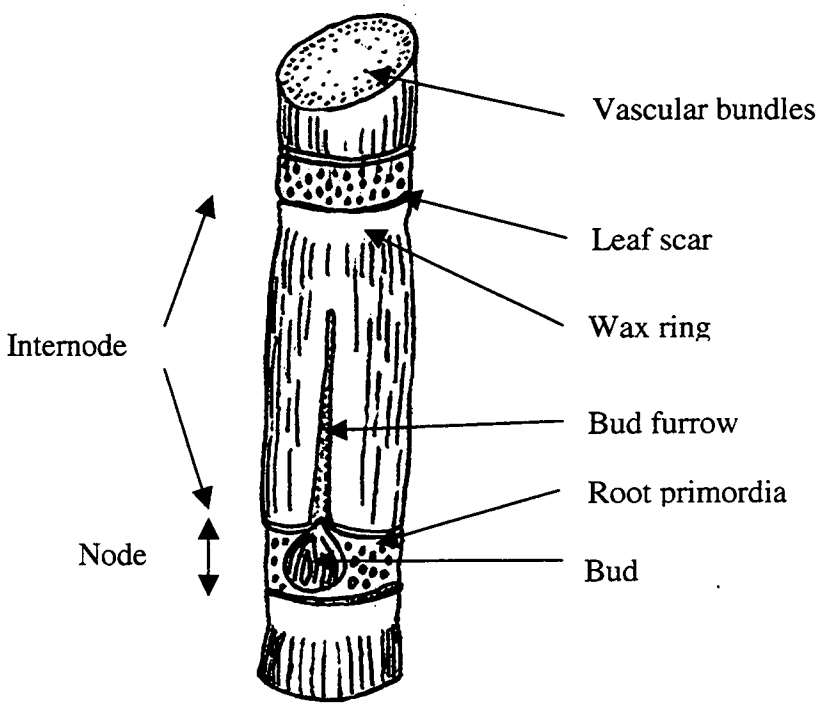

Figure 2.2 Drawing of a section of cane stalk (redrawn from Artschwager 1940)

Artschwager and Brandes (1958) stated: 'The indispensable sugar-bearing component of essentially all the numerous and widespread varieties of domesticated sugarcane, which form a complex of polyploid hybrids, is provided by a few selected representatives of the tropical, thick-stemmed horticultural species *Saccharum officinarum'. S. officinarum* generally shows high weight per stalk, high tonnage per unit area, resistance to some diseases, adaptability to harsh climates, low fibre content, high purity (% sucrose), and low proportion of invert sugar (Artschwager and Brandes

1958). This species has many of the qualities that make sugarcane such an excellent producer of sugar. In order to make sugarcane better adapted to temperate zone conditions and for resistance to other diseases (e.g. mosaic and root rot), *S. officinarum* was crossed with other species, notably *S. spontaneum. S. spontaneum* is a wild species with high vigour but low sucrose content (Barnes 1974). High sugar content was restored through repeated backcrossing with *S. officinarum*, a process termed nobilisation (Jannoo *et al.* 1999). Most current commercial cultivars are hybrids derived from the few original crosses of *S. officinarum* and *S. spontaneum.*

The lack of variation between current commercial cultivars in the Australian sugar industry is a concern to plant breeders. Berding *et al.* (1998) stated that a long-term strategy of breeding for improved productivity as well as genetic diversity is required in the Australian industry. Genetic uniformity increases crop vulnerability (Berding *et al.* 1998). Increasing genetic variability may be possible by backcrossing with the original *Saccharum* species (*S. officinarum, S. spontaneum, S. robustum, S. barberi, S. sinense, S. edule*), as well as other related species.

It is generally thought that the original *Saccharum* species came from the South Pacific. Wild forms evolved in isolated parts of Asia, New Guinea, and insular Melanesia (Alexander 1973). The centre of origin and diversity for *S. spontaneum* is thought to be in India (Roach 1989). It is presumed to be a product of introgression among members of the *Saccharum* complex (Daniels and Roach 1987). *S. officinarum's* origin is thought to be in New Guinea about 8000-15000 B.C. (Artschwager and Brandes 1958). *S. officinarum* is the product of selection as a chewing cane in 'gardens' in this region. It was presumed these chewing canes were selected on the basis of sweetness (Grassl

1974). Artschwager and Brandes (1958) hypothesized that much of the dispersal of sugarcane, throughout the region, was done by man, and that natural hybridisation occurred in the different regions.

2.1.2 Sugarcane agriculture within Australia

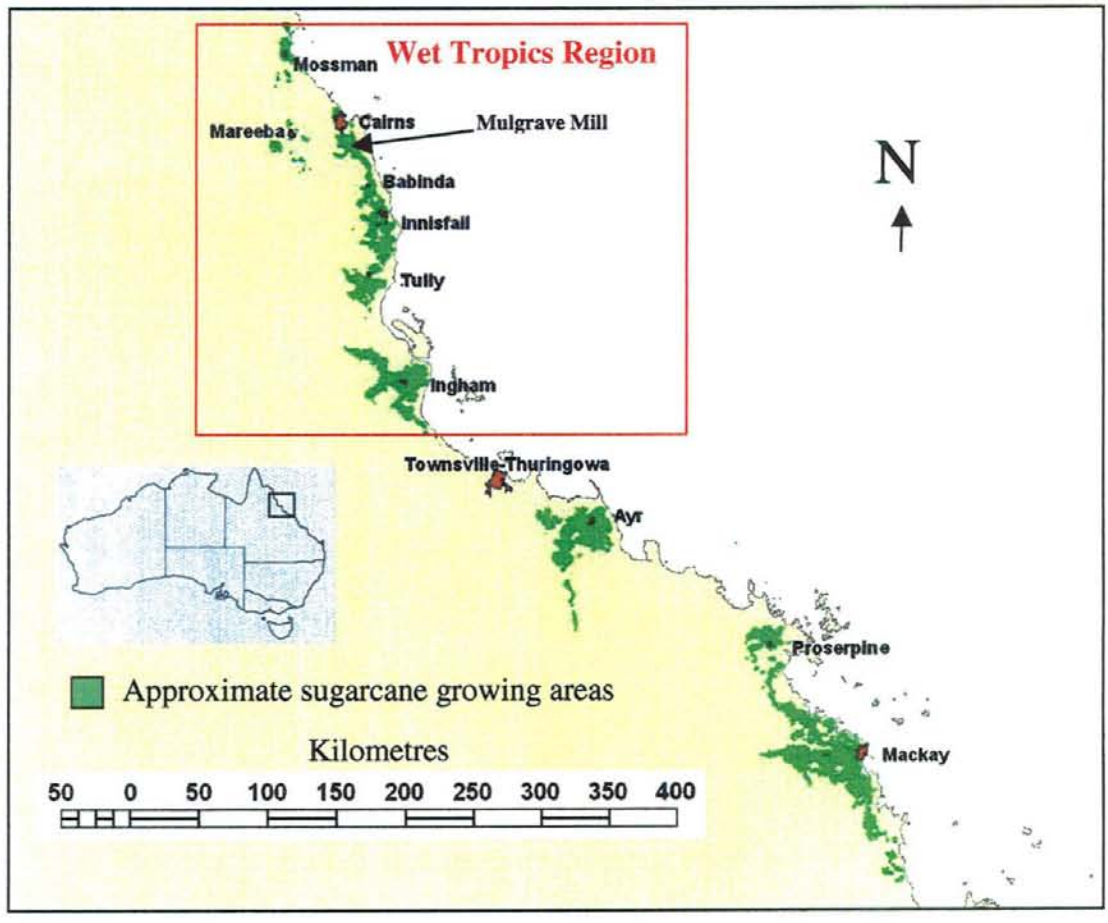

Figure 2.3 Sugarcane agricultural regions in North-eastern Australia indicating the wet tropics region and the Mulgrave Mill discussed in Chapter 1 (data from the Queensland Department of Natural Resources 1995).

In Australia, the majority of sugarcane is grown on the coastal plains and river valleys along 2100 kilometres of the eastern coastline between Mossman in northern Queensland (QLD) and Grafton in the northern part of the adjoining state of New South Wales (NSW).There is a small industry in the Ord River region of the state of Western

Australia. This study concentrates on the wet tropics of Australia. Isbell and Edwards (1988) described this as being an area that receives more than 1500 mm annual average rainfall. The regions between Mossman in the north and Ingham in the south are located in this wet tropics region (Figure 2.3).

2.1.3 Agronomic practices

Sugarcane is propagated asexually. Sections of stalk, setts, are planted and the buds on the sett give rise to the primary stalks. Initially, the primary stalk produces many short internodes, each of which contains an axillary bud. This mass of underground buds gives rise to secondary stalks, which in turn give rise to tertiary stalks and so on (Figure 2.4). This process of underground branching is termed tillering, and results in numerous stalks being produced from the original bud(s) located on the planted sett. This mass of stalks, originating from a single bud, is often referred to as a stool.

Sugarcane is generally grown in single rows around 1.5 m apart. Dual rows (pairs of rows 0.5 m apart with 1.8 m between centres) are sometimes used but are not common practice. Increases in yield have been reported with the use of high-density planting (HDP), 0.5 m rows (Bull and Bull 2000). However, this practice has not been widely adopted.

Sugarcane is harvested mechanically, after approximately 9 – 15 months, in the wet tropics of Australia. Stalks are cut into billets (small sections of stem) and are transported to the mill either by rail or road. After harvesting, the underground section of the stool remains in the soil and the next crop generation grows from it. The new

crop is termed a ratoon, and generally a crop is allowed to ratoon 3-4 times before it is ploughed out and replanted. Hand harvesting is no longer practised in Australia, due to high labour costs. Hand harvesting is still practised in some countries (South Africa, China).

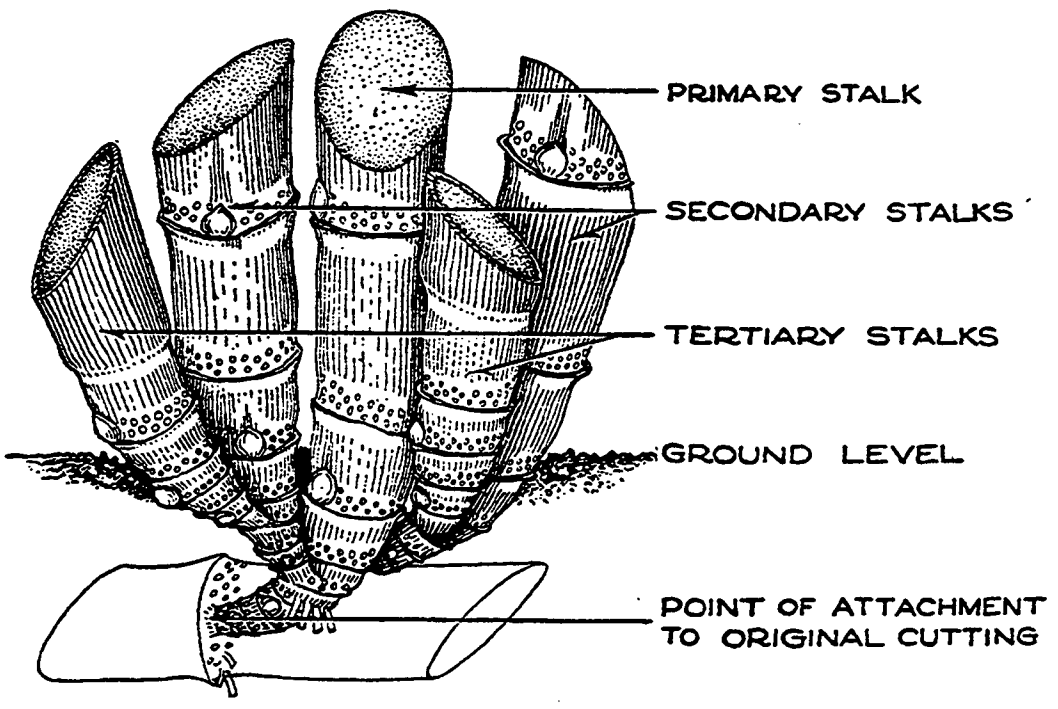

Figure 2.4 The underground portion of a cane stool (from Martin 1938)

The change from hand harvesting to mechanical harvesting occurred over a fifteen-year period (1957-1972). In 1968, 50% of all cane was harvested mechanically, and by 1972, 100% of cane in the wet tropics was harvested mechanically (Leslie and Wilson 1996). More recently there has also been a shift from harvesting burnt cane to harvesting green cane (1978-present, Leslie and Wilson 1996). This was due to both the adoption of mechanical harvesting and environmental concerns. Harvesting burnt cane is rarely practised in the wet tropics. However it is still conducted in other sugarcane growing regions in Australia. Green cane harvesting (GCH) has brought about the practice of green cane trash blanketing (GCTB), where the unwanted leaf and immature

stalk is left on the paddock as a trash blanket. Trash blankets retain moisture in the soil, reduce soil temperature (Chapman *et al.* 2001), and return nutrients and organic matter to the soil (Robertson and Thorburn 2000). The majority of growers in the wet tropics of Australia use trash blanketing.

Fertilisation of the crop usually takes place in the months following planting or ratooning. Nitrogen recommendations for the Herbert River district (centred on Ingham Fig 2.4) in 2000 were between 80 and 200 kg N/ha (Anon. 2000), depending on land quality and cultivar selection. Irrigation (either full or supplementary) is applied to crops in dry areas. This practice is not usually required in the wet tropics regions.

2.2 Productivity trends in sugarcane grown in the wet tropics of Australia

2.2.1 Factors thought to contribute to CCS decline

Many factors have been proposed as contributing to CCS decline in the wet tropics. A review of these can be found in Leslie and Wilson (1996). Extraneous matter (EM) in the harvest is any material with low sucrose content; it usually refers to green leaf, trash (dead leaves), and immature stalk from the plant. Extraneous plant matter, and soil, increase the total biomass of the harvest but contribute very little sucrose. They therefore dilute the sucrose concentration in the harvested material. Brotherton (1980) proposed that for every percentage point of EM in the harvest, a loss of 0.15 units of CCS would be incurred. There has been an increase in extraneous matter going into the mill in recent years. Initially this increase was due to a change from hand harvesting to

mechanical harvesting. This resulted in a change from clean hand harvested whole stalks to stalks accompanied by some tops and trash being delivered to the mill. Mechanical harvesting appears to be associated with an average 5% increase in EM (Leslie and Wilson 1996). More recently the switch from burnt cane to GCH has resulted in a further increase in EM (Smith *et al.* 1984; Pope 1997). EM levels are also partly associated with weather conditions, as the harvesters' ability to clean stalks, and therefore reduce EM, is not as good under wet conditions. Mechanical harvesting also results in losses of cane, 7.4 t/ha green cane and 3.4 t/ha burnt cane (Linedale and Ridge 1996).

While there is little evidence to suggest that the introduction of GCTB has contributed to CCS decline, its introduction has coincided with the decline in CCS (Leslie and Wilson 1996). Therefore, there has been some suggestion that it is, in part, a causal factor.

While there have been changes in the sugarcane cultivars grown in far-north Queensland, it is not believed that the decline in CCS in the wet tropics is due to inadequate cultivars (Leslie and Wilson 1996). Cultivars grown today tend to be thinner and taller than those when hand harvesting was practised. The trend to grow cane for higher yield, has led to cultivars with large numbers of thin tall stalks and an increase in its tendency to lodge (Leslie and Wilson 1996). Cane usually lodges (falls over) due to windy and/or wet conditions, once it has reached sufficient height. In an experiment in the wet tropics, lodging was shown to cause an 18 - 22% reduction in sugar yield in 1999 (Singh *et al.* 1999) and 15 – 35 % in 2000 (Singh *et al.* 2000). The trials were conducted at Feluga, just north of Tully (Figure 2.4). Harvesting a lodged crop also

increases EM, as topping does not occur on the non-erect stalks. Topping is the process of removing the unwanted leaf and immature stem from the top of the stalk. Therefore, if windy and wet conditions are experienced when the cane is relatively tall, these newer cultivars may contribute to the decline in CCS at the mill. There is also an industry perception that lodged crops have a higher occurrence of suckers.

2.2.2 Suckering in sugarcane

The presence of suckers in sugarcane crops at harvest is a major factor contributing to the decline of CCS in the wet tropics (Leslie and Wilson 1996). As noted previously, the low sucrose content of suckers dilutes the sucrose content of the harvested material. Suckers also increase EM in the harvest, which further dilutes the sucrose content.

Data from Borden (1948) indicated that suckers appear after approximately nine months (Figure 1.2), whereas primary stalks and tillers (main stalks) were produced within the first six months, with the majority being produced in the first three months of the crops growth. These data are from Hawaii, where crops are grown on a 24-month cycle.

Hes (1954) states: 'Although everyone more or less acquainted with cane will recognise such a sprout (sucker) in time, a description of it is not so simple'. While underground buds produce both suckers and tillers, suckers appear to have a different morphology to other stalks. van Dillewijn (1952) and Barnes (1974) described suckers as often being thicker than maturing cane, succulent, and as stalks that grow faster with

well-developed buds and longer internodes than the main crop. Sucker leaves are also thought to be shorter than normal cane leaves (Hes 1954). Hes described the difference between suckers and other stalks as 'striking'. No data were presented or cited to back up these statements.

In two-year crops, such as those in northern New South Wales, it has been shown that suckers produced in the first year contribute positively to the sugar content at harvest. Suckers appear to accumulate sucrose at a similar rate to normal stalks if allowed to grow for this extra period of time (Hughes and Muchow 2000). Hes (1954) indicated that suckers' influence on sucrose yield was initially negative, but after sufficient growth, they contribute positively. However, this observation was not supported by any data. Two-year crops are generally grown in cooler regions, where at the end of two years, the majority of the stalks grown in the first year are still present. Growing cane crops on a two-year cycle is not practised in the wet tropics. Stand-over cane in the wet tropics (cane grown for two years due to inability to harvest after one year) is usually low in sugar content and low in yield, as most of the stalks grown in the first year have deteriorated in some way.

In countries where cane crops are harvested by hand, suckers are not included in the harvest. They are therefore not considered to be as problematic as in countries where cane is mechanically harvested. Barnes (1974) stated that it was common for suckers to be left uncut in a hand harvested field so that they could continue to grow into marketable cane in the next season. In Java, suckers that are over two metres tall were included in the harvest as it is at this point that they are considered to be beneficial to the harvest (Hes 1954).

The apparent rapid growth rate of sugarcane suckers, sometimes under a dense canopy, suggests that suckers may draw upon more than their own photosynthetic source in order to grow. This carbon would be used in sucker growth, at the expense of sugar content in mature stalks. Hes (1954) found an average increase of 0.5 ton of sucrose produced per hectare, when suckers were constantly removed from the crop. Although this result indicated that sucker growth may lower the sugar content of the mature stalks from which they grow, it is not known whether this was a statistically significant response, and the average sucrose produced per hectare was not reported. Hes (1954) stated 'Cutting the suckers did not prove to be of great value'.

Bull and Glasziou (1963) proposed that natural selection for increased sucrose content of cane may have occurred due to high sucrose canes being able to rapidly mobilize sucrose to support sucker growth. These canes, through promoting suckering, would have a competitive advantage over canes with lower sucrose content. Bull and Glasziou implied that suckering is a trait brought to modern canes through the *S. officinarum* genome. The relative levels of suckering in *S. officinarum*, *S. spontaneum*, and other species do not appear to have been determined, and therefore, the hypothesis of the origins of suckering remains untested. To my knowledge, there have been no measurements at the individual stalk level that shows suckers are significantly supported by the stalk from which they are growing. It does appear logical that at least until the sucker is green and potentially autotrophic, that this would be the case.

Chemical ripeners have been shown to increase the sucrose yield of suckers (Andries and DeStefano 1979; Andries and DeStefano 1980). These data were from Florida USA, and may not be applicable to the Australian sugarcane cultivars or climatic conditions. Chemical ripeners are not currently used in Australia mainly due to mixed results in field trials (McDonald *et al.* 2000). The opportunity to use ripeners to increase the sucrose yield of main stalks and suckers may be a viable option if further research is conducted and demonstrates benefits under Australian conditions.

The propensity to sucker has been shown to be highly variable across clones, ranging from 0.7 – 31 tonnes sucker stalks per hectare in 1998 final assessment trials, and 0.7 – 43 in 1999 trials (Berding and Hurney 2000). It has also been suggested (N. Berding unpubl.) that propensity to sucker is not correlated to ratooning propensity in all cases. This genetic variation for the trait, uncoupled from ratooning ability, would be likely to prove valuable for breeding programs that are aimed at reducing suckering. It allows breeders to select cultivars that have good ratooning and tillering capacity without inadvertently selecting for suckering.

In Australia, the Bureau of Sugar Experiment Stations (BSES) conducts the majority of sugarcane breeding. BSES cultivar guides rate suckering in terms of whether it is a high, medium or low suckering cultivar. This is sometimes followed by a comment such as 'late in season' or 'numerous small suckers'. It has been suggested that the present increase in suckering is due to cultivars being bred for their high tillering capacity, and as a consequence of this, suckering has been selected for unintentionally. Unintentional selection for suckers may occur if they are included in the cane yield (tonnes) of trials, while CCS is measured from mature sound stalks only. According to

Berding and Hurney (2000), this results in a plus-plus scenario, where the clone is reported as high yielding with high CCS. Barring other factors, a clone could be selected on this basis. It is only when the cultivar reaches the mill that the penalty is realised, as the high yield is partly due to a high proportion of low sucrose material. Berding and Hurney (2000) stated that BSES upgraded their penalty for sucker culm content in 1998. This should result in cultivars with lower suckering propensity being released in the future.

2.3 Tillering in sugarcane and other grasses

The factors that lead to the emergence of suckers from the crop are not understood. Many ideas have been put forward, but are mainly based on very limited evidence. Andries and DeStefano (1979) stated that suckers appear when cane becomes recumbent (lodged) or when temperature and rainfall create good growing conditions late in the season. Generally, it is thought that light, nitrogen and water are likely to be important in the growth of suckers, but there is a lack of data to support these suggestions. Since suckering is likely to be similar to tillering, at least in some respects, it is important to understand the role that these factors, and others, play in the tillering process.

2.3.1 Light

Light has been implicated in the process of suckering. This is mainly due to the observation that suckers are more numerous in the outside rows (Bonnett *et al.* 2001),

ends of rows, and in lodged areas within the sugarcane crop. These regions of the crop would typically have greater exposure to light due to reduced light interception by surrounding plants.

The light environment is a determinant of growth and development of sugarcane and other grass species. Generally, grasses go through two phases of vegetative growth, a phase of stooling or tillering, and a phase of stem elongation. There is some overlap of these two phases, and this sometimes results in the youngest tillers not being able to survive, as they are not able to compete with the older, larger tillers for light. If cane is given enough space and light to grow, it will continue to initiate new tillers resulting in stools with several hundred stalks (van Dillewijn 1952). However, this does not usually occur in commercial fields. Under commercial field conditions, there is a more clearly defined phase of tillering and elongation.

Tillering has been shown to decrease with reduced light intensity in sugarcane (Verret and McLennan 1927; Martin and Eckart 1933), barley (Ellen and van Oene 1989), ryegrass (*Lolium perenne*) (Spiertz and Ellen 1972), wheat (Wattal and Asana 1974) and various other grass species (Eussen 1981; Everson *et al.* 1988; Deinum *et al.* 1996).

Light quality has also been shown to affect tillering. Plants have a mechanism by which they can determine the light environment in which they are located and the presence of other plants around them (Ballare *et al.* 1987). Plants do this by detecting differences/changes in the red to far-red ratio of light. Red light is used by the plant in photosynthesis; far-red light is not used in this process, and is partly reflected off leaves

and stalks (Kasperbauer and Karlen 1994). Therefore, as a crop grows the ratio of red to far-red light beneath the canopy decreases. This change in light quality has been observed under sugarcane canopies (Ludlow *et al.* 1990). A decrease in the red to far-red ratio of light has been shown to reduce tillering in barley (Davis and Simmons 1994), ryegrass (*Lolium multiflorum*) (Casal *et al.* 1985; Casal *et al.* 1987a), wheat (Casal 1988), and other grass species (Deregibus *et al.* 1985; Skalova and Krahulec 1992). Changes in the red/far-red ratio of light are detected by the pigment phytochrome (Borthwick 1972). The relative levels of the different forms of phytochrome, determined by the red/far-red ratio of light, can cause biochemical changes within the plant. Therefore, this mechanism allows the plant to detect changes in light quality and react to them.

Increased availability of light within the field, such as ends of rows, outside rows and lodged areas within the crop, could result in more suckers as a result of either greater light intensity or altered light quality when compared to the rest of the crop. However, other factors may also be involved as increased availability of light may also result in increased soil temperatures.

2.3.2 Nitrogen

It is thought that nitrogen availability may play a role in suckering, but there is limited available evidence to support these claims. Borden (1948) demonstrated that plots with high N applications produced more suckers than plots with low N applications (cultivar 32-8560 in Hawaii). However, it is not clear whether this was a statistically significant difference. Hurney and Berding (2000) found no increase in suckering when nitrogen

was applied at four rates (0, 70, 140 and 210 kg N/ha) to three cultivars (Q117, Q120 and Q138). Hurney and Berding's experiment differed from that of Borden's, as they applied nitrogen between 95 and 134 days after planting, whereas Borden's experiment included nitrogen applications up to 11 months after planting. The availability of nitrogen in the soil at the time when suckers are initiated may have differed between these two experiments. Suckering has been found to be greater under GCTB than burnt trash management (Chapman *et al.* 2001). GCTB has been shown to increase total soil N, but this may only become available to the plant in the long term, after many seasons of trash blanketing (Robertson and Thorburn 2000).

Nitrogen has been shown to increase tillering in sugarcane in numerous studies (Borden 1945, 1948; Eavis and Cumberbatch 1977; Singh 1977, 1978a, 1978b; Abayomi 1987; Shrivastava and Kumar 1984; Ng Kee Kwong *et al.* 1999), as well as other agriculturally important grasses, like barley (Garcia del Moral *et al.* 1984) and wheat (Mahmoud and Osman 1981; Silberbush and Lips 1991). If nitrogen becomes available late in the growing season, tillering may be re-activated, resulting in late tillers (suckers).

Nitrogen is the only element (to date) that has been suggested as having an important role in stimulating suckering in sugarcane. Therefore, other macro and micro nutrients have not been discussed or investigated in this study.

2.3.3 Moisture

Suckering is thought to be more prevalent in unusually wet seasons and also more of a problem in the wet tropics than in drier areas of cane production in Australia. Olmstead (1941) found that both rate and amount of tillering in *Bouteloua curtipendula* (range grass) decreased with decreasing soil moisture. Gardner (1942) showed that wheat produced more tillers when the soil was at 50 % water holding capacity than when it was at 25 % water holding capacity. However, Gosnell (1971) reported significantly more stalks per drum (pot) when the water table depth was at 50-100 cm than when it was at 25 cm below ground level. Deren and Raid (1997) found significantly fewer stalks in plots that were flooded for ten days, three days after planting. The experiments conducted by Gosnell (1971) and Deren and Raid (1997) indicated a possible negative impact of waterlogging on tillering. Therefore, it appears that tillering is stimulated by increased water content of the soil, but not when water is in excess and waterlogging becomes a problem.

Berding and Hurney (2000) stated that the greater sucker stalk content in crops in recent times has been due to climatic change marked by wet episodes during harvest. The observation that suckers are more numerous in wet years does not necessarily mean that soil moisture is directly responsible. It is during wet years that damage to crops from lodging is most likely to occur. Therefore the greater suckering may be due to increased light conditions as a result of lodging. However, increased suckering due to high moisture availability alone cannot be overlooked, as high moisture availability may well contribute to sucker initiation.

2.3.4 Temperature

The importance of temperature in the process of suckering is not known. van Dillewijn (1952) stated that next to light, temperature is the most important climatic factor that influences tiller formation. Rands and Dopp (1938) found an increase in tillering from 20 °C to 30 °C in sugarcane. However, this result may be dependent on the cultivar used, as Glasziou *et al.* (1965) found significantly greater tiller numbers at 18 °C and 22 °C compared to 25 °C, 30 °C and 34 °C for the sugarcane cultivar Pindar. Ebrahim *et al.* (1998) found that tiller formation was greatest at 45 °C and least at 15 °C for *Saccharum officinarum* cultivar H50-7209. Mongelard and Mimura (1971) reported that tiller production was less at temperatures below 24.5 °C.

Interaction effects between light, nitrogen, moisture and temperature may also effect suckering in sugarcane. Langer (1963) stated that the effect of temperature on tillering is influenced by a number of other environmental factors, in particular light intensity. Templeton *et al.* (1961) found that photoperiod x temperature, temperature x age of plant, and temperature x duration of treatment all affected tiller development in *Festuca arundinacea.* Escalada and Plucknett (1975) found an interaction between temperature and photoperiod on tillering in sorghum.

Source – sink relationships have been used to model tillering in sorghum (Lafarge and Hammer 2002). In these models, tillering is controlled by source and sink strengths within the plant. Environmental stimuli have their effect by influencing these source

and sink strengths. Such an approach may also prove valuable for modelling normal tillering in sugarcane. However, such a model may not adequately predict suckering in sugarcane, as suckers are produced at a physiologically different stage of the crop growth cycle. Further, as noted in previous discussion, there are reports of other, as yet unquantified differences between tillers and suckers. Source – sink models also appear to work most successfully with annual plants, so that their use for modelling tillering in perennials such as sugarcane may require further investigation. Due to these limitations, a source – sink model would not seem an appropriate model for investigating suckering in sugarcane.

2.3.5 Plant hormones

Suckering occurs from the underground buds of mature stalks that have a growing apex. This means that apical dominance is still intact. Phillips (1975) discussed the nutritive and hormonal theories of apical dominance. The nutritive theory is based on the inability of dormant buds to compete with other parts of the plant for organic and inorganic nutrients and water. Phillips argued that while nutrition and water status are important, it is more likely that the mechanism of control of apical dominance is due to changes in the local concentration of plant hormones.

Auxin produced by the stem apex and leaves is thought to inhibit the growth of lateral buds. Leopold (1949) found that tillering in barley was controlled by auxin produced by the apical bud. Removal of the apical bud resulted in increased tillering, but when auxin was applied, following apical bud removal, tillering was inhibited. The

application of 2,3,5 tri-iodobenzioc acid (TIBA) was shown to increase tillering in barley (Woodward and Marshall 1988; Suge and Iwamura 1993) and oats (Harrison and Kaufman 1980). Galston (1947) showed that TIBA inhibited the action of auxin.

Woodward and Marshall (1988) found that applications of Terpol and Cerone increased tillering in barley. They argued that this reflected an effect of ethylene as both Terpol and Cerone contain ethephon, an ethylene-releasing compound. Harrison and Kaufman (1982) found that ethylene promoted the swelling of tiller buds in oats. Ethylene has been found to inhibit auxin transport (Morgan and Gausman 1966).

In a number of grasses, gibberellic acid (GA) inhibits tillering, and along with auxins, is thought to play a major role in grass growth patterns (Scurfield 1959; Fejer 1960; Evans *et al*, 1964). Kirby and Faris (1972) suggested that the initial growth of tiller buds in barley is controlled by levels of endogenous gibberellins, whereas whether the tiller survives depends largely on competition for light. Application of GA to sorghum resulted in fewer tillers in all cultivars (Morgan *et al.* 1977). While stem extension was increased in some cultivars as a result of the GA application, two groups, the Redlan group and the Hegari group, were relatively insensitive in terms of stem extension. These two groups showed decreases in tillering with GA application and therefore this does provide evidence of the control of tillering without the influence of stem extension. Isbell and Morgan (1982) applied GA to sorghum at sufficiently low levels to reduce tillering but not increase stem elongation. They argued that this provided proof that GA inhibits tiller bud growth directly and not via the promotion of stem elongation.

Harrison and Kaufman (1980) found that kinetin, a synthetic cytokinin, also increased tillering in oats. They suggested that the cytokinin to auxin ratio played a major role in regulating the release of tillers, and that abscissic acid and gibberellins may act as modulator hormones in this system. Suge and Iwamura (1993) found that cytokinin (N-[2-isopnetenyl] adenine) increased tillering, and anticytokinin (4-chloro-2-cyclobutylamino-6-ethylamino-s-triazine) retarded tillering in barley.

2.4 Role of plant physiology in plant/crop improvement

Eliminating or reducing the occurrence of suckers in sugarcane crops grown in the wet tropics is of great importance, as their detrimental effect on CCS, and therefore productivity, has already been demonstrated. Understanding the physiological processes that result in sugarcane plants producing suckers is important for crop improvement. Crop improvement generally occurs in two ways, firstly by manipulation of genetic material to optimise production relative to the constraints imposed by the environment (plant breeding), and secondly by manipulating the environment to optimise production relative to the constraints imposed by the available genetic material (agronomy) (Lawn 1980). Understanding the physiological mechanism allows for informed decisions to be made by both plant breeders and farmers.

2.4.1 Plant breeding

Selection for yield, pest and disease resistance, and crop quality are the broad objectives of a crop improvement program (Austin 1993). These general principles are certainly used in the Australian sugarcane industry. Donald (1968) stated that the majority of plant breeding is based on 'defect elimination' or 'selection for yield'. Austin (1993) concurred with the statement by Donald, indicating that the majority of selections in plant breeding are based on yield, and that even though efficiency of selection for characters other than yield has improved, few have been adopted. Donald (1968) proposed the inclusion of a third element in breeding selections, model plants or ideotypes. Austin (1993) stated that whether or not plant breeders explicitly recognize it, they have an ideotype in mind when evaluations of material are made. The ideotype takes into account the target environment, agronomic practices, crop quality characteristics, and the need for pest and disease resistance. Skinner (1967) described a grading system for clonal assessment in sugarcane that incorporated appearance. While these characters were not detailed, Berding and Hurney (2000) indicated that in practice these include habit, propensity for flowering, canopy cover, and propensity to produce sucker culms (stalks). These characters are all facets of an ideotype. The ideotype is only likely to be reliable if it is based on good understanding of the growth and development of the crop and its responses to environmental factors, rather than intuition and prejudice (Austin 1993). This requires knowledge of the physiology of the character/trait being selected.

Berding and Hurney (2000) have proposed a more stringent selection process in regards to suckering in sugarcane in the wet tropics of Australia. When assessing suckering in

sugarcane, clones are essentially assessed on the number and weight of sucker stalks, and their resulting effect on CCS. Selection by this approach is logical. However, in years or environments where the conditions are such that few suckers are produced, this type of selection becomes limited, as the genetic variation between clones is not expressed. Without any knowledge of why suckering occurs, it is during these years that inadvertent selection for suckering may occur, particularly if there is little understanding of the trait. In these situations it is the understanding of what causes suckering that allows selection of other traits to be made that will result in cultivars with reduced suckering. These other characters may already be part of the proposed ideotype, but only a good understanding of the physiology of suckering will reveal all the traits that could be selected for or against in order to minimise suckering.

Understanding of the physiological bases of suckering may also allow the use of managed environment trials in breeding programs. Essentially, once a good understanding of suckering is obtained, trials can be established where all the physiological requirements for suckering are supplied through agronomic means. These trials are specifically designed to let the genetic variation between clones for the trait to be expressed. This method allows for selection against suckering to be achieved even in years and environments where the genetic differences would normally not be expressed.

In the future, screening of clones for particular traits may take place in the laboratory using molecular markers, resulting in a dramatic decrease in the amount of time it takes for a clone to be tested and later released. While molecular markers for suckering have been found (L. McIntyre, pers. comm.) an understanding of the physiological processes

is still needed in order to select all the associated traits, and their markers, that result in suckers being produced.

2.4.2 Agronomy

Knowledge of plant physiology does not just aid plant breeders, it also allows for informed crop management decisions to be made by farmers. If aspects of the light, nitrogen and soil moisture environments are found to stimulate suckering, then row spacings, fertiliser applications, irrigation, drainage and cultivar selection are all decisions that could potentially be altered, on-farm, in order to reduce suckering in the future. Without the physiological knowledge these decisions can only be made on a trial and error basis.

2.5 Concluding remarks

The information presented in the introduction and literature review indicates that there is a trend of decreased CCS at the mill for sugarcane crops grown in the wet tropics of Australia. Sugarcane suckers have been highlighted as a major causal factor in this trend, as their inclusion in the harvest results in low sucrose material being processed at the mill.

There is general lack of knowledge on the biology of sugarcane suckers. They appear to have different morphology to other stalks, withdraw sucrose from the main stem in order to maintain their own growth and are thought to be stimulated by light, nitrogen, moisture and other environmental factors. However, there is limited evidence to

support these claims. Light, nitrogen, moisture, and temperature have been shown to affect tillering in numerous grass species, and therefore they could potentially have a similar effect on suckering. These environmental factors could act by manipulating the ratio of plant hormones or other biochemical processes within the plant.

The work conduced in this thesis was done in order to overcome some the weaknesses in the understanding of suckering in sugarcane, and the observations are likely to have important implications for crop improvement for sugarcane grown in the wet tropics of Australia.

In Chapter 3 and 4 differences in the morphology and gene expression of suckers and tillers are explored with a view to establishing a better definition of sugarcane suckers and why these differences in morphology may occur. In Chapter 5, the relation between the sucker and its parent stalk is explored to establish the extent to which the mature stalk supports sucker growth. In Chapter 6 and 7 the effects of two key environmental stimuli, soil nitrogen and light, on suckering are explored experimentally and their interaction with soil water is examined in Chapter 8. Finally, the implications of the thesis findings in terms of the options for sugarcane production and improvement are discussed in Chapter 9.

Part B: Biology of sugarcane suckers

Chapter 3. Sucker Morphology

3.1 Differences in morphology between sucker and 'normal' stalks

3.1.1 Introduction

In the Australian sugar industry, the term sucker usually refers to a tiller that has been formed late in the growing season. However, in the literature the term sucker is often used to describe tillers in general. Hartt *et al.* (1963) described suckers as shoots which develop from the buds at the base of the stalk. This definition would mean that all tillers are in fact suckers. Due to the differences in terminology and the practical need to describe suckers consistently when trialing agronomic or plant breeding solutions, a formal definition of what the Australian industry refers to as a sucker would be beneficial. This definition would need to be able to distinguish suckers from 'normal' tillers by factors other than their time of emergence.

van Dillewijn (1952) and Barnes (1974) described suckers as often being thicker than maturing cane, succulent, and as stalks that grow faster with well-developed buds and longer internodes than the main crop. Sucker leaves are also thought to be shorter than normal cane leaves (Hes 1954). However, data were not presented, nor cited, to support these statements in any of these reports.

Hes (1954) stated, '...although everyone more or less acquainted with cane will recognise such a sprout (sucker) in time, a description of it is not so simple'. In this

chapter, the morphology of suckers are characterised by comparing them to primary stems in a plant crop and tillers in a ratoon crop (normal stalks) of similar age and grown in close proximity. By quantifying some of the morphological differences between suckers and 'normal' cane, identification of suckers in the field will be able to be done with greater certainty. It is also a valuable step in gaining a better understanding of what suckers are, and perhaps why they are formed.

3.1.2 Methods

3.1.2.1 Field experiment design and data collection, 1998

Three crops of Q138 were selected in 1998. The crops were: (i) mature second ratoon crop, where suckers were present (last harvest, 4th September 1997), (ii) plant crop (planted, 17th July 1998), (iii) young second ratoon crop (last harvest, 14th August 1998). The crops were located within close proximity (less than 300 m from each other) at the Tully BSES research station. All crops were grown on similar soil types; the plant and young ratoon crops were grown on a Bulgun series soil, and the mature crop containing suckers was grown on a Hewitt type soil. A description of these soil types can be found in Murtha (1986).

On 3rd September 1998, 100 suckers in the mature crop and 100 tillers in each of the plant and ratoon crops were selected. Selection of suckers was based on the definitions of van Dillewijn (1952) and Barnes (1978). The height to the dewlap of the last fully expanded (LFE) leaf on each stalk, and the number of this leaf, were recorded. The leaf number was recorded on the leaf with a permanent marker pen and each stalk was

marked with flagging tape. Leaf one was defined as the first leaf greater than 20 mm in length. All leaf data, other than leaf sheath, refers to the lamina. All small shoots in the mature crop appeared to be suckers. Stalks in the ratoon crop, that appeared to have leaves cut by the harvester, were not chosen. These shoots were initiated before harvest and therefore may have been suckers.

Twenty stalks from each crop were destructively sampled on three occasions, 4th September 1998, 12th-13th October 1998, and 9th-10th November 1998. A further sample was taken from the ratoon crop on 2nd December 1998. This was done as the ratoon crop was slightly younger than the other crops and therefore an additional harvest was needed to obtain data for the higher leaf numbers. Stalks were placed in plastic bags for transport back to the laboratory and were then placed in buckets filled with water in order to prevent leaf rolling. Measurements of leaf lamina length, maximum leaf lamina breadth, leaf lamina area, leaf dry weight, leaf sheath length, internode diameter, and stalk height above ground to the dewlap of the LFE leaf. Leaf area was measured with a Paton Electronic Planimeter (Paton Industries, South Australia).

3.1.2.2 Field experiment design and data collection, 1999

Two crops of cultivar Q152 were selected in 1999: (i) mature crop containing suckers, and (ii) a young ratoon crop. The two crops were located within 50 m of each other at A. Zappalla's farm in the Babinda district (17° 30´S, 145° 50´E).

On 15th September 1999 sixty sucker and young ratoon stalks were selected. The height to the dewlap of the LFE leaf on each stalk, and the number of this leaf, were recorded as for Q138 in 1998. On 23rd - 24th September 1999 and 20th October 1999, 20 stalks were cut at ground level and measurements of leaf lamina length, maximum breadth and area were taken as described previously. A further sample was not taken due to an earlier than predicted commercial harvest of the mature crop.

3.1.2.3 Pot experiment design and data collection, 2001

A short commercial harvesting season in 2000, due to poor yields, resulted in the mature crops that contained suckers being harvested before an adequate comparison between sucker, plant cane and ratoon cane could be made. Consequently, a pot experiment was used to compare suckers and plant cane in order to ensure that data would not be lost in 2001 if the commercial harvesting season was once again short in duration.

Cultivars Q117, Q138 and Q152 were grown in pots (38 cm diameter and 30 cm depth) at CSIRO Davies Laboratory, Townsville (19° 15´S, 146° 46´E). Single eye sets were originally planted in trays on 2nd August 1999, and following germination, individual plants were planted into separate pots. Each pot contained a mixture of peat, coarse sand and fine sand (1:2:2 v/v/v). Shoots initially germinated in a glasshouse, however, once in pots, the cane was grown in the open air. On 3rd September 2000 the stalks were cut at the base and allowed to ratoon. These plants produced suckers in 2001. Fifteen suckers per cultivar were marked with flagging tape in order for measurements of morphology to be taken as the suckers grew.

To compare suckers with young plant cane, single eye sets of cultivars Q117, Q138 and Q152 were planted in trays on the 6th April 2001. Following germination, the young plants were placed into pots (38 cm diameter and 30 cm depth). Three plants were grown per pot, with five pots per cultivar. Each pot contained a mixture of peat, coarse sand and fine sand (1:2:2 v/v/v).

All plants were automatically irrigated three times a day. Fertiliser was applied at regular intervals: liquid fertiliser (Wuxal®, Schering Pty Ltd, NSW, Australia, 300 ml of 15 ml/l) approximately every fortnight; granular, slow release fertiliser (Osmocote®, Scotts Australia Pty Ltd, NSW, Australia, 14:6.1:11.6 N:P:K, 50 g pot^{-1}) approximately every eight weeks. Plants were prevented from lodging by wire supports suspended either side of the row of pots.

Measurements of leaf lamina length, maximum breadth, leaf sheath length and the height of the dewlap of each leaf above ground were taken from both the plant cane stalks and the sucker stalks for all three cultivars.

3.1.2.4 Statistical analysis

All statistical analyses were conducted using the statistical package SYSTAT 9 (SPSS Inc. Chicago, USA). Leaf data were analysed using two-way ANOVA with the stalk type and leaf number as independent variables and the morphological characteristic as the dependent variable. Comparisons between stalk types, for individual leaf numbers, were made following single factor ANOVA. The internode diameter data was analysed

using single factor ANOVA. *Post-hoc* comparisons of means were conducted using Fisher's least significant difference (LSD) ($p \leq 0.05$).

3.1.3 Results

3.1.3.1 Field experiment 1998, cultivar Q138

Leaves of suckers exhibited a significantly different morphology to those of plant cane and ratoon tillers. They were significantly shorter in length and had a significantly greater maximum breadth, and this resulted in a significantly different leaf length to breadth ratio (Figure 3.1). The leaf sheaths of leaves from suckers were significantly longer than those of plant cane and ratoon tillers (Figure 3.2). No significant difference was found between plant cane and ratoon tiller leaves.

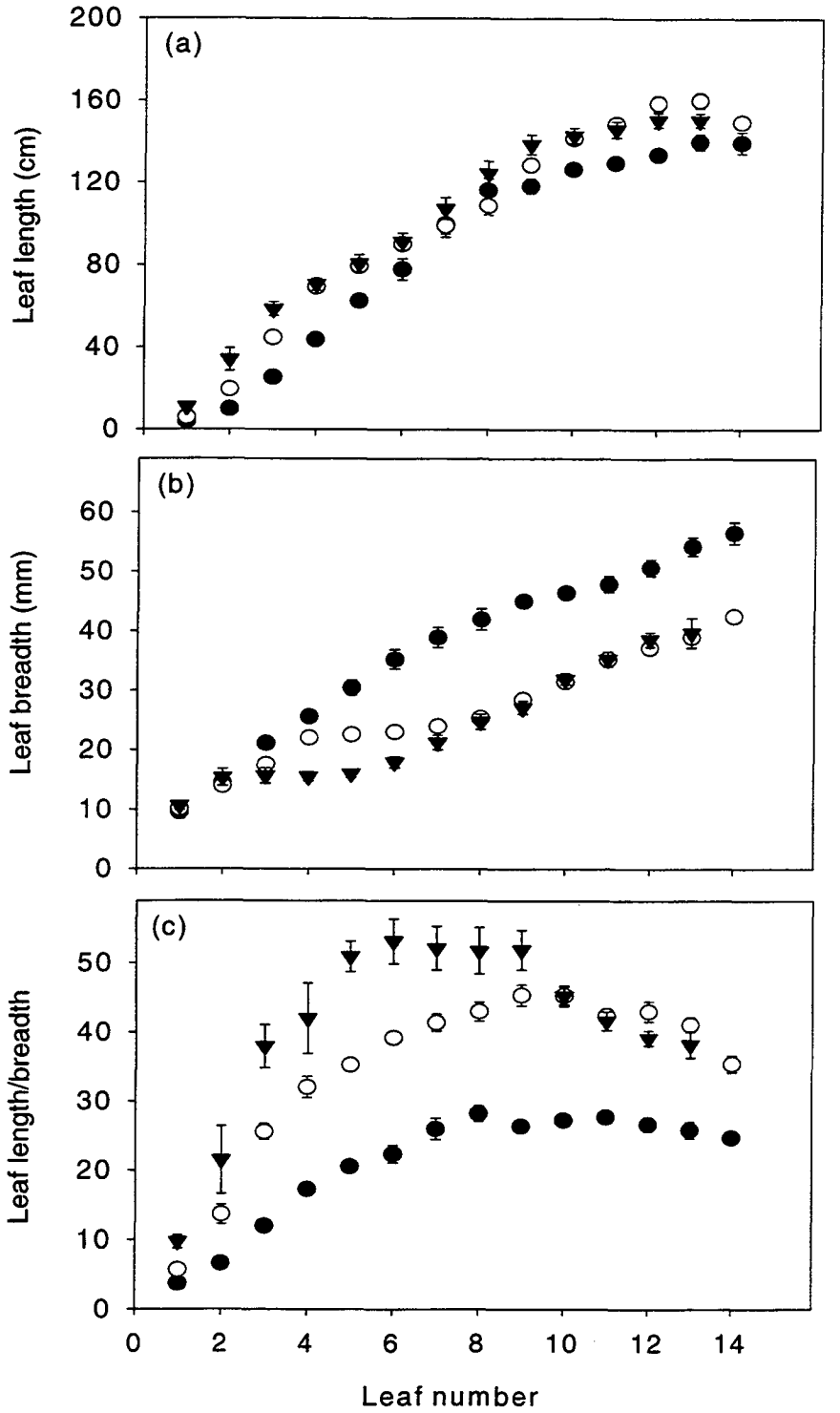

Figure 3.1 Leaf length (a), leaf maximum breadth (b) and leaf length to breadth ratio (c) for suckers ●, ratoon stalks ▼ and plant cane stalks ○. Error bars represent the standard error of the mean, and are shown where they are larger than the size of the symbol.

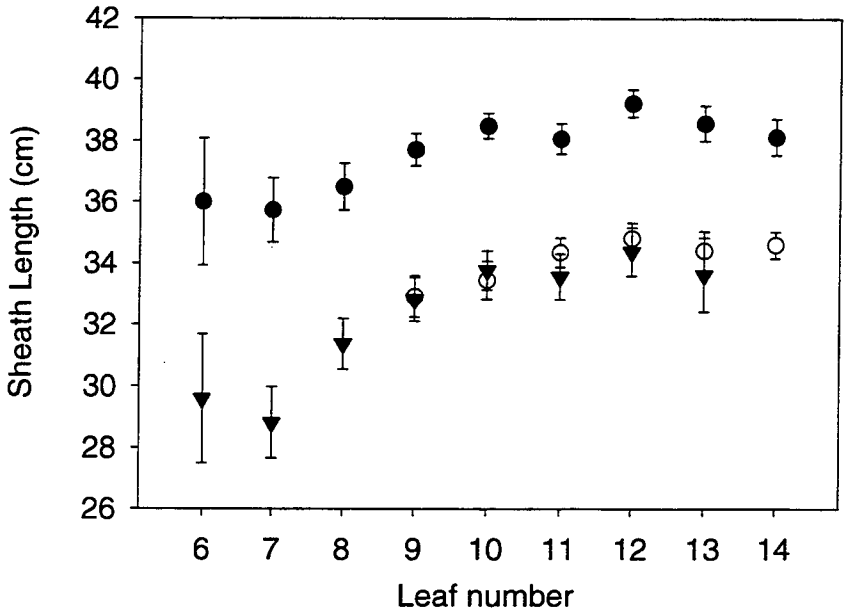

Figure 3.2 Leaf sheath length for suckers ●, ratoon stalks ▼ and plant cane stalks ○. Error bars represent the standard error of the mean. Note y axis does not start at 0.

Sucker leaves had significantly greater leaf area than both plant cane and ratoon tiller leaves from leaf 6 onwards (Figure 3.3). The difference between the plant and ratoon crops was only significant for leaves 2, 4, and 5.

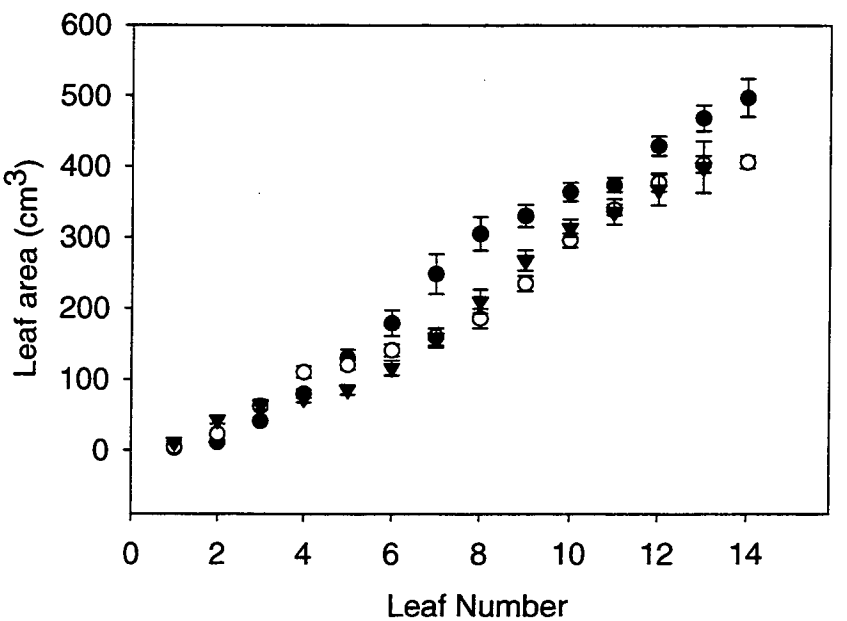

Figure 3.3 Leaf area of suckers ●, ratoon stalks ▼ and plant cane stalks ○. Error bars represent the standard error of the mean.

Specific leaf area (SLA), the mass of leaf per unit area, can be used as a measurement of leaf thickness. Sucker leaves had significantly lower SLA than both ratoon shoots and plant cane shoots for the first three leaves (Figure 3.4). There was no difference between sucker and ratoon shoots for the further leaves. However, the difference between sucker and plant cane shoots remained for leaves 4 - 13. This means that sucker leaves were denser on an area basis and therefore, possibly thicker than plant cane leaves.

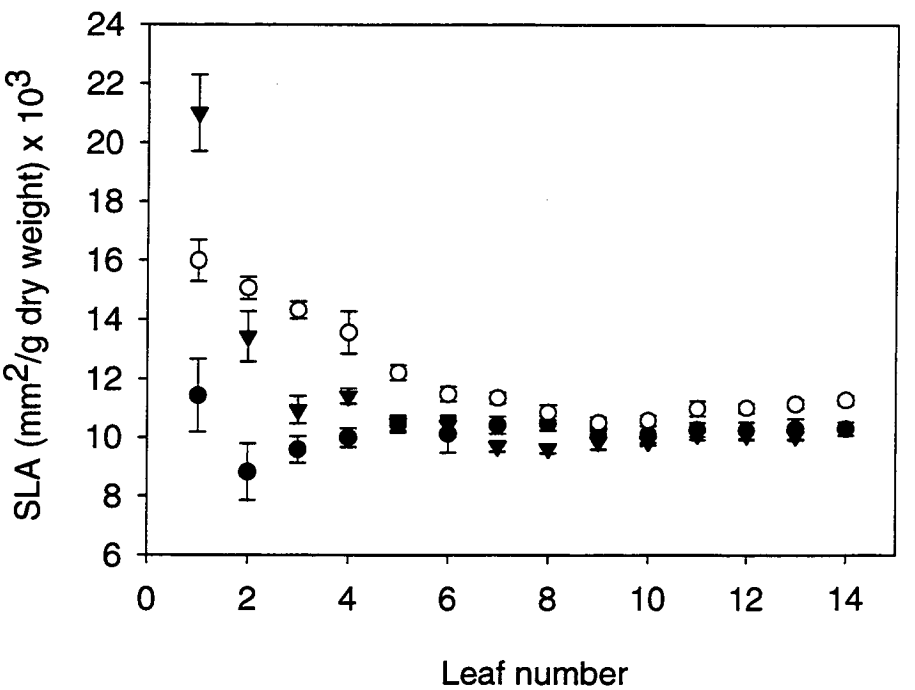

Figure 3.4 Specific leaf area (SLA) of suckers ●, ratoon stalks▼ and plant cane stalks ○. Error bars represent the standard error of the mean.

Suckers were found to be significantly taller than both ratoon shoots and plant cane shoots after having produced a similar number of leaves (Figure 3.5). The diameter of the internodes at leaf 7 and leaf 8 were significantly thicker for sucker stalks compared to ratoon shoots and plant cane shoots (Figure 3.6). Data for internodes below leaf 7 were not collected as these internodes were below ground level in the plant crop. Data

for internodes above leaf 8 were not included as they were deemed to be immature, and had not reached their full size.

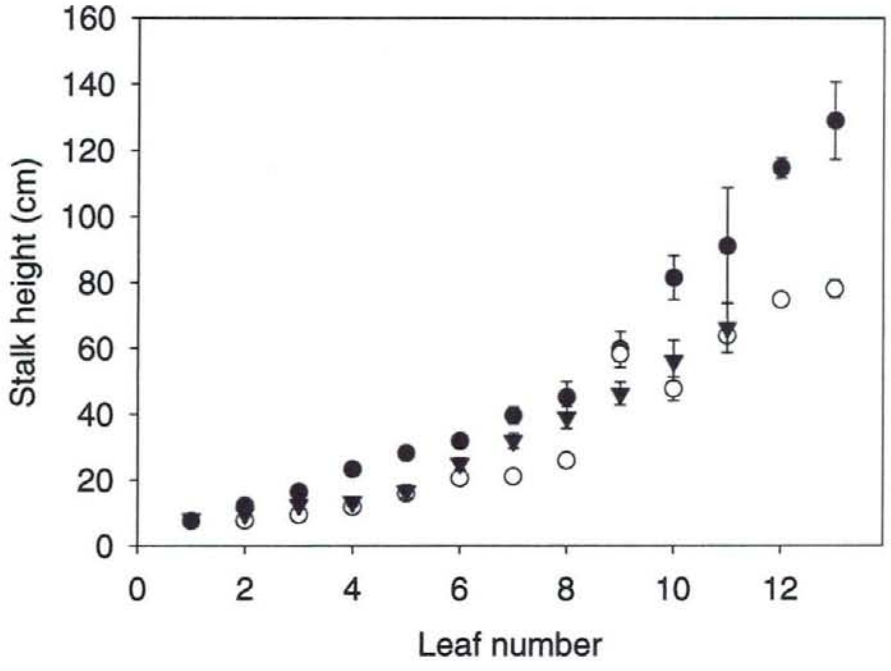

Figure 3.5 Stalk height (cm) to the dewlap of the leaves of suckers ●, ratoon stalks ▼ and plant cane stalks ○. Error bars represent the standard error of the mean.

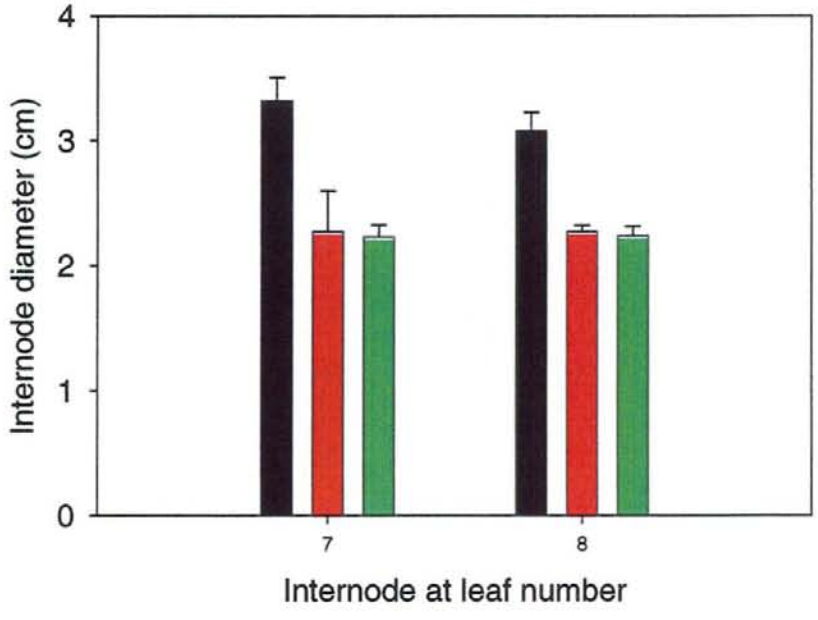

Figure 3.6 Internode diameter of sucker stalks (■), ratoon stalks (■) and plant cane stalks (■). Error bars represent the standard error of the mean.

The growth of suckers was highly variable, when compared to the plant cane crop. Figure 3.7 shows the total increase in height (cm) between 4th September, 1998 and 4th November, 1998 for 50 suckers and 56 stalks in the plant crop. The columns represent the proportion of stalks that had increased in height within the ranges defined by the x-axis. Sucker growth ranged from zero to just over a metre, whilst growth in the stalks from the plant cane crop was more consistent. More than 60% of these stalks grew between 50.1 and 70.0 cm.

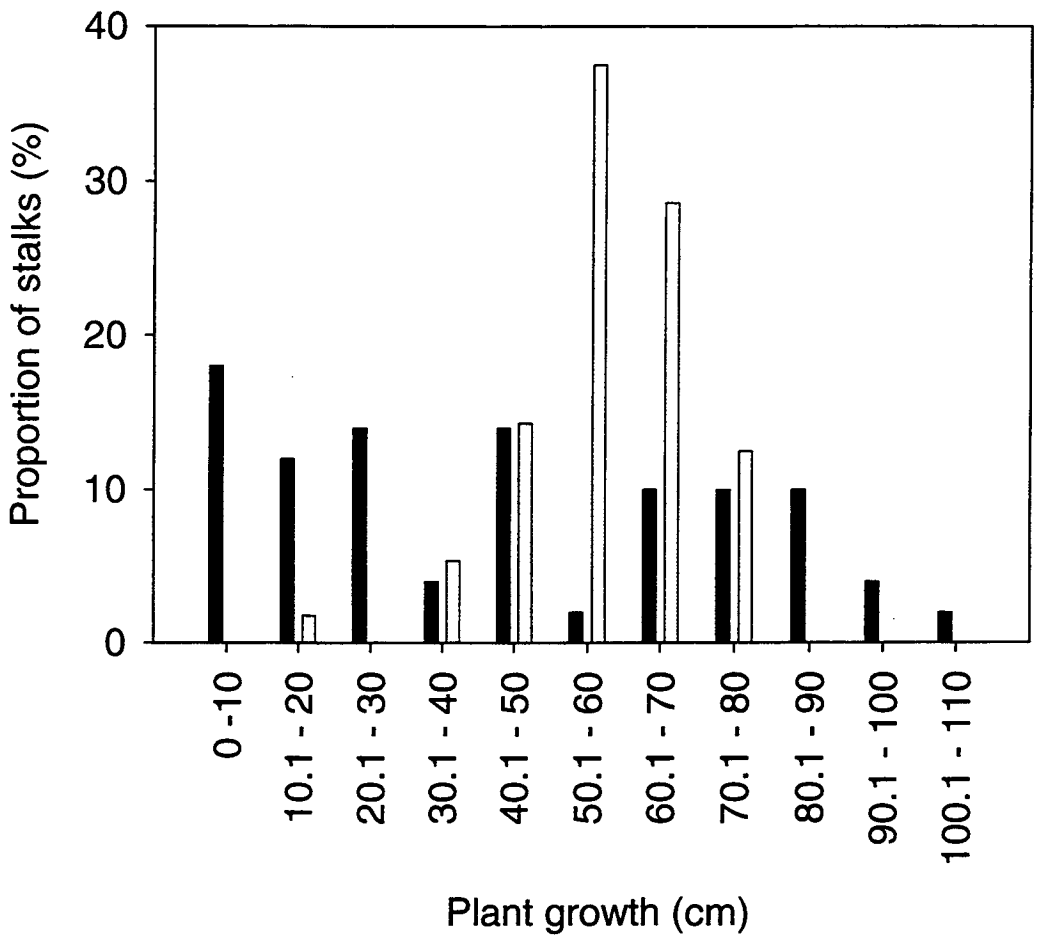

Figure 3.7 Growth of suckers (■) and plant cane shoots (□) between 4th September 1998 and 4th November 1998.

3.1.3.2 Field experiment 1999, cultivar Q152

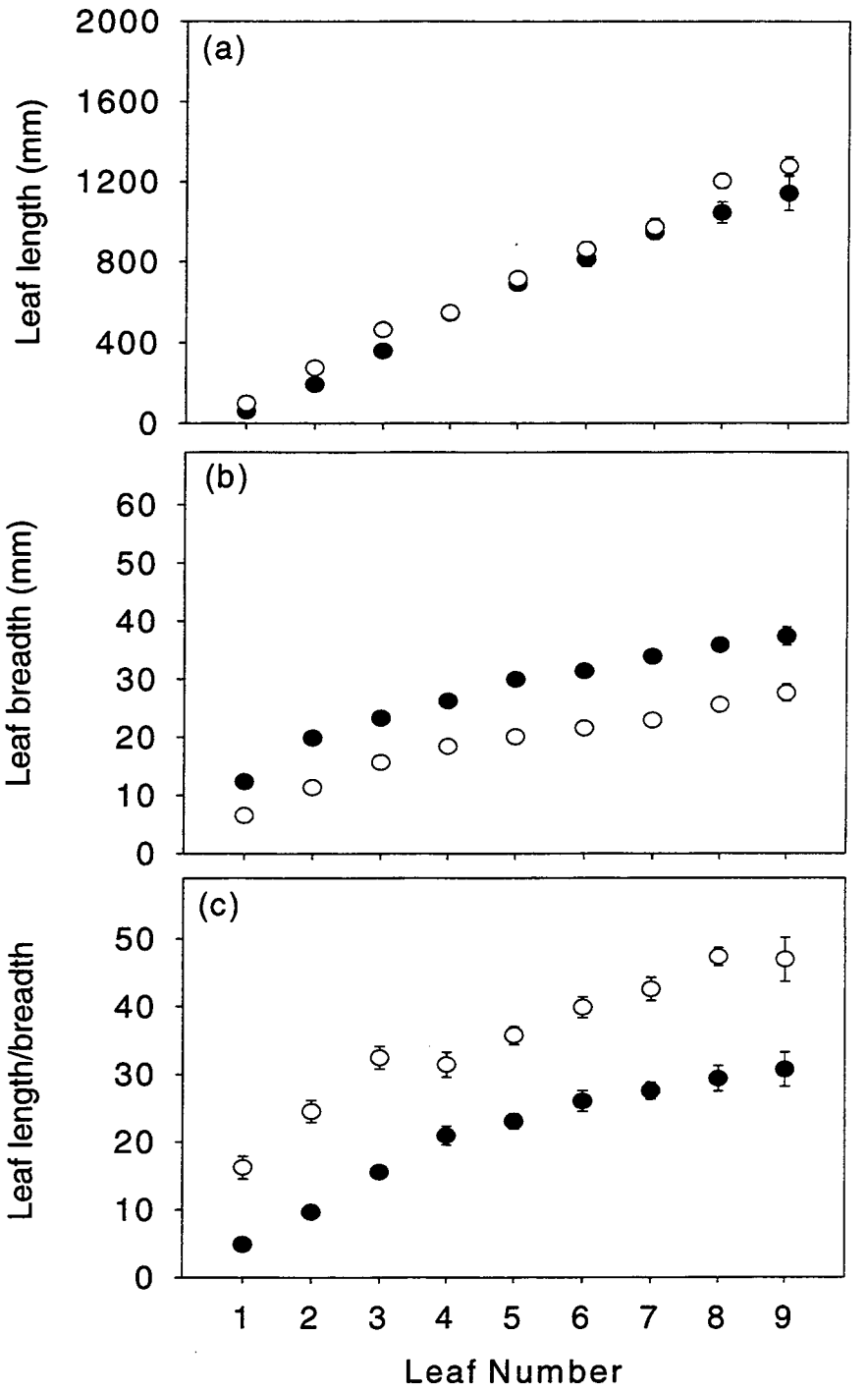

Figure 3.8 Leaf length (a), leaf maximum breadth (b) and leaf length to breadth ratio (c) for suckers ● and ratoon stalks ○ of cultivar Q152. Error bars represent the standard error of the mean, and are shown when larger than the symbol.

Sugarcane suckers of cultivar Q152 were found to have significantly shorter leaf lengths, significantly greater leaf breadth and as a result of this, a significantly different

leaf length to breadth ratio than ratoon stalks in the field experiment conducted in 1999 (Figure 3.8). The sucker leaves were also found to have significantly greater leaf area than leaves of ratoon stalks (Figure 3.9).

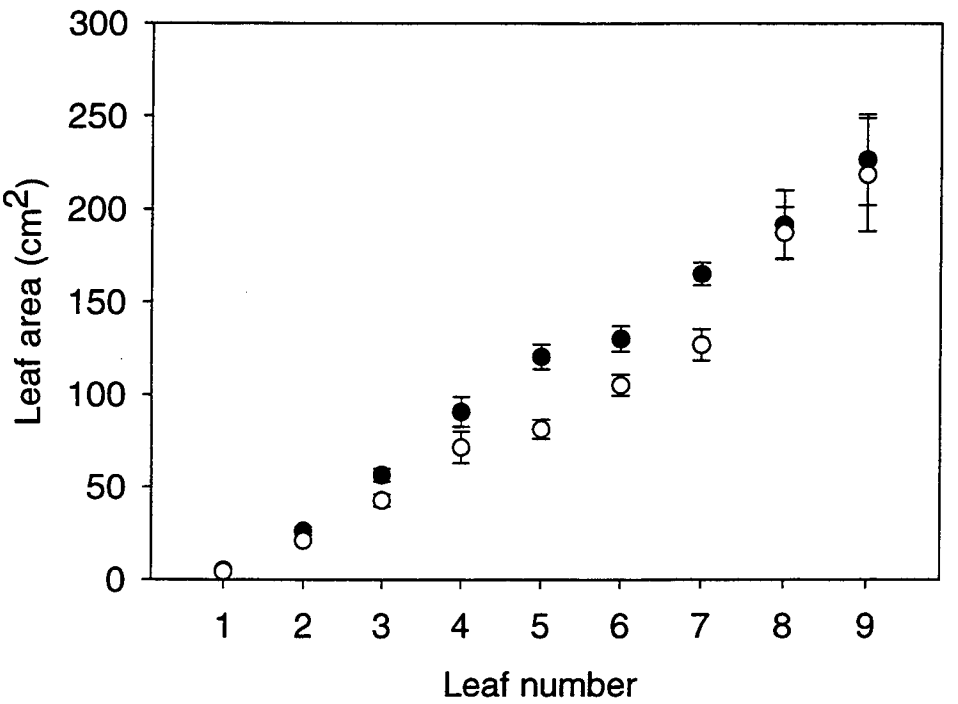

Figure 3.9 Leaf area (cm^2) for suckers ● and ratoon shoots ○ of cultivar Q152. Error bars represent the standard error of the mean.

3.1.3.3 Pot experiment, 2001

Suckers of cultivar Q117 had significantly greater leaf length than plant cane stalks, significantly wider maximum leaf breadth and a significantly lower leaf length to breadth ratio (Figure 3.10).

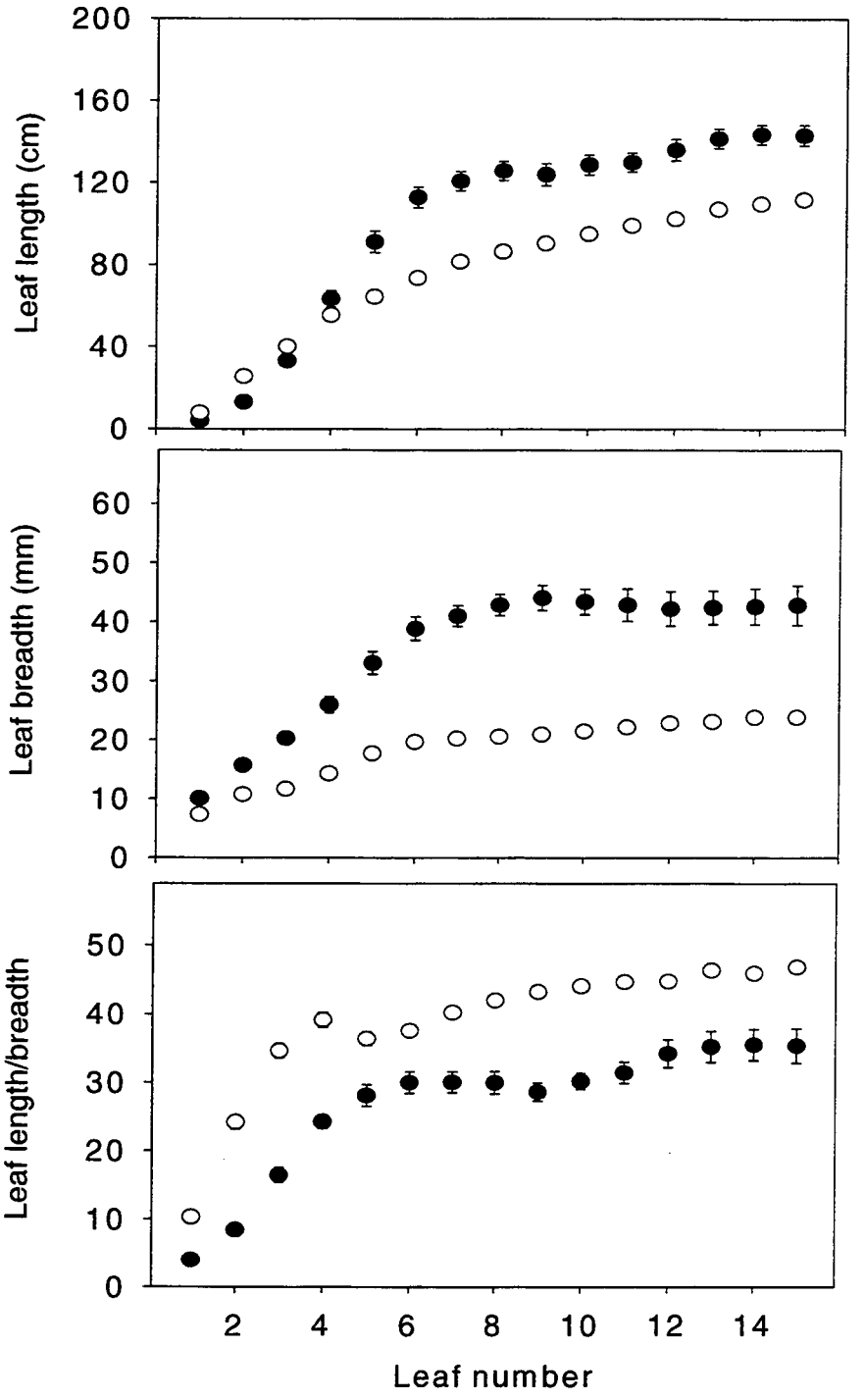

Figure 3.10 Leaf length (a), leaf breadth (b) and leaf length to breadth ratio (c) for suckers (●) and plant cane stalks (○) of cultivar Q117. Error bars represent ± standard error of the mean, and are shown when larger than the symbol.

Suckers of cultivar Q138 (Figure 3.11) and Q152 (Figure 3.12) likewise had significantly greater leaf length than plant cane stalks, significantly wider maximum leaf breadth and a significantly lower leaf length to breadth ratio.

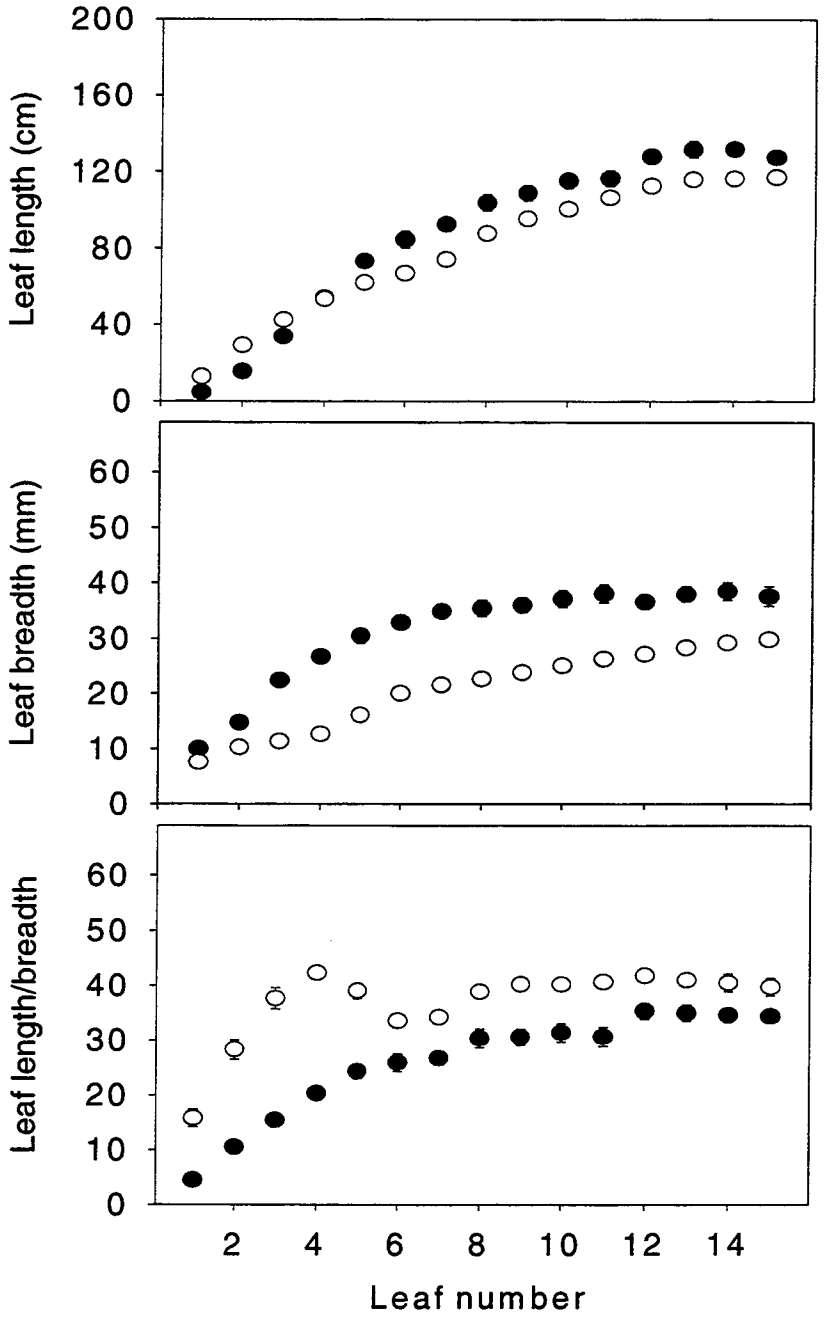

Figure 3.11 Leaf length (a), leaf breadth (b) and leaf length to breadth ratio (c) for suckers (●) and plant cane stalks (○) of cultivar Q138. Error bars represent ± standard error of the mean, and are shown when larger then the symbol.

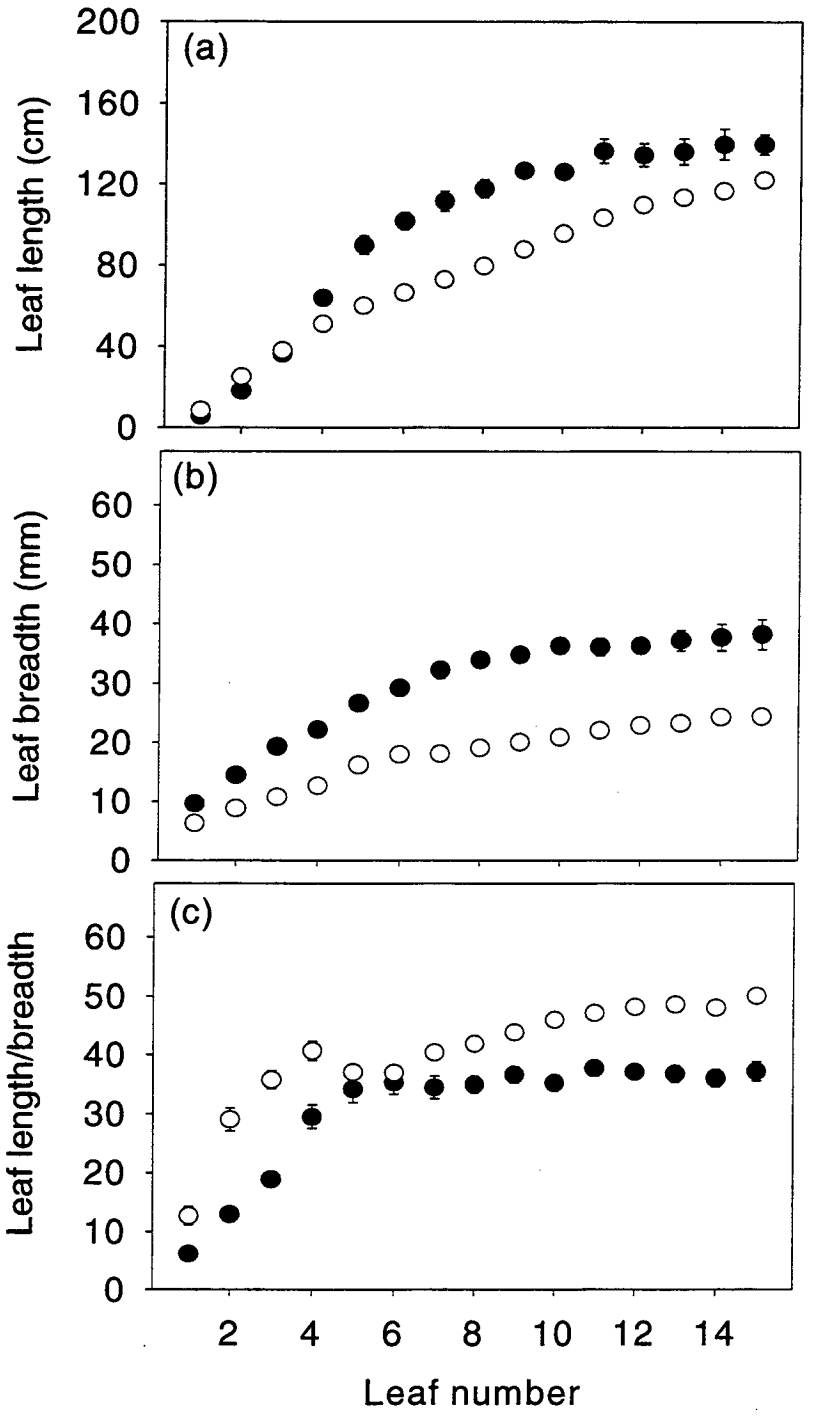

Figure 3.12 Leaf length (a), leaf breadth (b) and leaf length to breadth ratio (c) for suckers (●) and plant cane stalks (○) of cultivar Q152. Error bars represent ± standard error of the mean, and are shown when larger than the symbol.

The diameter at the base of the stalks was measured once they had produced 15 leaves. Sucker stalk bases were found to be significantly ($p < 0.01$) wider than plant cane stalk bases for all three cultivars (Figure 3.13)

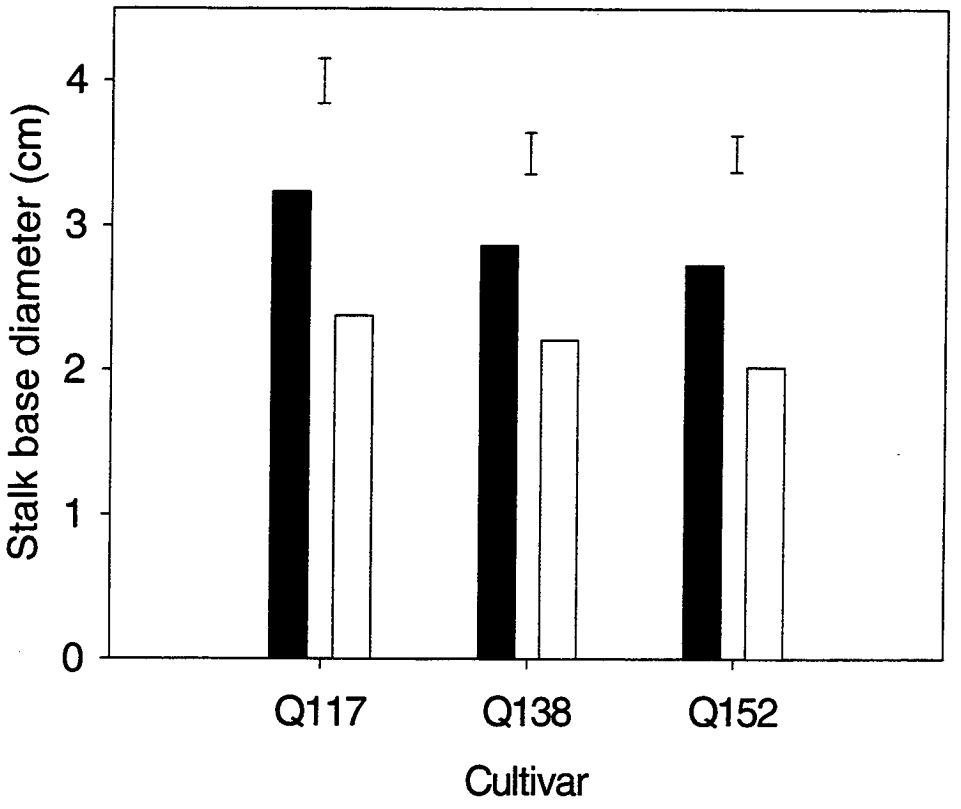

Figure 3.13 Stalk base diameter (cm) for sucker (■) and plant cane stalks ( ) of cultivars Q117, Q138 and Q152. Error bars represent LSD ($p < 0.05$).

3.1.4 Discussion

Sugarcane suckers exhibited significantly different leaf and stem morphology when compared to normal stalks. These results quantify and extend the observations of van Dillewijn (1952), Hes (1954) and Barnes (1974). The results also, in part, contradict the assertion that sucker leaves are shorter than normal cane leaves.

Leaf maximum breadth of suckers was shown to be significantly greater than that of normal stalks in all environments and all cultivars. Leaf length of suckers was shown to be significantly shorter than those of normal stalks for cultivars Q138 and Q152 when the experiments were conducted in the field. However, leaf length of suckers was

shown to be significantly greater than those of normal stalks when the experiment was conducted in pots. The reason for this contradiction is not known. Despite this, the leaf length to breadth ratio of suckers was found to be significantly smaller than that of normal stalks in all environments and all cultivars. This result does suggest that the major difference between sucker leaves and those of normal stalks is the difference in leaf maximum breadth.

The lengths of the leaf sheaths of suckers were significantly greater than those of normal stalks. Since the suckers were growing beneath a canopy, the light environment in which they were growing was most likely different to that of the normal stalk crops. This could potentially explain the differences in leaf morphology. Casal *et al.* (1987b) found that leaf sheaths of *Lolium multiflorum* were significantly longer when receiving an end of day pulse of far-red light. The red/far-red ratio of light is reduced by crop canopies, and Ludlow *et al.* (1990) reported a lower red/far-red ratio beneath sugarcane canopies. However, sucker stalks still appear to have their distinctive morphology even when located in a lodged area within the crop. These areas would, presumably, have a higher red/far-red ratio of light due to the disruption in the crop canopy.

The first three leaves produced by a sucker had significantly greater SLA than the other two stalk types. This suggests that, at least initially, the internal structure of sucker leaves may be different to that of the other stalk type leaves. This would need further study to confirm.

Internode thickness and the diameter at the base of stalks was found to be significantly greater in suckers than normal stalks in all cultivars and environments tested. A similar

result was found for cultivar Q117 by Bonnett *et al.* (2001), and is also in accordance with the postulations of van Dillewijn (1952), Hes (1954) and Barnes (1974).

Lodging of stalks in the Q138 crop in which the suckers were growing in the field in 1998, may be one of the causes behind the highly variable growth of suckers. Following lodging, some suckers may have been in an unfavourable position for growth (shade), and some were noticeably damaged. The reasons why some suckers are capable of high growth are also unknown. It has been proposed (Hes 1954) that suckers receive nutritional support from the mature stalks above them. This may result in the higher growth rate, but presumably all suckers are capable of receiving this benefit. Therefore, this does not explain the low growth rates of some suckers, unless the sucker itself was damaged by lodging or the main stalk to which they were attached deteriorated in some way (e.g. due to damage from lodging, rats).

The height above ground at which suckers produce their leaves was found to be significantly greater than that of normal stalks. This may be due to etiolation, as the suckers were growing beneath a canopy, which would have altered the light environment in which they were growing. Low light intensity has been shown to cause etiolation in sugarcane (Martin and Eckart 1933).

The data presented in this chapter show that suckers have significantly different morphology to other 'normal' stalks. While these differences have been alluded to in older references, this is the first time that data have been presented to quantify and support the assumptions.

3.2 Morphology of shoots grown from buds on sucker stalks

3.2.1 Introduction

Some of the morphological differences between sugarcane suckers and 'normal' sugarcane stalks were established in section 3.1. These included leaf length and maximum breadth, leaf sheath length and internode diameter. Why late-formed tillers, suckers, should have a different morphology is unknown. This changed morphology of sucker stalks is probably due to altered expression of genes in the stalk as it is growing. Though how the expression of genes is changed is as yet unknown. This altered expression of genes in the sucker stalk may also have an effect on growth from buds produced by the stalk, or alternatively the pattern of gene expression may be reset in the next generation.

When a crop containing suckers is harvested, some of the new shoots that appear in the following ratoon crop may have grown from the stubble remaining from the suckers as well as the mature stalks. There was some speculation in the industry that the stalks that were produced from buds born on suckers would have low sucrose concentration, as it was believed that suckers stalks accumulated sucrose at a lower rate than normal stalks. However, recent research has shown that suckers appear to accumulate sugar at a similar rate to normal stalks in crops grown in northern New South Wales, Australia (Hughes and Muchow 2000).

An experiment was conducted to address the question: do buds on sucker stalks produce shoots that have sucker morphology or normal stalk morphology, and is this dependent on bud age/maturity?

3.2.2 Methods

3.2.2.1 Experiment 1, plant growth and experimental design

On 23rd October 1998, single eye setts of cultivar Q138 were planted in trays at CSIRO Davies Laboratory. The setts were taken from two stalk types, suckers and normal stalks, which were both collected from the Q138 crop grown in Tully in experiment 3.1.2.1. Buds of five different ages were planted for each stalk type: the youngest visible bud at the stem apex, the 3rd youngest bud, the 5th youngest bud, the 7th youngest bud and the 9th youngest bud from the stem apex. All buds were initially grown in trays in a glasshouse.

Following sprouting, the young shoots were planted into pots (38 cm diameter and 30 cm depth) containing a mixture of peat, coarse sand and fine sand (1:2:2 v/v/v). Three young shoots were planted into each pot, and there were three pots per bud age for each stalk type. The pots were placed in a single row and were irrigated automatically for five minutes, three times a day. Fertilizer was applied at regular intervals: liquid fertiliser (Wuxal®, Schering Pty. Ltd., NSW, Australia, 300 ml of 15 ml/l) approximately every fortnight; granular, slow release fertiliser (Osmocote®, Scotts Australia Pty Ltd, NSW, Australia, 14:6.1:11.6 N:P:K, 50 g pot^{-1}) every eight weeks. Plants were prevented from lodging by wire supports, as described earlier.

Days to emergence were recorded and leaf lamina length and breadth measured on the first twenty fully expanded leaves. Periodically the number of fully expanded leaves (last visible dewlap) was recorded.

3.2.2.2 Experiment 2, plant growth and experimental design

On 2nd August 1999, single eye setts of cultivars Q117, Q138 and Q152 were planted in trays at CSIRO Davies Laboratory. The setts were taken from two stalk types, suckers and normal stalks, which were all taken from crops on A. Maifredi's farm near Tully (18° 0´S, 145° 55´E). Buds of three different ages were planted for each stalk type: the youngest visible bud at the stem apex, the 3rd youngest bud from the stem apex and the 5th youngest bud from the stem apex. All buds were initially grown in trays in a glasshouse. Later the buds were planted into pots as described previously and grown outside. The pots, soil, fertiliser applications, watering and scaffolding were all as described previously. Pots were placed in two rows with cultivars and bud age groups being randomly distributed throughout. Similar measurements to experiment 3.2.2.1 were taken as the plants grew.

3.2.2.3 Statistical analysis

Leaf data were analysed using two-way ANOVA with the leaf number and bud origin as independent variables. This was done separately for each cultivar. Comparisons between the origin of the buds, for individual leaf numbers, were made following single factor ANOVA. The rate of leaf appearance was analysed using two-way ANOVA with

the number of fully expanded leaves and time as the independent variables, for each cultivar. The bud ages were compared using two-way ANOVA with bud age and bud origin as independent variables, leaf length to breadth ratio data were used as the dependent variable in this analysis. *Post-hoc* comparisons of means were conducted using Fisher's least significant difference (LSD) ($p \leq 0.05$).

3.2.3 Results

3.2.3.1 Experiment 1

The analysis showed that the shoots produced by buds taken from suckers had significantly shorter leaf length ($p > 0.01$) than shoots produced by buds taken from normal stalks (Figure 3.14). However, the difference between means was only 1.5 cm, and Figure 3.14 (a) shows that the leaf lengths were very similar. Presumably, these small differences were statistically significant due to a high degree of precision, perhaps afforded by the large sample size. No difference was found between the two stalk types for the leaf maximum breadth ($p < 0.05$) or leaf length to breadth ratio ($p < 0.05$) following two-way ANOVA but some significant differences were found at the individual leaf level (Figure 3.14).

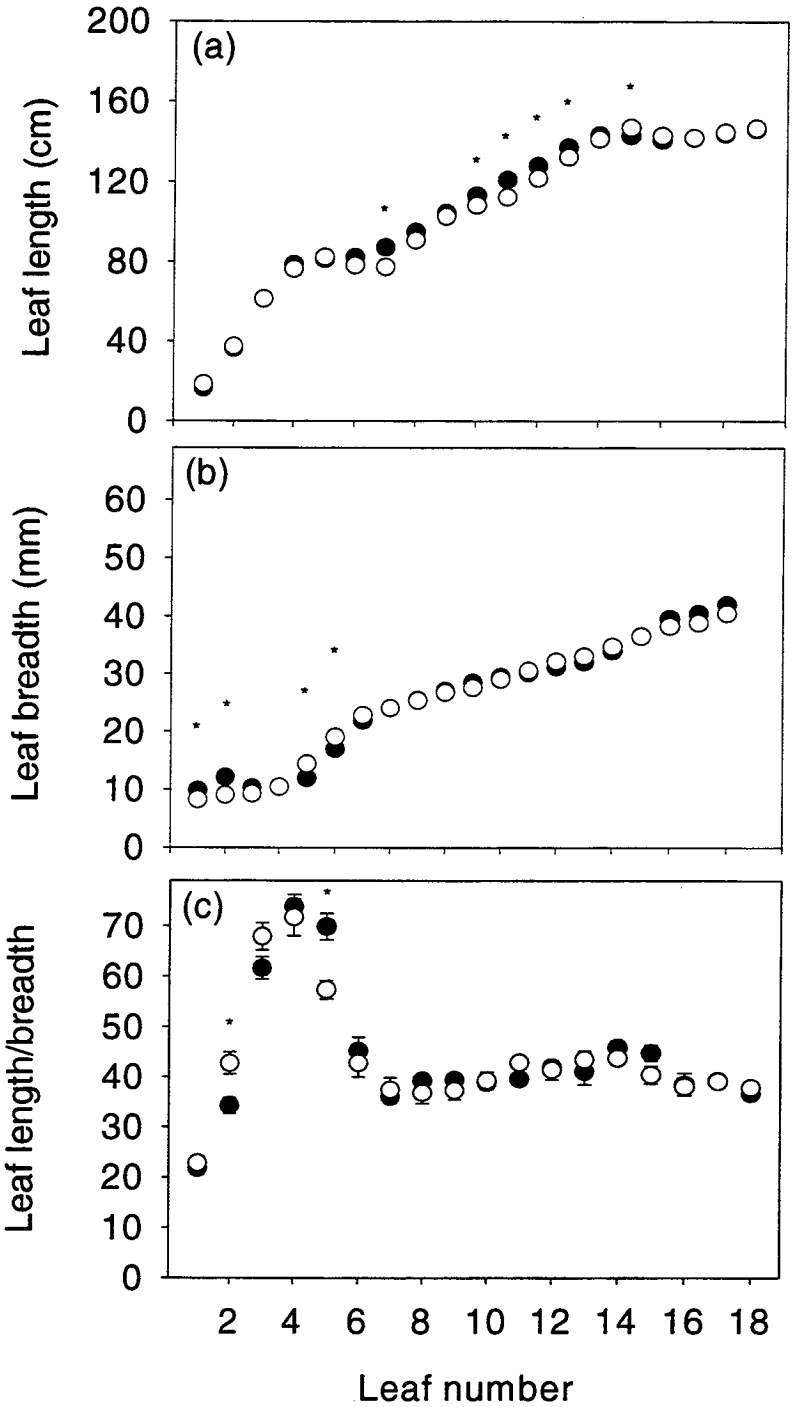

Figure 3.14 Leaf length (a), leaf maximum breadth (b) and leaf length to breadth ratio (c) from stalks grown from buds produced by suckers (●) and normal stalks (○).Error bars represent ± the standard error of the mean, and are shown when they are larger than the size of the symbol. * indicates a significant difference ($p < 0.05$) following single factor ANOVA.

There was no significant difference in the rate of leaf appearance for shoots grown from buds taken from suckers and normal stalks (Figure 3.15).

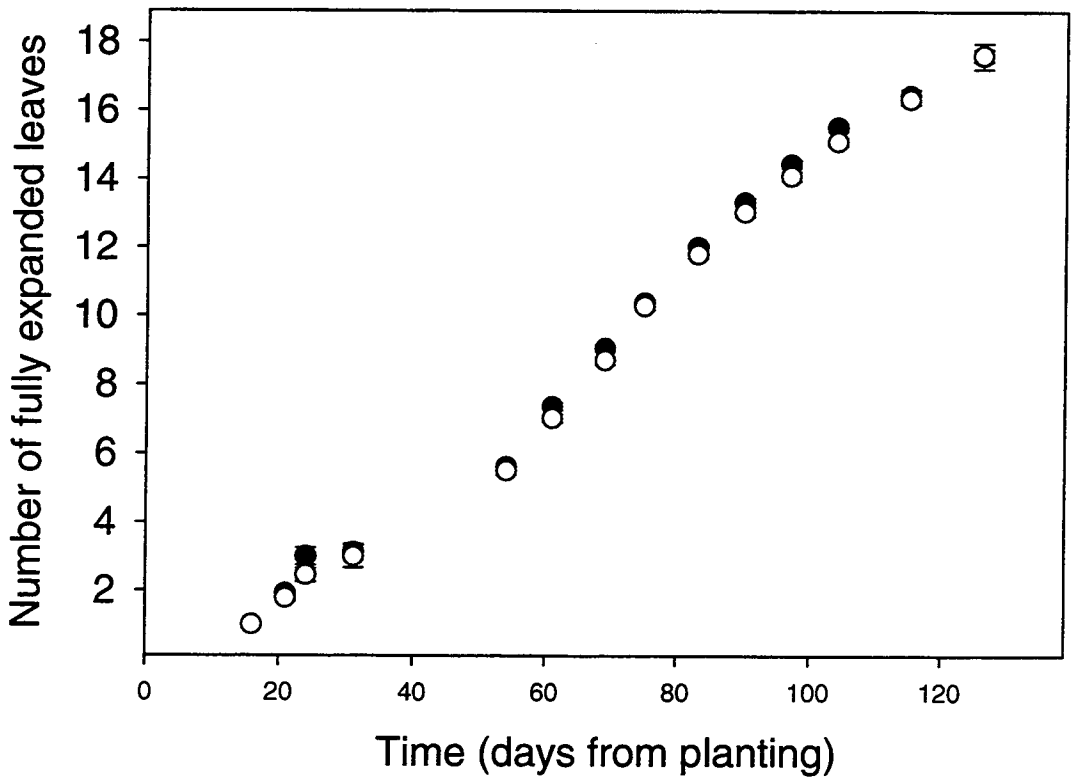

Figure 3.15 Leaf appearance of shoots grown from buds taken from suckers (●) and normal stalks (○) over time. Error bars represent ± the standard error of the mean. All bud ages combined.

Analysis of the different bud ages showed that there was a significant effect of bud age on the leaf length to breadth ratio of shoots ($p < 0.05$), but there was no significant difference in leaf length to breadth ratio due to stalk type for all bud ages. The older buds produced shoots with a significantly lower leaf length to breadth ratio than the young buds (Table 3.1). It was noted that none of the youngest buds taken from the sucker stalks grew, whereas the youngest buds taken from the normal stalks did.

Table 3.1 Mean leaf length to breadth ratio for shoots grown from sucker and normal stalk buds of five ages. Means followed by the same letter are not significantly different ($p > 0.05$).

Stalk type	Bud age	Mean leaf length to breadth ratio	
		Stalk type	Bud age
Sucker	Youngest		No shoots emerged
Sucker	3rd		46.5^{cd}
Sucker	5th		46.3^{bcd}
Sucker	7th		44.2^{abc}
Sucker	9th	44.7	42.4^{ab}
Normal	Youngest		49.0^{d}
Normal	3rd		44.9^{bc}
Normal	5th		43.2^{abc}
Normal	7th		45.3^{bcd}
Normal	9th	44.6	40.5^{a}
		ns	

ns - F test not significant ($p > 0.05$)

3.2.3.1 Experiment 2

The percentage germinations of the different bud ages for each cultivar are shown in Table 3.2. The youngest sucker buds of cultivars Q117, Q138 did not emerge while cultivar Q152 showed very low emergence. Shoots grown from buds taken from suckers of cultivar Q117 had significantly shorter leaves ($p < 0.05$) and significantly smaller maximum leaf breadth ($p < 0.05$) than shoots grown from buds taken from normal stalks (Figure 3.16). There was no significant difference in the leaf length to breadth ratio ($p > 0.05$, two-way ANOVA). While significant differences were found in leaf length and leaf maximum breadth, the differences were small when compared to the difference between sucker leaves and plant cane leaves (Figure 3.10).

Table 3.2 Germination of twenty single-eye sets of cultivars Q117, Q138 and Q152 taken from sucker and normal stalk buds of three ages.

Cultivar	Bud age	Germination (%)	
		Sucker	Normal stalk
Q117	Youngest	0	70
	3rd youngest	55	95
	5th youngest	95	85
Q138	Youngest	0	80
	3rd youngest	80	100
	5th youngest	85	100
Q152	Youngest	15	90
	3rd youngest	100	90
	5th youngest	100	90

Shoots grown from buds taken from suckers of cultivar Q138 had significantly smaller maximum leaf breadth ($p < 0.05$) than shoots grown from buds taken from normal stalks (Figure 3.17). There was no significant difference in the leaf length and the leaf length to breadth ratio ($p > 0.05$, two-way ANOVA). Again, while significant differences were found in leaf maximum breadth , the differences were small when compared to the difference between sucker leaves and plant cane leaves (Figure 3.11).

Shoots grown from buds taken from suckers of cultivar Q152 had significantly smaller maximum leaf breadth ($p < 0.05$) than shoots grown from buds taken from normal stalks (Figure 3.18). There was no significant difference in the leaf length and the leaf length to breadth ratio ($p > 0.05$, two-way ANOVA). Yet again, while significant differences were found in leaf maximum breadth , the differences were small when compared to the difference between sucker leaves and plant cane leaves (Figure 3.12).

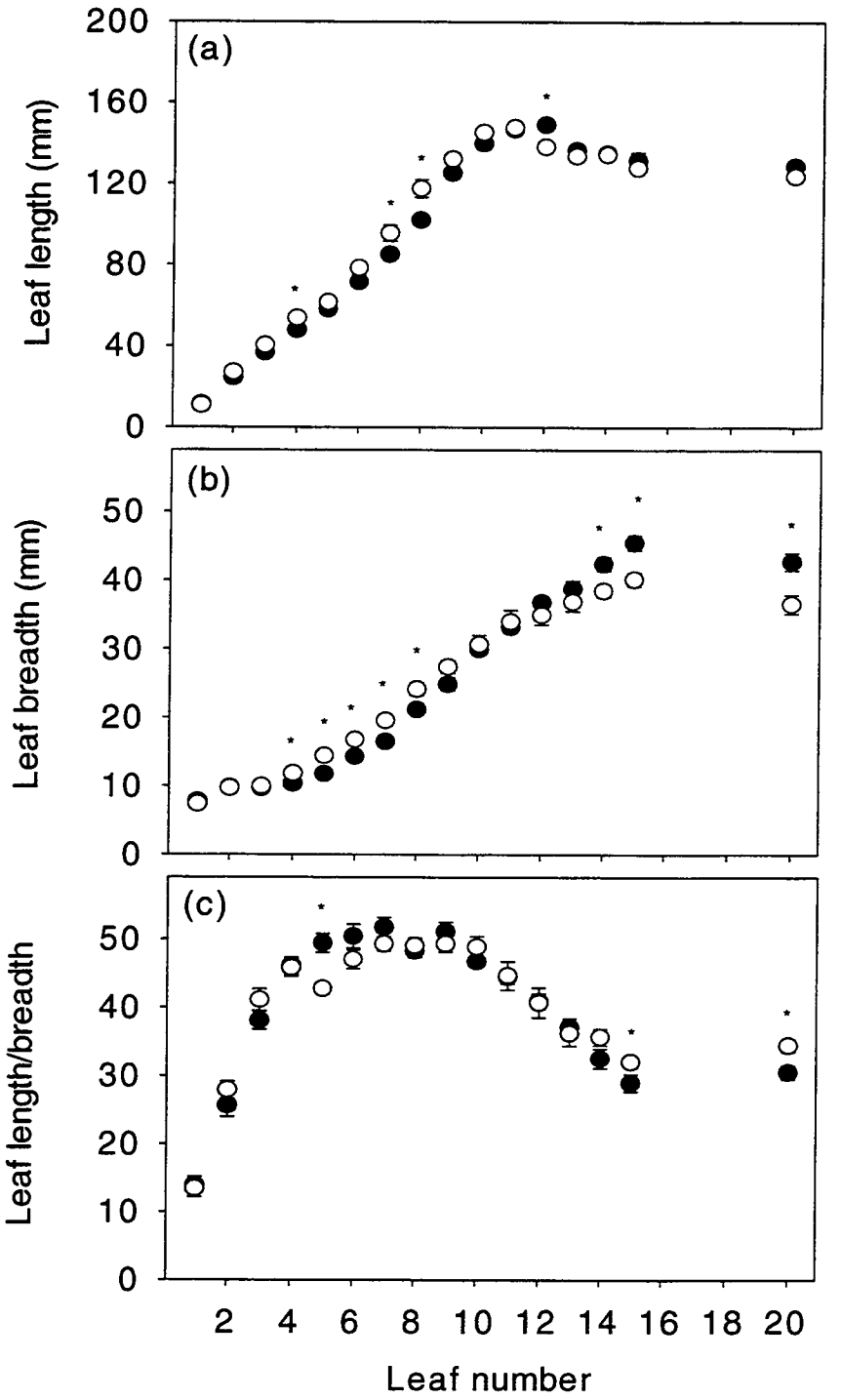

Figure 3.16 Leaf length (a), leaf maximum breadth (b) and leaf length to breadth ratio (c) for shoots of cultivar Q117 grown from buds taken from suckers (●) and normal stalks (○). Error bars represent ± the standard error of the mean, and are shown when they are larger than the size of the symbol. * indicates a significant difference ($p < 0.05$) following single factor ANOVA.

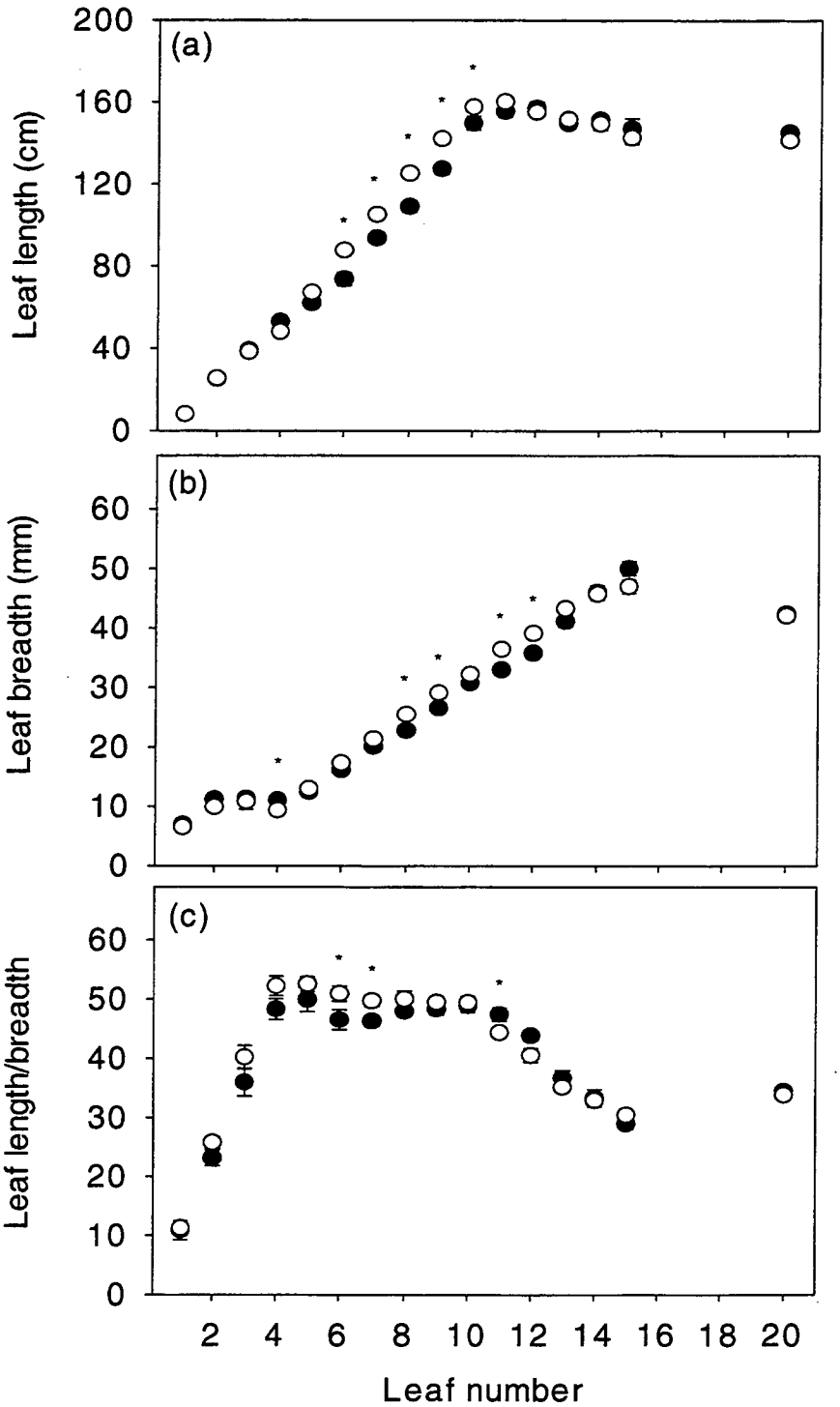

Figure 3.17 Leaf length (a), leaf maximum breadth (b) and leaf length to breadth ratio (c) for shoots of cultivar Q138 grown from buds taken from suckers (●) and normal stalks (○). Error bars represent ± the standard error of the mean and are shown when they are larger than the size of the symbol. * indicates a significant difference ($p < 0.05$) following single factor ANOVA.

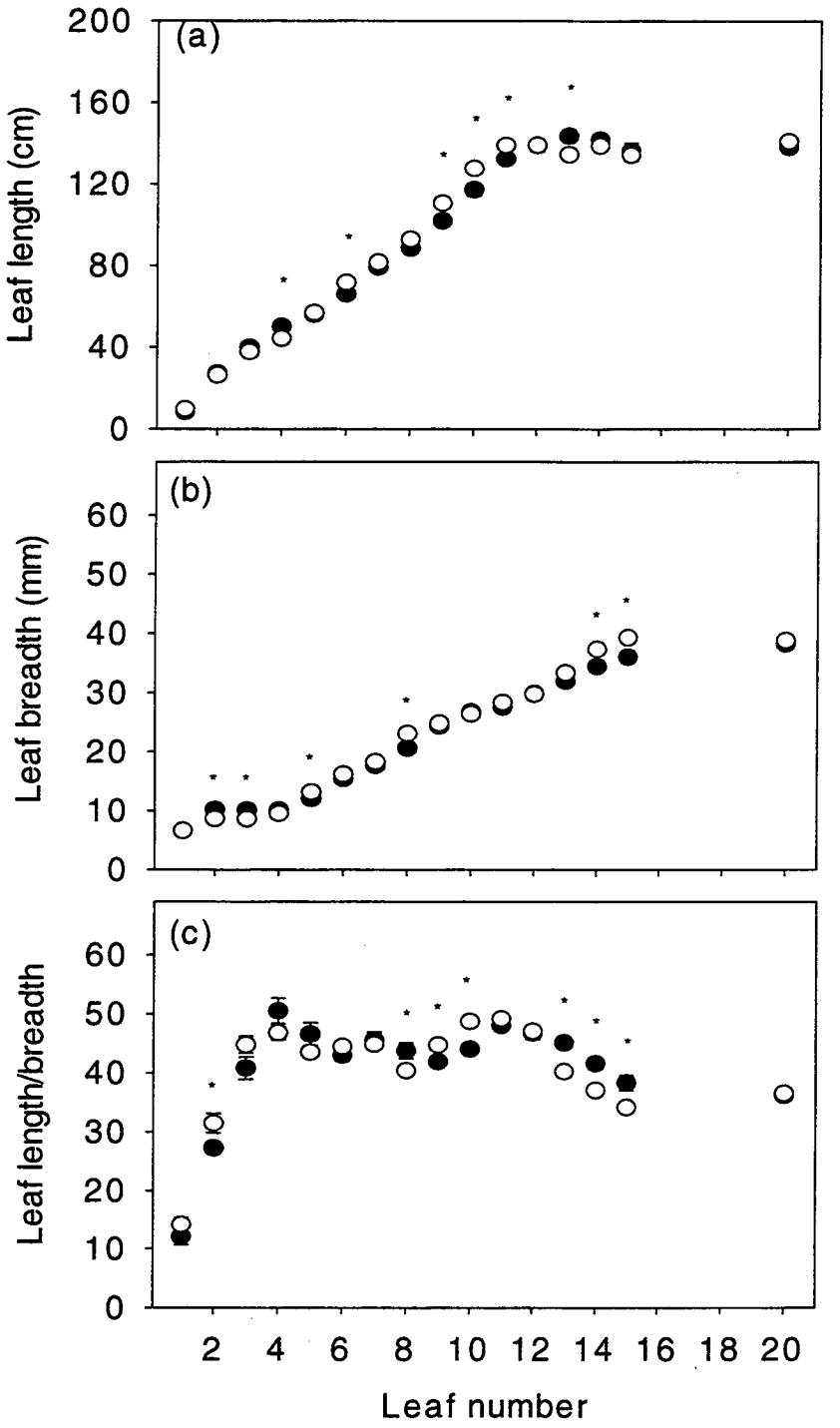

Figure 3.18 Leaf length (a), leaf maximum breadth (b) and leaf length to breadth ratio (c) for shoots of cultivar Q152 grown from buds taken from suckers (●) and normal stalks (○). Error bars represent ± the standard error of the mean and are shown when they are larger than the size of the symbol. * indicates a significant difference ($p < 0.05$) following single factor ANOVA.

There was no significant difference in the leaf length to breadth ratio of shoots grown from buds taken from suckers and normal stalks, for the 3rd youngest bud and the 5th youngest bud, for cultivars Q138 and Q152 (Table 3.3).

Table 3.3 Mean leaf length to breadth ratio for shoots grown from sucker and normal stalk buds of three ages. Means followed by the same letter are not significantly different ($p > 0.05$).

Cultivar	Bud age	Stalk type	Mean leaf length/breadth ratio
Q117	Youngest	Sucker	No emergence
	3rd	Sucker	40.9^{b}
	5th	Sucker	40.4^{b}
	Youngest	Normal	42.4^{c}
	3rd	Normal	40.4^{b}
	5th	Normal	37.8^{a}
Q138	Youngest	Sucker	No emergence
	3rd	Sucker	40.4^{ab}
	5th	Sucker	40.0^{a}
	Youngest	Normal	44.1^{b}
	3rd	Normal	40.9^{ab}
	5th	Normal	38.2^{a}
Q152	Youngest	Sucker	Low emergence
	3rd	Sucker	41.2^{b}
	5th	Sucker	40.5^{ab}
	Youngest	Normal	41.9^{b}
	3rd	Normal	40.5^{ab}
	5th	Normal	39.5^{a}

However, there was a significant difference in the average leaf length to breadth ratio of shoots grown from sucker and normal stalks for the 5th youngest bud of cultivar Q117, but not for the 3rd youngest bud. No comparison between the shoots produced by the youngest buds could be made due to the lack of emergence of the youngest sucker buds. Shoots grown from buds of increased age had a significantly lower average leaf length to breadth ratio.

The rate of leaf appearance for shoots grown from buds taken from suckers was not significantly greater than that of normal stalks for all three cultivars. However, the rate of leaf appearance tended to be slower for shoots grown from older buds (Table 3.4).

Table 3.4 The number of fully expended leaves over time for shoots grown from buds taken from suckers and normal stalks. Means followed by the same letter are not significantly different ($p > 0.05$).

Cultivar	Stalk type	Bud age	Time (days after planting)					
			24	58	87	107	128	143
Q117	Sucker	Youngest						
		3rd	0.1^{a}	4.9^{c}	9.1^{c}	11.7^{b}	15.7^{c}	18.0^{b}
		5th	0.6^{b}	4.3^{ab}	7.7^{b}	11.3^{b}	14.9^{bc}	17.3^{ab}
	Normal	Youngest	0.6^{b}	4.1^{b}	7.9^{b}	11.0^{b}	14.4^{bc}	16.8^{ab}
	stalks	3rd	0.4^{b}	3.4^{a}	7.6^{b}	10.4^{ab}	13.4^{ab}	16.2^{ab}
		5th	0.0^{a}	2.9^{a}	6.4^{a}	9.7^{a}	12.8^{a}	15.3^{a}
Q138	Sucker	Youngest						
		3rd	0.4	5.1^{bc}	8.8^{b}	11.1	15.2^{b}	17.2^{b}
		5th	0.9	5.3^{c}	9.0^{b}	11.2	14.9^{b}	17.4^{b}
	Normal	Youngest	0.8	4.7^{bc}	8.8^{b}	11.4	15.0^{b}	17.3^{b}
	stalks	3rd	0.7	4.2^{a}	8.4^{ab}	11.1	13.8^{ab}	16.0^{a}
		5th	0.8	4.2^{a}	7.8^{a}	10.6	14.5^{a}	16.9^{ab}
			ns			ns		
Q152	Sucker	Youngest						
		3rd	0.9	5.1^{c}	9.1^{c}	11.7	14.6	17.7
		5th	0.9	4.6^{b}	8.4^{b}	12.1	15.3	18.0
	Normal	Youngest	0.8	4.3^{b}	8.4^{b}	11.6	14.7	17.6
	stalks	3rd	1.0	4.0^{ab}	7.9^{ab}	11.5	14.9	17.8
		5th	0.7	3.7^{a}	7.7^{a}	11.0	14.8	17.5
			ns			ns	ns	ns

ns - F test not significant ($p > 0.05$)

3.2.4 Discussion

The leaf morphology of shoots grown from buds taken from suckers was broadly similar to that of shoots grown from buds taken from normal stalks. Therefore, the difference in leaf and stalk morphology displayed by suckers is not displayed by the

buds on the sucker stalk. While some significant differences were found in leaf morphology, when compared with the difference between sucker leaves and normal stalk leaves, the differences found were very small and not biologically meaningful. The observation that plants developed in a similar way from buds on suckers and buds on normal stalks was the same for buds of different ages and for three different cultivars.

None of the youngest buds taken from suckers emerged. This may imply that there is a difference in bud maturity for the bud at the stem apex compared to a normal stalk. It was thought that if any difference between buds produced by suckers and normal stalks was found, that it may only be found in the younger buds. These buds were closer to the apex of the stalk. Presumably, there may be some difference in gene expression between sucker and normal stalks in this region, due to the differences found in sucker stalk and normal stalk morphology.

No differences were found in the rate of leaf appearance for shoots grown from buds taken from suckers and normal stalks. However, the older buds tended to produce leaves at a slower rate than the youngest buds. van Dillewijn (1952) stated that cuttings from the top of the stem generally germinate more rapidly than cuttings from lower down the stem. The buds at the top of the stem are usually very soft and are therefore not suitable for planting a commercial field. The older buds also produced shoots with leaves with a lower leaf length to breadth ratio than the shoots of younger buds. Older buds were located on thicker parts of the stem. This means that when planted, the setts containing the older buds were larger than the setts containing the younger buds. van Dillewijn (1952) presents data from an anonymous source that showed that the growth

of buds is affected by the size of the section of stalk from which a bud is grown. The larger the section of stalk, the better the shoot growth.

Since buds on suckers produce shoots of normal appearance, when a crop containing suckers is harvested, sucker like shoots should not emerge in the following ratoon crop. This was feared by some farmers, as sucker stalks were thought to be low in sugar content. Recent work has shown that suckers accumulate sugar at a similar rate as normal stalks, and therefore as long as suckers have sufficient time to grow, they can contribute positively to a sugarcane crop (Hughes and Muchow 2000). Therefore, even if sucker like stalks were produced in the ratoon crop, they would have a similar sugar content as the normal stalks when the crop is harvested in the following year. Some sucker-like stalks can be observed in young ratoon crops. However, these shoots usually have leaves that appeared to have been damaged by the harvester, and were obviously very young suckers at the time of harvest. An insight into how these shoots develop is presented in Chapter 5. The differences in the meristems of suckers and mature stalks that are associated with the different morphologies observed in this chapter is investigated in Chapter 4.

Chapter 4. Comparison of gene expression in stem tissue of sucker and 'normal' stalks

4.1 Introduction

Differences between the morphology of suckers and the morphology of normal stalks have been shown in Chapter 3. The differences in morphology between the stalk types were presumably due to differential expression of genes in the different tissue types. An understanding of which genes show differential expression between the stalk types may allow for a better understanding of the actual causes of this altered expression, and possibly a better understanding of suckering and the inter-stalk relationship between a sucker and the main stalk to which it is attached.

Microarray analysis was used in order to compare gene expression in the sucker and young cane stem apex tissue. This method allows for the expression of thousands of genes to be screened simultaneously. The microarray used, canearray, was developed at CSIRO Plant Industry in Brisbane based upon expressed sequence tag (EST's) sequences from separate complementary deoxyribonucleic acid (cDNA) libraries of immature and mature cane of cultivar Q117 (Casu *et al.* 2001).

4.2 Methods

4.2.1 Sampling

In order to compare gene expression in suckers to 'normal' stalks, stem tissue was harvested from young suckers and young plant cane on 25th October 1999. The suckers were taken from a mature Q117 crop on E.S. Tua's farm, near Abergowrie, in the Ingham district (18° 40´S, 146° 10´E). The young plant cane shoots were harvested from a young Q117 crop growing opposite to this mature crop. The crops were located within 20 m from each other, separated by a headland. Suckers that had produced a similar number of leaves as the plant cane shoots were selected. The plant cane shoots had produced between 5 and 8 leaves. The close proximity of these two crops, and the young age of both shoot types, meant that it was likely that both shoot types had been growing in similar conditions for a similar period of time.

Suckers and plant cane shoots were cut at the base and stripped of all leaf laminas and leaf sheaths. About 8 cm of stem tissue (youngest immature internodes) was harvested from the apex of the stalk, snap frozen in liquid nitrogen and then stored at – 80 °C. Immediately prior to fixing, this young stem apex tissue was still growing and developing, and therefore genes that are responsible for the differences observed in stem and leaf morphology, between suckers and 'normal' stalks, should have been differentially active. Ribonucleic acid (RNA) was extracted from this tissue.

4.2.2 RNA extraction

The method used for RNA extraction was an adaptation of the method devised by Chomczynski and Sacchi (1987). For each RNA extraction, four stalks of the same type were ground together to a fine powder in liquid nitrogen with a mortar and pestle. Approximately 7 g of powder was then placed in a 50 ml Falcon tube which contained 15 ml denaturing solution (Appendix 4.1), 100 mg N-lauroysarcosine, and 100 µl 2-mercaptoethanol. The tubes were shaken to submerge the powder in the buffer. Six such tubes were made per extraction. The remainder of the protocol appears below.

1. The contents of each tube were blended with a Polytron for 1 min.
2. Tubes were centrifuged (Sigma 4K15) at 3500 rpm (2600 g) at 4 °C, for 15 min using a swing-out rotor.
3. The supernatant was poured through Miracloth into a clean 50 ml Falcon tube. 3.5 ml of 5.7 M cesium chloride (Appendix 4.1) was pipetted into a Beckman Ultra-Clear Ultracentrifuge tube. Each sample was layered on top of the cesium chloride. The tubes were balanced using denaturing solution.
4. The tubes were spun in an ultracentrifuge (Beckman L8-80M) at 23500 rpm (90 000 g) at 20 °C for 20 hr using a SW28 rotor.
5. All visible debris and buffer were removed as quickly as possible using a wide bore transfer pipette. Tubes were immediately inverted on to a clean pad of tissues and allowed to drain.
6. 400 µl of diethylpyrocarbonate (DEPC) treated (Appendix 4.1) milliQ water (MQW) (Millipore, USA) was added to each pellet. The pellet was allowed to re-

suspend for 45 min at room temperature. The re-suspended RNA was transferred to an Eppendorf tube.

7. 400 µl of phenol/chloroform/isoamyl alcohol (25:24:1 v/v/v) was added. Tubes were vortexed and then centrifuged (Sigma 3K15) at 14000 rpm (18 000 g) for 5 min. The upper layer of the solution was removed and the aqueous phase precipitated with 40 µl 3 M sodium acetate, pH 5.3 (DEPC treated).
8. 800 µl of absolute ethanol (- 20 °C) was added and the tubes were centrifuged (Sigma 3K15) at 14000 rpm (18 000 g) at 4 °C, for 30 min. The supernatant was removed with a pipette. The pellet was washed with 500 µl 70 % ethanol (-20 °C), vortexed, and then centrifuged at 18 000 g for 5 min. The excess ethanol was removed from the pellet, and the pellet was allowed to air dry for about 15 min.
9. The pellet was re-suspended in 400 µl DEPC-MQW and stored at – 80 °C.

Four RNA extractions were performed, two from the sucker stem apex tissue samples and two from the young plant cane apex tissue samples.

4.2.3 Spectrophotometric determination of RNA concentration

Following the extraction of the RNA from the stem tissue, the concentration was determined. The ratio between the optical density (OD) at 260 nm and 280 nm (OD260 / OD280) provided an estimate of the purity of the nucleic acid (Sambrook *et al.* 1989) (Table 4.1). RNA quality was also checked by agarose gel electrophoresis.

Table 4.1 RNA concentrations of extracts taken from sucker stalk apices and young plant cane apices.

Tissue sample	Date Extracted	Ratio 260/280 nm	RNA Conc. ug/mL
Sucker 1 (S1)	5/6/00	1.45	2252
Young cane 1 (YC1)	6/6/00	1.47	2707
Sucker 2 (S2)	7/6/00	1.43	1177
Young cane 2 (YC2)	8/6/00	1.45	1725

The ratios found are typical of those found for sugarcane RNA at CSIRO Plant Industry in Brisbane (R. Casu CSIRO Plant Industry Brisbane pers. comm. 2001)

4.2.4 RNA clean-up/preparation

The RNA was purified (removal of DNA and other compounds) using a RNeasy Plant Mini Kit (50) (Qiagen, Valencia, CA, USA). The first three steps of the protocol are described in the Qiagen RNeasy Mini Handbook, second edition, May 1999, p. 48. Steps 4-11 are described in the Qiagen RNase free Dnase Set for use with RNeasy Plant Mini Kit (50).

Following the RNA clean-up, the RNA concentration was again determined using the method described above, and the RNA quality was checked by agarose gel electrophoresis.

4.2.5 Labelling of the probes

The method used to label the probes was from the arrayTRACKER^{tm} Standard labelling kit, Kit manual version 1.3, 14th April 2000 (Display Systems Biotech Inc., Vista, CA, USA). Each microarray slide was probed with 20 µg reference RNA and 20 µg test

RNA. Four hybridisations were conducted. On each slide, the reference probe contained 10 µg YC1 and 10 µg YC2. The test probe was made from 20 µg RNA from S1, S2, YC1 or YC2. The reference RNA was labelled with Cy-3 dUTP and the test RNA was labelled with Cy-5 dUTP (Amersham Pharmacia Biotech AB, Uppsala, Sweden).

4.2.6 Hybridisation of microarrays

Pre-hybridisation of the slides was performed as recommended by the manufacturers (Corning Microarray Technology CMT-GAPS^{tm} coated slides, Instruction manual pp 3-7, Corning Inc., USA, 2000).

1. The probe was pipetted onto the slide surface adjacent to the array. It was then covered with a clean cover slip, which forced the solution over the array surface.
2. The slide was then placed in an Arraylt™ Hybridisation Cassette (Telechem International).
3. 10 µl 3 X SSC (Appendix 1) was added to the wells at each end of the chamber.
4. The hybridisation chamber was sealed by tightening the four sealing screws.
5. The chamber was placed in a 42 °C water bath over night.

Post-hybridisation washes were conducted as recommended by the manufacturers of the slides. Following the completion of the post-hybridisation washes, the slide was scanned with a GenePix 4000A scanner (Axon Instruments, Inc., Union City, CA, USA) at wavelengths of both 635 nm (to quantify the signal due to the fluorescence of Cy-5) and 532 nm (to quantify the signal due to the fluorescence of Cy-3).

4.2.7 Statistical analysis

Data from the slides were analysed using tools for R microarray analysis (tRMA) (version 1.5.1). This software was developed by Wilson and Buckley (2001) and enquiries for its use should be referred to trma@cmis.csiro.au. The statistical software package called R was downloaded from http://www.r-project.org/

The median value for the fluorescence of the pixels within each spot as measured by the red channel (R, 635 nm) was divided by the median value as measured by the green channel (G, 532 nm) to give the ratio of the medians. Typically, a gene that is equally expressed in the two tissue types being compared appears yellow as it has equal amounts of both red and green fluorescence (R/G = 1). The data for all the spots were $\log_n$ transformed, as it was highly skewed. Following transformation, the data was 'spatially' normalised. This removed variation in expression due to spatial differences across the slide. This method of normalisation was recommended above all others (Wilson and Buckley 2001). The detection of differentially expressed genes was performed using the FindDiffExpGenes function. This method works by comparing the $\log_n$ (R/G) data with a normal distribution (with mean 0 and standard deviation 1). The outliers, or highly differentially expressed genes, make the tails of the distribution of the $\log_n$ (R/G) data appear heavier than the normal distribution. The outliers are removed one by one, starting with the outlier with the largest absolute p-value (outlier furthest from the mean). As each data value is removed, the data are again compared to the normal distribution. At some point the distribution of the subset of ln (R/G) data will have tails that are not heavy enough when compared to the normal distribution.

When this occurs the algorithm terminates, suggesting that at that point all of the data values that have been removed so far can be considered as outliers, and hence represent highly differentially expressed genes (Wilson and Buckley 2001).

4.3 Results

Inspection of the slides following scanning revealed a 'curious' green colour on the bottom edge of most blocks on the array (Figure 4.1).

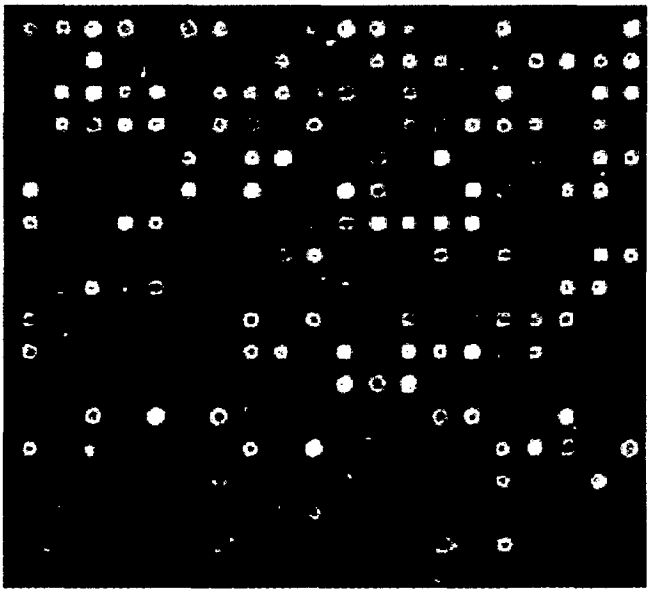

Figure 4.1 A single block on a microarray slide following hybridisation with RNA of two tissues labelled with red and green fluorescence. Each spot represents a different gene. Note the number of spots with green fluorescence at the bottom of the block.

This phenomenon was present on all four slides, to some extent. The spots on the bottom edge of the slide, and some others, were also found to be green prior to hybridisation on unused slides (R. Casu pers comm. 2001). Therefore, since the spots were predisposed to fluorescing green, even before hybridisation, the genes represented at these positions were removed from the data set prior to statistical analysis. It appeared that this may have been due to the use of a different buffer when the spots

were placed on the array as this was the most obvious difference between the green fluorescing and normal spots.

The genes that were found to be differentially expressed in the apex region of the sucker stem are shown in Table 4.2. These genes were found to be differentially expressed on both slides where sucker tissue was compared to the control. Many genes were found to have significantly lower expression in sucker stem tissue than in the control tissue, but only five genes were found to have significantly higher expression in the sucker tissue. The genes that were found to be differentially expressed in the apex tissue of young cane stalks compared to the control are shown in Table 4.3.

The genes in Table 4.4 were found to be differentially expressed irrespective of what tissue was being compared to the control. They appear in both Tables 4.2 and 4.3. These genes should be considered with caution. The two slides where YC1 and YC2 were compared to the pooled control (YC1 + YC2) should not show large differences in expression. Therefore, it appears that there may have been some fault with the spots associated with these genes. Interestingly, all these genes were located on the 15^{th} and 16^{th} rows of the blocks on the microarray slide. Each block contained 18 rows. This is in the same region where problems were found with spots fluorescing green (discussed earlier). However, the genes in Table 4.4 were treated in a similar manner to those on the rest of the slide and were not produced using the buffer that was thought to cause the other genes to fluoresce green.

Table 4.2 Differentially expressed genes in the apex tissue of sucker stalks compared to young plant cane stalks. The genes listed were found to be differentially expressed on both slides where sucker tissue was compared to the control.

Gene ID	Name	Median LogR/G	StdDev
MJR019E04	translation initiation factor	9.22	4.39
MCSA176G02	not significant	6.63	0.48
MJR017F10	significant but no function assigned	6.46	3.62
MCSA111G04	significant but no function assigned	4.31	1.26
MJR012G06	not significant	3.64	0.76
MCSA174C06	not significant	-2.91	2.51
MJR015H03	not significant	-3.73	0.50
MJR011D09	glucose dehydrogenase	-4.06	1.60
MJR012A09	significant but no function assigned	-4.32	1.18
MJR015F08	not significant	-4.37	3.35
MJR011C03	significant but no function assigned	-4.77	1.32
MCSA062F05	not significant	-4.82	6.41
MJR014E02	zinc finger protein	-5.02	2.41
MCSA115C02	ascorbate oxidase promoter-binding prote	-5.42	2.95
MCSA209D07	transcription factor	-5.49	2.33
MJR011D06	thiosulfate sulfurtransferase	-5.64	1.89
MJR016A06	cullin	-5.66	3.00
MJR011E10	photosystem II protein	-5.73	2.21
MJR013B02	GTP-binding protein	-5.81	0.28
MJR014E06	XAP-5 protein	-5.94	0.65
MJR014H11	GST	-6.18	11.60
MJR012A02	not significant	-6.20	1.32
MJR014G07	significant but no function assigned	-6.30	2.08
MJR018A02	not significant	-6.43	1.20
MJR014G02	not significant	-6.90	1.07
MJR014F06	significant but no function assigned	-6.94	2.46
MJR013G03	not significant	-7.54	2.23
MJR015A02	reverse transcriptase	-7.85	3.67
MJR015E10	UDP-glucose pyrophosphorylase	-8.19	3.99
MJR014D10	not significant	-9.00	1.13
MJR016F05	significant but no function assigned	-9.09	3.71
MJR014E04	not significant	-9.54	1.26
MJR016F02	not significant	-9.65	2.69
MJR015D09	integral membrane protein	-10.70	6.29
MJR014F07	lysophospholipase	-10.91	1.90
MJR014F12	not significant	-11.35	2.60
MJR014C12	significant but no function assigned	-13.15	5.14
MJR014G03	not significant	-13.62	1.71
MJR014E11	significant but no function assigned	-13.82	4.23
MJR014E10	chalcone synthase	-14.05	4.41
MJR014D08	not significant	-14.20	3.33
MJR014F10	not significant	-14.32	4.23
MJR016E06	not significant	-14.97	5.50
MJR014F03	GST	-18.02	4.72
MJR014D11	not significant	-18.91	3.24
MJR014G01	proteasome	-21.12	6.20

Table 4.3 Differentially expressed genes in the apex tissue of young cane stalks compared to the control. The genes listed were found to be differentially expressed on both slides where the young cane stem tissue was compared to the control.

Gene ID	Name	Median LogR/G	StdDev
MJR019E04	translation initiation factor	9.53	1.85
MJR017F10	significant but no function assigned	7.08	2.68
MJR017E11	not significant	5.90	2.30
MJR014H08	not significant	-5.17	0.56
MJR014H11	GST	-5.84	10.61
MJR018A02	not significant	-6.81	2.29
MJR014G02	not significant	-6.87	2.36
MJR011E10	photosystem II protein	-6.88	3.74
MJR013C08	heat shock protein	-7.36	1.29
MJR015F08	not significant	-7.44	2.75
MJR014G06	significant but no function assigned	-7.63	2.82
MJR014G07	significant but no function assigned	-8.50	4.24
MJR016F05	significant but no function assigned	-8.55	4.79
MJR014F06	significant but no function assigned	-8.59	3.02
MJR013G03	not significant	-8.75	3.90
MJR014E04	not significant	-8.79	4.75
MJR016F02	not significant	-9.53	1.91
MJR015E10	UDP-glucose pyrophosphorylase	-9.65	3.47
MJR014F07	lysophospholipase	-10.51	1.71
MJR015A02	reverse transcriptase	-10.96	4.77
MJR014D10	not significant	-11.46	5.69
MJR014D08	not significant	-12.35	1.60
MJR014F12	not significant	-12.41	5.23
MJR014E10	chalcone synthase	-13.50	4.05
MJR015D09	integral membrane protein	-13.84	5.20
MJR014C12	significant but no function assigned	-14.79	3.23
MJR014G03	not significant	-15.63	4.05
MJR014F10	not significant	-16.03	5.59
MJR014E11	significant but no function assigned	-16.12	4.85
MJR016E06	not significant	-16.82	5.91
MJR014D11	not significant	-18.36	5.02
MJR014F03	GST	-20.99	4.61
MJR014G01	proteasome	-24.61	6.89

When this occurs the algorithm terminates, suggesting that at that point all of the data values that have been removed so far can be considered as outliers, and hence represent highly differentially expressed genes (Wilson and Buckley 2001).

4.3 Results

Inspection of the slides following scanning revealed a 'curious' green colour on the bottom edge of most blocks on the array (Figure 4.1).

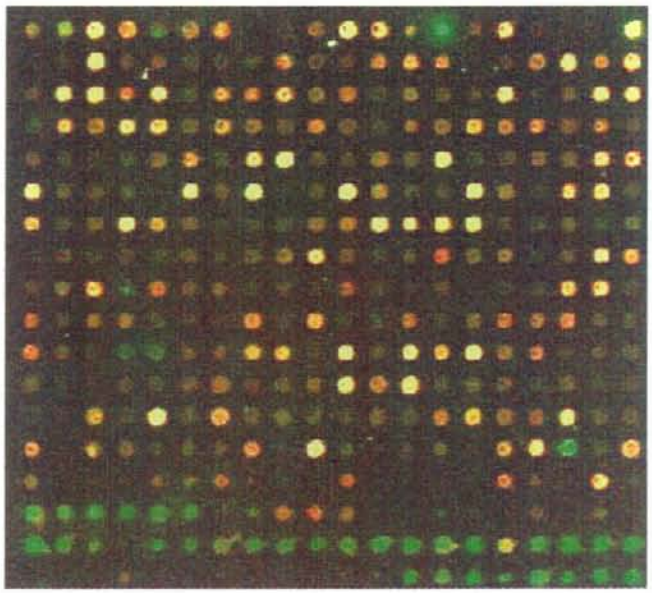

Figure 4.1 A single block on a microarray slide following hybridisation with RNA of two tissues labelled with red and green fluorescence. Each spot represents a different gene. Note the number of spots with green fluorescence at the bottom of the block.

This phenomenon was present on all four slides, to some extent. The spots on the bottom edge of the slide, and some others, were also found to be green prior to hybridisation on unused slides (R. Casu pers comm. 2001). Therefore, since the spots were predisposed to fluorescing green, even before hybridisation, the genes represented at these positions were removed from the data set prior to statistical analysis. It appeared that this may have been due to the use of a different buffer when the spots

Table 4.4 Genes found to be differentially expressed on all slides, irrespective of tissues being compared.

Gene ID	Name
MJR019E04	translation initiation factor
MJR017F10	significant but no function assigned
MJR014H11	GST
MJR018A02	not significant
MJR014G02	not significant
MJR011E10	photosystem II protein
MJR015F08	not significant
MJR014G07	significant but no function assigned
MJR016F05	significant but no function assigned
MJR014F06	significant but no function assigned
MJR013G03	not significant
MJR014E04	not significant
MJR016F02	not significant
MJR015E10	UDP-glucose pyrophosphorylase
MJR014F07	lysophospholipase
MJR015A02	reverse transcriptase
MJR014D10	not significant
MJR014D08	not significant
MJR014F12	not significant
MJR014E10	chalcone synthase
MJR015D09	integral membrane protein
MJR014C12	significant but no function assigned
MJR014G03	not significant
MJR014F10	not significant
MJR014E11	significant but no function assigned
MJR016E06	not significant
MJR014D11	not significant
MJR014F03	GST
MJR014G01	proteasome

As there was some doubt about the genes found to differentially expressed in Table 4.4 the analysis was re-done following the removal of these genes from the data files. This was done by re-loading the original files into tRMA and then using the RemoveGene function to remove each gene. The data was then spatially normalized and differentially expressed genes were found and compared (as discussed earlier). Those genes that were found to be differentially expressed on both slides where sucker stem apex tissue was compared to the control are shown in Table 4.5.

Table 4.5 Differentially expressed genes in the apex tissue of sucker stalks compared to young plant cane stalks following the removal of 'bad' genes. The genes listed were found to be differentially expressed on both slides where sucker tissue was compared to the control .

Gene ID	Name	Median LogR/G	StdDev
MCSA176G02	not significant	6.70	0.49
MCSA111G04	significant but no function assigned	4.35	1.28
MJR012G06	not significant	3.68	0.78
MCSA174C06	not significant	-2.94	2.54
MJR015H03	not significant	-3.77	0.51
MJR011D09	glucose dehydrogenase	-4.11	1.61
MJR011E09	not significant	-4.31	1.78
MJR012A09	significant but no function assigned	-4.36	1.18
MJR015G08	not significant	-4.57	2.07
MJR011C03	significant but no function assigned	-4.82	1.33
MCSA062F05	not significant	-4.87	6.47
MJR014E02	zinc finger protein	-5.06	2.43
MCSA115C02	ascorbate oxidase promoter-binding prote	-5.47	2.98
MCSA209D07	transcription factor	-5.54	2.35
MJR011D06	thiosulfate sulfurtransferase	-5.70	1.91
MJR016A06	cullin	-5.73	3.03
MJR013B02	GTP-binding protein	-5.86	0.28
MJR012A02	not significant	-6.27	1.34
MJR014E06	XAP-5 protein	-6.34	0.72
MJR014G09	not significant	-7.80	3.92

4.4 Discussion

Of the twenty genes that were found to be differentially expressed in the stem apex of sucker stalks compared to the stem apex of young plant cane stalks, only three were up-regulated. Unfortunately, at this time, the partial sequences available for these EST's did not match any of the genes and EST's lodged in public access databases. However, they still have some use. Northern blots using MCSA176G02, MCSA111G04 and MJR012G06 would be the next step to see whether or not the result from the microarray analysis might be confirmed using this different technique. If such further investigation did confirm that these genes were significantly up-regulated in sucker stem tissue, the EST's could then be fully sequenced and used to find full length clones and their function investigated further. These genes might also potentially be used as a marker for sucker stalks. High expression of these genes in a sample may indicate that

the sample was taken from a sucker stalk, and not from a 'normal' stalk. This may provide a molecular way of identifying suckers in the future. Ultimately a marker for propensity to sucker would be of great use to sugarcane technologists.

Similarly, further investigations using northern blots would be required for the genes that were found to be significantly down-regulated in the apex tissue of sucker stalks in this study.

Some information on the function of the genes that were shown to be differentially expressed in sucker stem apices was found. Glucose dehydrogenase (MJR011D09) activity has been shown to decrease during the breaking of dormancy and the initial stages of germination in seeds of *Tollius ledebouri* following GA_3 treatment (Bailey *et al.* 1996). High ascorbate oxidase activity is associated with tissues containing rapidly expanding cells in a wide range of plants (Smirnoff, 1996), but why ascorbate oxidase promoter-binding protein (MCSA115C02) should be down-regulated in sucker tissue is not known. GTP-binding proteins have been implicated in auxin signal transduction in rice (Zaina *et al.* 1990) a reduction in apical dominance, dwarfism and abnormal flower development in tobacco (Kamada *et al.* 1992) and were shown to be regulated by phytochrome in pea (Clark *et al.* 1993). While these genes (and other unknowns) have been shown to have altered expression in the stem apex tissue of sucker stalks, it would be premature to speculate as to how such difference could relate back to the differences found between sucker and 'normal' stalk morphology.

During the analysis of the microarray slides it was noticed that the slide to slide variation, and even variation between replicate spots on the same slide, was high.

Therefore, it is strongly recommended that a greater number of replicates (slides) should be used for this type of analysis in the future. This could potentially result in the detection of many other genes that show altered expression, as would a more comprehensive set of spots on the array. Furthermore, the array used does not contain any genes that are only present in sucker tissue. This was due to the fact that none of the EST's used to create the array were sourced from a cDNA library from sucker tissue. In time, the identity and function of some of the genes found to have altered expression in this study will be made. This could provide further evidence as to the differences in sucker and normal stalk morphology and the possible causes of these differences.

Chapter 5. The relationship between suckers and main stems

5.1 Is sucrose lost from the main stalk to support sucker growth?

5.1.1 Introduction

Suckers arise from underground buds on the mature 'parent' stalk. Therefore a sucker starts its existence presumably when the mature stalk dictates, by allowing/making the bud burst and providing it with at least the essential nutrients until the new stalk can become self sufficient. However, the duration of the relationship, and the totality of its effect were not known.

The presence of low sucrose billets, from suckers, at the mill, causes a dilution of the sugar content of the harvested material. The loss of profitability this causes in turn has been highlighted by Wilson and Leslie (1997), and has been discussed in previous chapters. This negative impact on productivity was the reason why attention was currently focused on the biology of suckering. However, dilution at the mill may not be the only negative contribution that suckers afford on profitability. Hes (1954) stated "*It (sucker) is born from the base of a stalk, which has already gotten an appreciable length and foliage, able to realise a good photosynthesis. The products of the photosynthesis are, as we know, for the greater part stored in the base of the stalk. We may therefore suppose that a sucker is well fed right from its appearance out of the stalk from which it has derived.*" This proposed support would presumably result in the mature stalk having a lower sucrose content than if it had not produced a sucker. Since this translocated material would be used in sucker growth, it would not be available to

be recovered at the mill, and would therefore further impact on productivity. As further evidence for this theory, Hes introduces an observation by Kuyper. Kuyper described a cane stool with two well-developed suckers, both almost two metres tall. One sucker was an albino, containing no chlorophyll and the other was normal. Kuyper hypothesised that the albino sucker could not have contributed to its own growth and therefore must have been built up from material exclusively from the parent shoot or at least from the whole stool to which it was attached.

Clements (1980) found that the sucrose content of the first two internodes (above ground level) of cultivar H31-1389 decreased and hexose content increased after rain in June and early July 1941 in Kailua. Clements suggested that this was due to sucrose being moved to growing parts of the plant - stem tip, roots and suckers. This data also provides support for the theory that sucker growth may be supported by the stalk to which it is attached.

An early experiment conducted by Hes (1954) to quantify the influence of suckers on yield involved the removal of suckers from a portion of a crop at two-week intervals. The sucrose content of the harvested material was then compared to harvested material from plots where the suckers were allowed to grow. Hes stated that the removal of suckers did not appear to be of great value. The crop which had suckers frequently removed had an average sucrose yield 0.5 ton per ha greater than the crop where suckers were allowed to grow. This was due to a slightly higher sucrose content of the juice but a lower average yield of cane (3 ton per ha). It is not clear from the paper if these differences were statistically significant, and the mean yields were not presented.

It is also not clear whether the experiment conducted by Hes gave any indication of the loss of sucrose from the mature stem to aid sucker growth. If only the mature stalks were harvested at the end of the experiment, then the slightly higher sucrose yield in plots where suckers were removed might mean that sucrose was lost from the mature stalks when suckers were allowed to grow. However, if the suckers were harvested with the mature stalks at the end of the experiment, the comparison between the plots would just show that the low sucrose material from the harvested suckers was diluting the overall sucrose concentration of the crop. Hes did not state exactly what was sampled in order to calculate sucrose yield per ha at the end of the experiment. Therefore the loss, if any, of stored assimilate from the mature stem to the sucker, to aid growth, remains unknown.

The term sucker is often used in other agricultural crops such as banana and tobacco. Banana suckers are similar to sugarcane suckers as they appear from the underground buds. Various de-suckering experiments in banana crops have shown that sucker growth has a negative effect on yield, with excessive sucker growth resulting in a lower bunch weight on the mature stalk than if suckers were removed (Turner 1972; Robinson 1987; Chundawat and Patel 1992; Mondal 1993). Consequently, de-suckering is thought to be one of the most important management practices in order to achieve maximum yield in banana crops (Robinson, 1987). Sucker control in tobacco is considered important as the suckers are thought to initially withdraw carbohydrate from the plant at a point when leaf carbohydrate levels are required to be high (Akehurst 1981). Yield increases of 40% have been measured as the benefit of clean suckering over no sucker control (Akehurst 1981). Tobacco suckers are produced in the upper leaf axils, as a response to topping. Tobacco suckers therefore differ to both banana and

sugarcane suckers, as tobacco suckers are a result of the loss of apical dominance. Tobacco suckers would therefore be more similar to side shoots in sugarcane, which appear from the buds above ground if the apex of the stalk is damaged.

In both the banana and tobacco industries, suckers are removed in order to increase yield. The growth of the suckers acts as a sink for carbohydrate and results in lower yield on the mature stem. Whether sugarcane suckers act as a sink in the sugarcane plant is as yet unknown, but evidence from the banana and tobacco plants suggests that it is possible. However, the fact that sugarcane, banana, and tobacco plants have tissue that are termed suckers, does not mean that they are botanically equivalent or act and respond in a similar way.

The relationship between stalks in a grass plant is complex. Translocation between tillers has been shown in a number of plant species, including oats (Labanauskas and Dungan 1956), sugarcane (Hartt *et al.* 1963) and *Lolium multiflorum* (Marshall and Sagar 1968a, 1968b; Gifford and Marshall 1973). Labanauskas and Dungan (1956) argued that the unit in a field of oats is the plant and not any individual stem. The performance of any stem is influenced by the benevolence of conditions surrounding the other stems of the same plant. This is in agreement with Marshall and Sagar (1968) who stated that the evidence points to a highly organised system rather than a collection of individuals. If this is the case, translocation to a sucker, which might be in unfavourable light conditions, could occur. Hartt *et al.* (1963) found that translocation in sugarcane does occur between stalks. When blade 3 of one stalk was fed with $^{14}CO_2$, radioactive photosynthate had reached blade 2 of all other stalks within 20 h. Sucrose was found to be the principal compound translocated throughout the sugarcane plant.

It is known that assimilates are initially translocated to young developing tillers, and as these tillers grow, the level of support declines (Sagar and Marshall 1966; Marshall 1967). This is due to young developing tillers not initially being autotrophic (Evans *et al.* 1964). Once the shoot becomes photosynthetically independent it no longer requires nutritional support from the mature stalk, but is capable of receiving translocated material and translocating material to other stalks if required. It is not known whether suckers become photosynthetically independent early in their growth, as they appear to have a faster growth rate than 'normal' stalks, while often being shaded by the canopy to some extent. The assertion that suckers have rapid growth rates is made in the earlier summaries of suckering (Hes 1954; Barnes 1974). This faster growth rate may be a shade avoidance response as it is important for these new shoots to reach favourable light conditions for growth. This may mean that suckers require prolonged support from other tillers and therefore loss of sucrose from these stalks to suckers may be appreciable.

The aim of this investigation was to determine whether stored assimilate is lost from the mature 'parent' stalks to suckers. As well as describing some basic sucker biology, this was performed to determine if the full extent of the problem of suckering in sugarcane and its negative impact on profitability has been taken into account by the industry.

5.1.2 Methods

5.1.2.1 Treatments and sampling

Two treatments were initiated on 23rd April 1999, using cultivar Q152. The crop was located near Euramo, 20 km South of Tully, in far-north Queensland. The first treatment was the removal of suckers from mature stalks, by cutting at ground level. The second treatment was the removal of every second leaf that had appeared on suckers. This was an attempt to increase the dependency of those suckers on the mature stalk, thereby possibly increasing any loss of stored sucrose from the mature stem.

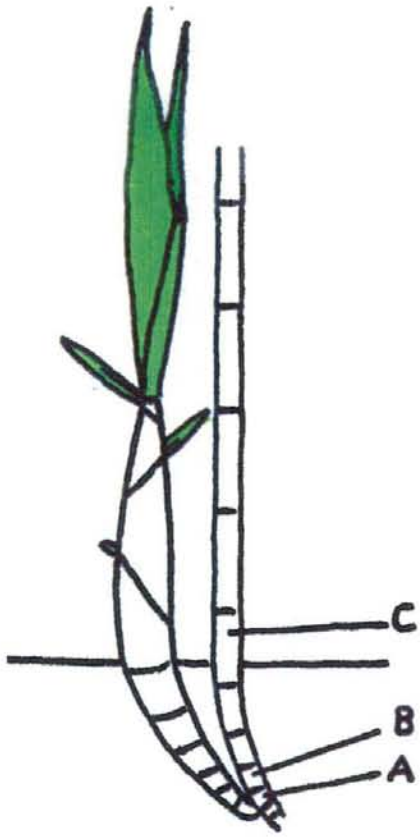

Figure 5.1 Internodes sampled on the mature stalk on 9th June 1999. **A**. Internode to which sucker was attached **B**. Internode above that to which the sucker was attached **C**. First internode above ground level. On 7th September 1999, the internodes described above plus the 5th, 10th, 15th and 20th internode above ground were also sampled. Note the figure may not represent the number of below ground internodes on both stalk types accurately.

On 9th June 1999 and 7th September 1999, 5 mature stalks that had either (i) no sucker growing on them, (ii) a sucker initiated but later removed, (iii) a sucker that had every second leaf removed or (iv) a sucker that had been allowed to grow normally were randomly chosen, harvested and processed separately. From all of the stalks harvested on 9th June 1999, a series of internodes was sampled. These comprised the internode from which the sucker had emerged, the next internode up the stem and the first internode above ground (Figure 5.1) .

The first internode above ground was generally 2-5 internodes above the internode from which a sucker had emerged.

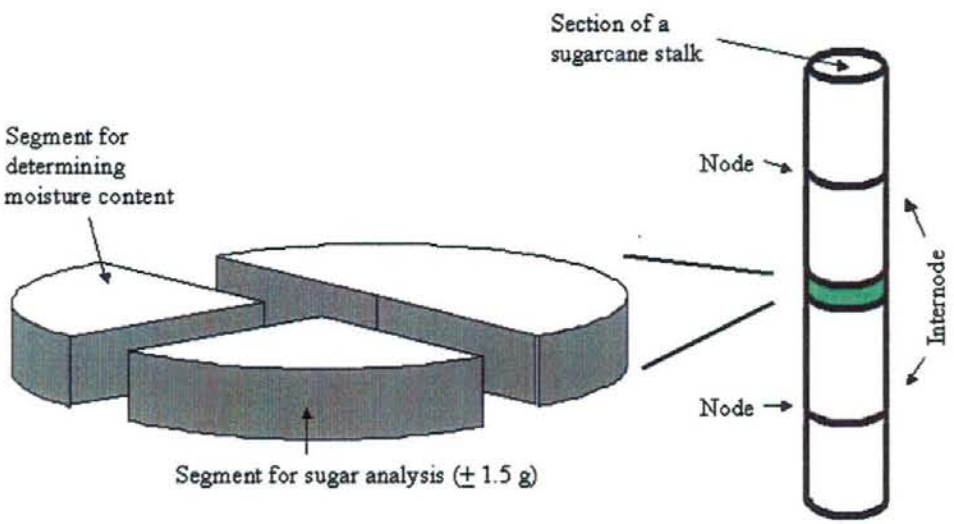

Figure 5.2 Position of tissue sample taken from each internode from the mature stem for sugar analysis.

From the middle of each internode a 2-5 mm slice was cut, the slice was quartered and placed in a plastic tube (Figure 5.2). This was placed in liquid nitrogen, stored on dry ice and then at –80°C prior to extraction.

On 7th September 1999, in addition to the internodes harvested at the first sampling, every 5th internode up the above-ground stem was also sampled. A Q138 crop, adjacent to the Q152 crop, was also sampled on 7th September 1999. However, only stalks with and without a sucker were sampled, as described for Q152.

5.1.2.2 Sugar extraction and analysis

Sugars from a sub-sample of the tissue samples (approximately 1.5 g) were extracted by adding 10 ml 80 % ethanol and incubating in a water bath (75 °C) for 2 h. Liquid was decanted and stored at 4 °C. A further 10 ml of 80% ethanol was added to samples and the tubes were again placed in the water bath for 6 h. After this re-extraction the liquid was decanted and the two extractions were combined and stored at 4 °C.

A sub-sample of the combined extracts (1.2 ml) was then dried under vacuum in a microcentrifuge. The resulting pellet was resuspended in 1 ml water and 100 µl was removed and diluted to 10 ml. The drying step was performed only for samples from the first harvest date. For the second set of samples, 25 µl of the combined extract was made up to 10 ml. For both sets of samples, concentration of sucrose was then determined by high performance liquid chromatography (HPLC).

The 10 ml diluted samples were filtered through a 0.45 μm filter. 10 μl of the filtered sample was injected into a HPLC system (controller, Waters 600s; pump, Waters 626; autosampler, Waters 717; Waters, Milford, MA, USA). The non-metallic system performed separations with a carbohydrate column (RCX 10, Hamilton, Reno, Nevada, USA) and a mobile phase of 75 mM NaOH at 1 ml min^{-1}. Eluting sugars were detected by pulsed amperometric detection (Waters 464; Waters, Milford, MA, USA). The potentials for the pulsed amperometric detector were (E1 = +80 mV; E2 = + 730 mV; E3 = -570 mV) as described by Papageorgiou *et al.* (1997). Sucrose was quantified by comparison with an external standard.

A further sub-sample of the internode tissue was weighed and dried in an oven at 60°C then re-weighed to determine moisture content.

5.1.2.3 Statistical analysis

Sucrose concentration and sample moisture contents were analysed using two-way ANOVA with treatment and internode as dependent variables. Orthogonal comparisons of sucrose content were used following the two-way ANOVA. *Post-hoc* comparisons of moisture content means were conducted using Fisher's least significant difference (LSD) ($p \leq 0.05$).

5.1.3 Results

Treatments were imposed in an attempt to quantify the amount of sucrose removed, if any, from mature stalks to support sucker growth. The mean sucrose contents are shown in Table 5.1. Two-way ANOVA showed a significant effect due to both treatment and internode. Orthogonal comparisons between treatments showed there was a highly significant difference between stalks that did not produce a sucker and those that had ($p < 0.01$); no difference between stalks that had an attached growing sucker and stalks that had the sucker removed ($p > 0.05$); and no difference between the stalks that had a normal growing sucker and stalks that had a sucker growing with half the leaves removed ($p > 0.05$). The difference between the stalks that had not produced a sucker and those that had accounted for over 97% of the variation due to treatments. Sucrose content was significantly greater in the first internode above ground.

Table 5.1 Sucrose concentration of stalks (standard error in brackets) of cultivar Q152 which did not have an attached sucker, those with an attached sucker, those with an attached sucker with every second leaf removed from that sucker, and those stalks that had produced a sucker which was removed. Samples taken on 9th June 1999.

Treatment	Sucrose concentration (mg g^{-1} fresh mass)			
	Internode attached to sucker	Internode above attachment of sucker	First internode above ground	Mean (treatments)
No sucker	161.5 (10.2)	194.3 (5.3)	187.8 (15.1)	181.2 (7.0)
Sucker	109.8 (7.1)	127.6 (19.2)	155.2 (8.1)	130.9 (8.4)
Sucker with ½ leaves	118.5 (10.4)	113.4 (11.6)	147.3 (18.7)	126.4 (8.5)
Sucker removed	115.9 (4.4)	109.2 (9.6)	134.0 (9.4)	119.7 (5.2)
Mean (internodes)	126.4 (6.1)	136.1 (9.7)	156.1 (7.7)	

The differences in sucrose concentration were not due to differences in moisture content. The mean moisture content of mature stalks of Q152 for plants with no sucker, a sucker with half leaves present, a sucker allowed to grow normally and a sucker removed were 67.1%, 70.7%, 68.0% and 66.9%, respectively. Two-way analysis of variance gave a non-significant F ratio. There was also no significant difference in moisture content between internode positions.

In the second sampling, two-way analysis of variance (cultivar Q152) demonstrated that there was a significant difference between treatments and internodes on the stem, but no significant interaction between internode position and treatment. The mean sucrose contents are shown in Table 5.2. Orthogonal comparisons showed that there was no significant difference between stalks that had not produced a sucker and those that had ($p > 0.05$); no significant difference between stalks with an attached growing sucker and stalks that had the sucker removed ($p > 0.05$); but a significant difference between stalks that had a normal growing sucker and stalks that had a sucker growing

with every second leaf removed ($p = 0.012$). This effect accounted for 55% of the variation due to treatments.

The lack of a significant difference between stalks (Q152) that had not produced a sucker and those that had, was in contradiction with the results found at the first sampling. In Q138, the average sucrose concentration was 167 mg g^{-1} fresh mass for stalks with a sucker and 136 mg g^{-1} fresh mass for stalks without a sucker. This is the opposite effect to what was found for Q152 at the first sampling. Two-way analysis of variance demonstrated no difference between the internodes along the stem but a significant difference between the two treatments.

Table 5.2 Sucrose concentration of main stalks of cultivars Q138 and Q152 with manipulated sucker growth. Samples taken on 7th September 1999. Means followed by the same letter in the same column are not significantly different ($p > 0.05$).

Cultivar	Sucrose concentration (mg g^{-1} fresh mass)							
	Internode attached to sucker	Internode above attachment of sucker	1st above-ground	5th above-ground	10th above-ground	15th above-ground	20th above-ground	Mean
Q152								
No sucker	116.1	150.2	156.4	176.8	148.4	175.6	150.1	153.4
Sucker	130.9	149.0	181.4	176.2	179.5	190.8	187.1	170.7
Sucker with 1/2 leaves	124.1	151.2	145.7	170.0	186.9	134.3	140.7	150.4
Sucker removed	157.2	154.2	164.4	168.8	176.5	214.1	163.9	171.3
Mean	132.1[a]	151.2[ab]	162.0[bc]	173.0[c]	172.8[c]	178.7[c]	160.5[b]	
Q138								
No sucker	126.9	145.6	148.5	152.7	133.4	108.5	138.8	136.3[a]
Sucker	152.2	149.1	182.5	169.3	165.1	178.3	172.9	167.1[b]
Mean (ns)	139.5	147.4	165.5	161.0	149.2	143.4	155.9	

ns - F test not significant ($p > 0.05$)

The differences in sucrose concentration were not due to differences in moisture content. The mean moisture content of mature stalks of Q152 for plants with no sucker, a sucker with half leaves present, a sucker allowed to grow normally and a sucker removed were 64.5%, 64. 8%, 64.3% and 61.8%, respectively. Two-way analysis of variance gave a non-significant F ratio. For Q138, the mean moisture contents of the internodes of the mature stalks with suckers and without suckers were 66.0% and 66.5%, respectively. Again, two-way analysis of variance gave a non-significant F ratio.

5.1.4 Discussion

The results indicated that allocation of carbon to suckers potentially causes losses in profitability through two effects. Firstly, carbon allocated to suckers is not being directed towards sucrose storage in main stalks, and secondly it is having a further negative impact, when harvested, on the sucrose content that is stored in the mature stalks by dilution. The degree to which the carbon used in sucker growth would be directed to sucrose storage in the main stalk, in the absence of suckers, was not clear.

Sucrose from the mature stalk appeared to be consumed in the process of initiation of a sucker. This statement is supported by the initial results. This does not seem unreasonable, as developing tillers are not initially autotrophic (Evans *et al.* 1964). It is known that assimilates are initially translocated to young developing tillers from established ones, and as these tillers grow, the level of support declines (Sagar and Marshall 1966; Marshall 1967). The period of time required before developing tillers become autotrophic has yet to be determined for sugarcane suckers. As suckers are

initiated below ground level they cannot be autotrophic until they emerge from the soil. Suckers often grow, at least initially, below the main canopy. In these cases any photosynthetic contribution to their own growth would be a result of intercepting light not captured by the mature stalks.

For the second set of sucrose measurements, taken later in the year, interpretation of the data was less certain. The mature stalks that had suckers with half their leaves removed had significantly less sucrose than stalks that had initiated a sucker that was not altered. This seems logical as a sucker with half its leaves removed would be less able to be self-sufficient in photosynthetic products. One possible reason why this difference was not found following the first set of samples was the short period of time between the first sampling and the implementation of the treatments (47 days). There were 139 days between the implementation of the treatments and taking the second set of samples.

The second data set showed no significant difference in sucrose concentration in main stalks that had not initiated a sucker. This was different from the first sampling, three months earlier. In the intervening three months the numbers of suckers increased dramatically. The number of mature stalks that did not have a sucker present was few. van Dillewijn (1952) stated that suckers "appear when the other tillers are already more or less full-grown". There does appear to be a certain maturity requirement for a stalk before it can initiate a sucker. Taking the increased number of suckers and the apparent requirement of a level of maturity of an individual stalk before it can produce a sucker together, the second set of sucrose experiments could be explained. If younger order tillers were the only main stalks that had not initiated suckers then the stalks used as a control would by virtue of their younger age contain less sucrose. Consequently the

stalks without suckers in the second sampling would not be a suitable control in this experiment. This was also demonstrated in the mean sucrose content for the internodes of stalks that had not produced a sucker on the two sampling dates. At the first sampling, the mean sucrose content for stalks which had not produced a sucker was 181.2 mg g^{-1} fresh mass, at the second sampling the mean for the lowest three internodes was 140.9 mg g^{-1} fresh mass. This difference was found to be highly significant when the data were analysed using two-way ANOVA with time and internodes as independent variables.

While suckers may withdraw stored assimilate from the stem of the main stalk to which they are attached, they may also rely on current assimilate that is being produced in the leaves of that mature stalk. If some of this assimilate is lost to the sucker, the newly developing internodes at the top of the mature stalk could have a significantly reduced sucrose concentration. This is one of the reasons why internodes from further up the stem were harvested in the second sampling. When looking at the 15^{th} and 20^{th} internodes above ground (Q152), the stalks with no sucker (175.6 and 150.1 mg g^{-1} fresh mass) and the sucker with half leaves (134.3 and 140.7 mg g^{-1} fresh mass) had much lower sucrose concentrations than the stalks with a normal sucker attached (190.8 and 187.1 mg g^{-1} fresh mass) and the stalks with the sucker removed (214.1 and 163.9 mg g^{-1} fresh mass). The lower sucrose concentration in these upper two internodes for the stalks that had not produced a sucker, may be further indication to them being higher order, younger tillers, which would be less mature. While the lower sucrose concentration in the upper two internodes of the stalks, which had produced a sucker from which every second leaf was removed, may have been due to greater amounts of

assimilate being lost from the leaves to the sucker, further evidence would be needed before this conclusion could be made.

The data allows for some speculation as to the amount of sucrose that suckers may withdraw from mature stalks. Suckers with every second leaf removed may be considered a worst case scenario, possibly similar to a sucker that is heavily shaded by the canopy. This sucker could require more nutritional support than others. These data can be compared to that of a stalk with a normal sucker. Ideally, it would be compared to a stalk with no sucker attached, but as shown before this control was not adequate at the second sampling. The mean values for these two treatments were 150.4 and 170.7, respectively. This means that removing every second sucker leaf resulted in an 11.89 % reduction in sucrose content in the main stalk. Even if the actual difference between a stalk with no sucker and a stalk with a sucker attached is only half this amount, it is still large and may reflect a real commercial loss for the industry. Therefore, there is a need to take this into account when assessing the importance of suckering to the industry.

To fully determine how much assimilate is drawn from stored sucrose and from current assimilate to support sucker growth will require further experimentation. Showing the movement of labelled carbon from main stalks to suckers would provide a definitive answer. The size of sugarcane crops and the duration of the crop cycle however, presents challenges for this kind of experimentation. Consequently it may require analysis of different stalks of the same age, manipulated with environmental cues that promote or inhibit suckering without altering carbon assimilation to establish unequivocally the carbon supplied to suckers. The results of the initial sampling and the stalks with suckers attached with half their leaves removed sampled later in the season

indicated that suckers withdraw carbohydrate from the stalks that they are attached to. Until the level of dependence of suckers on parental stalks is defined however, the full impacts of suckering on profitability of sugarcane production will remain unknown. Some work also needs to be directed at finding out whether only stalks with 'excess' sucrose, or sucrose concentrations above a certain 'trigger' level, produce suckers. This is alluded to by Bull and Glasziou (1963). If suckering were somehow prevented could the modern day cultivars actually store this excess?

5.2 Morphology of suckers after the detachment of the parent stalk

5.2.1 Introduction

In Chapter 5.1, some evidence for the translocation of sucrose from the mature 'parent' stalk to the sucker was shown. This nutritional support has been alluded to in some references (Hes 1954; Barnes 1974), but as yet, has not been conclusively shown. This nutritional support from the mature stalk to the sucker may partly be responsible for the differences found in morphology between sucker and normal stalks (Chapter 3), as it may allow smaller investment in photosynthetic capacity and greater investment in growth. It is not known whether plant hormones or other biochemical compounds are also transported to the sucker from the mature stalk. Potentially, these compounds may also provide a signal that could affect the growth of the sucker stalk.

After a crop is harvested, often young stalks can be seen with cropped upper leaves. These stalks must have been initiated prior to harvest and therefore were probably initiated as suckers. Later in the year these stalks are indistinguishable from all the other ratoon stalks. This raises the question, is sucker morphology dependent on the presence of the mature stalk to which it is attached?

In order to test whether the morphology of a sucker is influenced by the presence of the mature stalk, an experiment was established where parent stalks were removed following the initiation of the sucker. Stalk morphology was then compared to suckers that had the mature stalk attached.

5.2.2 Methods

5.2.2.1 Plant growth and experimental design

On 23rd October 1998 three single eye sets of cultivar Q138 were planted in each of 24 pots at CSIRO Davies Laboratory. Pots were placed in a single row. On 26th May 1999, the mature stalks were removed from 12 of the 24 pots, selected at random. Suckers had initiated prior to this date. Suckers of two age groups were marked and subsequently monitored: those that had just emerged or produced only one leaf (>20 mm), and those that had produced three or four leaves at the time of the main stalk removal. Leaf lamina length and maximum breadth were collected from the marked suckers as they grew, up to leaf 12 for the younger suckers and leaf 18 for those with 3-4 leaves emerged at the time of mature stalk removal (Experiment 1).

In a similar experiment at the same location, plants of three sugarcane cultivars (Q117, Q138 and Q152) were grown. Fifteen pots of each cultivar were planted on 2nd August 1999. Three single-eye sets were planted into each pot. Pots were placed in two rows with cultivars being randomly distributed throughout. On 24th May 2000, mature stalks were removed from every second pot in each row. This was done in order to minimise any effect due to light. Suckers were initiated prior to this date. Leaf length and maximum breadth were collected from young suckers that had their mature stalk removed and young suckers that had their mature stalk attached. Suckers that had produced 4, 5 or 6 leaves were marked and followed. Data were collected until twenty leaves had been produced (Experiment 2).

Plants in both experiments were grown in pots (38 cm diameter and 30 cm depth) containing a mixture of peat, coarse sand and fine sand (1:2:2 v/v/v). The plants were automatically irrigated three times a day. Fertilizer was applied at regular intervals: liquid fertiliser (Wuxal®, Schering Pty Ltd, NSW, Australia, 300 ml of 15 ml/l) approximately every fortnight; granular, slow release fertiliser (Osmocote®, Scotts Australia Pty Ltd, NSW, Australia, 14:6.1:11.6 N:P:K, 50 g pot^{-1}) every eight weeks. Plants were prevented from lodging by wire supports, as described in previous chapters.

5.2.2.2 Statistical analysis

Leaf data were analysed using two-way ANOVA with treatment and leaf number as dependent variables. This was done separately for both sucker age groups at the time of stalk removal in experiment 1, and for the three cultivars in experiment 2. Individual analysis of treatments at each leaf number was also performed using one-way ANOVA. Post hoc comparison of means was performed using Fisher's LSD ($p < 0.05$).

5.2.3 Results

5.2.3.1 Experiment 1

Removing the mature stalks in pots of cultivar Q138, following the initiation of suckers, had a significant effect on the leaf morphology of suckers. The data refer to the leaf lamina. Following the removal of the mature stalk, the suckers produced leaves

that had a significantly greater leaf length ($p < 0.05$) and significantly smaller leaf maximum breadth ($p < 0.05$). This caused the suckers with their mature stalks removed to have a significantly greater leaf length to breadth ratio ($p < 0.05$) than suckers that had their mature stalks attached. This result was found to be consistent for suckers of both age groups, those that had produced one leaf or less when the mature stalks were removed (Figure 5.3), and those that had produced 3 or 4 leaves when the mature stalk was removed (Figure 5.4). For both age groups, there was a 'lag' period (2-3 leaves) following the removal of the mature stalks, where the leaf morphology of suckers in each treatment remained similar. For both age groups the graphs are very similar. The differences in lengths became less 7-8 leaves after they initially showed differences, to the point that they became similar. Though the breadths of leaves in both treatments did not become similar for the later leaves, for both age groups the differences increased for 4-5 leaves and then decrease. Combined, this resulted in a very marked increase in the length to breadth ratio and then a distinct decrease.

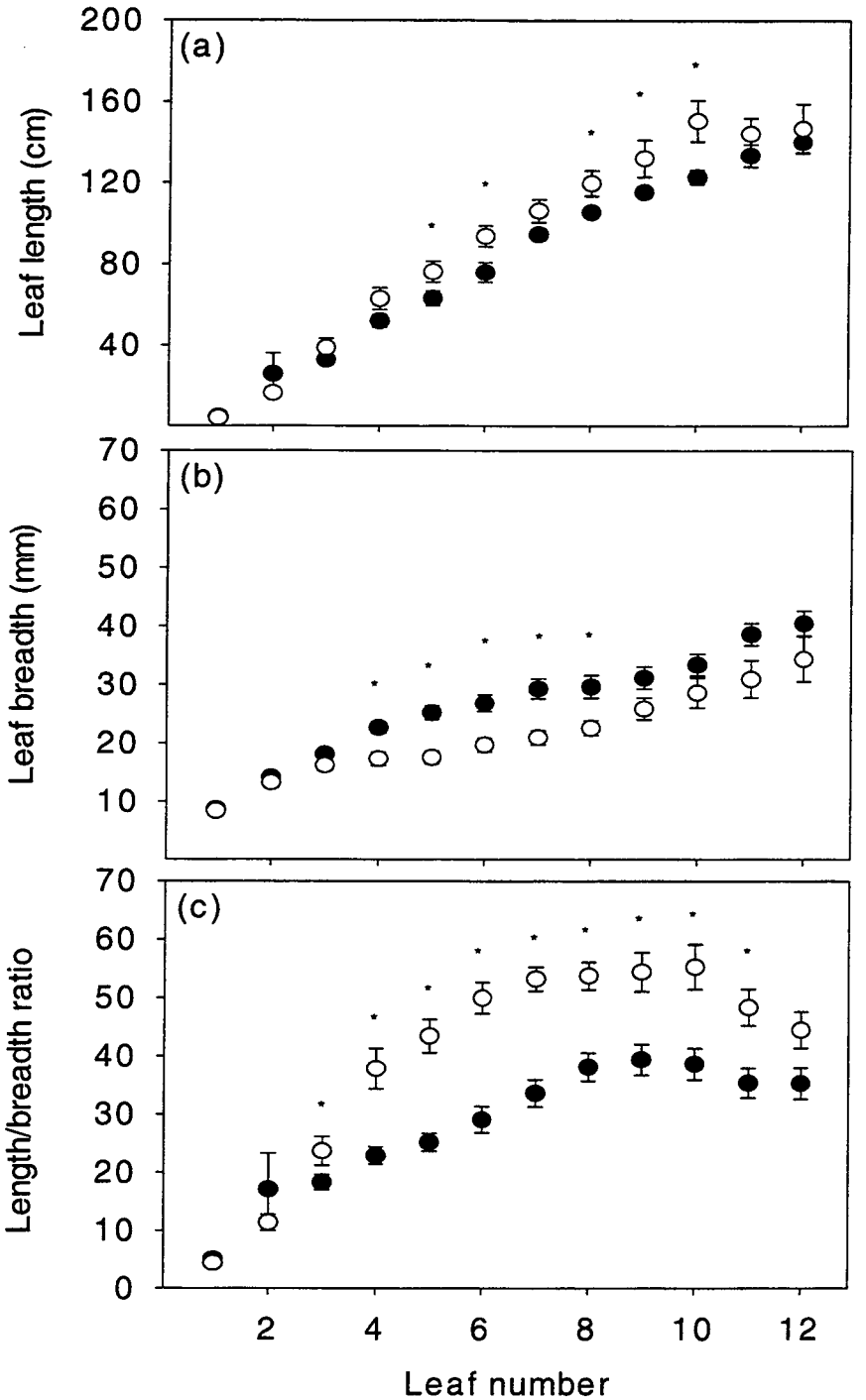

Figure 5.3 Leaf length (a), maximum breadth (b) and length to breadth ratio (c) of suckers of cultivar Q138 with the main stalks attached (●) and mature stalks removed (○). The suckers had just emerged or produced one leaf prior to the removal of the mature stalks. Error bars represent ± the standard error of the mean. * indicates a significant difference ($p < 0.05$) following single factor ANOVA.

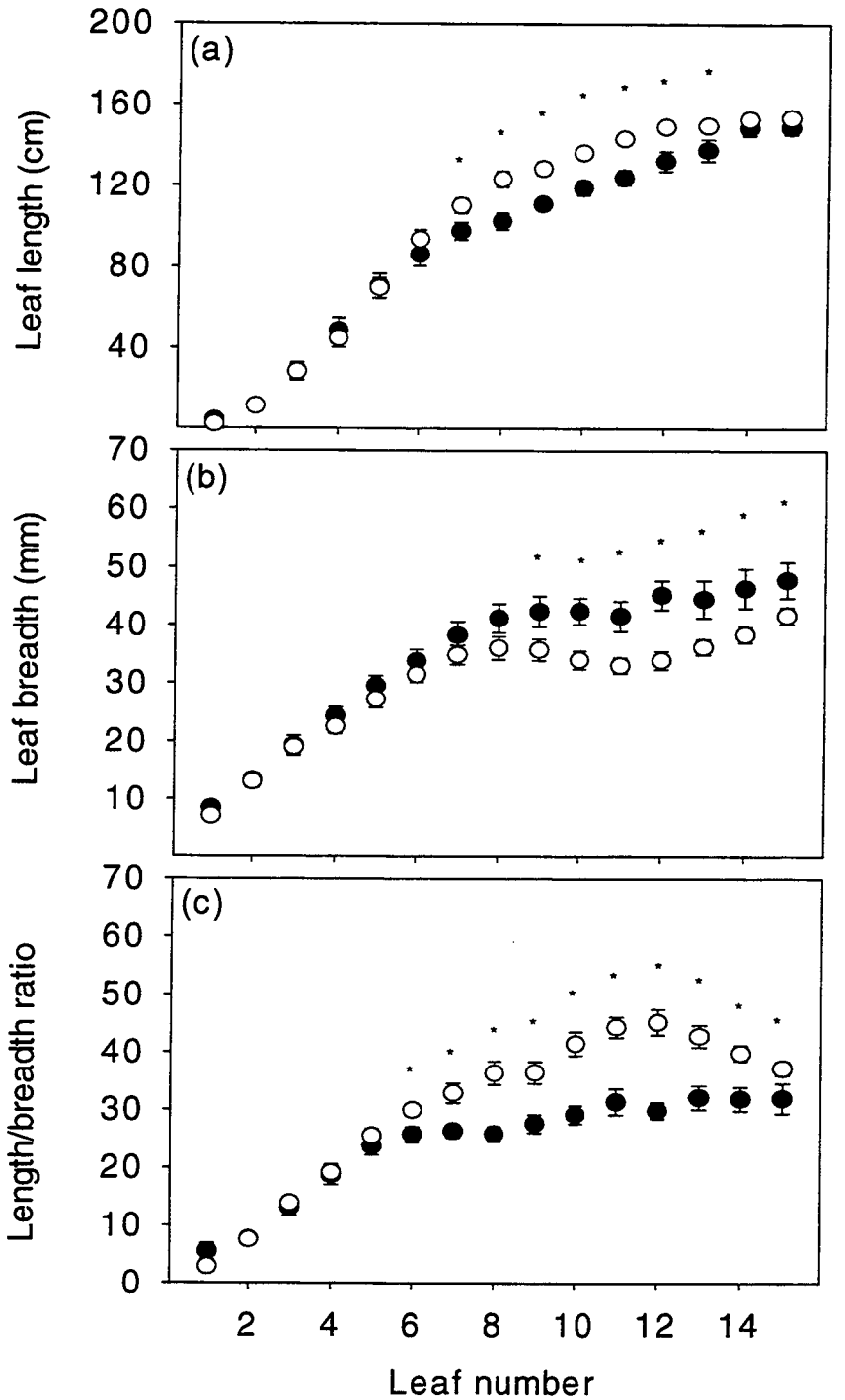

Figure 5.4 Leaf length (a), maximum breadth (b) and length to breadth ratio (c) of suckers of cultivar Q138 with the main stalks attached (●) and mature stalks removed (○). Suckers had produced 3 or 4 leaves prior to removal of mature stalks. Error bars represent ± the standard error of the mean. * indicates a significant difference ($p < 0.05$) following single factor ANOVA.

5.2.3.2 Experiment 2

Removing the mature stalks from pots which contained suckers again had a significant effect on sucker leaf morphology. For cultivar Q117 (Figure 5.5) and cultivar Q152 (Figure 5.7), suckers with their mature stalks attached had significantly greater leaf maximum breadth ($p < 0.05$) than suckers with their mature stalks removed. This resulted in a significant difference between treatments for the leaf length to breadth ratio. The treatments did not have a significant effect on leaf length ($p > 0.05$), and the interaction effects were not significant. For cultivar Q138 (Figure 5.6), the suckers with their mature stalks removed had significantly greater leaf length and leaf breadth than those with the mature stalk attached ($p < 0.05$). There was no significant difference in leaf length to breadth ratio, but there was a significant leaf x treatment interaction for both leaf length and leaf length to breadth ratio ($p < 0.05$).

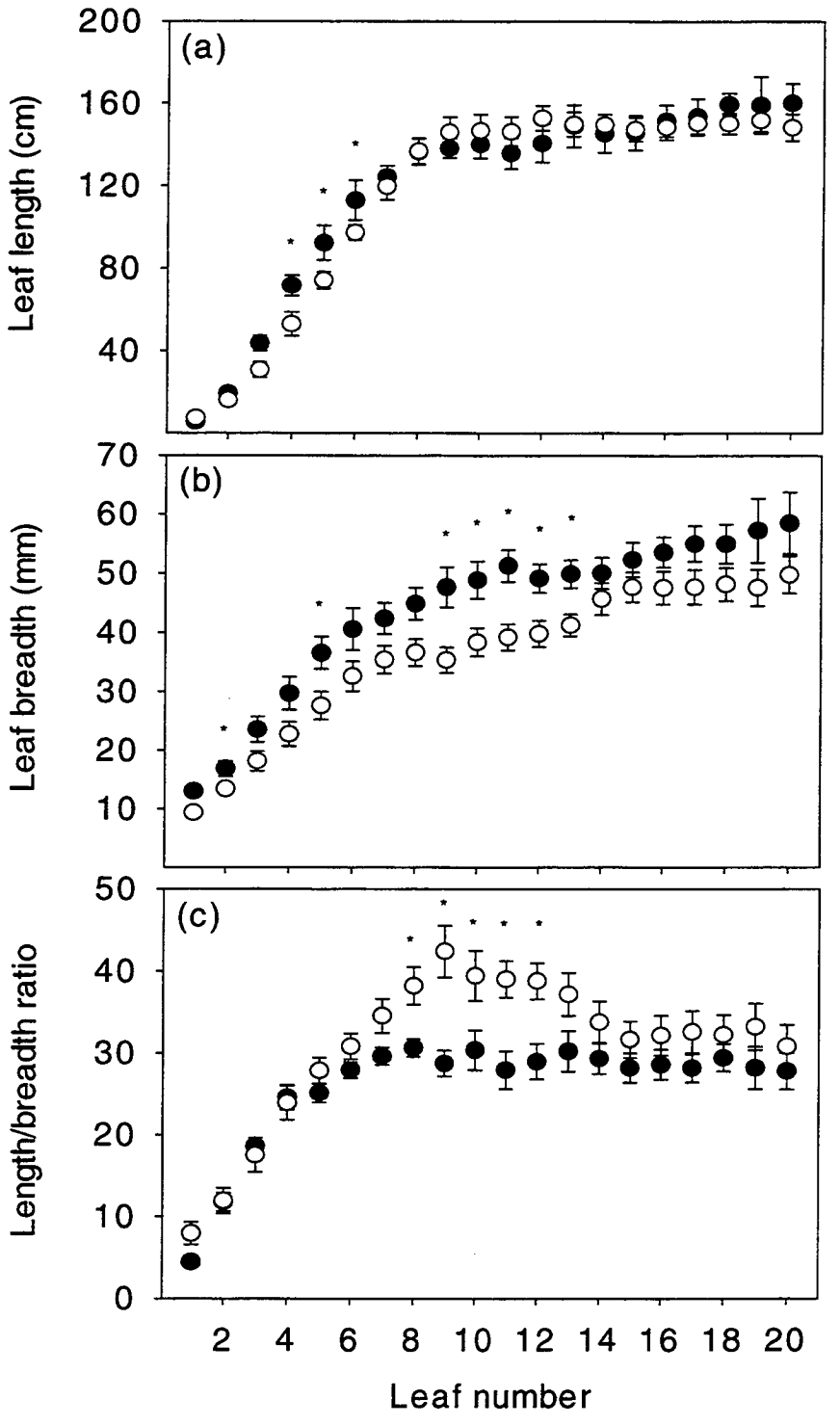

Figure 5.5 Leaf length (a), maximum breadth (b) and length to breadth ratio (c) of suckers of cultivar Q117 with the mature stalks attached (●) and mature stalks removed (○). Error bars represent ± the standard error of the mean. * indicates a significant difference ($p < 0.05$) following single factor ANOVA.

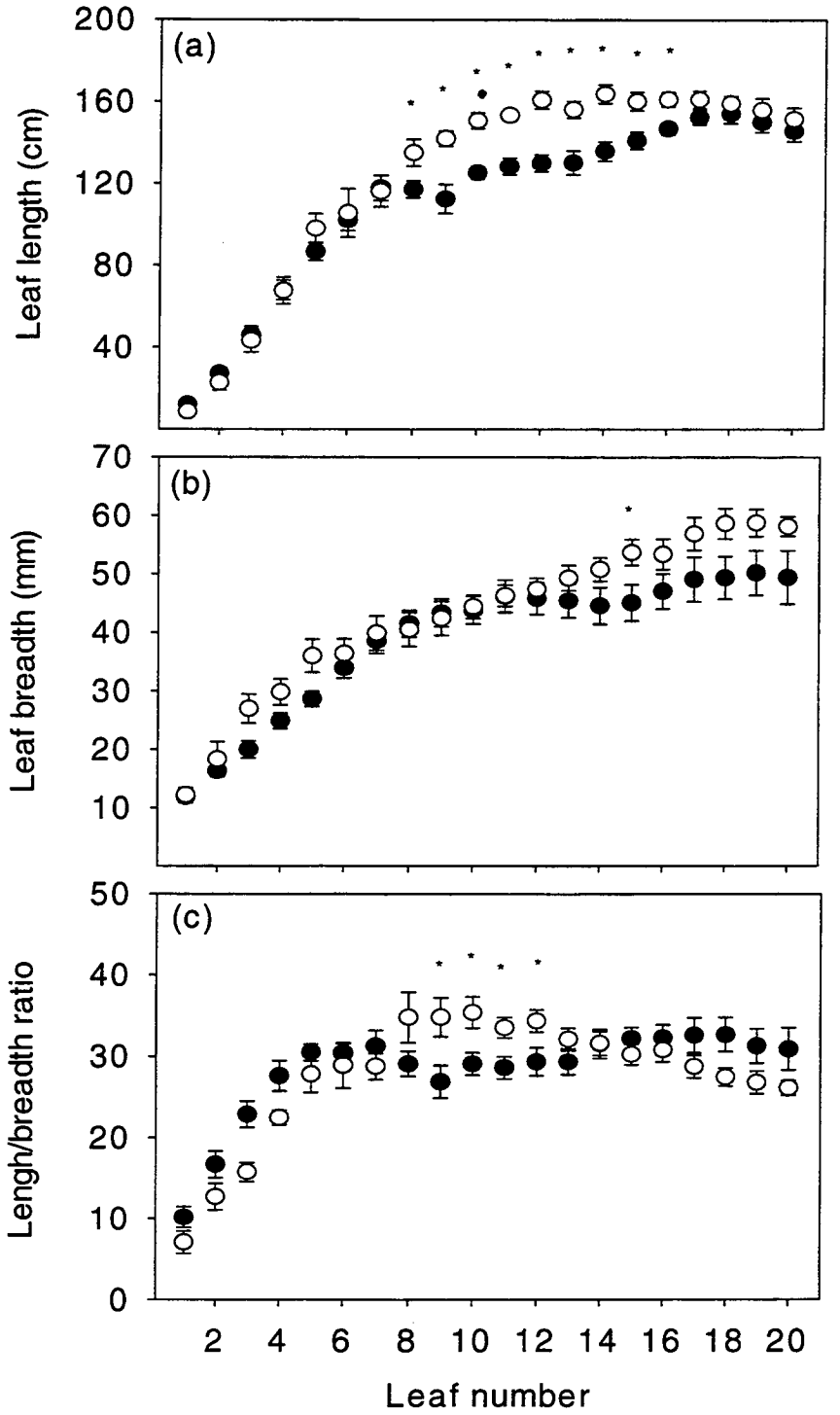

Figure 5.6 Leaf length (a), maximum breadth (b) and length to breadth ratio (c) of suckers of cultivar Q138 with the mature stalks attached (●) and mature stalks removed (○). Error bars represent ± the standard error of the mean. * indicates a significant difference ($p < 0.05$) following single factor ANOVA.

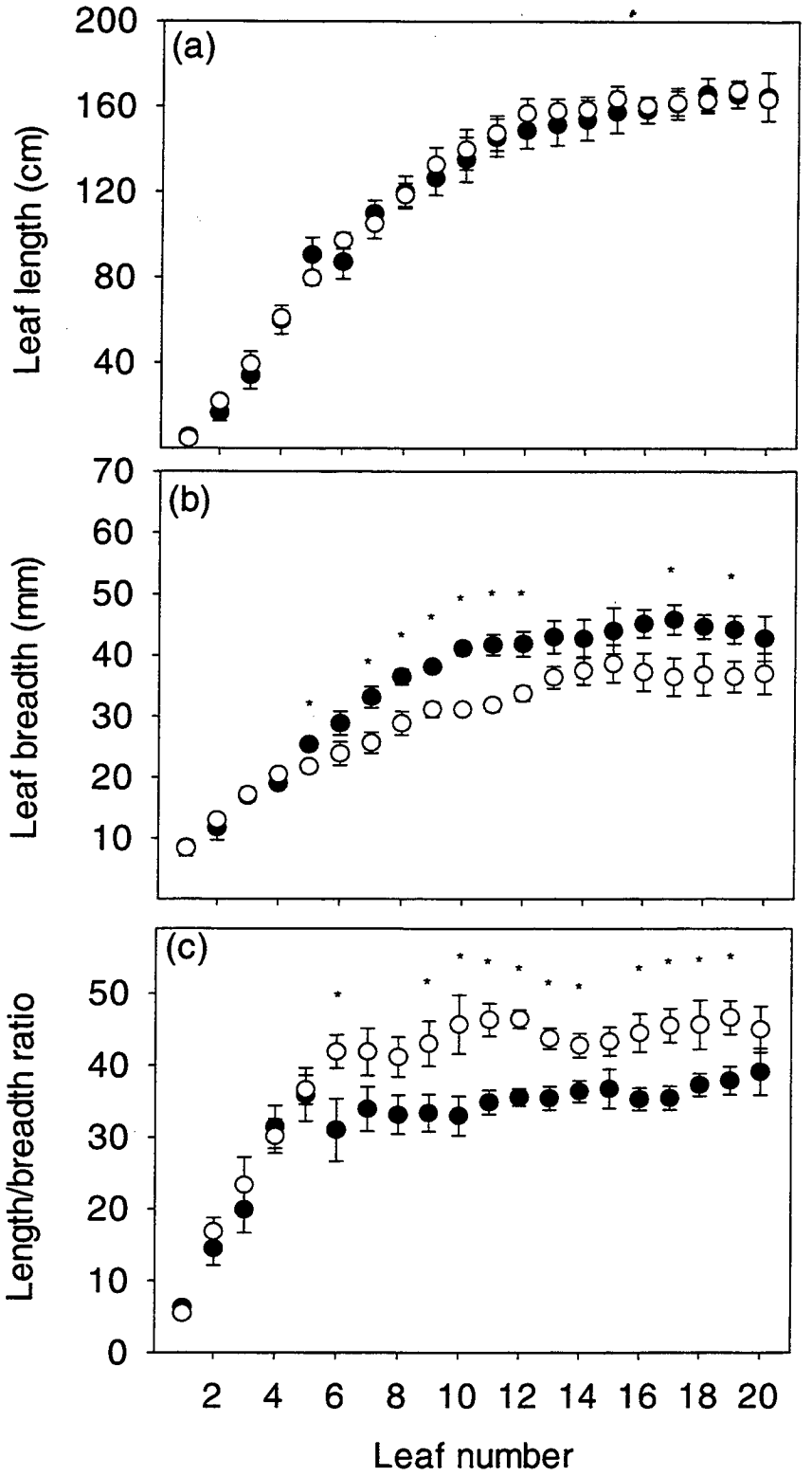

Figure 5.7 Leaf length (a), maximum breadth (b) and length to breadth ratio (c) of suckers of cultivar Q152 with the mature stalks attached (●) and mature stalks removed (○). Error bars represent ± the standard error of the mean. * indicates a significant difference ($p < 0.05$) following single factor ANOVA.

5.2.4 Discussion

The leaf morphology of suckers of cultivar Q138 was shown to change following the removal of the mature stalks to which they were attached in the first experiment. The morphology changed to being more similar to normal stalk leaves (Chapter 3), with thinner maximum breadth and a greater leaf length to breadth ratio, when compared to suckers with the mature stalks attached. This was also found for cultivars Q117 and Q152 in the second experiment. However, in the second experiment, suckers of cultivar Q138 with the mature stalk removed, had leaves with significantly greater leaf maximum breadth than suckers with the mature stalks attached. These contradictory results for Q138 are not easily explained. In the second experiment the mature stalks were removed when the suckers had produced 4 - 6 leaves whereas in the first experiment they were removed when the suckers had produced either 0 - 1 or 3 – 4 leaves. Perhaps the sucker size/age at the time of mature stalk removal may have had an effect. If sucker size/age was important then why cultivars Q117 and Q152 did not show a similar response to cultivar Q138 is not known.

When one-way ANOVA was used to analyse the effect of the treatments at each leaf number, a significant increase in the leaf length to breadth ratio of suckers with the mature stalk removed was found for cultivar Q138 in the second experiment (leaves 9 – 12), as expected. However, this result was due to the difference in leaf length, whereas in cultivars Q117 and Q152, the difference in the leaf length to breadth ratio was mainly due to the difference in leaf breadth.

It is not known why significant differences between treatments were found in leaves that were initiated prior to the removal of the mature stalks in experiment 2. It appears that it was not due to an inadvertent systematic error in selection of stalks for each treatment, as in cultivar Q117 the maximum leaf breadth of suckers with the mature stalks attached was significantly greater than suckers with the mature stalk removed (leaf 2 and 5), whereas in cultivar Q138, the maximum leaf breadth of suckers with the mature stalks attached was significantly less than suckers with the mature stalk removed (leaf 3). If there were a systematic error in selection of stalks for each treatment, it would presumably have been similar for both cultivars.

In both experiment 1 and 2, for all cultivars, the difference between treatments decreased around leaf 14-15. It is not clear why this occurred but could indicate that the main stalk was having less of an effect on sucker growth at these later leaf numbers. Therefore this may indicate when the sucker is beginning to become a photosynthetically self-reliant stalk.

The results suggested that there is some form of inter-stalk control of sucker morphology via the movement of compounds from the mature stalk to the sucker. One such compound could be sucrose. Potentially, this translocated sucrose could come from both stored sucrose in the stem of the mature stalk (discussed in Chapter 5.1) or current assimilate being produced in the leaves of the mature stalk. Hartt *et al.* (1963) showed that, in sugarcane, radioactive labelled sucrose can move from a leaf on one stalk in a stool to the top of all the other stalks in the stool within 24 hours. Sucrose has been shown to affect leaf shape. Montaldi (1974) showed that 0.14 M sucrose in a nutrient solution resulted in significantly shorter leaves in *Cynodon dactylon*. This

effect of sucrose was countered by gibberellic acid as well as ammonium nitrate. Montaldi (1974) proposed that sucrose decreased the rate of cell division and the number of cells in the leaf basal meristem.

This nutritional support from the mature stalk may allow the sucker to grow in a different manner to a stalk that is not supported by another larger stalk. It appears that leaf breadth is most affected by the presence of a mature stalk. Possibly, an increase in leaf breadth may allow for increased photosynthetic capacity via an increase in leaf area. Together with the nutritional support from the main stem, this may be important in achieving a fast growth rate, which may allow a sucker to reach favourable light conditions more quickly.

Potentially, it is not just sucrose that may move from the mature stalk to the sucker. Even though there is little evidence to suggest that other compounds move from the mature stalk to the sucker, there is also little to suggest that they do not. Montaldi (1974) has shown that the plant hormone gibberellic acid and other compounds can effect leaf shape. Radioactively labelled compounds may again be required to find out whether other chemicals do move from the main stalk to the sucker and how these chemicals may affect leaf shape will need to be investigated further.

It is not expected that the difference found between treatments were due to differences in the availability of light. The experiments were designed in order to reduce this possibility. Furthermore, when suckers are found in fields that are lodged, they do not appear to be different to those in a field that has not lodged.

Part C: Putative environmental factors affecting sucker formation

Chapter 6. Nitrogen

6.1 Introduction

Increased nitrogen availability has been thought to result in increased suckering in sugarcane. This is mainly due to the reported increase in sucker numbers with the use of green cane trash blankets (GCTB) (Leslie and Wilson 1996; Chapman *et al.* 2001). The decomposition of the trash results in nitrogen being released into the soil. Initially, the nitrogen is absorbed by the soil microflora responsible for decomposing the trash. However, after the period of some years of GCTB culture, there is a build-up of plant-available soil nitrogen, which has brought about speculation that fertilizer applications may need to be reduced (Robertson and Thorburn 2000). There are also older reports in the literature from Hawaii that high nitrogen rates caused increased suckering (Borden 1948; Stanford 1963). Whether these results are applicable to Australian conditions and cultivars is not known. Hurney and Berding (2000) found no effect of nitrogen rate on sucker numbers. However, all the nitrogen was applied at the early stages of the crop's growth, and therefore there may not have been any difference in nitrogen availability at the time when suckers were developing. Interestingly, no effect of nitrogen was found on cane yield and therefore must raise doubts as to whether losses of nitrogen due to leaching or denitrification were high in the period immediately after application. Increased nitrogen has been shown to increase tillering in sugarcane and other grasses (see Chapter 2).

The aim of the following experiments was to determine whether high concentrations of plant-available nitrogen in the soil, late in the growing season, would increase the number and/or size of suckers. The experiments also looked at whether a strongly and weakly suckering cultivar differed in their response to available nitrogen. Nitrogen, available late in the growing season, may act as a trigger for the development of suckers. This process may be occurring naturally in the field via the breakdown of organic matter built up through the practice of GCTB. However, the experimental treatments were designed to test the hypothesis that increased available nitrogen, late in the season, would result in increased suckering, rather than to mimic the release of nitrogen via the breakdown of organic matter.

6.2 Methods

6.2.1 Effect of a late nitrogen application on suckering in cultivar Q152 at Tully

6.2.1.1 Location and experimental design

The experiment was conducted on A. Maifredi's farm, at Euramo, south of Tully, in 1999, using cultivar Q152 (second ratoon). Plot size was four rows by 5 m. Only the middle two rows were used to collect data. Row spacing was 1.5 m, and there was a 1 m buffer between plots. All plots were located in the outside four rows of the crop. It was thought that this would be the area least affected by lodging. Lodging has been speculated to increase the presence of suckers and if this were true, lodging could affect the interpretation of results. Plots were arranged in a randomised block design,

replicated five times (Figure 6.1). Rainfall data were sourced from the Bureau of Meteorology station in Tully (Weather station no. 32042).

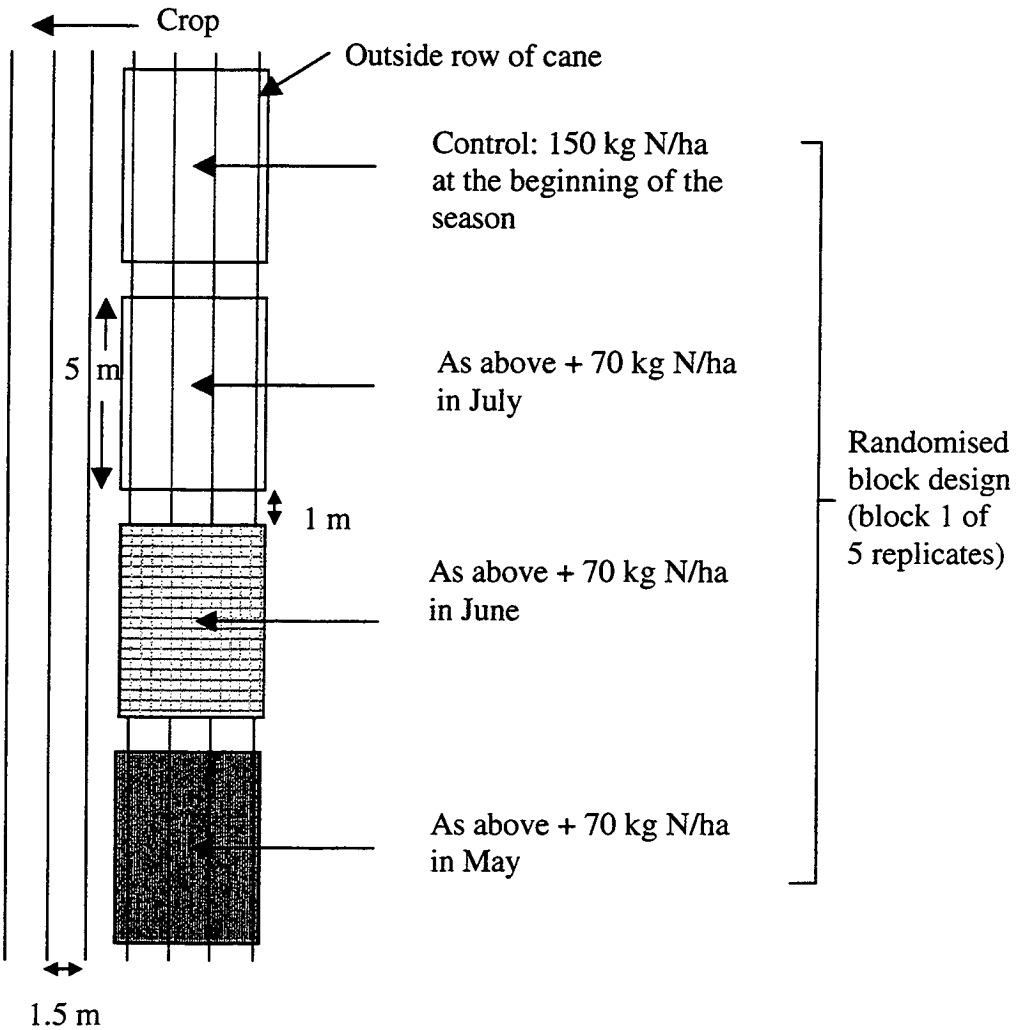

Figure 6.1. Experimental design for the late application of nitrogen to cultivar Q152 in Tully. Three treatments (70 kg N/ha in May, June or July 1999) and a control were established. All plots received 150 kg N/ha following ratooning on 4th October 1998.

6.2.1.2 Treatments

All plots received 150 kg N/ha after ratooning on 4th October 1998. Three treatments were initiated where an additional 70 kg N/ha equivalent was applied. For treatment 1, the additional nitrogen was applied in May (10th May 1999); treatment 2, in June (8th

June 1999); and treatment 3, in July (20th July 1999). The additional nitrogen was applied as ammonium nitrate (34.5% N, Pivot Ltd.), and was spread on the soil surface by hand.

6.2.1.3 Sampling

Sucker numbers were counted in each plot, after the additional N applications, until the crop was harvested. Suckers were defined as all young shoots emerging from the crop. All young shoots appeared to have sucker-like morphology. A final sample was conducted on 17th September 1999. All suckers in row 2 (3 replicates), were cut out of the plots, counted and weighed. Sampling suckers in all replicates and rows was not possible due to short notice of an unexpected commercial harvest. Soil samples were taken on four occasions (28th May 1999, 23rd June 1999, 20th July 1999 and 25th August 1999) to determine whether the nitrogen application increased soil nitrate levels, an indication of plant available N. On each occasion two soil cores (50 mm diameter) to a depth of 50 cm were taken. Cores were separated into 10 cm increments (0-10 cm, 11-20 cm, 21-30 cm, 31-40 cm and 41-50 cm). Only 0-10 cm, 21-30 cm, and 41-50 cm increments were collected at the final soil sampling.

6.2.1.4 Soil nitrate-N analysis

Soil samples were kept at 4 °C after sampling, and were later air-dried. Soil nitrate-N was extracted using 2 M KCl solutions. Each extraction consisted of 4 g soil in 40 ml KCl. Samples were shaken mechanically, end over end, for one hour at 25 °C. Soil nitrate concentration was determined using an adaptation of the method of Best (1976) (Appendix 6.1)

6.2.1.5 Statistical analysis

Sucker counts were analysed using single factor ANOVA, with treatment as the independent variable, on each sample date. Blocks were not used in the analysis as preliminary analysis showed that they did not have a significant effect on sucker number. The sucker number data were square root transformed as probability plots and histograms showed that the data were not normally distributed. Following transformation the data met the assumption of a normal distribution. Soil nitrate-N data were analysed using single factor ANOVA for each soil depth, on each sample date. Mean monthly temperature was analysed using single factor ANOVA. *Post-hoc* comparisons of means were conducted using Fisher's least significant difference (LSD) ($p \leq 0.05$).

6.2.2 Effect of a late nitrogen application on a strongly and a weakly suckering cultivar

6.2.2.1 Location and experimental design

The experiment was conducted on A. Zappalla's farm, approximately 15 km north of Babinda (17° 30'S 145° 50'E), in far-north Queensland in the 1999/2000 season. Two cultivars were chosen, Q152 (1st ratoon, last harvest 1st September 1999) and Q181 (1st ratoon, last harvest 1st September 1999). Both crops received 100 kg N/ha following ratooning. Cultivar Q152 is known to have a high propensity to sucker, whereas Q181 is known to have a low propensity to sucker. This comparison is based on observations rather than counts.

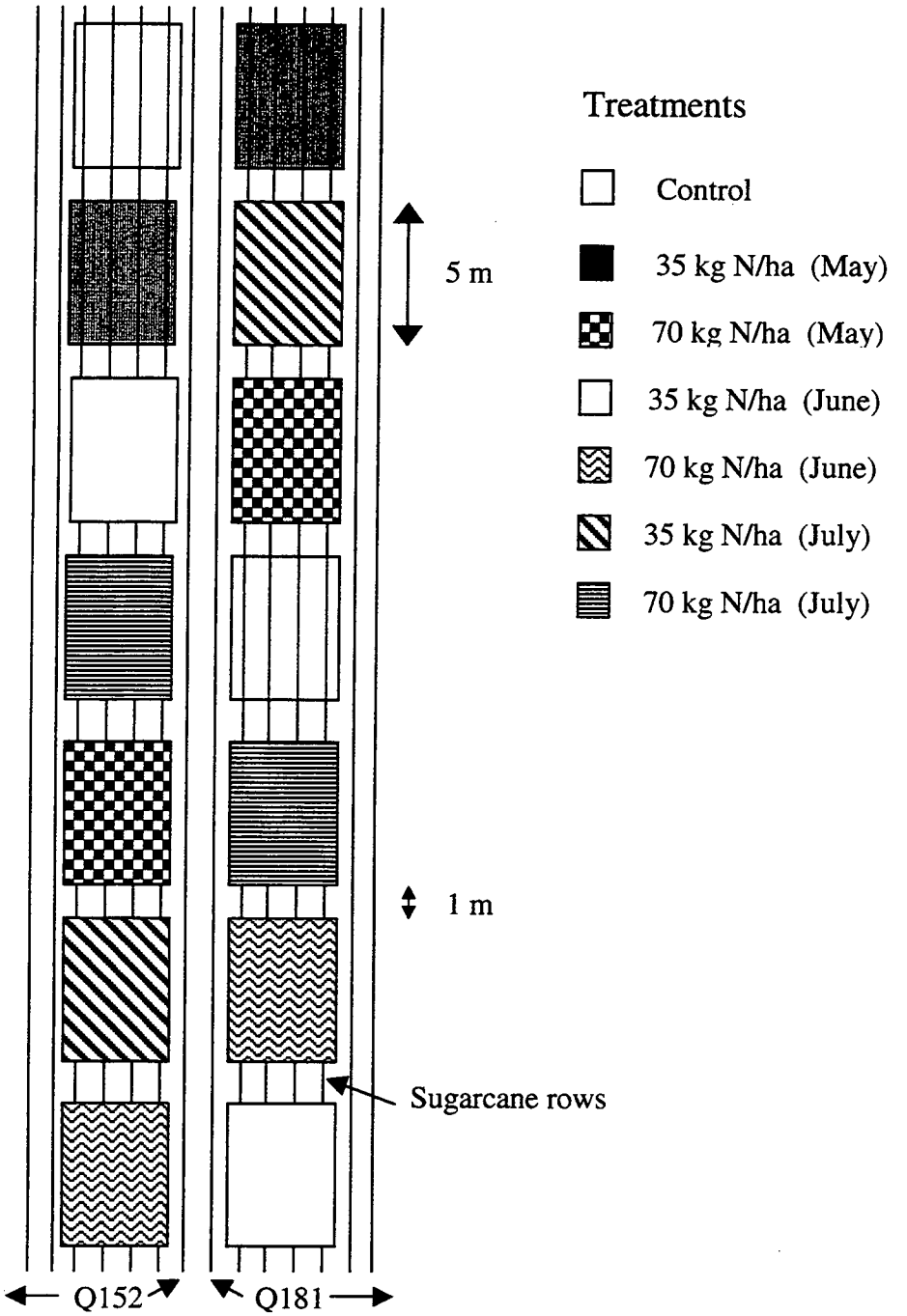

Figure 6.2. Experimental design and plot layout for late nitrogen application to a strongly and weakly suckering cultivar. Seven treatments were initiated: 35 kg N /ha and 70 kg N/ha was added to different plots in May, June and July, as well as a control, which received no additional nitrogen. The figure depicts one of five replicate blocks.

In both crops, plot size was 5 m by 4 rows, with a 1 m gap between plots. Plots were arranged in a randomised block design. Blocks were arranged linearly, in the same four

rows of cane. It was not possible to use the outside four rows of the crop in this experiment. However, neither crop lodged throughout the duration of the experiment. The two cultivars were grown adjacent to each other and experimental plots were separated by two rows of cane (Figure 6.2)

6.2.2.2 Treatments

Seven treatments were initiated: 35 kg N /ha and 70 kg N/ha was added to different plots in May, June and July, as well as a control, which received no additional N. Fertiliser was applied to plots on 17th May 2000 (May applications), 23rd June 2000 (June applications) and 26th July (July applications). Nitrogen was applied as ammonium nitrate (34.5% N, Pivot Ltd.) and was spread by hand.

6.2.2.3 Sampling

Sucker counts were conducted in the middle two rows of each plot. A preliminary sucker count was taken on 17th May 2000, before any additional nitrogen had been applied. Further sucker counts were conducted on 23rd June 2000 and 26th July 2000. No sucker counts were taken for the July application treatments due to the early commercial harvest of the crop. Early commercial harvest prevented a final sample of the experimental plots to determine sucker mass, mature stalk counts and nitrogen content of mature stalks. Commercial harvests occurred earlier than expected due to a short harvest season length in 2000. This was primarily due to poor yield in the wet tropics region.

Soil samples were taken on 11th May 2000, 31st May 2000, 5th July 2000 and 16th August 2000 using an auger. On 11th May 2000, ten cores (20 mm diameter) to 50 cm were taken at random within the experimental area. This was done to ascertain the background level of nitrate-N and ammonium-N prior to the establishment of the experiment. On the other three sample dates, three cores (20 mm diameter) to 50 cm, were taken from each plot. Cores from each plot were divided into 0 – 25 cm and 25.1 – 50 cm increments, pooled and stored at 4 °C, until soil N extraction was conducted.

6.2.2.4 Soil N analysis

Soil nitrate-N and ammonium-N were extracted from the soil using 2 M KCl solutions as described in section 5.2.1.4. Soil nitrate concentration was determined using the method described in Appendix 6.1. Soil ammonium concentration was determined using an adaptation of the method described by Nelson (1983) (Appendix 6.2).

6.2.2.5 Statistical analysis

Sucker counts were analysed using a general linear model (GLM) for randomised block designs, with treatment and blocks as dependent variables, on each sample date. The sucker number data were square root transformed in order to meet the assumption of a normalised distribution. Sucker number data were compared using orthogonal comparisons. Soil nitrate-N and ammonium-N data were analysed using two-way ANOVA on each sample date, with treatment and soil depth as factors. *Post-hoc* comparisons of means were conducted using Fisher's LSD ($p \leq 0.05$).

6.3 Results

6.3.1 Effect of a late nitrogen application on suckering in cultivar Q152 in Tully

The application of nitrogen, late in the growing season, resulted in an increase in sucker number in all treatments (Table 6.1).

Table 6.1 Sucker number in cultivar Q152 in Tully following the addition of 70 kg N/ha on 10th May 1999, 8th June 1999 and 20th July 1999. Means followed by the same letter are not significantly different ($p > 0.05$).

Treatment	Time (days after 10th May 1999)				
	1	18	44	71	114
Control	5.80	8.00	31.80[a]	93.80[a]	115.00[a]
May application		17.00	53.40[b]	139.60[b]	154.40[b]
June application			28.00[a]	95.20a	153.60[b]
July application					139.00[b]
		ns			

ns - F test not significant ($p > 0.05$)

The applications in May and July were followed by significant increases in sucker number, when compared with the control, approximately 40 days after application. The June application only resulted in significant increases in sucker number 85 days after application. The sucker numbers following the May, June and July applications were not significantly different from each other on the 1st September count (day 114, Table 6.1). When analysed individually, both the first row of the plot and the second row of the plot showed similar sucker numbers and were significantly different to the control at the same sample dates. This indicated that any light that was penetrating through the outside row was not having a differential effect on the rows in the plot.

Sucker numbers nearly doubled between 1st and 17th September 1999. There was no significant difference between the number of suckers, total mass of suckers and average size of suckers for any of the treatments at the final sampling (Table 6.2).

Table 6.2. Sucker number, fresh mass and average fresh mass per sucker at the final sampling (17th September 1999, day 131) of cultivar Q152 in Tully. Data represent an average of three replicates from row 2 (half total plot).

Nitrogen application	Sucker no.	Mass (kg)	g sucker^{-1}
May	152.0	12.1	79.8
June	118.7	10.3	86.8
July	142.7	12.4	86.5
Control	129.3	11.3	86.8
	ns	ns	ns

ns - F test not significant ($p > 0.05$)

The soil nitrate-N concentrations are shown in Table 6.3. While soil nitrate-N does not represent total nitrogen in the soil, it does give an indication as to whether or not the nitrogen applications were having an effect on plant-available N. Soil nitrate-N after the May application was significantly higher than the control, in the first 10 cm of soil, on 28th May 1999. The soil nitrate-N concentration after the June application was significantly higher than the control, in the first 10 cm of soil, on 20th July 1999. The soil nitrate-N concentration after the July application was significantly higher than the control, in the first 10 cm of soil, 25th August 1999. These data show that the late nitrogen applications did initially raise the levels of soil nitrate-N in the soil, near the surface. The variations in nitrate-N for soil deeper in the profile were not significant.

Table 6.3 Soil nitrate-N concentration (mg g^{-1} dry weight) following the application of 70 kg N/ha on 10th May 1999, 8th June 1999 and 20th July 1999 to cultivar Q152 in Tully. Means followed by the same letter are not significantly different ($p > 0.05$).

Sample Date	N application	Depth below soil surface (cm)				
		0-10	10.1-20	20.1-30	30.1-40	40.1-50
28 May (day 18)	Control	18.5[a]	15.9	7.9	7.1	8.2
	May	83.6[b]	17.9	12.3	10.5	9.2
			ns	ns	ns	ns
23 June (day 44)	Control	12.0	10.4	11.2	6.8	6.7
	May	45.9	31.2	15.6	11.7	8.0
	June	67.7	47.4	34.5	19.6	20.2
		ns	ns	ns	ns	ns
20 July (day 71)	Control	13.1[a]	10.6	11.6	8.6	6.2
	May	31.2[a]	19.2	12.7	9.1	9.9
	June	51.9[b]	36.0	21.1	13.9	14.1
			ns	ns	ns	ns
25 August (day 107)	Control	17.0[a]	not	15.8	not	11.5
	May	18.2[a]	sampled	20.4	sampled	14.3
	June	30.1[a]		28.4		24.6
	July	62.7[b]		30.6		16.1
				ns		ns

ns - F test not significant ($p > 0.05$)

Figure 6.3 shows rainfall for the Tully region for the study period. There were 122.2 mm, 64.4 mm, and 141.4 mm of rain in the 25 days following May, June and July N applications respectively.

The mean monthly maximum and minimum temperatures for Tully for the period over which the experiment was conducted are shown in Table 6.4. There was a significant increase in temperature from July to September.

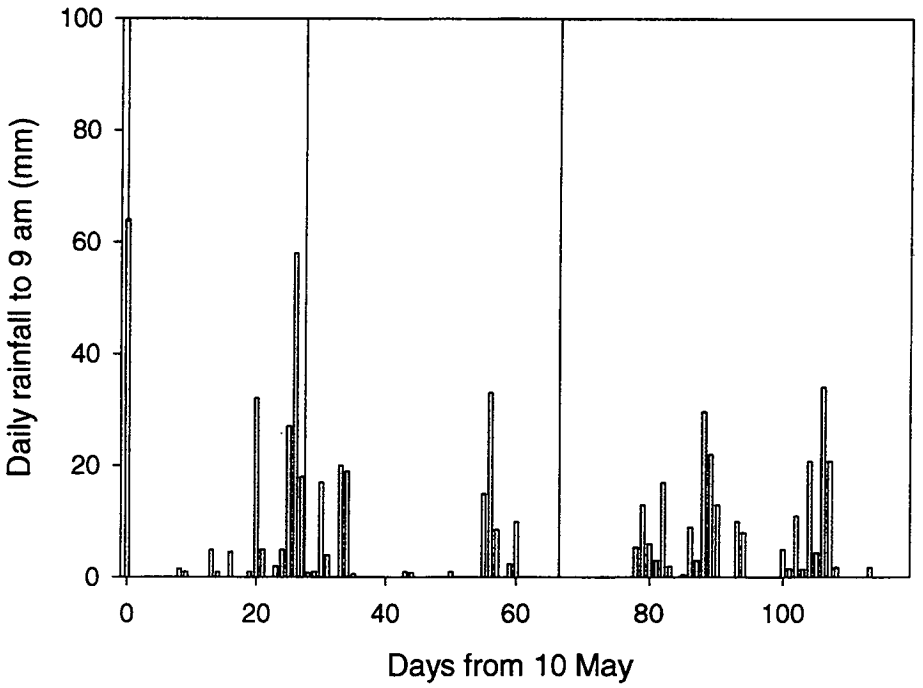

Figure 6.3 Daily rainfall to 9 am for the Tully region from 10th May (Day 0) to 31 August 1999. Lines represent nitrogen application dates. Soils were sampled on days 18, 44, 71 and 107. Data from Tully Meteorological Bureau station (32042).

Table 6.4 Mean monthly maximum and minimum temperatures for Tully in 1999. Means followed by the same letter are not significantly different ($p > 0.05$).

Month	Mean temperature (°C)	
	Maximum	Minimum
May	26.2[a]	17.7[a]
June	24.4[b]	15.9[b]
July	24.0[b]	13.3[c]
August	23.7[b]	15.4[b]
September	26.9[a]	16.6[ab]

6.3.2 Effect of a late nitrogen application on a high and a low suckering cultivar

The mean sucker numbers following a late nitrogen application of 70 kg N/ha and 35 kg N/ha to cultivars Q152 and Q181 are shown in Table 6.5. GLM analysis found a significant effect due to treatments and blocks on 28th June and 26th July for both cultivars.

Table 6.5. Sucker number per plot following the late application of nitrogen to cultivars Q152 and Q181 on 17th May 2000 and 28th June 2000.

Cultivar	Treatment	Sample date		
		17th May	28th June	26th July
Q152	Control	39.2	104.6	158.2
	35 kg N/ha in May		130.2	184.2
	70 kg N/ha in May		153.4	220.4
	35 kg N/ha in June			186.0
	70 kg N/ha in June			196.4
Q181	Control	5.8	12.4	26.2
	35 kg N/ha in May		26.2	42.2
	70 kg N/ha in May		31.6	54.4
	35 kg N/ha in June			26.8
	70 kg N/ha in June			34.4

Orthogonal comparisons for the data taken on 28th June showed that there was a significant increase in sucker number due to nitrogen application, but there was not a significant difference ($p > 0.05$) between the two application rates. This was found for both cultivars.

As up to ten comparisons can be made from the data taken on 26th July 2000 for each cultivar, the probability of a type I error is high. To control for this, the data were analysed using orthogonal comparisons. For both cultivars there was a significant

increase in sucker number, over the control, due to the addition of nitrogen (Table 6.6). There was a significant difference in sucker number due to the application dates for Q181, but not for Q152, and there was a significant difference due to the application rates for Q152 in May, but not Q181. There was no significant difference in either cultivar due to application rate in June.

Table 6.6 Orthogonal comparisons between means for sucker number data taken on the 26th July 2000.

Comparison					Significance	
Control	May 35 kg N/ha	May 70 kg N/ha	June 35 kg N/ha	June 70 kg N/ha	Q152	Q181
-4	1	1	1	1	*	*
0	-2	-2	2	2	ns	*
0	1	-1	0	0	*	ns
0	0	0	1	-1	ns	ns

* - F test significant ($p \leq 0.05$)
ns - F test not significant ($p > 0.05$)

No significant difference due to treatment was found in soil nitrate-N and soil ammonium-N on 31st May 2000 (Table 6.7). Significant differences due to treatment were found for both soil nitrate-N and ammonium-N on 5th July 2000. This was due to a significant increase in concentration in the 70 kg N/ha plots applied in June. This significant difference had dissipated by the final sample on 16th August 2000.

Table 6.7 Soil nitrate-N and ammonium-N following the additional application of nitrogen at three rates. Means followed by the same letter are not significantly different ($p > 0.05$).

Effect	Soil nitrate-N and ammonium-N (mg g^{-1} dry weight)							
	11 May		31 May		5 July		16 August	
	NO_3-N	NH^4-N	NO_3-N	NH^4-N	NO_3-N	NH^4-N	NO_3-N	NH^4-N
Depth (cm)								
0 - 25	1.2	8.2^a	14.3	5.9^a	12.5^a	12.2^a	13.4	4.5
25.1 - 50	4.4	3.8^b	9.6	2.5^b	5.9^b	3.1^b	10.6	3.4
	ns		ns				ns	ns
Treatment								
Control	2.8	6.0	11.4	3.4	7.8^a	4.5^a	11.3	3.9
35 kg N/ha May			10.4	4.6	6.2^a	4.3^a	12.8	3.2
70 kg N/ha May			14.2	4.6	5.9^a	4.8^a	9.1	3.6
35 kg N/ha June					5.8^a	5.6^a	9.9	4.5
70 kg N/ha June					20.5^b	18.8^b	16.8	4.6
			ns	ns			ns	ns

ns - F test not significant ($p > 0.05$)

6.4 Discussion

6.4.1 Effect of a late nitrogen application on suckering in cultivar Q152 in Tully

The results are consistent with the hypothesis that an increase in the amount of plant-available nitrogen following the wet season increases sucker number. Soil nitrogen is therefore potentially a factor that predisposes, initiates or stimulates the development and/or growth of suckers.

There was some delay in the increase in sucker numbers after nitrogen application. While significant increases in soil nitrate were found approximately two weeks after application, significant increases in sucker number were not found until after 44 days

for the May application, 85 days for the June application and 43 days for the July application. This delay may be due to the time taken for the nitrogen to enter the soil, the time taken for the plant to detect increased nitrogen levels, bud growth to be stimulated, and for the sucker to emerge. Why this process took longer following the June application is not known. The process is likely to be affected by several environmental factors such as soil temperature, rainfall and humidity. There was roughly half the amount of rain in the 25 days following the June application compared with the May and July applications. Interestingly, soil nitrate concentrations indicated that the added nitrate was present in the soil roughly two weeks after the June application, but was only significantly greater than the control at the following sample (20^{th} July 1999). Other factors such as temperature could have influenced the delay seen in the suckering response. Temperature data indicated that there were significantly warmer temperatures (max and min) following the May application, compared to both June and July applications. Temperatures following the June and July applications were very similar. Temperatures in September were significantly higher than those in June, July and August. The increase in temperature may partly explain the huge increase in sucker numbers towards the end of the experiment.

While there was a general increase in sucker number through the season following nitrogen application over and above the increase seen in the control, sucker number at the end of the season showed no significant differences between treatments. Sucker numbers roughly doubled in the period 1^{st} September 1999 to 17^{th} September 1999. This massive increase in suckering may have been due to something other than plant available nitrogen and therefore the significant differences due to nitrogen may have been obscured.

Counting of suckers *in situ* during the season may have underestimated the actual number. Counting was difficult as suckers were numerous, small and often obscured by stalks and leaf material. Removing suckers prior to counting would probably have resulted in a more accurate account of sucker number. This could partly explain the increase in sucker number between 1st September 1999 and the final harvest on 17th September 1999. However, it is likely that any error in counting would be uniform across all plots. At the final sampling, large numbers of very small suckers were present in all plots, indicating that they were young and only recently emerged.

Nitrate levels in the control plots were between 10 and 20 μg/g soil. This is high when compared with data from Keating *et al.* (1994) and Garside *et al.* (1998). It is not known whether or not this relatively high base level of nitrate in the soil is the cause of the high sucker numbers in the controls in this experiment. Adding additional nitrogen to a soil with a lower base level of soil nitrate may have shown larger differences in sucker number between the treatments and the control.

6.4.2 Effect of late nitrogen application on a strongly and a weakly suckering cultivar

Increases in suckering due to late nitrogen applications were again demonstrated. The response to nitrogen was present in both a high and a low suckering cultivar, but even after the application of nitrogen, the difference in the number of suckers between the two cultivars remained, with Q152 producing more than Q181. This suggests that the

genetic differences in suckering propensity between these two cultivars is independent of the response to nitrogen

Sucker numbers were found to increase following the May application despite significant differences in soil nitrogen not being found on 31st May 2000. This suggests that the plant responded to increased soil nitrogen but other processes such as loss of nitrogen due to leaching, volatilisation and loss of nitrogen to the plant may have reduced the levels by the time the soil samples were taken. The soil samples were also only a small sub-sample of the whole plot, this may not have allowed small changes to be detected above the natural variation within the plot. The application of 35 kg N/ha did not increase soil nitrate-N or ammonium-N above the levels found in the control plots on any of the sample dates. However, both cultivars had significantly more suckers in the May 35 kg N/ha treatment than in the control by the final count. This also shows that while significant increases in soil nitrogen were not detected, the plants were responding to the treatment, and must have had access to the additional nitrogen at some stage.

Q152 is a cultivar that produces high numbers of thin stalks whereas Q181 produces fewer, thicker stalks. This may mean that one of the major differences in suckering potential between these two cultivars is the number of available buds beneath the ground. Potentially the number of suckers per stalk for both cultivars may have been similar. The unexpected early commercial harvest prevented the collection of data that may have elucidated the issue.

The effect of different nitrogen rates applied at the beginning of the season and the interaction of nitrogen with other environmental stimuli are discussed in Chapter 8. The increase in suckering due to the availability of nitrogen has important implications for crop improvement (see discussion in Chapter 9).

Chapter 7. Light

Many authors (van Dillewijn 1952; Hes 1954; Barnes 1974) have made the assumption that suckering is partly a result of increased or high levels of available light beneath the crop canopy. However, none of the authors provided any data or citation to provide evidence for their assumption. There is speculation that light may cause suckering as suckers have been observed to be more prevalent on the edges of the crop (Bonnett *et al.* 2001) and where the crop has lodged or has a disrupted canopy. Since suckers are late tillers, with altered morphology, it is possible or even likely that factors that influence tillering may also control suckering. Light quantity and light quality have been shown to affect tillering in a number of grasses, as was discussed in Chapter 2.

7.1 Manipulation of the light in the outside row of sugarcane

7.1.1 Introduction

The outside row of a sugarcane crop was shown to contain a greater number of suckers than the second row of the crop (Bonnett *et al.* 2001). Edges of a crop, and areas with a disturbed canopy, would typically have higher available light than the middle of a well-grown crop. However, the edges of the crop may also have increased access to nutrients and water due to their roots being able to exploit a greater area of soil. This means that a simple comparison between sucker number in the outside row of a crop and the middle of the crop is not sufficient to fully determine the role that light may have in suckering.

To test the hypothesis that increased suckering in the outside row of a cane crop was due to high light availability, an experiment was established where the below canopy region of the outside row of cane was shaded. Treatments were also designed to try to determine what part of the sugarcane stem was responsible for detecting changes in the light environment below the canopy.

7.1.2 Methods

7.1.2.1 Experimental design

The experiment was conducted at three sites Tully (18° 0′S, 145° 55′E), Babinda (17° 30′S 145° 50′E) and Mulgrave (17° 5′S, 145° 42′E), using outside rows of commercial crops of cultivars Q138 and Q152. These cultivars were chosen as they both have a high propensity to sucker. This meant that suckering was highly likely to occur during the season. By shading the outside row, it could be determined if increased light was the cause of high suckering reported by Bonnett *et al.* (2001) in this region of the crop.

Five treatments were established: T1. Shade cloth (99% visible light, 97% UV, Z16 Black, Knittex, South Africa) was erected alongside (as close as possible) the outside row of the crop (5 m per plot). This was done in order to prevent light from entering from the side, and possibly changing the light characteristics of that row to something more similar to an inside row. The cloth was suspended between two posts (PVC pipe) with wire, at the height of the oldest green leaf. PVC pipes were placed over star pickets in order to prevent them from bowing. The height of the shade cloth was

adjusted as the crop grew. This was done by sliding the PVC pipe up the star picket, with wire used to prevent the pipe from sliding back down.

Table 7.1 Cultivar, previous harvest date, aspect, nitrogen fertilizer application, and date of treatment establishment for the six crops where light was manipulated in the outside row of cane.

District	Cultivar	Previous harvest	Aspect	Fertiliser	kg N/ha	Treatments established
Tully	Q138	20/7/98*	West	GF Organo130	60	30/3/99
	Q152	15/8/98	East	GF 402 Urea (GF)	140	30/3/99
Babinda	Q138	not available	East	not available	not available	1/04/99
	Q152	1/10/98	West	Incitec CK220	110	1/04/99
Mulgrave	Q138	10/98	East	GF 501	145	8/4/99
	Q152	10/98	West	GF 501	145	8/4/99

* indicates planting date
GF – Grow Force (Grow Force Australia Ltd.)

T2. Individual stalks were shaded with shade cloth (same type as T1) to the lowest clasping leaf (2.5 m of row per plot). This was done by wrapping a strip of shade cloth around the stalk. The shade cloth was held in place with staples. The shade cloth was maintained at the height of the oldest green leaf.

T3. All nodes on the stalk were wrapped with black insulation tape (2.5 m of row per plot). This was done in an attempt to determine which part of the stalk was responsible for detecting changes in light (should a response be found). Nodes of senesced leaves were wrapped with tape at regular intervals This treatment was only established at the Tully sites because it was particularly labour intensive.

T4. Dead leaf (trash) was removed from stalks, possibly increasing light reaching the stalk, and controlling for the removal of dead leaf in order to establish T2 and T3. For each T2 and T3 treatment, there was 2.5 m of row where trash was removed. Leaves were removed as they senesced during the study.

T5. Control, the crop was not altered (5 m of row per plot).

The treatments were arranged in a randomised block design replicated four times (Figure 7.1).

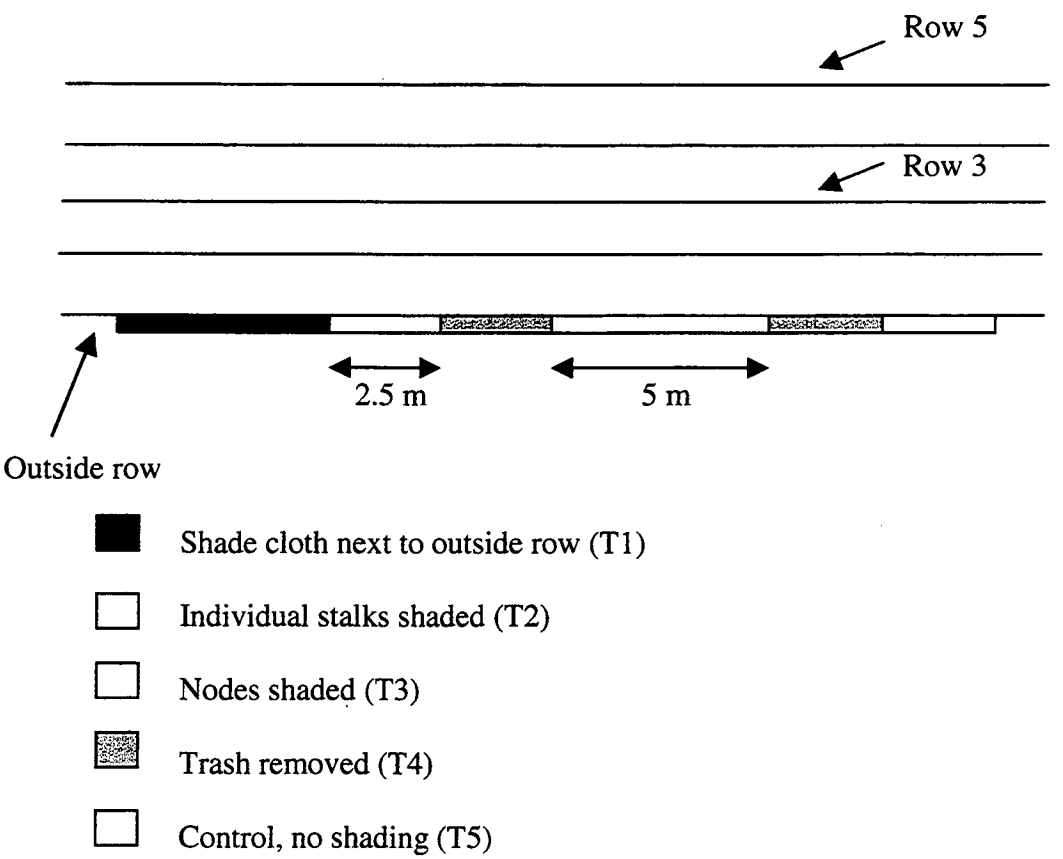

Figure 7.1 Experimental design used to manipulate light in the outside row of sugarcane crops. T3 was only established in Tully. The treatments were arranged randomly within each block.

7.1.2.2 Sucker counts

Sucker counts were conducted approximately every two weeks for all sites. Prior to commercial harvest, the suckers were cut out, counted and weighed. On 19th July 1999, the sucker numbers in rows 3 and 5 in the 5 m sections directly adjacent to the control plots were counted. This was conducted at the Tully and Babinda sites in order to ascertain whether or not there was an outside row effect in the experimental crops.

7.1.2.3 Light measurements

Measurements of photosynthetically active radiation (PAR) were taken on 11th August 1999 in Tully, 11th August 1999 in Babinda and 10th August 1999 in Mulgrave. PAR was measured with a AccuPAR Linear PAR Ceptometer, Decagon Devices, USA. Two measurements were taken at both 10 cm and 100 cm above ground in the control (T5) and side shade treatments (T1). The measurements were taken directly behind the shade cloth, and in the inter-row space between rows one and two (Figure 7.2). Measurements were taken in equivalent positions for the control plots. The two measurements per height were averaged to give one reading per plot at each height in each position. For all measurements, an external probe was used to take a measurement of PAR outside the crop at the same time as the measurement was being taken inside the crop. PAR within the canopy was expressed as a proportion of the total incident PAR (sunlight).

The red (660 nm – 680 nm)/far-red (720 nm – 740 nm) ratio of light was measured on the day of the final harvest for all crops, except Tully Q152 and Babinda Q152, where equipment failure prevented data collection. The ratio was determined by scanning

between 300 nm and 1100 nm with a Licor LI-1800 portable spectroradiometer (Licor Inc. Nebraska, USA). The amount of light between 660 nm and 680 nm was then divided by the amount between 720 nm and 740 nm to give the ratio. Measurements were taken at 10 cm above ground, directly behind the shade cloth, for T1, and in an equivalent position for the control plots (T5) and trash removed plots (T4). Two scans were performed per plot, which were automatically averaged by the spectroradiometer.

On the 3rd March 1999 the red/far-red ratio of light passing through the shade cloth, leaf sheath (cultivar Q138) and green leaf (cultivar Q138) were also measured by placing the cloth or leaf material over the sensor and then scanning from 300 nm – 1100 nm. Measurements of the red/far-red ratio of sunlight were also taken as a control.

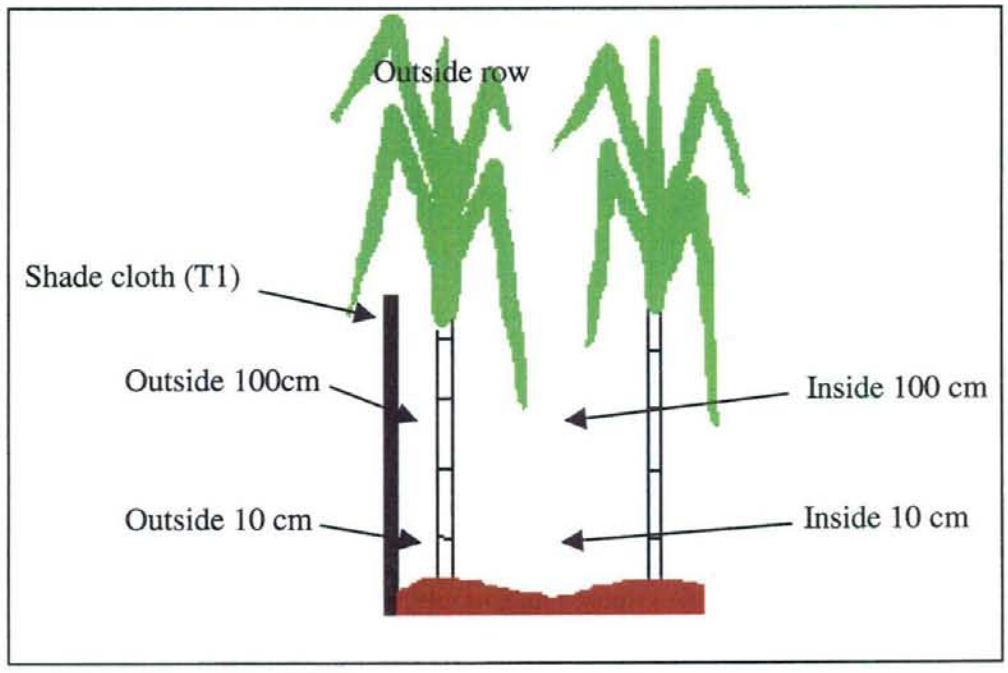

Figure 7.2 Position of PAR measurements taken on 11th August 1999 in Tully and Babinda and 10th August 1999 in Mulgrave. Red/far-red ratio measurements were taken at the outside 10 cm position

7.1.2.4 Temperature measurements

Temperature probes were used at the Tully Q152 site (block 2) to determine if the shading treatments affected temperature. Three thermocouples (type K) were placed c. 10 cm above ground directly behind T1 (side shade), three thermocouple were placed c. 5 cm below ground directly behind T1 (side shade), three thermocouples were placed c. 10 cm above ground in the control plot (T5), three thermocouple were placed c. 5 cm below ground in the control plot (T5), two thermocouple were placed c. 5 cm below ground away from the crop on a headland as an outside control, and two thermocouples were placed at c. 1.5 m above ground to give an indication of air temperature (outside control). The thermocouples were installed on 26th August 1999 and were removed on 17th September 1999. Thermocouples were wired to a data logger (CR10X, Campbell Scientific, Logan, Utah, USA). Temperature was sampled every 10 seconds and an hourly average recorded.

7.1.2.5 Statistical analysis

Sucker counts were expressed on a per metre basis, and were square root transformed prior to analysis in order to meet the assumption of a normalized distribution. Analysis was performed using a general linear model (GLM) for randomised block designs with repeated measures. Least significant differences were calculated to compare means following the analysis. LSDs were calculated by hand using the method described by Steel and Torrie (1980) for split plot designs (Appendix 7.1). Light quantity data were analysed using ANOVA with position within the row, height above ground and treatment as factors. The red/far-red ratio of light data was analysed using single factor

ANOVA with treatments as factors. Temperature data were analysed using ANOVA with time of day as a repeated measure.

7.1.3 Results

7.1.3.1 Sucker numbers

The mean number of suckers per metre for the two crops grown in Tully is shown in Table 7.2. No significant difference was found between treatments for cultivar Q138 ($p > 0.05$) and cultivar Q152 ($p > 0.05$). The mean number of suckers per metre for the two crops grown in Babinda is shown in Table 7.3. Significant differences were found between treatments for cultivar Q138 ($p < 0.01$) and Q152 ($p < 0.05$). The mean number of suckers per metre for the two crops grown in Mulgrave is shown in Table 7.4. Significant differences were found between treatments for cultivar Q138 ($p < 0.01$) and Q152 ($p < 0.05$). Sucker number was found to increase significantly with time for all crops in all regions ($p < 0.01$).

The sucker counts taken on 19th July 1999 from rows three and five, for the 5 m directly adjacent to the control plots, showed that the sucker number in row one was not significantly different from rows three and five at the Tully sites ($p > 0.05$) (Table 7.5) but significant differences were found at the Babinda sites ($p \leq 0.05$).

Table 7.2 Sucker number per metre appearing with time following the shading of the outside row of cane, cultivars Q138 and Q152, at Tully. Treatments were: Side shade (T1); Stalk shade (T2); Stalk clear (T4); Node shade (T3); Node clear (T4) and Control (T5). Means followed by the same letter are not significantly different ($p > 0.05$).

Tully	Time (Day of the year)												
Treatment	91	112	131	146	159	175	190	200	216	236	244	250	260
Q138													
Side shade	0.0	0.0	0.2	0.2	0.7	1.7	3.4	3.7	4.7	5.5	7.5		
Stalk shade	0.0	0.0	0.1	0.2	0.4	1.0	2.0	2.3	3.4	5.6	5.7		
Stalk clear	0.0	0.0	0.0	0.0	0.5	1.9	3.3	3.4	5.8	5.6	7.2		
Node shade	0.0	0.0	0.0	0.0	0.4	1.5	3.0	3.8	4.9	7.2	8.3		
Node clear	0.0	0.0	0.0	0.0	0.3	1.2	2.1	3.1	3.6	6.0	7.9		
Control	0.1	0.1	0.1	0.1	0.4	1.2	2.3	2.9	4.0	5.4	6.4		
(ns)													
Q152													
Side shade	0.5	0.6	0.4	0.8	1.4	2.2	5.3	6.2	8.1	9.4		15.8	22.7
Stalk shade	1.6	1.3	1.3	2.3	3.7	3.5	7.3	6.0	11.3	13.1		19.0	21.4
Stalk clear	1.3	1.7	1.5	3.3	4.0	4.4	7.6	5.8	8.4	13.7		21.7	23.1
Node shade	1.0	1.4	1.2	2.4	2.8	2.8	6.4	6.4	10.1	12.1		19.6	21.7
Node clear	1.4	1.5	1.3	2.6	2.4	3.4	5.9	7.4	10.1	11.1		14.8	15.5
Control	0.7	1.2	1.1	1.8	1.7	2.3	5.4	6.1	7.1	9.4		17.5	21.5
(ns)													

ns - F test not significant ($p > 0.05$)

Table 7.3 Sucker number per metre appearing with time following the shading of the outside row of cane, cultivars Q138 and Q152, at Babinda. Treatments were: Side shade (T1); Stalk shade (T2); Stalk clear (T4); Node shade (T3); Node clear (T4) and Control (T5). Means followed by the same letter are not significantly different ($p > 0.05$).

Babinda	Time (Day of the year)												
Treatment	90	112	130	146	159	175	190	200	215	236	243	249	253
Q138													
Side shade	0.8^{a}	1.7^{a}	2.2^{a}	3.2^{a}	3.8^{a}	4.8^{a}	7.4^{a}	6.5^{a}	7.5^{a}	8.5^{a}	12.7^{a}		
Stalk shade	0.9^{a}	2.0^{a}	3.2^{a}	5.3^{b}	6.8^{b}	7.8^{bc}	10.3^{b}	10.3^{b}	9.8^{ab}	12.2^{bc}	15.0^{a}		
Stalk clear	0.3^{a}	2.0^{a}	2.9^{a}	4.4^{ab}	5.7^{ab}	8.4^{c}	10.0^{ab}	10.1^{b}	11.3^{b}	13.4^{c}	14.5^{a}		
Control	0.5^{a}	1.6^{a}	2.5^{a}	3.6^{ab}	4.2^{a}	5.6^{ab}	7.4^{a}	7.2^{a}	8.5^{ab}	$9.6a^{b}$	12.1^{a}		
Q152													
Side shade	0.7^{a}	1.4^{a}	2.2^{a}	3.1^{a}	3.8^{a}	4.6^{a}	6.7^{a}	7.3^{a}	7.3^{a}	8.9^{a}		10.8^{a}	13.8^{a}
Stalk shade	1.2^{ab}	2.3^{ab}	3.2^{a}	4.3^{ab}	5.0^{a}	5.8^{ab}	6.9^{a}	7.7^{a}	7.5^{ab}	8.9^{a}		11.0^{a}	13.7^{a}
Stalk clear	1.5^{b}	3.5^{b}	5.1^{b}	5.6^{b}	6.9^{b}	6.6^{b}	7.8^{a}	9.4^{a}	10.0^{b}	10.4^{a}		12.6^{a}	13.7^{a}
Control	0.7^{ab}	2.1^{ab}	3.6^{ab}	4.4^{ab}	4.9^{ab}	5.6^{ab}	7.7^{a}	8.3^{a}	8.6^{ab}	9.8^{a}		12.1^{a}	13.7^{a}

Table 7.4 Sucker number per metre appearing with time following the shading of the outside row of cane, cultivars Q138 and Q152, at Mulgrave. Treatments were: Side shade (T1); Stalk shade (T2); Stalk clear (T4); Node shade (T3); Node clear (T4) and Control (T5). Means followed by the same letter are not significantly different ($p > 0.05$).

Mulgrave	Time (Day of the year)											
Treatment	99	112	130	146	159	175	189	200	215	236	249	265
Q138												
Side shade	1.2^{a}	1.4^{a}	2.8^{a}	5.3^{a}	7.0^{a}	7.3^{a}	10.8^{a}	9.0^{a}	10.3^{a}	10.5^{a}	11.2^{a}	16.0^{a}
Stalk shade	3.2^{b}	3.8^{b}	6.4^{b}	10.2^{bc}	12.5^{bc}	11.7^{b}	15.1^{b}	13.1^{b}	16.1^{b}	15.7^{bc}	14.5^{ab}	22.1^{bc}
Stalk clear	4.6^{b}	7.1^{c}	9.6^{c}	13.7^{c}	15.6^{c}	15.8^{c}	15.3^{b}	15.0^{b}	16.3^{b}	17.5^{c}	16.8^{b}	24.0^{c}
Control	2.8^{b}	4.0^{b}	5.7^{b}	7.7^{ab}	9.9^{ab}	10.5^{ab}	12.7^{ab}	12.0^{ab}	12.0^{ab}	12.5^{ab}	13.3^{ab}	17.1^{ab}
Q152												
Side shade	0.5^{b}	0.8^{ab}	1.9^{a}	4.4^{a}	5.2^{a}	5.5^{a}	13.3^{a}	10.5^{ab}	14.1^{a}	16.5^{a}	15.4^{a}	21.9^{ab}
Stalk shade	0.5^{ab}	1.3^{ab}	2.8^{a}	7.9^{b}	10.2^{b}	13.3^{b}	18.9^{b}	16.9^{c}	21.6^{b}	22.9^{b}	20.7^{b}	26.8^{b}
Stalk clear	0.1^{a}	0.8^{a}	3.2^{a}	6.6^{ab}	8.1^{b}	7.9^{a}	13.2^{a}	9.9^{a}	13.4^{a}	14.9^{a}	14.9^{a}	17.9^{a}
Control	0.7^{b}	1.5^{b}	2.8^{a}	6.6^{ab}	7.3^{ab}	7.8^{a}	15.1^{ab}	13.9^{bc}	15.7^{a}	17.4^{a}	18.2^{ab}	24.7^{b}

Table 7.5 Mean sucker number per plot (5 m of row) in rows one (control plots), three and five at Tully and Babinda.

Site	Tully		Babinda	
Cultivar	Q138	Q152	Q138	Q152
Row				
1	14.25	30.25	35.75^{b}	41.50^{b}
3	19.75	24.50	27.75^{ab}	25.50^{a}
5	21.00	21.75	22.00^{a}	32.25^{ab}
	ns	ns		

ns - F test not significant ($p > 0.05$)

7.1.3.2 Light measurements

There was a significant difference in the available proportion of light between treatments (T1 and T5) at all sites for all cultivars (Table 7.6), except Q152 in Tully. There was significantly more light available at 100 cm than at 10 cm at all sites except for Q138 at Tully, where the difference was not significant. There was a significant row x treatment interaction at all sites except for Q152 in Tully. This was due to Tully Q152 having a lower amount of available light on the outside of the crop whether it was shaded or not. For the other five sites, the significant interaction was mainly due to the shade cloth significantly reducing the amount of available light immediately behind it (outside-shade). Inside-shade and inside-control were only significantly different at the Babinda Q138 site. Therefore, at the other sites, the shading treatment was only lowering the amount of available light on one side of the row, as the light in the inter-row space between rows 1 and 2 was not affected by the shading treatment.

There was a significant effect of the shading treatments on the red/far-red ratio of light at three of the four sites sampled (Table 7.7). At the Tully Q138 and the Mulgrave Q152 sites,

the side shade treatment (T1) had significantly lower red/far-red ratio than the control (T5) and stalk clear (T4) treatments, as well as sunlight. The control and stalk clear treatments had significantly lower red/far-red ratios than sunlight, but not from each other. At the Mulgrave Q138 site, the three treatments, side shade (T1), stalk clear (T4) and control (T5) all had a significantly lower red/far-red ratio than sunlight, but not from each other. There was no significant difference in red/far-red ratio at the Babinda Q138 site ($p > 0.05$).

Table 7.6 Measurements of PAR as a proportion of sunlight for cultivars Q138 and Q152 at the Tully, Babinda and Mulgrave sites. Measurements were taken at 10 cm and 100 cm above ground on the outside of the crop and in the inter-row space between rows 1 and 2 in the inside of the crop. Means followed by the same letter are not significantly different ($p > 0.05$).

Site	Tully		Babinda		Mulgrave	
Cultivar	Q138	Q152	Q138	Q152	Q138	Q152
Row						
Inside	0.47	0.48^{b}	0.22^{a}	0.49	0.65^{b}	0.61
Outside	0.51	0.32^{a}	0.29^{b}	0.53	0.43^{a}	0.55
	ns			ns		ns
Height						
10 cm	0.46	0.35^{a}	0.23^{a}	0.45^{a}	0.48^{a}	0.52^{a}
100 cm	0.52	0.45^{b}	0.28^{b}	0.58^{b}	0.59^{b}	0.63^{b}
	ns					
Treatment						
Shade (T1)	0.33^{a}	0.36	0.14^{a}	0.43^{a}	0.46^{a}	0.46^{a}
Control (T5)	0.65^{b}	0.43	0.37^{b}	0.60^{b}	0.62^{b}	0.72^{b}
		ns				
Row*Treatment						
Inside-Shade	0.45^{b}	0.45	0.17^{b}	0.46^{ab}	0.62^{bc}	0.60^{b}
Outside-Shade	0.21^{a}	0.28	0.12^{a}	0.40^{a}	0.30^{a}	0.32^{a}
Inside-Control	0.48^{b}	0.51	0.28^{c}	0.52^{b}	0.69^{c}	0.62^{b}
Outside-Control	0.81^{c}	0.36	0.46^{d}	0.67^{c}	0.55^{b}	0.78^{c}
		ns				

ns - F test not significant ($p > 0.05$)

Table 7.7 Mean red/far-red ratio of light following the shading of the outside row of sugarcane. Means followed by the same letter are not significantly different ($p > 0.05$).

Site	Tully	Babinda	Mulgrave	Mulgrave
Cultivar	Q138	Q138	Q138	Q152
Treatment				
Side shade (T1)	0.98^a	1.04	1.06^a	0.98^a
Stalk clear (T4)	1.11^b	0.97	1.17^a	1.09^b
Control (T5)	1.15^b	1.03	1.17^a	1.12^b
Sunlight	1.33^c	1.27	1.31^b	1.27^c
		ns		

ns - F test not significant ($p > 0.05$)

The red/far-red ratio of light passing through shade cloth and dry leaf sheath was measured in order to see whether light quality was affected by the treatments (Table 7.8). The shade cloth was found to be spectrally neutral, with a red/far-red ratio of the light passing through it being similar to that of sunlight. Both green leaf and dry leaf sheath significantly reduced the red/far-red ratio, but green leaf reduced the ratio more than dry leaf sheath.

Table 7.8 Mean red/far-red ratio of sunlight and that of light passing through shade cloth, dry leaf sheath and green leaf ($n = 4$). Means followed by the same letter are not significantly different ($p > 0.05$).

Treatment	Red/Far-red ratio
Shade cloth	1.28^c
Dry leaf sheath	0.89^b
Green leaf	0.04^a
Sunlight	1.30^c

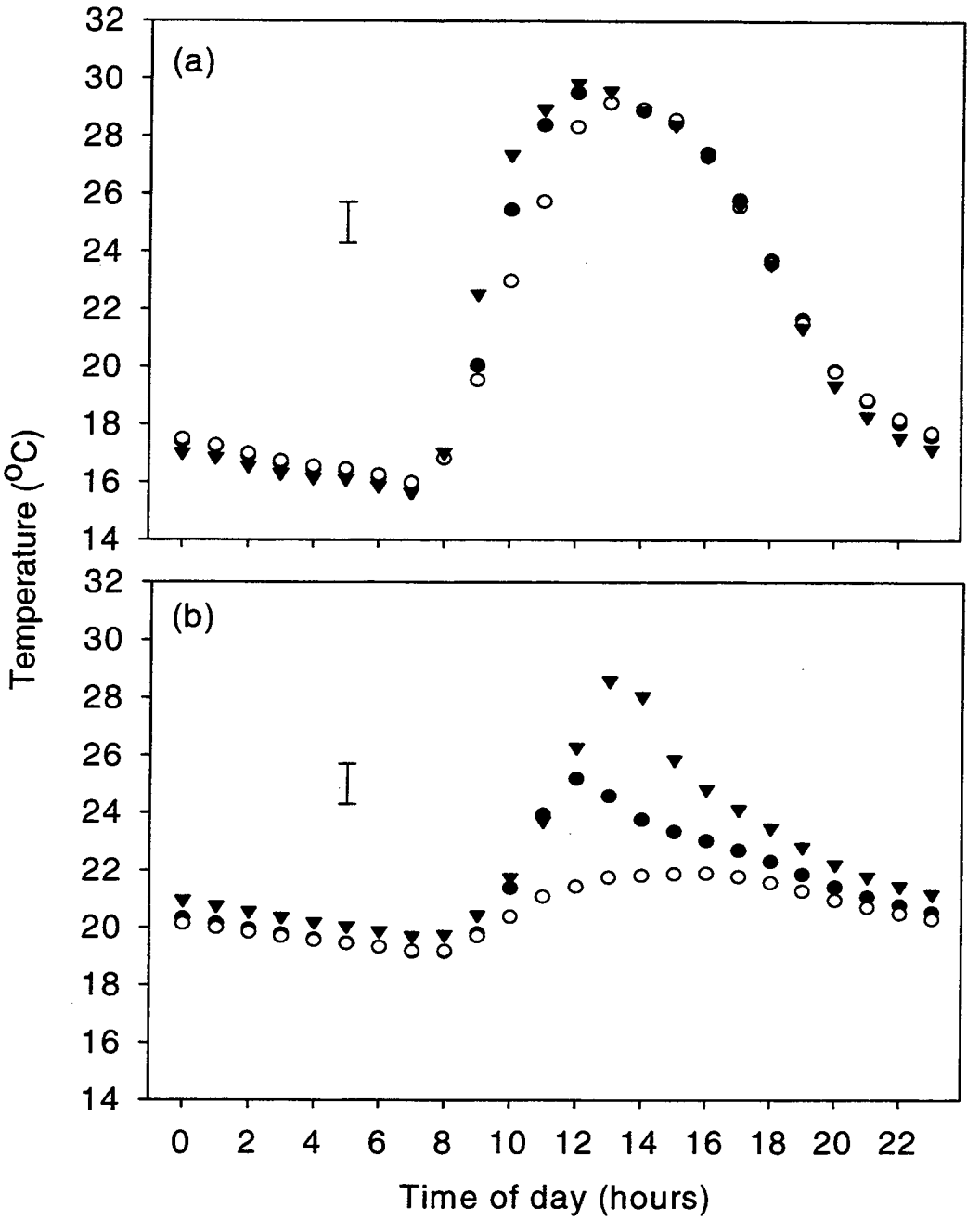

Figure7.3 Effect of shading the outside row of cane on (a) air and (b) soil temperature as measured by thermocouples at the Q152 site in Tully. Treatments were: ● Control (T5) ○ Side shade (T1); and ► Outside temperature. Average of 22 days. Error bars represent LSD ($p < 0.05$)

7.1.3.3 Temperature measurements

Shading the outside row of cane (T1) significantly reduced the temperature below ground (c. 5 cm) compared to the control (T5) treatment (Figure 7.3). The below ground temperature for treatments T1 and T5 were both significantly cooler than the outside control, which was not shaded by sugarcane during the day. Shading of the outside row of cane also significantly reduced the air temperature behind the shade cloth (T1). This effect on air temperature was small when compared to the effect on ground temperature. The outside control and the air temperature in T5 were significantly different at 9 and 10 am only.

7.1.4 Discussion

Shading the outside row of sugarcane by placing shade cloth alongside the row (T1) had very little effect on the number of suckers behind the shade cloth. There was virtually no difference between the T1 treatment and control for any of the crops. Where there was a difference, Mulgrave Q138, it appeared that this may have been due to a significant difference between the two treatments when the treatments were first established. This significant difference was initially maintained, but was then lost when sucker numbers increased later in the year.

The light measurements showed that the amount of light directly behind the shade cloth was significantly reduced, but they also revealed that for all sites except Babinda Q138,

there was no difference in the amount of light in the inter-row space between rows 1 and 2. Therefore, while the treatment may have lowered light on one side of the stool, the light characteristics on the other side of the stool remained similar to the control. The treatment was designed to prevent light from entering the crop from the side. However, if sufficient light was passing through the canopy, then possibly there was sufficient light to prevent any differences between T1 and the control being expressed.

Differences were found in the red/far-red ratio of light between T1 and the control. However the measurements were only taken immediately behind the shade cloth, and given the PAR measurements, it appears likely that no difference would have been found in the inter-row space between rows 1 and 2. The red/far-red ratio difference may have been due to the T1 treatment reducing the amount of sprawling of the canopy, and thus the light would have passed through more green leaf before reaching the base of the stalks. Sprawling may have been reduced by the wire supports that were used to hold the shade cloth in place.

The sucker counts taken on 19th July 1999 showed that there was not a significant edge effect at the Tully site for both cultivars, but there was an edge effect at the Babinda site for both cultivars. The edge effect at the Babinda sites was not as pronounced as that found by Bonnett *et al.* (2001) in a crop grown in the Burdekin region. Bonnett *et al.* found that the outside row of the crop had on average 21 suckers per 3 m whereas the 2nd row did not produce any suckers. This too could provide evidence for sufficient amounts of light passing through the crop canopy. Placing shade cloth alongside the outside row of a crop that had a good canopy such as those found in the Burdekin district may result in reduced

suckering as the main source of light incident on the stalks in the outside row would be reduced due to the presence of the shade cloth. It should be noted that the Burdekin district generally produces big crops with good canopies, whereas crops in the wet tropics often have poor canopies due to poor weather conditions. Consequently the light environment of the outside and inside rows in the tropics would have been more similar than in the Burdekin. The lack of an edge effect at Tully is interesting considering that no effect of treatment was found for either cultivar at this site.

Placing shade cloth along the outside row of cane also had an effect on both soil and air temperature behind the shade cloth. This was not associated with differences in the number of suckers. While soil temperature was similar for T1 and T5 at night, during the day, T5 reached a maximum mean temperature of 25.2 °C whereas the maximum mean temperature behind the shade cloth only reached 21.9 °C. There were also small differences in air temperature between treatments T1 and T5. This was mainly due to the shade cloth reducing the temperature behind it during the morning. Once the sun was directly overhead (midday), the air temperature differences were lost. The crops facing west may have experienced this difference during the afternoon, as the temperature measurements were taken from a crop that faced east.

Rands and Dopp (1938) found an increase in tillering from 20 °C to 30 °C in sugarcane. However, this result may be dependent on the cultivar used, as Glasziou *et al.* (1965) found significantly higher tiller numbers at 18 °C and 22 °C compared to 25 °C, 30 °C and 34 °C for the sugarcane cultivar Pindar. Ebrahim *et al.* (1998) found that tiller formation was greatest at 45 °C and least at 15 °C for cultivar H50-7209. The tillering response of

cultivars Q138 and Q152 to different temperature treatments has not been reported. Therefore, it is difficult to determine whether temperature changes, found during the day only, following the shading of the outside row of cane, could have contributed to changes in suckering. Furthermore, it is not known whether suckering responds to temperature in a similar manner as tillering in sugarcane.

It was noted from this experiment that there was a trend that the two treatments where the dead leaf (trash) was removed from the stalk had a higher number of suckers than the treatments where dead leaf was left attached to the stalk. This was despite T2 being shaded after the trash was removed. Removing dead leaf would potentially expose more of the stalk to light, which could explain why the trash removed treatment tended to have higher number of suckers, but this would not be the case for the T2 treatment.

One possible explanation for this trend may be light quality. The data in Table 7.8 shows the red/far-red ratio of light passing through dead leaf, shade cloth, green leaf and the red/far-red ratio of sunlight. These data show that while green leaf caused a significant reduction in the red/far-red ratio of the light passing through it due to the absorption of red light in photosynthesis, dead leaf also significantly reduced the ratio from that of sunlight and light passing through the shade cloth. The data only represents the reduction in red/far-red ratio due to one leaf sheath. Removing all the dead leaves from stalks in a crop could increase the red/far-red ratio even more than the data indicates due to incident light on stalks being effected by many dead leaves. Therefore, removing trash may have brought about an increase in the red/far-red ratio of the light incident on the stalks. Shading the stalks with shade cloth would have decreased the amount of light incident on the stalks but

the red/far-red ratio would have remained high due to the removal of dead leaf and the shade cloth being spectrally neutral. Holmes and Wagner (1980) have shown that a number of phytochrome mediated responses can occur when the amount of light was very low (night sky).

7.2 Trash stripping and its influence on suckering

The trend toward increased suckering with the removal of dead leaf from the stalk noted above was possibly due to changes in the light incident on the stalks. In order to test this hypothesis further, an experiment was established where dead leaf (trash) was removed from stalks of several sugarcane cultivars.

7.2.1 Methods

7.2.1.1 Experimental design and data collection

The experiments were established in existing BSES cultivar x nitrogen fertilisation trials, at Mulgrave and Tully. The BSES trials contained five cultivars, four nitrogen rates (0, 60, 120 and 180 kg N/ha), with three replicates. The trials were arranged in a randomised block design. Plot size was four rows by 15 m. Trash (dead leaf) was removed from two 5 m sections of row in each plot. Two 5 m sections of row, where trash was left attached to the stalk, were marked as controls in each plot. The two 5 m sections per treatment were averaged before analysis. These sub-plots were established in the middle two rows of each plot. Dead leaf was left on the ground in the inter-row space.

Suckers were counted at the Mulgrave site on 14th June 2000 and 21st July 2000. Suckers were counted at the Tully site on 24th July 2000.

Table 7.9 Dates of crop planting, nitrogen application, and the application of leaf trash removal treatments, in BSES experiments at Tully and Mulgrave involving five sugarcane cultivars.

Crop	Planted	Nitrogen application	Trash removed	Cultivars
Tully	21/7/99	27/10/99	14/3/00[A]	Q117, Q120, Q152, Q186, Q187
Mulgrave	22/7/99	1/11/99	10/5/00[A]	Q113, Q120, Q152, Q186, Q187

[A] trash was removed at regular intervals following this date

Figure 7.4 Stalks with their trash removed, Tully 2000.

Measurements of PAR were taken at the base of the stalks (10 cm above ground) on the date of the final sucker count in each district. PAR was measured with a ACUPAR Linear PAR Captometer (Decagon Devices Inc., USA). The readings were taken in the middle of each subplot. The measurements for each subplot were averaged prior to analysis. This

gave one measurement for trash removed and trash present per plot. Measurements were also taken outside of the crop, and were used to calculate the proportion of light available beneath the canopy.

7.2.1.2 Statistical analysis

Sucker numbers were initially analysed using ANOVA. The data were also analysed using paired t-tests. This was done as there was large variation in sucker numbers between plots, but trash removed treatments tended to have a higher number of suckers than trash present, whether or not the plot had a high or low number of suckers. The paired t-test removed the variation found between plots, possibly due to environmental factors, from the analysis.

7.2.2 Results

7.2.2.1 Sucker numbers

Analysis of variance for the sucker counts taken on 14th June 2000 at Mulgrave indicated that there was a highly significant difference in the number of suckers due to cultivar ($p < 0.01$) and a significantly greater number of suckers in the trash removed sub-plots ($p < 0.01$). There was no significant difference in the number of suckers due to nitrogen application rate and no significant interaction effects (Table 7.10).

Analysis of variance for the sucker counts taken on 21st July 2000 at Mulgrave indicted that there was a highly significant difference in sucker number due to cultivar ($p < 0.01$) and a

highly significant difference due to the removal of trash ($p < 0.01$). There was no significant difference in sucker number due to nitrogen application rate and no significant interaction effects.

Analysis of variance for the sucker count from the Tully site indicated that there was a highly significant difference in sucker number due to cultivar ($p < 0.01$), but there was no significant difference due to the removal of trash or nitrogen application rates. There were no significant interaction effects. Suckering in all cultivars, except Q152, was very low at this site.

Table 7.10 Average sucker number per plot (5 m) in cultivar x nitrogen trials at Mulgrave and Tully. Data were square root transformed prior to analysis. Means followed by the same letter are not significantly different ($p > 0.05$).

Main effects	Mulgrave		Tully
	14/6/00	21/7/00	24/7/00
Cultivar			
Q113	0.79^{a}	8.65^{b}	
Q117			0.06^{a}
Q120	9.48^{c}	15.94^{c}	0.42^{a}
Q152	10.98^{d}	27.19^{d}	18.94^{b}
Q186	0.58^{a}	4.63^{a}	0.35^{a}
Q187	3.00^{b}	6.75^{ab}	0.08^{a}
Treatment			
Trash removed	5.67^{b}	14.73^{b}	3.97
Trash present	4.27^{a}	10.53^{a}	3.98
LSD			ns
Nitrogen (kg/ha)			
0	4.62	12.18	3.95
60	4.43	11.20	4.03
120	5.83	14.83	4.28
180	4.98	12.30	3.62
	ns	ns	ns

ns - F test not significant ($p > 0.05$)

Using paired t-tests, a significant difference was found between the trash removed and trash attached treatments at the Mulgrave site, but not at the Tully site (Table 7.11).

Table 7.11 Paired t-test of trash removed vs trash present at Mulgrave and Tully.

Site	Mulgrave				Tully	
Date	14/6/00		21/7/00		24/7/00	
Treatment	Trash Removed	Trash	Trash Removed	Trash	Trash Removed	Trash
Mean	5.667	4.267	14.725	10.533	3.967	3.975
Mean difference	1.4		4.192		-0.008	
S.D. difference	2.696		4.683		2.603	
t	4.022		6.933		-0.025	
df	59		59		59	
Prob.	0.000		0.000		0.980	

Paired t-tests were used to analyse the effect of removing trash on each cultivar. The results of this analysis from the Mulgrave site are shown in Table 7.12. At the last date all cultivars showed a significant effect ($p \leq 0.03$). No significant differences were found at the Tully site, and therefore they have not been included in the table.

7.2.2.2 Light measurements

Analysis of variance of the light measurements taken at Mulgrave and Tully revealed significantly more light reaching the stalk bases in the trash removed subplots than the trash present subplots (Table 7.13).

Table 7.12 Differences between trash removed (rem) and trash present for five cultivars at the Mulgrave site on two dates using paired t-tests.

Cultivar	Date	Treatment	Mean	S.D. difference	Prob.
Q113	14/6/00	Trash (rem)	1.125	1.303	0.104
		Trash	0.458		
	21/7/00	Trash (rem)	10.375	4.984	0.035
		Trash	6.917		
Q120	14/6/00	Trash (rem)	10.208	3.421	0.168
		Trash	8.750		
	21/7/00	Trash (rem)	17.625	3.061	0.003
		Trash	14.250		
Q152	14/6/00	Trash (rem)	12.542	3.920	0.019
		Trash	9.417		
	21/7/00	Trash (rem)	31.375	6.161	0.001
		Trash	23.000		
Q186	14/6/00	Trash (rem)	0.833	0.929	0.089
		Trash	0.333		
	21/7/00	Trash (rem)	6.208	2.683	0.002
		Trash	3.042		
Q187	14/6/00	Trash (rem)	3.625	2.148	0.069
		Trash	2.375		
	21/7/00	Trash (rem)	8.042	3.728	0.035
		Trash	5.458		

Table 7.13 Proportion of light reaching stalk bases in the trash removed and trash present subplots at Mulgrave and Tully. Means followed by the same letter are not significantly different ($p > 0.05$).

Effect	Mulgrave	Tully
Treatment		
Trash removed	0.14[b]	0.13[b]
Trash present	0.07[a]	0.08[a]
Cultivar		
Q117		0.13
Q152	0.10	0.10
Q186	0.11	0.09
	ns	ns
Nitrogen		
0	0.10	0.10
120	0.10	0.12
	ns	ns

ns - F test not significant ($p > 0.05$)

7.2.3 Discussion

Removing dead leaf from the stalks significantly increased suckering in all five cultivars in the Mulgrave district. However, the same treatment did not result in increased suckering at the Tully site. Four of the five cultivars were present at both sites. The lack of response at Tully seemed to be partly due to there being very limited suckering at the site, and therefore any difference between treatments was not expressed. However, cultivar Q152 which did sucker at Tully, did not show any significant difference in sucker number between trash removed and trash present treatments.

Light measurements indicated that removing dead leaf resulted in increased light availability at the base of the stalks at both sites. Therefore, this increase in available light

may have caused the increase in sucker numbers at the Mulgrave site. Tillering in sugarcane has been shown to be affected by the amount of available light (Verret and McLennan 1927; Martin and Eckart 1933). The results from this experiment also suggest that the formation of late tillers is also similarly affected by the amount of available light. Why no difference was found at the Tully site is not known, but possibly other factors, which were causing very low sucker number at this site in all cultivars except Q152, were involved. If the Tully crop had a good canopy, then possibly removing dead leaf would have had little effect on the light incident on the stalks, as the crop canopy would be responsible for filtering more light than the removal of dead leaf. However, the amount of light reaching the stalk bases was similar at both sites. This would indicate a similar canopy structure. The cause of the differences between the two locations is not known, but factors like temperature, or water availability may have been involved.

No effect on sucker number was found due to nitrogen application rate at either site. This evidence is similar to that found by Berding and Hurney (2000), where nitrogen application rates at the start of the growing season had no effect on sucker number. The Mulgrave site was partly waterlogged for much of the season, and this may have meant that differences between plots in terms of nitrogen application were lost due to leaching and other denitrification processes. There is evidence that nitrogen can play an important role in suckering and this was discussed in Chapter 6.

While removing dead leaf increased sucker numbers at Mulgrave, it was not the primary factor in determining sucker number. Sucker number was highly variable between plots, due to some unknown factor(s), and the removal of trash increased the sucker number only

slightly above this background level. The interaction of environmental factors affecting suckering are discussed in Chapter 8.

7.3 The effect of light quality on suckering in sugarcane

7.3.1 Introduction

Changes in light quality, in particular the ratio of red light to far-red light, have been shown to affect tillering in a number of grasses, and the red//far-red ratio has been proposed as a mechanism by which plants are able to detect changes in the light environment in which they are growing (Chapter 2). Due to this, and some evidence that removing trash may have resulted in an altered red/far-red ratio of light incident on stalks (section 7.1.3) and that this may have had an effect on suckering in experiment 7.2.1, it was thought that further investigation of the effect of the red/far-red ratio of light on suckering was warranted. In order to test the hypothesis that suckering is stimulated by light with a high red/far-red ratio, an experiment was established where the red/far-red ratio of light incident on stalk bases was manipulated.

7.3.2 Methods

7.3.2.1 Plant growth

Cultivar Q138 was grown in pots (38 cm diameter and 30 cm depth) at CSIRO Davies Laboratory. Single eye sets were originally planted in trays on 2nd August 1999, and following germination individual plantlets were planted into separate pots. Each pot contained a mixture of peat, coarse sand and fine sand (1:2:2 v/v/v). Shoots initially

germinated in a glasshouse. However, once in pots, the cane was grown in the open air. On 3rd September 2000 the stalks were cut at the base and allowed to ratoon.

Plants were irrigated and fertilized as described in previous chapters, and were prevented from lodging by wire supports as described in previous chapters.

7.3.2.2 Manipulation of red/far-red ratio and PAR

Manipulation of the red/far-red ratio of light has been achieved by using combinations of light sources and filters specific to the desired wavelengths by numerous authors (Tucker 1976; Child *et al.* 1981; Casal *et al* 1987a; Casal 1988; Chow *et al.* 1990; Skinner and Simmons 1993). However, due to the size of the sugarcane plant, using this approach would have been extremely difficult. Three treatments were sought: (i) a control which had high PAR and high red/far-red ratio (no shading); (ii) low PAR and high red/far-red ratio; and (iii) low PAR and low red/far-red ratio. Pre-experiment testing showed that these treatments could be established with combinations of coloured cellophane and coloured shade cloth (Table 7.14). The pre-experiment tests were conducted at midday on a sunny day, in direct sun outside the glasshouse. These treatments were designed in order to distinguish between any effects of light quantity and light quality on suckering. Smith (1982) showed that phytochrome is particularly sensitive to small changes in red/far-red ratio in range from 1.15 to 0.05. These ratios are typical of vegetational shade. Holmes and Wagner (1980) have shown that a number of phytochrome mediated responses can occur when the amount of light is very low.

The shade cloth used in the experiment was Black Z16, 99% visible light, 97% UV, Knittex, South Africa and Coolaroo knitted shade cloth, heritage green, extra heavy 84-90% cover factor, Gale Pacific Pty Ltd, Australia. The cellophane used was: Clear, Cello sheets, Big W, Australia and Emerald green, Hallmark, Australia.

Table 7.14 Quality and quantity of light passing through shade cloth and cellophane sheets of different colour, for three shading treatments designed to alter the quantity and the red/far-red quality of light reaching the lower stalks of sugarcane grown in pots in a glasshouse (n = 4). Means followed by the same letter are not significantly different ($p > 0.05$).

Treatment	Shade cloth /cellophane	Measured red/far-red ratio	PAR (% of sunlight)
High Ratio High PAR	Unshaded	1.23^{b}	100^{b}
High Ratio Low PAR	Black Cloth Clear cellophane	1.19^{b}	1.12^{a}
Low Ratio Low PAR	Green cloth Green cellophane	0.39^{a}	2.21^{a}

7.3.2.3 Experimental design and sampling

On 12th December 2000 thirty pots were moved into a controlled environment glasshouse. The pots were placed in three rows with ten pots per row. There was a 2 m space between rows and 0.9 m between pots (centre to centre). These pot and row spacings were designed in order to allow high amounts of light beneath the canopy, and a high red/far-red ratio beneath the canopy. Plants were initially irrigated automatically for 4 min three times a day (9 am, 1 pm and 5 pm). This was changed to 6 min, three times a day, at the end of December 2000. The temperature and humidity settings of the glasshouse are shown in Table 7.15. On 19th February 2001 the treatments were initiated with 10 replicates per

treatment. Stalks were shaded from the base to a height of 2 m. This was done by wrapping all stalks with a layer of cellophane followed by the shade cloth (Figure 7.5). Treatments were arranged randomly.

Figure 7.5 Sugarcane plants growing in the glasshouse. The stalks were shaded with black shade cloth and clear cellophane, green shade cloth and green cellophane or an unshaded control.

Stalk and sucker counts were taken on 19th February 2001 and at the final harvest on 4th September 2001. Measurements of sucker morphology were also taken at the final harvest in order to ascertain whether light quality/quantity was affecting sucker morphology.

Table 7.15 Glasshouse temperature settings and the mean air temperatures (duration of the experiment) within the glasshouse compartment in which the plants were grown.

Time	Temperature (°C)	Air temperature (°C)
6:00	21	21.2
9:00	26	26.9
13:00	30	31.4
15:00	30	31.5
18:00	27	27.1
20:00	23	23.4
23:00	21	21.4

7.3.2.4 Light measurements

Measurements of the red/far-red ratio were taken on 6th March 2001, 10th July 2001 and 4th September 2001. On 6th March 2001, one pot of each treatment was chosen randomly and the red/far-red ratio was calculated from a scan from 300 nm – 1100 nm taken by a Licor LI-1800 portable spectroradiometer (Licor Inc. Nebraska, USA). The scans was taken at 1 m above ground (ground level in the pot), within the cellophane/shade cloth wrapped stool. This measurement was taken in order to ensure that the shading treatments were actually altering the light quality and quantity in a similar manner as the pre-experiment tests. Only

one pot per treatment was chosen in order to reduce damage to the cellophane layer. On 10th July 2001 and 4th September 2001 scans were taken with the same instrument from all pots. These tests were done to ensure that the treatments were maintaining the altered light characteristics. More rigorous sampling was not conducted as the placing of the light sensor within the stool caused some damage to the cellophane, and it was noted that the cellophane did not fade as it was located within the shade cloth layer. An estimation of the total amount of light was obtained from the scans by summing the amount of light between 400 – 700 nm. This too was done in order to minimise the amount of damage to the treatments, as the AccuPAR Linear PAR Ceptometer (Decagon Devices USA), used to measure PAR in previous experiments, could not be placed inside the cellophane/shade cloth screening without external light interfering with the measurement and damage being done to the cellophane layer. All light measurements were conducted at around midday on sunny days. Measurements were taken from all treatments and also from outside the glasshouse (sunlight) on all sample dates. The light measurements inside the glasshouse were expressed as a proportion of what was available outside the glasshouse in full sunlight. The polycarbonate walls and roof of the glasshouse filtered out approximately 54% of the light which was available outside. However, the spectrum of light remained similar (data not presented).

7.3.2.5 Statistical analysis

Stalk numbers were analysed using ANOVA, and ANOVA with repeated measures to determine the effect of time on the number of suckers. Paired t-tests and ANOVA were used to compare stalk morphology of suckers in each treatment.

7.3.3 Results

7.3.3.1 Stalk numbers

There was no significant difference in the number of mature stalks and sucker stalks prior to the establishment of the treatments (Table 7.16). It was noted that many of the suckers initiated prior to the establishment of the treatments died following shading.

Table 7.16 Average number of mature stalks and suckers prior to the establishment of the treatments designed to explore the effects of light quality and quantity on sucker formation on 19th February 2001.

Treatment	Mature stalks	Suckers
High ratio High PAR	5.8	6.6
High ratio Low PAR	6.3	6.2
Low Ratio Low PAR	5.1	5.9
	ns	ns

ns - F test not significant ($p > 0.05$)

Table 7.17 Stalk numbers, following the exposure to shading treatments using shade cloth and cellophane designed to affect the quality and quantity of light reaching the lower parts of the plant on the 4th September 2001.

Treatment	High ratio High PAR	High ratio Low PAR	Low Ratio Low PAR	
Stalk type				
Mature stalks	5	4.9	4.6	ns
Secondary stalks	3.9	3.7	3.3	ns
Mature + Secondaries	8.9	8.6	7.9	ns
Sucker	8.1	12.0	11.9	ns
Suckers + Secondaries	12	15.7	15.2	ns

ns - F test not significant

Using analysis of variance with repeated measures, there was no significant effect of treatment on sucker number (Table 7.17), although as expected, there was a significant increase in sucker number with time. There was no significant effect of treatment or time on the number of mature stalks, but there was a significant increase in stalk number with time when mature stalk initial was compared with the number of mature stalks + secondary stalks at final harvest. Secondary stalks were those that were of similar size to the mature stalks at the final harvest but were not mature when the experiment was established. Some of these stalks may have been originally counted as suckers when the experiment was established. However, there was no significant effect of treatment on sucker + secondary stalk number.

7.3.3.2 Stalk morphology

The suckers that emerged from each pot grew in the light conditions imposed by the treatments. This may have caused differences in sucker stalk morphology. The diameter at the base of the main stalks was significantly smaller than that of the secondary stalks. The diameter at the base of the sucker stalks was also significantly wider than that of the main stalks (Table 7.18). There was no significant difference in stalk base diameter for the secondary and sucker stalks. This is evidence that the secondary stalks were initiated as suckers. These comparisons were done by first averaging the diameters of the main, secondary and sucker stalks for each pot, and then analysing the data using paired t-tests. Mean width in Table 7.18 varies depending on the comparison being made, as some pots did not contain all three stalk types.

Table 7.18 Comparison of stalk base diameters of different stalk classes following the shading of pots with coloured cellophane and shade cloth. Comparisons were made by paired t-tests

Comparison	Mean	SD difference	p
Main Secondary	2.108 2.505	0.390	0.000
Main Sucker	2.198 2.613	0.294	0.003
Sucker Secondary	2.510 2.610	0.433	0.535

There was no effect of treatment on the differences between main and secondary stalk widths ($p > 0.05$). This was established by conducting an ANOVA of the difference between main stalk diameter and secondary stalk diameter with treatment as the independent variable. A comparison was not made for sucker stalks due to the small sample size for each treatment. Most sucker stalks were small and the diameter at the base of these stalks had not developed fully.

There was a significant effect of shading on the height above ground of the dewlap of the leaves produced by the suckers (Figure 7.6). The leaves of shaded suckers were produced at a greater height above ground than those of the unshaded control. There was no difference between the two low PAR treatments (except leaf 4), even though the red/far-red ratio of light was different between treatments. Therefore, the etiolation effect seems to be due to light quantity not quality.

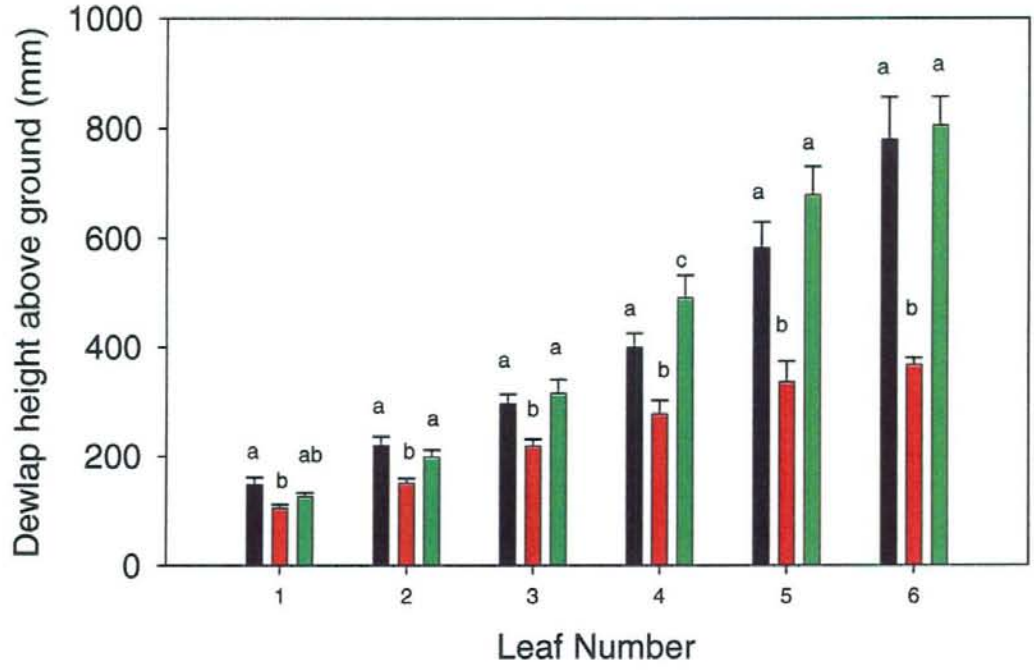

Figure 7.6 Leaf dewlap height above ground. Treatments were: ■ Low PAR high ratio; ■ High PAR high ratio; and ■ Low PAR low ratio. Error bars represent the standard error of the mean.

There was no significant effect of the three treatments on the maximum leaf breadth of the suckers ($p > 0.05$, all leaves). Shading of suckers had a significant effect on leaf length (Figure 7.7). This was mainly due to the low PAR high ratio treatment having significantly longer leaves than the other two treatments. However, this effect was not present for leaves 4 and 5, and at leaf 6 the two low PAR treatments were both significantly longer than the high PAR high ratio treatment. The shading treatments also had a significant effect on leaf length to breadth ratio. This effect was similar to that of leaf length, and as there were no significantly differences in leaf breadth, it is the differences in leaf length that were causing this significant effect on leaf length to breadth ratio.

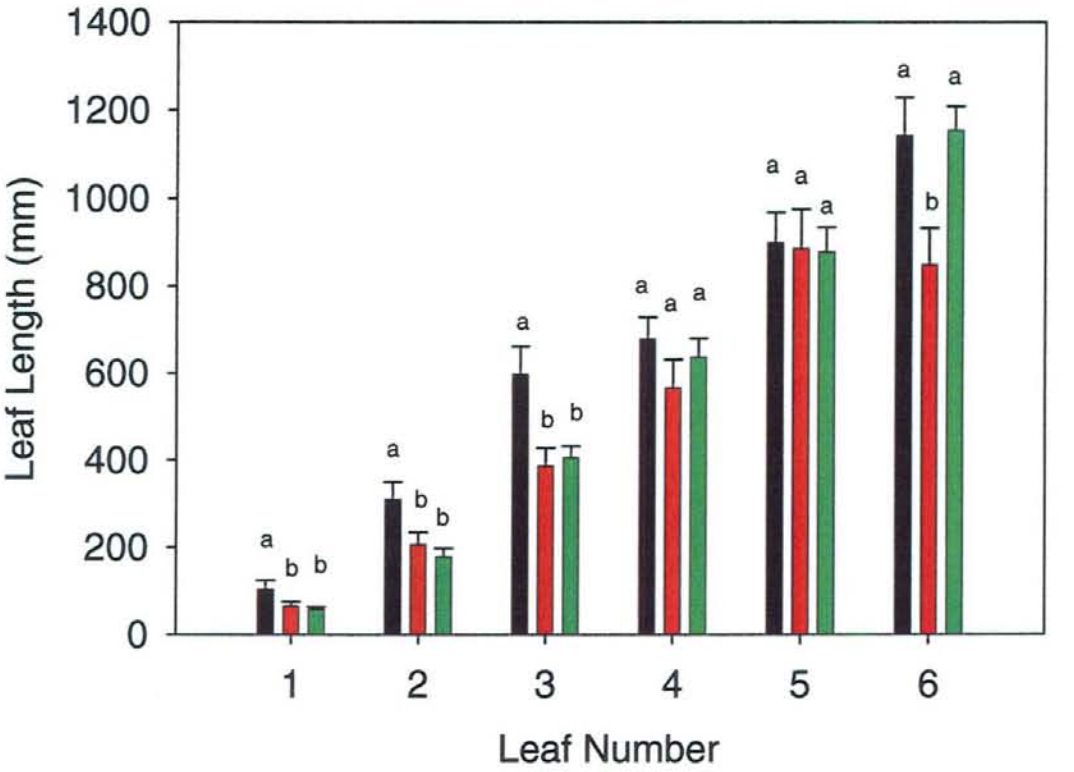

Figure 7.7 Leaf length of suckers grown under different light environments. Treatments were: ■ Low PAR high ratio; ■ High PAR high ratio; and ■ Low PAR low ratio. Error bars represent the standard error of the mean.

7.3.3.3 Light measurements

The measurements taken on 3rd June 2001 indicated that the proposed treatments had been successfully established. The measurements taken on 10th July 2001 and 4th September 2001 indicated that the desired treatments were still present at these dates (Table 7.19). The data were analysed using ANOVA with repeated measures. On both dates the high PAR high ratio treatment had significantly higher red/far-red ratio and amount of light than the low PAR low ratio treatment.

Table 7.19 Light measurements following the manipulation of the red/far-red ratio and amount of light incident on the lower parts of sugarcane stalks. Means followed by the same letter are not significantly different ($p > 0.05$).

	Red/Far-red ratio			Amount of light (400 – 700 nm, proportion of sunlight)		
Date	6/3/01	10/7/01	4/9/01	6/3/01	10/7/01	4/9/01
Treatment						
High PAR High ratio	1.15	0.73^{b}	0.98^{c}	0.1580	0.1491^{b}	0.0869^{b}
Low PAR High ratio	0.72	0.67^{b}	0.79^{b}	0.0007	0.0014^{a}	0.0010^{a}
Low PAR Low ratio	0.29	0.33^{a}	0.42^{a}	0.0011	0.0033^{a}	0.0019^{a}
Sunlight		1.22^{c}	1.21^{d}			

There was no significant difference in the amount of light between the two low PAR treatments, but the high ratio treatment had significantly higher ratio than the low ratio treatment on all dates. There was no difference in the ratio between the two high ratio treatments on 10th July 2001, but the difference was significant on 4th September 2001, where the high PAR high ratio treatment had a significantly greater ratio than the low PAR high ratio treatment. This may have been due to the presence of green leaves of suckers growing in the confined space under the shade cloth. The analysis also shows a significant increase in red/far-red ratio for all treatments with time. It appears that this may have been due to either the glasshouse or canopy structure within the glasshouse, as the red/far-red ratio of light outside the glasshouse remained constant, as expected.

7.3.4 Discussion

The lack of effect of light quantity on sucker number appeared contrary to what was found in the trash removal experiment, where removing dead leaf resulted in an increase in amount of light reaching the base of the stalk and an increase in suckering at one site. Low

levels of irradiance have been shown to reduce tillering in sugarcane (Verret and McLennan 1927; Martin and Eckart 1933). Whether this result was due to the cultivar used in this experiment is unknown. Ideally, several cultivars should have been tested. However, filling the glasshouse with large numbers of pots would have made it difficult to manipulate the red/far-red ratio of light as the thick canopy would have had a large effect on the ratio. Using shade cloth and cellophane outside the glasshouse would have been difficult as wind and rain would have damaged the treatments.

No evidence was found to show that sucker number is affected by the red/far-red ratio of light. However, the vast amount of evidence on the role of the red/far-red ratio in tillering and light perception in a number of species means that further experimentation is warranted. Ideally, this experiment would be done in a controlled environment where high levels of PAR are maintained but the red/far-red ratio is manipulated with wavelength specific filters. This would be an extremely expensive and difficult experiment to conduct given the size of the sugarcane plant when it is large enough to produces suckers. An experimental system that resulted in the manipulation of suckering could be of use when investigating the inter-stalk relationship between sucker and mature stalk. It would allow the comparison of mature stalks, of the same age, that had not produced a sucker to those that had. The information generated from this experiment could help resolve the issue of determining the full impact of suckering on the sugarcane industry as discussed in Chapter 5.

Ludlow *et al.* (1990) found no relationship between tillering and red/far-red ratio in sugarcane. However, they did not look at the same cultivar under different red/far-red ratio

conditions - they investigated different cultivars and the red/ far-red ratio of light at the base of stools as a result of the cultivars growth. This approach makes it very difficult to distinguish between inherent difference in tillering between cultivars and the role of the red/far-red ratio. It was not surprising that they found a trend where the cultivars with high tillering propensity had low red/far-red ratio at the base of their stalks, as potentially more light was filtered through a canopy with a larger number of stalks. A larger number of stalks may also result in more surfaces for the reflection of far-red light, which would also contribute to a lower red/far-red ratio. Kasperbauer and Karlen (1994) showed that a typical corn leaf reflected little red light but much of the far-red light that 'impinged' on its surface. A more interesting comparison would have been whether high tillering cultivars tillered at the same rate under different red/far-red ratio conditions. This might be achieved by growing the same cultivar at different planting densities. However, competition for other resources then becomes a contributing factor.

The difference in the height at which sucker leaves were produced due to shading does suggested that the stalks were etiolated, and that etiolation is a result of light quantity not quality. Low light intensity has been shown to increase plant height in sugarcane (Martin and Eckart 1933), *Festuca scabrella* (Willms 1988) *Sinapsis alba* (Ballare *et al.* 1991) and internode extension in *Helianthus annuus* (Garrison and Briggs 1972). However, many studies have also shown that the etiolation response in plants is due to changes in the red/far-red ratio of light (Kasperbauer *et al.* 1970; Child *et al.* 1981; Morgan and Smith 1981; Ballare *et al.* 1987; Casal and Smith 1988; Kasperbauer and Karlen 1994). There was some evidence that the low red/far-red ratio treatment was causing greater etiolation (Leaf 4). Ballare *et al.* (1991) suggest that the depression of light quantity and the red/far-red

balance are both involved in the process of internodal growth. This possibly explains why under field conditions sucker leaves are produced higher up the stalk than on normal stalks (Chapter 3). However, Morgan and Smith (1981) suggested that caution should be taken in drawing such conclusions, as light alone may not be causing this effect. Humidity, temperature and mechanical stress are all affected by vegetational shade. The data also showed that light quantity may also affect leaf length, but the relationship was not as clearly defined as the height at which the leaves are produced. No effect was found on leaf breadth, and therefore other factors may influence sucker leaf morphology more than the light environment in which they are growing. The presence of the parent stalk was shown in Chapter 5 to have an effect on leaf morphology. Since the suckers were attached to the parent stalk throughout the duration of the experiment, this might explain the lack of difference in leaf morphology.

7.3.5 Summary

The results reported in this chapter have provided some insight into the role of light in suckering. However, a number of inconsistency exist. In section 7.1 placing shade cloth along the outside of the crop did not result in fewer suckers in those regions of the crop. However, there was a trend of increased suckering in treatments where trash was removed from the stalk. In section 7.2 removing dead leaf from stalks caused an increase in sucker number in five cultivars, but only at one site. The removal of dead leaf caused an increase in the amount of light reaching the stalk bases at both sites. In section 7.3 reducing the amount of light incident on stalks and the red/far-red ratio of light incident on stalks did not result in reduced suckering. Light was shown to effect the morphology of sucker stalks.

These inconsistencies require further investigation.

Chapter 8. The interaction of environmental stimuli

8.1 Introduction

In Chapters 6 and 7 it was shown that the availability of nitrogen in the soil and light beneath the canopy (in some cases) can affect the number of suckers produced by a sugarcane crop. In this chapter a further environmental factor is introduced. The increased availability of moisture in the soil has also been thought to cause an increase in sucker numbers. Berding and Hurney (2000) claimed that the increase in sucker numbers in recent years was due to marked wet events late in the growing season. Increased water content in the soil has also been shown to increase tillering in grasses (Olmstead 1941; Gardner 1942).

Light, nitrogen and soil moisture may also interact with each other to affect suckering. These interactions along with other factors such as temperature may be the cause of some of the differences in sucker number already encountered. These include the differences between the two trash stripping sites and the lack of a significant difference in sucker number between nitrogen treatments in Tully at the final sampling. Therefore, an experiment was designed to further elucidate the role of nitrogen and light on suckering, to establish the role of soil moisture on suckering, and to determine the effect of the interaction of these factors on suckering in sugarcane. Temperature was not used as a treatment due to likely difficulties in manipulating temperature in the field.

8.2 Methods

8.2.1 Treatments and experiment design

A field experiment was established at Mr. T. Watters farm in the Mulgrave district by the BSES. The experiment contained three light treatments, three nitrogen treatments and two cultivars. The experiment was also conducted in two moisture regimes, one that was rain-fed, and one which was managed to receive additional irrigation following the wet season. The additional irrigation maintained field capacity at approximately 18 % moisture. This was determined during a drying cycle following heavy monsoonal rain earlier in the year. The additional irrigation commenced 323 days after planting (DAP) and 252 days after ratooning (DAR) in the plant and ratoon crops respectively. Each environment contained five replicates of a randomised complete block, three factor, factorial design. The two moisture environments were located next to each other. Plot size was six rows by 9.5 m. Measurements were taken in the middle 3 m of the two central rows. The experiment was conducted over two years, and data were taken from both the plant and first ratoon crops. The crop was planted on 15-28 July 1999, and ratooned on 5-8 September 2000. Sampling dates were calculated from 28th July 1999 and 8th September 2000. Treatments were established as follows:

Stool spacing: Cane was planted at three stool densities in order to attempt to manipulate the availability of light beneath the crop canopy. Two sections of stalk 0.5 m long, were bundled together and planted at intra-row spacings of 0.5 m, 1.0 m or 1.5 m spacings. The inter-row space was 1.5 m.

Nitrogen: The availability of nitrogen in the soil was manipulated by using three nitrogen application rates. The three rates were: 0 kg/ha, 140 kg/ha and 140 + 70 kg/ha. The extra 70 kg/ha was applied following the wet season to ensure that nitrogen was available in the soil late in the season. This was conducted 300 DAP and 241 DAR for the plant and ratoon crops respectively. The timing and amount of additional nitrogen was based on the results of the experiment conducted in Chapter 6. In the ratoon crop the 140 kg N/ha was replaced with 210 kg N/ha. This was done to ensure that the actual amount of nitrogen applied to treatments two and three was the same, but the timing of the application differed. In both crops the initial applications were conducted in November and the additional application was conducted in May.

Cultivars: The commercial cultivars Q138 and Q152 were used as they both tend to sucker profusely and are commonly grown in the region. The cultivars also have vastly different parentage.

8.2.2 Stalk counts

Stalk counts were conducted by BSES, in the core plot region, on regular occasions for both the plant and ratoon crops (Table 8.1). Both main stalks and sucker stalks were counted.

Table 8.1 Dates of stalk counts, soil samples and light measurements in the in the core plot area for the plant and ratoon crops at Mulgrave.

	Plant crop (DAP)	Ratoon crop (DAR)
Stalk counts	229, 285, 331, 392	181, 245, 287, 384
Soil samples	231, 286, 307, 342, 384	244, 297, 368
Light measurements	244, 302	195, 243, 298, 368

8.2.3 Soil nitrogen analysis

Soils samples were taken from all the plots that contained cultivar Q152 in the irrigated environment, in both the plant and ratoon crops (Table 8.1). Prior to the application of the additional 70 kg N/ha to the 140 + 70 kg N/ha treatments in May, only the 0 kg N/ha and 140 kg N/ha (210 kg N/ha in the ratoon crop) plots were sampled. Following the additional nitrogen application in May, soil samples were taken from plots of all three nitrogen treatments. Three soil cores to 50 cm below ground level were taken per plot with an auger (2.5 cm diameter). The cores were divided into two depths, 0 – 25 cm and 25.1 – 50 cm. The three cores per plot were pooled prior to soil N analysis. Soil nitrate-N and ammonium-N were determined as described previously in sections 6.2.1.4 and 6.2.2.4.

8.2.4 Light measurements

Light measurements (PAR and red/far-ed ratio) were taken from the 60 plots that received the 140 kg N/ha nitrogen application in the plant crop and the 210 kg N/ha nitrogen application in the ratoon crop (Table 8.1). Two measurements were taken at both 10 cm and 100 cm above ground in the inter-stool spaces in each plot. Scans were taken from 300 –

1100 nm using a Licor 1800 portable spectroradiometer (Licor Inc. Nebraska, USA). These scans were used to calculate the red (660 - 680 nm)/far-red (720 - 740 nm) ratio of light beneath the crop canopy. Measurements of PAR were taken with a ACUPAR Linear PAR Ceptometer (Decagon Devices Inc., USA). The data collected was compared to an external PAR reference which was located on a weather station next to the crop. The external reference recorded PAR every 5 minutes. As all light measurements were conducted on sunny days, there was very little change in PAR over the 5 minute period at the external reference. This allowed for the measurements taken within the canopy to be compared with the corresponding external measurement. The time on the ACUPAR Linear PAR Ceptometer was synchronised with that on the weather station prior to any measurements being taken. Simultaneous external measurements could not be taken directly above the crop canopy due to the difficulties with carry such a device through a fully grown sugarcane crop. The external reference allowed the light beneath the crop canopy to be expressed as a proportion of the total light incident on the crop.

8.2.5 Statistical analysis

All statistical analyses were conducted using SYSTAT 9 (SPSS Inc. Chicago, USA). The sucker number data were analysed using ANOVA with cultivar, nitrogen rate, stool spacing and moisture regime as independent variables. Sucker number was square root transformed prior to analysis as the data did not have a normal distribution. A histogram and probability plot confirmed that the transformation did result in a more normal distribution. Soil nitrogen was analysed using ANOVA with nitrogen rate, stool space and sample depth as independent variables. Light measurements were analysed using ANOVA with height of

sample above ground, cultivar, moisture regime and stool space as independent variables. Measurements of the percentage of available PAR beneath the canopy were $\log_n$ transformed as the data did not have a normal distribution. A histogram and probability plot confirmed that the transformation did result in a more normal distribution *Post-hoc* comparisons of means were conducted using Fisher's least significant difference (LSD) ($p \leq 0.05$).

8.3 Results

8.3.1 Plant crop

8.3.1.1 Sucker numbers

The mean number of suckers and significant differences for the main effects are shown in Table 8.2. Cultivar Q152 was found to have significantly greater number of suckers than cultivar Q138, 392 DAP. A significant effect due to nitrogen rate was found 331 and 392 DAP. At the final count both the 140 kg N/ha and 140 + 70 kg N/ha treatments had significantly greater number of suckers than the 0 kg N/ha treatment. The 140 + 70 kg N/ha treatment had significantly greater number of suckers than the 140 kg N/ha treatment. The rain-fed environment was found to have a significantly greater number of suckers than the irrigated environment 295 and 331 DAP. However, the irrigated environment had a significantly greater number of suckers than the rain-fed environment at the final count. The 1.5 m stool spacing had significantly greater number of suckers than the other stool

spacings 295 DAP. This effect was lost later in the year. No significant effects were found 229 DAP due to the very low number of suckers.

Table 8.2 Number of suckers in the plant crop 229, 295, 331 and 392 DAP for each of the main effect treatments. Late nitrogen application was conducted 300 DAP and additional irrigation commenced 323 DAP. Means followed by the same letter are not significantly different ($p > 0.05$).

Main effects	Number of suckers			
	229 DAP	295 DAP	331 DAP	392 DAP
Cultivar				
Q138	0.0	1.9	12.0	33.3[a]
Q152	0.1	2.0	11.1	38.1[b]
	ns	ns	ns	
Nitrogen (kg/ha)				
0	0.1	1.4	8.7[a]	26.8[a]
140	0.1	2.2	10.7[a]	34.3[b]
140 + 70	0.1	2.4	15.2[b]	45.9[c]
	ns	ns		
Moisture				
Irrigated	0.0	1.7[a]	9.7[a]	45.2[b]
Rain-fed	0.1	2.3[b]	13.4[b]	25.9[a]
	ns			
Stool spacing (m)				
0	0.0	1.8[a]	11.3	35.0
1.0	0.1	1.5[a]	10.9	37.2
1.5	0.1	2.7[b]	12.4	34.8
	ns		ns	ns

ns - F test not significant ($p > 0.05$)

Cultivar x moisture was found to have a significant interaction effect on sucker number per plot 331 DAP (Table 8.3). This was due to cultivar Q152 having significantly lower number of suckers than Q138 in the irrigated environment. No significant difference was found at the final count. The cultivar x stool space interaction was significant at both 331 and 392 DAP. Cultivar Q152 had a significant lower number of suckers in the 0.5 m

spacing than in the 1.0 m and 1.5 m spacings, 331 DAP. This difference was not present for cultivar Q138. At 383 DAP cultivar Q152 again had a significantly lower number of suckers in the 0.5 m spacing than in the 1.0 m and 1.5 m spacings. Cultivar Q138 had significantly lower number of suckers in the 1.5 m spacing than in the 0.5 m and 1.0 m spacings.

Table 8.3 Significant interaction effects on number of suckers in the plant crop 331 and 392 DAP. No interaction effects were found to be significant at the earlier sucker counts. Means followed by the same letter are not significantly different ($p > 0.05$).

Interaction effect	Contrast	Number of suckers	
		331 DAP	392 DAP
Cultivar x Moisture	Q138 x irrigated	11.4b	41.5
	Q152 x irrigated	7.9a	48.9
	Q138 x rain-fed	12.5b	25.0
	Q152 x rain-fed	14.3b	26.9
			ns
Cultivar x Space (m)	Q138 x 0.5	14.2b	35.8bcd
	Q138 x 1.0	11.2b	34.9bc
	Q138 x 1.5	10.4b	29.0a
	Q152 x 0.5	8.3a	34.1ab
	Q152 x 1.0	10.5b	39.4cde
	Q152 x 1.5	14.4b	40.5e
Cultivar x Moisture x Nitrogen (kg N/ha)	Q138 x irrigated x 0	6.8abc	28.0
	Q152 x irrigated x 0	6.7ab	39.7
	Q138 x rain-fed x 0	10.1bd	18.1
	Q152 x rain-fed x 0	11.2bd	21.3
	Q138 x irrigated x 140	13.5def	41.3
	Q152 x irrigated x 140	4.7a	43.7
	Q138 x rain-fed x 140	10.1bd	23.2
	Q152 x rain-fed x 140	14.9def	28.6
	Q138 x irrigated x 140 + 70	14.0def	55.3
	Q152 x irrigated x 140 + 70	12.3cde	63.5
	Q138 x rain-fed x 140 + 70	17.4f	33.7
	Q152 x rain-fed x 140 + 70	16.9ef	31.0
			ns

ns - F test not significant ($p > 0.05$)

The cultivar x moisture x nitrogen interaction was significant at 331 DAP. It was mainly due to cultivar Q138 producing significantly greater number of suckers than cultivar Q152 in the 140 kg N/ha irrigated environment, whereas cultivar Q152 produced a greater number of suckers than cultivar Q138 in the 140 kg N/ha rain-fed environment. This interaction was not significantly different at the final count.

It was expected that increasing the space between stools would result in an increased number of suckers due to an increase in the amount of light beneath the canopy of the crop.

Table 8.4 Suckers per mature stalk in the plant crop 229, 295, 331 and 392 DAP. Means followed by the same letter are not significantly different ($p > 0.05$).

Effects	Suckers per mature stalk			
	229 DAP	295 DAP	331 DAP	392 DAP
Cultivar				
Q138	0.000	0.027	0.165^{a}	0.483
Q152	0.001	0.028	0.149^{b}	0.506
	ns	ns		ns
Stool spacing (m)				
0	0.000	0.023^{a}	0.143^{a}	0.454^{a}
1.0	0.001	0.019^{a}	0.145^{a}	0.506^{b}
1.5	0.001	0.040^{b}	0.184^{b}	0.522^{b}
	ns			
Cultivar x Stool spacing (m)				
Q138 x 0.5	0.001	0.028^{ab}	0.182^{bc}	0.486^{b}
Q138 x 1.0	0.000	0.020^{ab}	0.155^{bc}	0.501^{b}
Q138 x 1.5	0.000	0.032^{bc}	0.159^{bc}	0.462^{ab}
Q152 x 0.5	0.000	0.017^{a}	0.102^{a}	0.422^{a}
Q152 x 1.0	0.002	0.019^{ab}	0.134^{b}	0.512^{b}
Q152 x 1.5	0.002	0.047^{c}	0.209^{c}	0.582^{c}
	ns			

ns - F test not significant ($p > 0.05$)

However, while cultivar Q152 showed an increase in sucker number in the 1.0 m and 1.5 m stool spacings, cultivar Q138 actually had significantly lower sucker number in the 1.5 m spacing than in the 0.5 m and 1.0 m spacings at the final count. This observation, prompted a calculation where suckers were expressed on a per mature stalk basis. This was done as it was expected that there would be more mature stalks in the 0.5 m spacing than in the 1.5 m spacing. Greater mature stalk number could mean that there were more buds with the potential to develop in to suckers.

The increased number of suckers found for cultivar Q152 at the final count appeared to be due to a higher number of mature stalks. Both cultivars produced a similar number of suckers per mature stalk at the final count. Expressing the sucker number on a per mature stalk basis resulted in significant effects due to stool spacing being found. The main difference was a higher number of suckers per mature stalk in the 1.5 m spacing than in the 0.5 m spacing. This effect was mainly expressed in cultivar Q152, as cultivar Q138 had a similar number of suckers per mature stalk at all three stool densities. At the final two sucker counts, cultivar Q152 had a significant linear increase in suckers per mature stalk with the increase in stool spacing.

8.3.1.2 Soil nitrogen

A significant increase in soil nitrate-N was found following the application of 70 kg N/ha 300 DAP (Table 8.5). The significant increase in soil nitrate-N was maintained to the final sampling 384 DAP. No significant difference in soil nitrate-N was found between the 0 and 140 kg N/ha treatments, applied in November 1999, at any of the sample dates. The top 25

cm of soil contained more soil nitrate-N than 25.1 – 50 cm below ground level. The additional N application in May resulted in a significant nitrogen x depth interaction, with a significantly greater increase in soil nitrate-N being found in the top 25 cm of soil compared to the 25.1 – 50 cm below ground level section.

Table 8.5 Soil nitrate-N (mg g^{-1} dry weight) 231, 286, 307, 342 and 384 DAP following the application of nitrogen at three rates. Means followed by the same letter are not significantly different ($p > 0.05$).

Effect	Soil nitrate-N (mg g^{-1} dry weight)				
	231 DAP	286 DAP	307 DAP	342 DAP	384 DAP
Nitrogen (kg N/ha)					
0	4.1	1.3	15.4^a	6.0^a	0.4^a
140	4.9	0.7	14.1^a	6.8^a	0.3^a
140 + 70			26.3^b	18.3^b	10.7^b
	ns	ns			
Stool space (m)					
0.5	5.1	0.4	18.1	9.6	3.2
1.0	4.3	1.6	19.0	10.0	5.0
1.5	4.0	0.9	18.6	11.6	3.3
	ns	ns	ns	ns	ns
Depth (cm)					
0 – 25	5.2	0.8	20.1^b	13.0^b	6.6^b
25.1 - 50	3.8	1.2	17.0^a	7.8^a	1.0^a
	ns	ns			
Nitrogen x Depth					
0 x 0 - 25	4.4	0.7	15.3^a	6.5^a	0.4^a
140 x 0 - 25	6.1	0.8	14.7^a	6.5^a	0.1^a
140 + 70 x 0 - 25			30.3^b	25.9^b	19.3^b
0 x 25.1 - 50	3.8	1.9	15.5^a	5.5^a	0.5^a
140 x 25.1 - 50	3.8	0.5	13.4^a	7.2^a	0.4^a
140 + 70 x 25.1 - 50			22.2^c	10.8^c	2.1^a
	ns	ns			

ns - F test not significant ($p > 0.05$)

Soil nitrate-N concentration between sample dates should not be compared. The apparent changes in the base soil nitrate-N concentration between sample dates may be due to loss of

nitrogen during storage as samples from each sampling date were stored for different periods of time prior to analysis. Storage time was consistent within each sample date as all soils from each individual sampling were analysed over a short period of time. This also applies for the ammonium-N analysis and the soil nitrogen analyses in the ratoon crop.

Table 8.6 Soil ammonium-N (mg g^{-1} dry weight) 231, 286, 307, 342 and 384 DAP following the application of nitrogen at three rates. Means followed by the same letter are not significantly different ($p > 0.05$).

Effect	Soil ammonium-N (mg g^{-1} dry weight)				
	231 DAP	286 DAP	307 DAP	342 DAP	384 DAP
Nitrogen (kg N/ha)					
0	6.7	10.0	5.2a	4.4a	5.9
140	5.9	9.6	6.1a	3.7a	6.1
140 + 70			12.2b	5.7b	6.1
	ns	ns			ns
Stool space (m)					
0.5	5.7a	10.4	7.3	5.1	6.2
1.0	7.8b	9.6	8.1	4.4	6.0
1.5	5.4a	9.4	8.0	4.3	5.9
		ns	ns	ns	ns
Depth (cm)					
0 – 25	5.8	10.1	8.8a	4.4	6.3
25.1 - 50	6.8	9.6	6.9b	4.8	5.8
	ns	ns		ns	ns
Nitrogen x Depth					
0 x 0 - 25	6.2	10.8	5.3a	4.3	6.4
140 x 0 - 25	5.4	9.3	6.0a	3.6	6.5
140 + 70 x 0 - 25			15.1b	5.3	6.0
0 x 25.1 - 50	7.2	9.1	5.1a	4.5	5.4
140 x 25.1 - 50	6.5	10.0	6.1	3.8	5.7
140 + 70 x 25.1 - 50			9.4c	6.1	6.2
	ns	ns		ns	ns

ns - F test not significant ($p > 0.05$)

A significant increase in soil ammonium-N was also found following the addition of 70 kg N/ha in May (Table 8.6). No significant difference in soil ammonium-N was found between

the 0 and 140 kg N/ha treatments, applied in November 1999, at any of the sample dates. The soil ammonium-N samples showed some differences from the soil nitrate-N samples. The significant increase in soil ammonium-N following the application of 70 kg N/ha in May was not found at the final sampling, the depth at which the samples were taken was only significantly different at the sample immediately after the additional nitrogen application, and the nitrogen x depth interaction was only present at the sampling immediately after the additional N application.

8.3.1.3 Light measurements

Measurements of the red (660 - 680 nm)/far-red (720 – 740 nm) ratio of light were taken 244 and 302 DAP (Table 8.7). Significant differences due to stool spacing were found in the red/far-red ratio of light 244 DAP. The overall effect of increased spacing resulted in an increase in the red/far-red ratio of light beneath the canopy. However, no significant differences were found 302 DAP.

Although no significant difference was found 302 DAP, a difference may still have been present in the period between 244 DAP and 302 DAP. A significant cultivar x moisture interaction was also found 244 DAP. Cultivar Q152 had a significantly higher red/far-red ratio than cultivar Q138 in the rain-fed environment but not in the irrigated environment. No other significant differences were found.

Table 8.7 Red/far-red ratio of light beneath the canopy of sugarcane grown at three stool densities, 244 and 302 DAP. Means followed by the same letter are not significantly different ($p > 0.05$).

Effect	Red (660 –680 nm)/Far-red (720 – 740 nm) ratio of light	
	244 DAP	302 DAP
Stool space (m)		
0.5	0.37[a]	0.51
1.0	0.47[a]	0.54
1.5	0.56[b]	0.62
		ns
Cultivar		
Q138	0.46	0.52
Q152	0.47	0.60
	ns	ns
Moisture		
Irrigated	0.47	0.51
Rain-fed	0.45	0.60
	ns	ns
Cultivar x Moisture		
Q138 x irrigated	0.51[b]	0.49
Q152 x irrigated	0.44[ab]	0.52
Q138 x rain-fed	0.41[a]	0.54
Q152 x rain-fed	0.51[b]	0.67
		ns

ns - F test not significant ($p > 0.05$)

Measurements of PAR were taken 302 DAP (Table 8.8). This measurement gives an indication of light quantity, whereas the red/far-red ratio gives an indication of light quality. There was a significant increase in PAR due to the height above ground at which the measurement was taken, stool spacing and the moisture environment. There were also significant moisture x height and space x height interactions. There was no significant difference between the two cultivars.

Table 8.8 Photosynthetic active radiation (PAR) measured beneath the canopy of a sugarcane crop grown at three stool spacings 302 DAP. Means followed by the same letter are not significantly different ($p > 0.05$).

Effect	PAR (% of sunlight)
Height above ground (cm)	
10	4.3^{a}
100	10.0^{b}
Stool spacing (m)	
0.5	4.3^{a}
1.0	6.5^{b}
1.5	10.7^{c}
Moisture	
Irrigated	6.0^{a}
Rain-fed	8.3^{b}
Moisture x Height (cm)	
Irrigated x 10	2.7^{a}
Irrigated x 100	9.3^{c}
Rain-fed x 10	5.9^{b}
Rain-fed x 100	10.8^{c}
Stool space (m) x Height (cm)	
0.5 x 10	1.3^{a}
1.5 x 10	3.8^{b}
1.5 x 10	7.7^{c}
0.5 x 100	7.2^{c}
1.0 x 100	9.2^{cd}
1.5 x 100	13.7^{d}

8.3.2 Ratoon Crop

8.3.2.1 Sucker numbers

Growth of the crop following ratooning 5th – 8th September 2000 was noted to be different to that of the plant crop.

Table 8.9 Sucker numbers in the ratoon crop 181, 245, 287 and 384 DAR. Late nitrogen application was conducted 241 DAR additional irrigation commenced 252 DAR. Means followed by the same letter are not significantly different ($p > 0.05$).

Main effects	Sucker number			
	181 DAR	245 DAR	287 DAR	384 DAR
Cultivar				
Q138	0.2^a	7.4^a	21.4^a	37.0^a
Q152	1.4^b	17.0^b	40.8^b	66.6^b
Nitrogen (kg/ha)				
0	0.8	9.2^a	18.9^a	40.0^a
210	0.8	11.7^b	31.8^b	52.6^b
140 + 70	0.8	15.6^c	42.5^c	62.7^c
	ns			
Moisture				
Irrigated	0.0^a	9.9^a	33.4^b	69.0^b
Rain-fed	1.5^b	14.5^b	28.7^a	34.3^a
Stool spacing (m)				
0	0.7	12.1	27.9^a	52.6
1.0	0.7	12.1	33.3^b	53.3
1.5	1.0	12.4	31.9^{ab}	49.4
	ns	ns		ns

ns - F test not significant ($p > 0.05$)

Sucker-like shoots were noticed in the crop early in 2001, particularly in the rain-fed environment. The morphology of these shoots appeared to change from being sucker-like to

normal stalk-like as the shoots grew. In the analysis presented below, these shoots were included as sucker stalks, although why their morphology should change is not known.

The mean number of suckers and significant differences for the main effects are shown in Table 8.9. Cultivar Q152 had significantly greater sucker numbers than cultivar Q138 at all four counts. 245 DAR the 210 kg N/ha and 140 + 70 kg N/ha treatments had significantly greater sucker number than the 0 kg N/ha treatment. The 140 + 70 kg N/ha treatment had significantly greater sucker number than the 210 kg N/ha treatment. Similar results were found for the 287 DAR and 384 DAR sucker counts. Initially the rain-fed environment had significantly greater suckers numbers than the irrigated environment. However, by 287 DAR the irrigated environment contained significantly greater sucker numbers than the rain-fed environment, and this was also the case at the final count. No significant differences were found due to stool spacing, except at the 287 DAR count, where the 1.0 m stool space had significantly greater sucker numbers than the 0.5 m stool spacing.

A significant cultivar x moisture interaction was found at the first three sucker counts (Table 8.10). This was mainly due to cultivar Q152 producing significantly more suckers than cultivar Q138 in the rain-fed environment. While cultivar Q152 had greater sucker number than Q138 in the irrigated environment, the difference is not as large as in the rain-fed environment. A significant moisture x nitrogen interaction was found at the final three counts. In the irrigated environment, the 210 kg N/ha treatment had a significantly greater number of suckers than the 0 kg N/ha treatment and a significantly lower number of suckers than the 140 + 70 kg N/ha treatment.

Table 8.10 Significant interaction effects on sucker number in the ratoon crop 181, 245, 287 and 384 days after ratooning. Means followed by the same letter are not significantly different ($p > 0.05$).

Interaction effect	Sucker number per plot			
	181 DAR	245 DAR	287 DAR	384 DAR
Cultivar x moisture				
Q138 x irrigated	0.0^{a}	7.3^{a}	25.4^{b}	51.3
Q152 x irrigated	0.1^{a}	12.6^{b}	41.4^{c}	86.7
Q138 x rain-fed	0.4^{b}	7.5^{a}	17.5^{a}	22.7
Q152 x rain-fed	2.7^{c}	21.6^{c}	40.2^{c}	46.1
				ns
Moisture x nitrogen (kg N/ha)				
Irrigated x 0	0.0	4.2^{a}	14.4^{a}	48.8^{a}
Irrigated x 210	0.0	11.1^{b}	36.6^{b}	72.7^{b}
Irrigated x 140 + 70	0.1	14.5^{cd}	49.3^{c}	85.6^{c}
Rain-fed x 0	1.6	14.1^{bcd}	23.5^{d}	31.1^{d}
Rain-fed x 210	1.6	12.4^{bc}	26.9^{d}	31.7^{d}
Rain-fed x 140 + 70	1.5	16.7^{d}	35.7^{b}	39.9^{e}
	ns			
Cultivar x nitrogen (kg N/ha)				
Q138 x 0	0.3	4.3	10.8	23.4^{a}
Q138 x 210	0.2	7.2	21.6	41.3^{b}
Q138 x 140 + 70	0.1	10.7	31.9	46.3^{c}
Q152 x 0	1.3	14.0	27.1	56.5^{d}
Q152 x 210	1.3	16.5	42.4	64.2^{d}
Q152 x 140 + 70	1.5	20.5	53.1	79.1^{e}
	ns	ns	ns	
Space (m) x nitrogen (kg N/ha)				
0.5 x 0	0.8	9.5	18.1	42.0^{ab}
0.5 x 210	0.4	11.1	29.0	57.1^{d}
0.5 x 140 + 70	1.0	15.5	36.8	59.0^{d}
1.0 x 0	0.3	8.8	19.7	38.6^{a}
1.0 x 210	1.0	11.0	32.0	49.0^{bc}
1.0 x 140 + 70	0.7	16.7	48.4	72.3^{e}
1.5 x 0	1.3	9.3	19.1	39.4^{a}
1.5 x 210	1.0	13.1	34.4	51.9^{cd}
1.5 x 140 + 70	0.8	14.7	42.4	57.0^{cd}
	ns	ns	ns	

ns - F test not significant ($p > 0.05$)

In the rain-fed environment the 210 kg N/ha treatment had a similar number of suckers as the 0 kg N/ha treatment. A significant cultivar x nitrogen interaction was found at the final

stalk count. Cultivar Q152 had similar sucker numbers for the 0 and 210 kg N/ha treatments, whereas cultivar Q138 had significantly different sucker numbers for all three nitrogen treatments. A significant spacing x nitrogen interaction was found at the final count. In the 0.5 and 1.5 m stool spacings, the 210 and 140 + 70 kg N/ha treatments had similar sucker numbers. However, in the 1.0 m stool spacing, all three nitrogen application rates had significantly different sucker numbers. The plots with a 1.0 m stool space which received the 140 + 70 kg N/ha nitrogen application had significantly higher sucker number than all other plots.

The analysis was repeated using sucker number per main stalk as the dependent variable. This was done due to the lack of effect of stool spacing. However, the results were similar to those when number of suckers per plot was used as the dependent variable. Stool spacing only had a significant effect on sucker numbers per main stalk at the third sucker count (287 DAR).

8.3.2.2 Soil nitrogen

Analysis of soil ammonium-N and nitrate-N from samples taken 244, 297 and 368 DAR showed no effect due to nitrogen rate, stool spacing or depth (Table 8.11). The samples taken 297 and 368 DAR were after the additional 70 kg N/ha applied in May. A significant difference due to nitrogen treatments was expected.

Table 8.11 Soil nitrate-N and ammonium-N 244, 297 and 368 days after ratooning. Means followed by the same letter are not significantly different ($p > 0.05$).

Effect	Soil nitrate-N (mg g^{-1} dry weight)			Soil ammonium-N (mg g^{-1} dry weight)		
	244 DAR	297 DAR	368 DAR	244 DAR	297 DAR	368 DAR
Nitrogen (kg N/ha)						
0	12.04	5.81	9.52	12.84	3.14	6.13
210	13.95	6.73	9.07	12.72	2.84	5.56
140 + 70	15.07	8.04	10.03	11.02	3.11	6.82
	ns	ns	ns	ns	ns	ns
Stool space (m)						
0.5	13.85	6.44	8.86	13.41	3.19	6.62
1.0	13.30	7.51	8.43	11.98	3.22	5.91
1.5	13.92	6.63	11.39	11.20	2.68	5.97
	ns	ns	ns	ns	ns	ns
Depth (cm)						
0 – 25	13.69	7.08	10.14	12.19	3.24	7.08
25.1 - 50		6.64	8.93		2.82	5.25
		ns	ns		ns	ns

ns – F test not significant ($p > 0.05$)

8.3.2.3 Light measurements

The red/far-red ratio of light was measured beneath the canopy on four occasions (Table 8.12). There was no effect of stool spacing or cultivar on the red/far-red ratio of light, but the rain-fed environment had a significantly higher red/far-red ratio of light than the irrigated environment on all four sample dates.

Table 8.12 Red/far-red ratio of light beneath the crop canopy taken 195, 243, 298 and 368 days after ratooning. Means followed by the same letter are not significantly different ($p > 0.05$).

Effect	Red (660 –680 nm)/Far-red (720 – 740 nm) ratio of light			
	195 DAR	243 DAR	298 DAR	368 DAR
Stool space (m)				
0.5	0.44	0.43	0.57	0.69
1.0	0.48	0.44	0.55	0.78
1.5	0.48	0.44	0.53	0.79
	ns	ns	ns	ns
Cultivar				
Q138	0.50	0.46	0.53	0.78
Q152	0.43	0.41	0.57	0.73
	ns	ns	ns	ns
Moisture				
Irrigated	0.35[a]	0.33[a]	0.42[a]	0.70[a]
Rain-fed	0.58[b]	0.54[b]	0.69[b]	0.81[b]

ns - F test not significant ($p > 0.05$)

Measurements of PAR were taken on three occasions (Table 8.13). There was a significant effect of stool space, moisture environment and the height above ground at which the measurement was taken on the percentage of available PAR beneath the crop canopy. A significant difference was also found between cultivars 243 DAR. There was a significant cultivar x moisture interaction 243 DAR. Cultivar Q152 had a greater percentage of available PAR in the rain-fed environment than in the irrigated environment. There was no difference between environments for cultivar Q138. A significant cultivar x space interaction was found 368 DAR. This was due to cultivar Q138 having the greatest amount of available PAR in the 1.0 m spacing and similar amounts in the 0.5 and 1.5 m spacings, whereas cultivar Q152 had a significantly greater amount of available PAR in the 1.0 and 1.5 m spacings than in the 0.5 m spacing.

Table 8.13 Photosynthetic active radiation (% of sunlight) beneath the canopy of a sugarcane crop grown at three stool spacings 195, 243 and 368 days after ratooning. Means followed by the same letter are not significantly different ($p > 0.05$).

Effect	PAR (% of sunlight)		
	195 DAR	243 DAR	368 DAR
Height above ground (cm)			
10	7.8^{a}	3.5^{a}	11.4^{a}
100	10.3^{b}	6.0^{b}	21.7^{b}
Stool space (m)			
0.5	7.1^{a}	3.8^{a}	13.2^{a}
1.0	8.6^{b}	3.9^{a}	18.4^{b}
1.5	11.6^{c}	6.6^{b}	17.8^{b}
Moisture			
Irrigated	5.4^{a}	3.1^{a}	12.6^{a}
Rain-fed	12.7^{b}	6.5^{b}	20.6^{b}
Cultivar			
Q138	8.9	2.8^{a}	17.3
Q152	9.2	6.8^{b}	15.8
	ns		ns
Cultivar x moisture			
Q138 x irrigated	4.8	2.6^{a}	15.0
Q138 x rain-fed	13.1	3.0^{a}	19.6
Q152 x irrigated	6.1	3.6^{a}	10.2
Q152 x rain-fed	12.3	10.0^{b}	21.7
	ns		ns
Cultivar x space (m)			
Q138 x 0.5	5.9	1.9	12.0^{c}
Q138 x 1.0	9.1	3.0	24.9^{a}
Q138 x 1.5	11.8	3.4	15.0^{bc}
Q152 x 0.5	8.2	5.6	14.6^{c}
Q152 x 1.0	8.0	4.9	12.0^{b}
Q152 x 1.5	11.4	9.8	20.6^{ab}
	ns	ns	

ns - F test not significant ($p > 0.05$)

There were significantly more suckers produced by the ratoon crop than by the plant crop. This was particularly evident for cultivar Q152 and was expressed in both moisture environments (Table 8.14).

Table 8.14 Differences in sucker numbers (at final count) between the plant and ratoon crops. Means followed by the same letter are not significantly different ($p > 0.05$).

Effect	Number of suckers
Crop	
Plant	35.6^{a}
Ratoon	51.7^{b}
Crop x cultivar	
Plant x Q138	33.3^{a}
Plant x Q152	38.1^{b}
Ratoon x Q138	37.0^{b}
Ratoon x Q152	66.6^{c}
Crop x moisture	
Plant x irrigated	45.2^{c}
Plant x rain-fed	25.9^{a}
Ratoon x irrigated	69.0^{d}
Ratoon x rain-fed	34.3^{b}

8.4 Discussion

In both the plant and ratoon crops the increased rate of application of nitrogen resulted in an increase in sucker numbers. A significantly greater number of suckers was found in the 140 + 70 kg N/ha (plant and ratoon crops) treatments compared to the 140 kg N/ha (plant crop) and 210 kg N/ha (ratoon crop) treatments. This result is similar to those reported in Chapter 6 of this thesis and to early work done by Borden (1948). It shows that the availability of nitrogen in the soil late in the crop's growth cycle causes increased initiation of suckers. Significantly greater number of suckers were also found in the 140 kg N/ha (plant crop) and

210 kg N/ha (ratoon crop) treatments compared to the 0 kg N/ha treatment. This result showed that the amount of nitrogen applied at the early stages of the plants development can have an effect on sucker numbers later in the year. However, the result differs to those of Hurney and Berding (2000) and to those reported in Chapter 7 of this thesis. Hurney and Berding (2000) found no effect of nitrogen application rate on sucker number when they applied nitrogen early in the crop growth cycle, as is normal commercial practice. While a difference was found in the number of suckers between the 0 kg N/ha and 140 or 210 kg N/ha treatments, no significant difference was found in the amount of available nitrogen in the soil between these two latter treatments. This suggests that the nitrogen status of the plants may have differed between these two treatments. A high early application of N may result in some form of luxury uptake, which allows the plant to produce more suckers later in the year when other conditions are favourable.

The addition of 140 + 70 kg N/ha in May in both the plant and ratoon crops resulted in significant increases in sucker number. This treatment had significantly greater sucker numbers than a treatment of 210 kg N/ha applied following the ratooning of the crop. While total nitrogen added to the system was of equal amounts, having nitrogen available in the soil (even though it was not detected in the ratoon crop) at the time suckers were initiated, resulted in greater sucker number. This means that preventing nitrogen from becoming available late in the crop's growth could be of greater importance in reducing the number of suckers than the amount of nitrogen applied during the early stages of growth of the crop. The two cultivars differed in their response to this additional nitrogen, cultivar Q138 showed a significant difference between the 210 kg N/ha and the 140 + 70 kg N/ha treatments, but there was no significant difference for cultivar Q152.

At the final harvest of both the plant and ratoon crops, the irrigated environment had significantly greater sucker numbers than the rain-fed environment. This result confirms the view of Berding and Hurney (2000) that wet conditions late in the crop's growth could result in increased suckering. Interestingly, the rain-fed environment contained a greater number of suckers than the irrigated environment at the early sucker counts, for both the plant and ratoon crops. This may have been due to inherent differences between the two sites despite their close proximity. In the ratoon crop, this difference may have been due to better establishment of the irrigated crop, a possible carry over effect of the irrigation in the plant crop.

A moisture x nitrogen interaction in the ratoon crop showed that the difference between the 0 kg N/ha and 140 + 70 kg N/ha treatments was much greater when the crop received additional irrigation. This interaction may be due to a loss of nitrogen in the rain-fed environment, better uptake of nitrogen by the plant in the irrigated environment or a better ability to produce suckers under high moisture conditions when nitrogen status is high. This interaction was not present in the plant crop. This may be due to differences between years. The crop grown in 1999/2000 (plant crop) received more precipitation than the 2000/2001 crop (ratoon). This may mean that the difference between the irrigated and rain-fed environments was greater in the ratoon crop, which allowed the interaction between moisture and nitrogen to be expressed.

Stool spacing had little effect on sucker number. In the plant crop, cultivar Q152 had significantly more suckers per mature stalk with an increase in stool spacing, but cultivar

Q138 did not. Light measurements showed that the percentage of PAR and the red/far-red ratio of light beneath the canopy were increased, at least initially, with the increased stool space. No difference in light beneath the canopy was found between the two cultivars. In the ratoon crop, the difference in sucker number due to stool spacing was only significant on one occasion. In this case the 1.0 m stool spacing had higher sucker number than the 0.5 m stool spacing. Light measurements showed that there was no difference in red/far-red ratio of light beneath the canopy between the three stool spacings, but there was a significant difference in the percentage of available PAR beneath the canopy. This was mainly due to a greater percentage of PAR available beneath the canopy in the 1.5 m stool space than in the 0.5 m stool spacing.

In the ratoon crop, there were significant differences in the red/far-red ratio of light and the percentage of PAR available beneath the canopy for the two moisture environments. More light, with a higher red/far-red ratio, was available beneath the canopy in the rain-fed environment than in the irrigated environment. This might explain why the rain-fed environment produced more suckers early in the year, prior to the irrigation of the irrigated environment. The low light environment of the irrigated crop was due to better establishment and growth of the crop resulting in increased light interception. This was due to a carry-over effect of irrigating the plant crop. Prior to the commencement of the irrigation (sucker counts 181 and 245 DAR), there was a significant cultivar x moisture interaction. This may be due to cultivar Q152 responding more to the increased light characteristics in the rain-fed environment than cultivar Q138. This result is consistent with the finding of increased suckering with stool spacing for cultivar Q152 in the plant crop.

The measurement of the red/far-red ratio of light beneath the canopy was unlikely to have provided as good an estimate of the actual light conditions beneath the canopy as the PAR measurements. This is because the spectroradiometer only had a single small sensor. The result obtained from a single scan would be highly dependent on the position in the highly variable environment beneath the crop canopy that the sensor was placed. The ACUPAR Linear PAR Ceptometer had a probe that contained 100 sensors. Each measurement was an average of that recorded by each of the sensors. It was not possible to increase the number of replications per plot for the red/far-red ratio of light measurements due to the large number of plots that needed to be measured.

The number of suckers produced by the ratoon crop was much greater than that produced by the plant crop. This was particularly evident for cultivar Q152. It is difficult to determine whether this is due to different environmental conditions between the years or whether ratoon crops are more prone to suckering than plant crops. Interestingly, the ratoon rain-fed crop produced more suckers than the plant rain-fed crop. This was despite the plant crop experiencing wetter conditions. This shows that there is an interaction of a number of factors which determines the number of suckers. This difference may have been due to more light beneath the canopy compared to the plant crop, crop age (plant or ratoon), nitrogen availability, or other factors not identified in the experiments.

A number of factors have been shown to effect suckering: nitrogen availability in the soil, late in the crop cycle, has once again been shown to cause an increase in the number of suckers; the initial rate of nitrogen application, applied to a young immature crop, has been shown to effect the number of suckers for the first time; a wet environment late in the

crop's growth has been shown to increase the number of suckers; initial differences in the number of suckers between environments could be due to the differences in the amount and quality of light beneath the canopy; and a number of interactions between factors have been found. In Chapter 9 the implications of these results are discussed with a view to understanding the process of sucker initiation and crop improvement.

Part D: Discussion

Chapter 9. Conclusions and implications for plant improvement and future work

9.1 The biology of sugarcane suckers

The morphology of sucker stalks was found to be different to that of normal sugarcane stalks in all cultivars studied. Sucker stalks had greater maximum breadth of leaf lamina, longer leaf sheaths, thicker internodes and produced each leaf at a greater height above ground than normal stalks. This altered morphology was not transmitted to the buds produced on the sucker stalk. The data provide evidence for, and extend, the descriptions of suckering in the literature (van Dillewijn 1952; Hes 1954; Barnes 1974).

The light environment in which a sucker grows, compared to a normal stalk, may be the cause of the differences in height at which each leaf was produced. The low light environment beneath a sugarcane canopy may cause an etiolation response in the stalk. Typically, etiolation results in maximized cell elongation in the shoot with little leaf development as the plant attempts to reach sufficient light conditions for photoautotrophic growth (von Arnim and Deng 1996). This may have follow-on effects on both leaf sheath length and internode length. Leaf sheath length has been shown to be increased by a low red/far-red ratio of light in *Lolium multiflorum* (Casal *et al.* 1987b). This could not be tested in sugarcane in Experiment 7.3 as the sample sizes were not sufficient for an adequate comparison to be made. It was shown in Experiment 7.3 that light had little effect on the

maximum breadth of the leaf lamina. It was the leaf lamina maximum breadth and the internode diameter that were consistently different between sucker and normal stalks. Therefore, it appears that at least for the leaf lamina maximum breadth, the difference between sucker and normal stalks is not due to the light environment in which they grow. This statement is also supported by observations in the field that suckers in lodged areas within the crop appear to have similar morphology as those under a closed canopy.

While the molecular data on gene regulation need to be interpreted with extreme caution, given the limitations discussed in the relevant chapter, there was some evidence that gene expression differed between suckers and normal tillers. This was not unexpected given the differences in morphology between the two stalk types. The identity and function of these genes has yet to be determined and requires further work. The comparison between suckers and normal stalks was also limited by the array that was used. For instance, the results could not show genes that were only expressed in sucker tissue as the array was made from sequences expressed in normal stalks.

Interestingly, removal of the mature stalk to which the sucker was attached had a significant effect on sucker morphology. Removal of the mature stalk resulted in suckers with thinner leaf maximum breadths, more similar to the normal stalks described in Chapter 3. This result was found for cultivars Q117, Q138 and Q152, but could not be repeated for Q138 in a second experiment, for unknown reasons.

Why the presence of a mature stalk should affect sucker morphology is not known, but it indicated the possible translocation of substances from the main stalk to the sucker. These

substances may include plant hormones, which are known to affect plant growth, and/or other biochemical compounds. Further investigation would be required to determine what these substances might be.

Evidence for the loss of sucrose from the main stalk to young suckers was obtained from a Q152 crop in Tully. Initially, stalks that had not produced a sucker had greater sucrose content than those that had. These data suggested that sucrose was lost from the mature stalk in the initiation of the sucker. This conclusion is plausible as support for young tillers from older more mature tillers has been reported in the literature (Sagar and Marshall 1966; Marshall 1967). Bull and Glasziou (1963) proposed that natural selection for increased sucrose content of cane may have occurred due to high sucrose canes being able to rapidly mobilize sucrose to support sucker growth.

The loss of sucrose from the mature stalk to support sucker growth has not been included when the negative effect of suckers on profitability has been assessed. To date, only the dilution effect at the mill has been included in this process. However, this loss may be of importance as the loss of sucrose from a mature stalk supporting the growth of a sucker could be as much as 12%. Even if the real value is half this amount it should still be of commercial concern.

A summary of what is now known about the biology of sugarcane suckers, and factors that may have an effect on their morphology is represented in Figure 9.1. Sugarcane suckers are shoots that appear late in the season when other stalks are more or less mature. They have distinctive morphology that differs from that of normal stalks of similar age. This

difference in morphology appeared to be largely associated with the presence of a mature stalk which could provide sucrose to aid sucker growth, the light conditions beneath the canopy within which young suckers grow and altered gene expression as a result of these and possibly other factors.

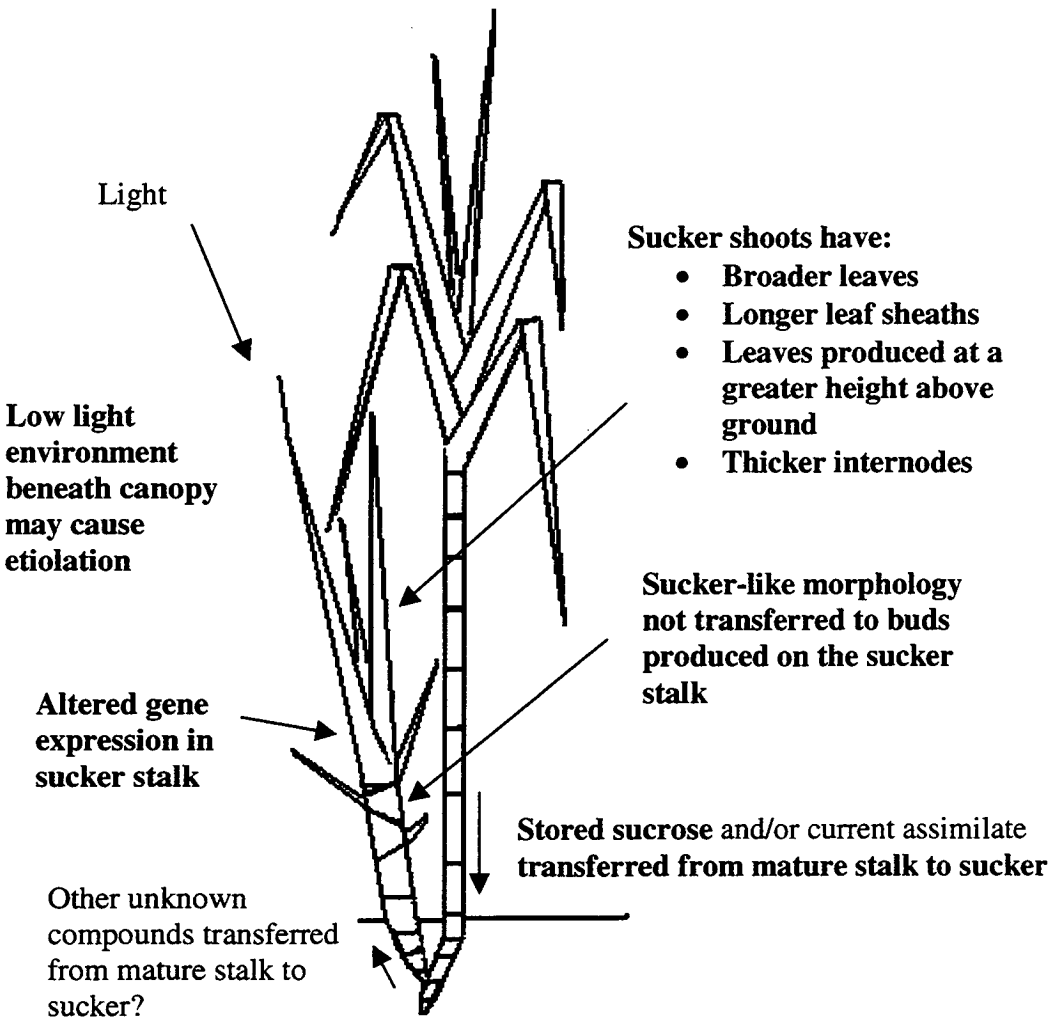

Figure 9.1 The morphology of sugarcane suckers and factors that may affect it. Text in bold indicates evidence that was generated in this thesis

The knowledge gained in this study will allow for better identification of sucker stalks in the field and a better definition and description of the trait. Suckers can now be identified by factors other than their late emergence with more certainty. This should aid research into suckering, and also means that further investigations can concentrate on factors other than the differences in stalk morphology, as this first step has now been well-described.

Future work on the molecular differences between suckers and normal stalks may result in development of (i) a marker(s) that can be used to identify clones likely to sucker or (ii) a strategy for controlling sucker expression by altering gene regulation. Knowing the differences in gene expression in sucker stalks compared to normal stalks may be of some use in plant improvement. Knowing the molecular changes that cause thicker stems and fast growth rates could potentially result in these attributes being introduced into normal stalks via genetic manipulation of the expression of these genes. This could result in an increase in productivity per unit area. Obviously this would be difficult to achieve if these differences in morphology are due to the presence of a mature stalk which is supplying current and/or stored assimilate.

The sugar industry should not be concerned that small suckers shoots will develop into large sucker stalks after the crop has been harvested and that buds produced on suckers will develop into sucker stalks after the crop has been harvested. This study has shown that buds on sucker stalks produce shoots similar to those on normal stalks and that if the mature stalk was removed (harvesting) an emerging sucker stalk would revert to being more similar to a normal stalk. Hughes and Muchow (2000) have also shown that sucker stalks

do accumulate sugar at a similar rate as normal stalks, and therefore if grown over a similar period of time should have similar sugar content at harvest.

9.2 Environmental factors affecting suckering

Several of the chapters have described experiments investigating the effect of environmental factors on suckering. Here the findings are synthesized and how the signals lead to suckering are discussed.

9.2.1 Nitrogen

Suckering was shown to be increased by the availability of nitrogen, late in the year, in three environments and three cultivars with differing suckering propensity. These results have some implications in terms of managing soil nitrogen. Fertilisers are most often applied much earlier in the season than the treatments used in the above experiments. However, there is the possibility that some of this early-applied nitrogen may become available to the plant later in the growing season. The plant may store the nitrogen due to 'luxury uptake', or alternatively, nitrogen may be held in the nitrogen cycle and become available to the plant at a later stage in its development. Evidence for early uptake stimulating later suckering was found (Chapter 8) where nitrogen applied at 140 kg N/ha at the start of the season resulted in significantly greater sucker number than 0 kg N/ha. No difference in the availability of nitrogen in the soil was found between the two treatments at the time that suckers were being initiated. This is in contrast to the results of Hurney and Berding (2000) and those found in Chapter 7. This may indicate that other environmental

conditions are required in order for early applied nitrogen to have an effect, and that these conditions were only present at the Mulgrave site.

While the practice of GCTB is thought to increase total soil nitrogen, it is not known whether there are any specific increases late in the season, possibly due to the breakdown of trash and other organic matter during the wet season. Work by Robertson and Thorburn (2000) suggested that release of nitrogen from trash blankets occurs at a uniform rate throughout the season. Furthermore, only very small amounts of nitrogen are released from trash into the top 5 cm of soil. On the other hand, recent findings at Tully indicate that with GCTB, there is a surge in crop nitrogen uptake after the end of the wet season (Klock unpubl., Cooperative Research Centre for Sustainable Sugar Production Annual Report for 2000/2001, p34). Trash blankets have also been shown to increase soil moisture (Chapman *et al.* 2001) which has now been shown to stimulate suckering.

Why in physiological terms high concentrations of available nitrogen might cause increased tillering and/or suckering is not fully understood. It is obvious that nitrogen is needed in order to produce amino acids required for plant growth. However, why increased nitrogen availability led to greater numbers of stalks being produced as opposed to a smaller number of larger stalks is not known. The answer may be that it is a complex system where various signals are received by the plant and the response to each individual stimulus is dependent on the relative proportions of the other stimuli. For example, a plant may produce more stalks in the presence of increased nitrogen if sufficient light was available, or it may result in luxury uptake of nitrogen if light was limiting.

There are various biochemical processes in the plant that allow it to detect high nitrogen concentrations and to respond by producing further tillers. Nitrate reductase activity has been shown to increase appreciably during the tillering phase compared to the pre-tillering phase in sugarcane (Solomon *et al.* 1988). Dwiveldi *et al.* (1984) reported concomitant peaks in glutamine synthatase, glutamate synthatase and nitrate reductase activity at the stage of shoot differentiation in sugarcane callus tissue.

Sugarcane suckers originate from buds that are under apical dominance, as they are attached to mature stalks that are still alive. Nitrogen nutrition has been shown to affect apical dominance in many studies. McIntyre (1972) found that root bud inhibition in *Euphorbia esula* was largely determined by the ability of root buds to compete with the dominant shoots for the limited nitrogen supply. It should be noted that root buds refer to buds that are located on the roots but develop into shoots. A similar result was found for *Cirsium arvensae* (McIntyre and Hunter 1975). McIntyre (1987) found that an interacting effect of nitrogen and humidity, on the water status of *Agropyron repens* buds, may play a role in the mechanism of apical dominance. Qureshi and McIntyre (1979) postulated that in *Agropyron repens*, stimulation of bud growth by high humidity when nitrogen is limiting may be due to the increased water potential of the bud accelerating protein synthesis, thereby enhancing the buds' capacity to compete for the limited nitrogen supply. In nitrogen deficient, low water stress environments, bud inhibition in *A. repens* was mainly determined by the nitrogen supply, whereas the relatively high concentrations of amino acids found in fully inhibited buds of field rhizomes suggested that water rather than nitrogen was more likely to be the limiting factor under field conditions (Nigam and

McIntyre 1977). This information suggested that nitrogen plays a role in the release of buds from apical dominance.

9.2.2 Light

The role of light in suckering is not clear. Preventing light from entering the outside row of a crop from the side, with shade cloth, did not result in fewer suckers for six crops grown in the wet tropics. Shading the bottom two metres of stalks of cultivar Q138 in a glasshouse experiment also did not reduce sucker numbers compared to an unshaded control. However, removing trash from five cultivars at Mulgrave increased the amount of light reaching the base of the stalks and sucker numbers. Likewise, planting sugarcane stools of cultivar Q152 at 1.5 m spacings resulted in significantly more suckers per main stalk than when stools were planted at 0.5 m. Finally, suckering was shown to be greater in an environment with increased PAR availability and red/far-red ratio of light beneath the crop canopy.

The edge of crop effect found in this study was not as pronounced as that found by Bonnett *et al.* (2001). The crop sampled by Bonnett *et al.* (2001) was from the Burdekin River district, which is not part of the wet tropics region. Typically, the Burdekin region has crops with dense canopies due to the good growing conditions. This district has high light, high temperatures and crops are fully irrigated. In the wet tropics region, light can often be limited due to the large number of overcast days, and rainfall can often be excessive. Potentially, this tends to result in poor crop canopies, which might explain why the outside row effect was reduced for crops in the wet tropics, if light is actually the cause of this edge

effect. The high rainfall and wind in the wet tropics also causes crop lodging and sprawling and this too increases light availability beneath the canopy. A combination of poor canopies and lodged/sprawled crops may contribute to high suckering in the wet tropics.

The variable nature of the light responses means that further experimentation is required or a new model of analysis is required. However, the size of the crop when it produces suckers makes experiments looking at the role of light difficult. The evidence does suggest that light may play a role in suckering and that a more appropriate experiment is needed in order to fully show its effect. The requirement for light in suckering may also be complicated by the interrelationship of the mature and sucker stalk. If a sucker stalk's growth is sufficiently supported by the mature stalk to which it is attached then adequate light conditions may be of less importance than other environmental factors in sucker initiation. This may explain why suckers, although few in number, are found beneath heavy crop canopies.

9.2.3 Moisture

The availability of moisture in the soil following the wet season was shown to increase sucker numbers. This means that crops are likely to produce more suckers in wet years, a conclusion which supports the view of Berding and Hurney (2000) that the increase in suckering over recent years was partly due to wet events late in the year. It may also provide evidence as to why suckering is more of a problem in the wet tropics region than in drier regions of cane production within Australia. Not only does the availability of moisture in the soil cause increased suckering but wet and windy conditions also have the additional

effect of causing sprawling and lodging which may result is increased light beneath the crop canopy.

How the moisture content of the soil acts physiologically to effect suckering is not known. Some of the possible physiological effects of moisture were discussed in the previous section looking at the physiological effect of nitrogen. Hsiao and McIntyre (1984) discussed the possibility of dormant buds of *Asclepias syriaca* not being able to compete against the main shoot for water due to the negative xylem water potential produced by transpiration from the mature plant. Under high soil moisture and humidity conditions the level of competition would be reduced. McIntyre (2001) wrote an interesting review of the control of plant development by limiting factors from a nutritional perspective. This paper discusses the role of nitrogen, water and other factors in the release of buds from inhibition. McIntyre (2001) proposed the need for further investigation on the metabolic and genetic effects of changes in the water status of plant cells as cell hydration had been shown to effect metabolic activity and gene expression in animal cells.

9.2.4 Temperature and other factors

The effect of temperature on suckering was not dealt with in this study, but it could potentially have a significant effect on sucker numbers. Some evidence for a possible effect of temperature was gained from the late nitrogen application experiment conducted in Tully on cultivar Q152 in 1999. Sucker numbers doubled in the final two weeks of this investigation. Temperature was also shown to increase significantly in this period. Temperature has been implicated in the control of suckering in experiments conducted by

L. McDonald (Cooperative Research Centre for Sustainable Sugar Production, pers. comm. 2001). These experiments were conducted by growing sugarcane crops on 12 month cycles, planted at different times of the year.

The possible role of other factors in the stimulation of suckering cannot be ruled out. Despite high levels of available nitrogen, moisture and low plant density, suckering in the environmental interaction experiment, particularly in the plant crop, was not as large as that reported by Crooke *et al.* (1999) and Berding and Hurney (2000). What these factors may be is not known.

Sucker numbers also increase rapidly late in the year when photoperiod is also increasing. Further investigation is needed in order to elucidate the role of such factors. It should be noted that photoperiod does not change markedly in the wet tropics region due to its proximity to the equator, but also that tropically adapted species are more sensitive to small changes in photoperiod.

9.2.5 Interaction of environmental stimuli

While it has been shown that suckering responds to the availability of nitrogen, light and moisture it also appears that these environmental stimuli interact with each other. Most of the crops used in the experiments had fairly open canopies, despite not having lodged. Whether or not the same response to nitrogen would have been found using a crop that had a heavy canopy, restricting the available light beneath canopy is not clear, and requires further research. While a significant spacing x nitrogen interaction was found (Chapter 8) it

is not clear whether or not this is a light x nitrogen interaction as light measurements showed that there was no detectable effect of spacing on the red/far-red ratio of light. However, there was a significant effect of spacing on the amount of PAR available beneath the canopy in the ratoon crop. A significant nitrogen x moisture interaction was also found in the ratoon crop of the environmental interaction experiment. This shows that multiple environmental factors are involved in the stimulation of suckering and they can operate in conjunction with each.

9.2.6 Perception of environmental stimuli

The exogenous environmental stimuli received by the plant must be translated into endogenous signals that result in the plant producing a sucker. Environmental factors have been shown to affect plant hormones. In sugarcane, the combination of light and hormones is thought to partly control tillering. van Dillewijn (1952) stated that under high light conditions apical dominance is reduced, stem elongation is slow while tiller initiation is high. Under low light, the reverse is true. Tucker (1976) suggested that in tomato far-red light causes increased auxin synthesis, which inhibited bud growth after it has induced the formation of abscissic acid (ABA). Leopold (1949) showed that production of diffusible auxin was affected by day-length in red-leaved *Coleus*. The photoperiodic conditions which increased auxin production in *Coleus* decreased tillering in barley. Chen *et al.* (1998) found tillering in wheat was promoted by the use of a combination of NH_4^+-N and NO_3 (30:70) than either of the forms of nitrogen alone. The mixed nitrogen source was found to increase the cytokinin/indoleacetic acid ratio and increase GA_{1+3} level in the shoot.

Shrivastava *et al.* (1992) postulated that in the right conditions, a tillering stimulus is produced in the leaves, which could be hormonal (ABA/GA_3 or auxin balance) and may be modified by temperature and light intensity. This is then translocated to the site of tiller initiation where nitrogen metabolism and processes related to P and K come into play and shoot differentiation takes place and tiller development begins. While this explanation shows how exogenous signals (environmental factors) may interact with endogenous signals to stimulate tillering, further evidence is needed to support this model.

The process of suckering in sugarcane is likely to differ from this model for tillering as suckers are produced under a canopy to some extent. The living leaves on a mature stalk would experience conditions vastly different to those of a sucker, at least until the sucker is large enough to reach the canopy. It therefore appears more logical that any effect of light would be mediated through detection of the signal beneath the canopy rather than in the leaves of the mature stalk. Casal *et al.* (1987a) provided some evidence for the perception of light at the base of stalks in *Lolium multiflorum*.

Suckers are initiated from below-ground buds, not from buds above ground. These buds usually have roots that have developed from the root primordia produced at each node, generally above ground nodes do not produce roots. This observation tends to support Shrivastava's model that nitrogen metabolism plays a role at the site of tiller initiation. It appears that with less access (no roots in the immediate area) to nutritional requirements (nitrogen and water) above ground buds are not able to respond to any signal that is being translocated down the stem.

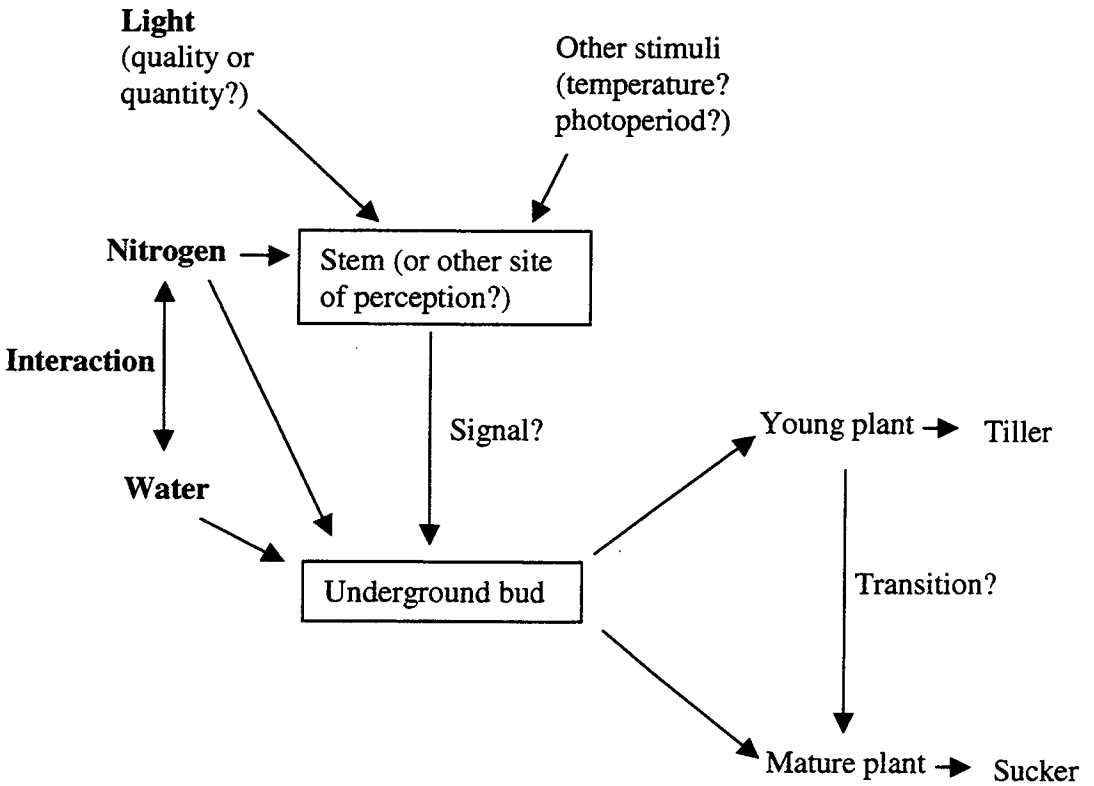

Figure 9.2 A model of the environmental stimuli for suckering in sugarcane. Text in bold indicates where evidence has been generated in this thesis.

Perhaps Shrivastava's model is deficient in that it does not allow for the possibility of just nutritional requirements causing shoot initiation without the presence of a light signal, but this may be something more appropriate to suckering than tillering given the possible support of sucker growth by the mature stalk. A model for suckering would also have to incorporate the interaction of environmental effects. In particular, suckering was stimulated more by nitrogen under high moisture conditions than under low moisture conditions. Whether this is a direct response of the plant to the presence of moisture or whether it is the moisture effecting nitrogen availability to the plant is not known. A model for suckering

would also have to include plant age as a factor. This is because only mature stalks produce suckers in the presence of nitrogen, light and moisture. Young sugarcane stalks produce tillers when exposed to high levels of nitrogen, light and moisture (Figure 9.2). In the period where tillers and suckers are not produced either the environmental stimuli are not present or the plant does not respond to them.

9.3 Implications for crop improvement

There are two ways in which this information can be used in attempts to produce crops with lower suckering, namely through agronomy and plant breeding. Each of these approaches are considered in turn below.

9.3.1 Agronomy and crop management

9.3.1.1 Adapting practices to minimise suckering

Excessive nitrogen rates are often applied to sugarcane crops in an attempt to guarantee a good return. An 'extra bag of fertiliser' is sometimes looked upon as a small cost compared to the much larger loss of having a nitrogen deficient crop in a good season. While the recent economic downturn in the sugar market and the environmental concerns of nitrogen leaching may have curbed this activity to some extent, it is likely that nitrogen rates used by some growers could still be reduced further. Evidence that excessive nitrogen use may stimulate suckering, and thus a loss in profitability, may give growers further cause to reduce nitrogen fertilisation rates closer to recommended rates.

Managing crop canopies may be one way in which the possible effect of light availability on suckering may be reduced. Planting crops at increased density could result in the reduced availability of light beneath the canopy. However, cultivars with low vigour, that remain erect under wet conditions, must be used (Bull and McLeod 2000), otherwise the higher plant density may result in many thin, long stalks that have a greater tendency to lodge. This could result in more light beneath the crop canopy. Increasing plant density may also require changes to farm machinery, and the cost of this change has resulted in minimal adoption of high density planting in many cane-growing districts.

Since the majority of crops are rain-fed in the wet tropics, it is difficult to manage the crop so that it does not experience wet conditions late in the year. However, the effect of soil moisture can potentially be reduced by reducing the interaction effects it may have with other factors. Therefore, prevention of light and nitrogen from becoming available to the crop, late in the year, when suckers normally appear, may also reduce the interaction effect of moisture availability with these factors. Therefore, the effect of moisture on suckering could potentially be reduced through the correct management of the crop to reduce the effect of the other environmental factors.

9.3.1.2 Better matching the crop to the production environment

Cultivar selection on a farm could be used in order to try reduce the occurrence of suckers. Cultivars that do not sucker profusely should be planted in areas that are known to have particularly fertile soils, or areas that are known to remain wet during autumn/winter.

9.3.2 Plant breeding and cultivar selection

Differences in the propensity of sugarcane clones and cultivars to sucker were shown in this thesis and have also been described by Berding and Hurney (2000). Therefore, breeding programs should be able to reduce the impact of suckering on the sugarcane industry in the wet tropics by selecting cultivars with low suckering propensity.

It is important that sucker stalk composition, in a selection trial, is incorporated into both the calculation of yield and sugar content. It has been suggested that previously yield was calculated with the sucker stalk content included, but sucrose was measured from just the main stalks (Berding and Hurney 2000). This method results in selection for high suckering cultivars, as they increase the yield of the crop, and their negative effect on sucrose content is not realised. It was stated that the penalty for sucker stalk composition has now been upgraded.

While direct selection against suckering continues, sugarcane breeding programs now have further information that can be used to weight the importance of other traits that may impact on suckering. As mentioned above, the adoption of high density planting will require cultivars that remain erect. Cultivars that are less likely to lodge and sprawl under wet conditions are needed. This could result in crops that do not suffer the loss in CCS associated with lodging as found by Singh *et al.* (1999, 2000), and may not sucker as profusely due to the lower availability of light beneath the crop canopy. This may also require breeding for increased stalk thickness, and better root structure which would

provide greater stability. Cultivars also need to ratoon and tiller profusely in order to establish a dense canopy and maintain high yield and also help fill in gaps associated with damage brought about by harvesting under wet conditions, another cause of poor canopies in the wet tropics. Clones with this proposed ideotype that also have high sugar content should rate highly in breeding programs. Managing the canopy in such a way would result in lower availability of light and a lower red/far-red ratio of light beneath the crop canopy.

Low suckering is one of several selection criteria of likely interest in a sugarcane breeding program. This means that there is still the potential for some high/moderate suckering clones to be released as commercial cultivars, as long as other selection criteria are met. These cultivars may have lower sucrose content when crushed at the mill, but this negative effect would need to be offset by high yield as a result of disease resistance or some other factor. Jackson *et al.* (2000) suggested a whole of industry approach when it comes to selection of traits. It was suggested that while millers may not benefit from reduced suckering the positive effect on grower profitability would result in a net gain for the industry.

9.3.2.1 Structuring an effective test environment

Breeding programs usually conduct trials in multiple environments in order to evaluate and select elite clones. However, there is the possibility that the genetic variation between clones for some traits is not expressed in some years under these conditions. For example, no difference in suckering propensity was found between cultivars Q120, Q186 and Q187 at the Tully site in the trash stripping experiment in 2000. However, differences in

suckering propensity existed, and were expressed at the Mulgrave site (Figure 9.3). Therefore, if conditions were similar to those in Tully in 2000, for many years, no selection decisions in terms of suckering propensity could be made between the three cultivars. In order to maximise the chance of genetic differences between clones being expressed, selection trials conducted in a managed environment could potentially be used.

In these trials, clones are provided with the environmental requirements for the suckering response. This provides the plant breeder the optimum conditions for evaluating suckering, clones that show low suckering propensity under these conditions, which sufficiently meet the other selection criteria, should score highly. The results show which cultivars will have low suckering propensity even in high suckering years and environments. Some recommendations for the requirements of such a trial can be made from the data in this investigation.

The results show that nitrogen applied to a mature crop in May at 70 kg N/ha caused increased suckering in all experiments. Therefore, one of the management practices that might be instigated is a late nitrogen application. While 70 kg N/ha was used in the experiments, greater rates could be used to ensure suckering was promoted. The timing of the application did not appear to be of major importance, but applying it too early may result in excessive loss of nitrogen due to leaching, and applying it too late may limit the amount of time for the crop to respond to its presence in the soil. Additional irrigation, following the wet season, would also be usefully applied to the crop in a managed environment selection trial, based on the effects of elevated soil moisture reported in Chapter 8 and the interaction effect of soil moisture and nitrogen.

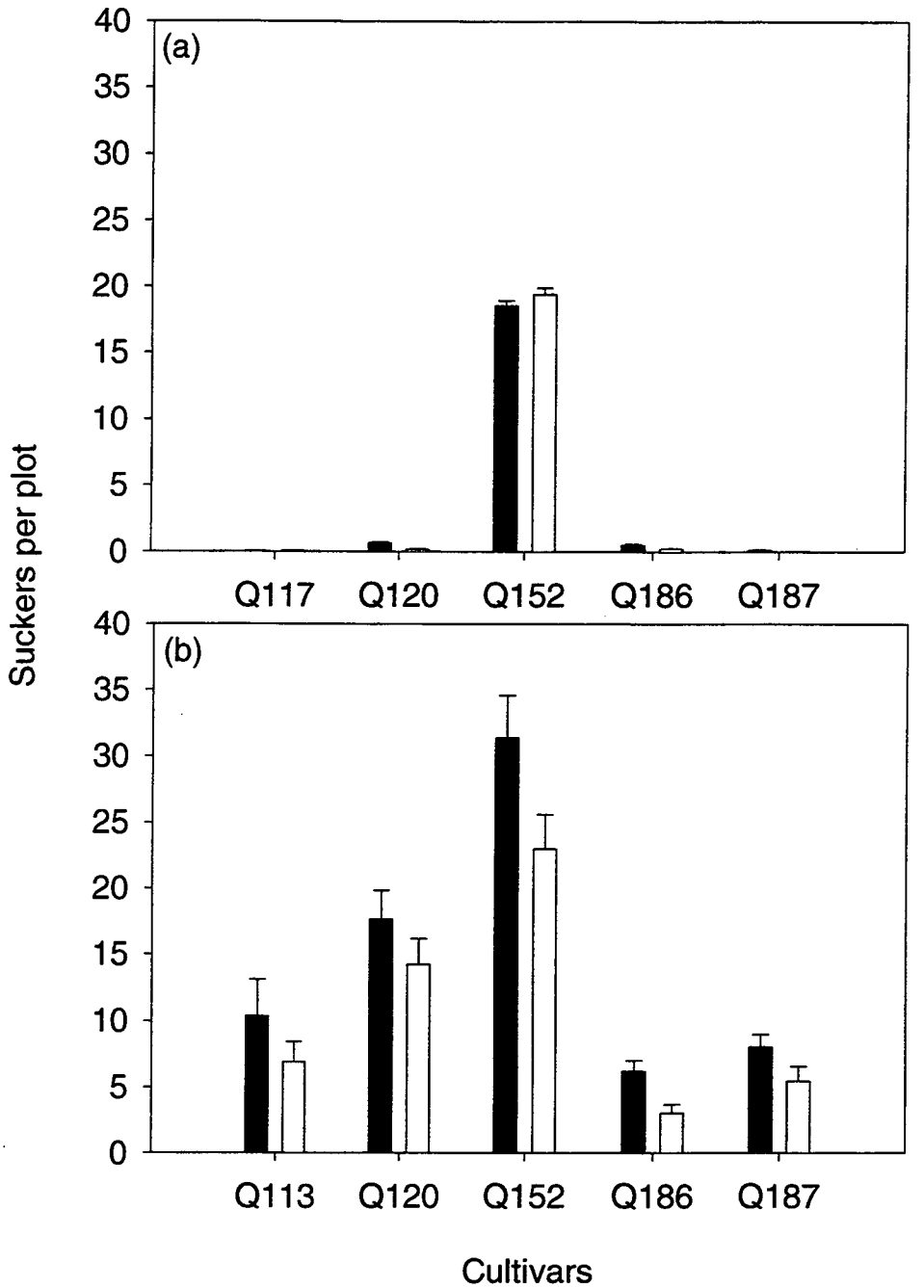

Figure 9.3 Sucker number in the Tully (a) and Mulgrave (b) regions for five cultivars with their trash removed (■) and trash present ( ). Error bars represent + the standard error of the mean.

The results of the environmental interaction experiment (Chapter 8) show that the greatest number of suckers was produced in the crop which received the late nitrogen application, additional irrigation, and was grown at a 1.0 m stool spacing. This was despite the 1.5 m spacing being expected to have greater sucker number due to increased availability of light. Therefore, it appears that the amount of light available in the 1.0 m spacing is sufficient for the other two factors, nitrogen and moisture, to have greatest effect. Light may limit the response for the crop grown at 0.5 m stool spacing, and other factors such as the number of available buds may limit the response of the crop grown at 1.5 m spacing.

While a managed environment selection trial may reveal very important information for plant breeders, it will obviously come at increased cost to the breeding program. Whether this extra trial is cost effective would have to be determined by evaluating it alongside the current process. If suckering was found to have a greater negative impact on profitability than first thought (withdrawal of sucrose from the mature stem), such a trial would become more cost effective.

9.4 Priorities for further research

This study has highlighted a number of areas of research that are still needed in order to better understand suckering in sugarcane and in order to reduce its effect on crops of sugarcane in the future.

In order to fully determine the effect of suckering on the profitability of sugarcane production an accurate estimate of how much sucrose is lost from the mature stalk to the

sucker is needed. It appears that a ^{14}C experiment may be required. The experiment should quantify the loss of carbohydrate from both stored sucrose and current assimilate from a mature stalk supporting sucker growth. Some evidence would also be needed to show that the mature stalk is capable of storing the sucrose that would otherwise have been used by the sucker. Once the negative effects of suckering have been fully quantified, then a better estimate of the effect of reducing the occurrence of the trait can be made. This then allows more informed decisions to be made when crop breeding priorities are discussed. Until such an experiment is performed it is possible that the effect of suckering may not be sufficiently penalised.

Further investigation of the possible link between suckering and GCTB is clearly needed. The process of nitrogen release from the breakdown of a trash blanket, the timing of this release and the effect of additional moisture in the soil need to be understood as they could all potentially impact on sucker numbers. Appropriate agronomic decisions, such as how to manage a trash blanket, can only be properly made if this relationship is completely understood.

The role of light in suckering needs further investigation as the results were not conclusive. This may require a controlled environment in order to reduce the effect of sprawling and lodging that could disrupt canopy structure in the field. How a light signal may be detected by the plant is not known, but given the amount of evidence that exists in relation to the role of the phytochrome system in tillering, this would appear to be a logical first point of investigation.

While environmental stimuli have been shown to affect suckering the mechanisms by which these signals are perceived by the plant need to be investigated further. This includes whether or not it is the whole plant nitrogen status that is important or whether it is each individual bud's access to soil nitrogen that causes shoot initiation. Furthermore, the downstream signals from the point of perception to the point of stalk initiation should be studied as they could potentially allow for manipulation of gene expression that may result in reduced suckering. Initially this could be done by comparing the expression of genes in dormant buds on a sugarcane stalk to that of buds which have started to show the first signs of initiating a sucker.

Source – sink relationships also need to be investigated in order to understand their role in suckering in sugarcane. All the environmental factors investigated in this thesis may have an effect on suckering via the manipulation of source - sink strengths. Such an investigation should allow suckering to be viewed in terms of a crop growth model, where growth is controlled by canopy effects interacting with genetic and environmental factors.

Selection against suckering in a breeding program needs to be done without inadvertent selection against high tillering and ratooning capacity. Cultivar Q152 is a high tillering cultivar and also has high suckering propensity, whereas cultivar Q181 is a low tillering cultivar that has low suckering propensity. Further work is needed to explore the relationship between suckering and tillering, and cultivars with low suckering propensity which tiller profusely need to be found. Berding (BSES, pers. comm. 2000) has indicated that such clones are likely to exist. The assoc has not yet been studied.

In the research proposed above an account of mature stalk number must be taken along with sucker number. This will allow the possibility of distinguishing between increased suckering per mature stalk and increased suckering per unit area. Mature stalk number may explain differences on an area basis but different levels of an environmental stimuli may explain differences in suckers per mature stalk.

9.5 Concluding remarks

This thesis has provided a better description and definition of suckering in sugarcane, and has highlighted the need for further research to be done on quantifying the possible loss of sucrose from the mature stalk to the sucker. This would allow the negative impact of suckering on the sugarcane industry to be established. The work has shown both differential genetic propensity of cultivars to sucker and differential sensitivity of cultivars to environmental stimuli. While it was not possible to comprehensively show what all the environmental stimuli might be, three stimuli, nitrogen, light and moisture were shown to have an effect on suckering. Combined, the differences in propensity to sucker, and differential sensitivity to environmental stimuli, lead to variation in the extent of suckering among crops and environments. While much remains to be done, this work has laid the groundwork for starting to manage the problem of suckering in sugarcane through agronomy and plant breeding.

References

Abayomi AY (1987) Growth, yield and crop quality performance of sugarcane cultivars Co 957 under different rates of application of nitrogen and potassium fertilizers. *Journal of Agricultural Science* **109**, 285-292.

Akehurst BC (1981) Tobacco. pp. 217-222. (Longman Group Ltd: Essex, United Kingdom.)

Alexander AE (1973) Sugarcane physiology: A comprehensive study of the Saccharum source-to-sink system. (Elsevier Scientific Publication Company: New York).

Andries HJ, DeStefano RP (1979) Chemical ripening of sugarcane suckers and variety CL41-191. *The Sugar Journal* **41**, 21-22.

Andries HJ, DeStefano RP (1980) Chemical ripening of sugarcane suckers. *The Sugar Journal* **43**, 26-27.

Artschwager E (1940) Morphology of the vegetative organs of sugarcane. *Journal of Agricultural Research* **60**, 503-549.

Artschwager EF, Brandes EW (1958) Sugarcane (*Saccharum officinarum* L.): origin, classification, characteristics, and descriptions of representative clones. (U.S. Department of Agriculture: Washington).

Austin RB (1993) Augmenting yield-based selection. In 'Plant breeding: principles and prospects'. (Eds Hayward MD , Bosemark NO, Romagosa I) pp. 391-405. (Chapman and Hall Ltd: London)

Bailey PC, Lycett GW, Roberts JA (1996) A molecular study of dormancy breaking and germination in seeds of *Trollius ledebouri*. *Plant Molecular Biology* **32**, 559-564

Ballare CL, Sanchez RA, Scopel AL, Casal JJ, Ghersa CM (1987) Early detection of neighbour plants by phytochrome perception of spectral changes in reflected sunlight. *Plant Cell and Environment* **10**, 551-557.

Ballare CL, Scopel AL, Sanchez RA (1991) Photocontrol of stem elongation in plant neighbourhoods: effect of photon fluence rate under natural conditions of radiation. *Plant Cell and Environment* **14**, 57-65.

Barnes AC (1974) The Sugar Cane. pp. 263-264. (Halsted Press: New York.)

Berding N, Hurney AP (2000) Suckering: a facet of ideotype selection and declining CCS in the wet tropics. *Proceedings of the Australian Society of Sugar Cane Technologists* **22**, 153-162.

Berding N, Owens WG, Le Brocq DG (1998) Genetic diversity: Breeding to avoid future vulnerability. *Proceedings of the Australian Society of Sugar Cane Technologists* **20**, 140-147.

Best EK (1976) An automated method for determining nitrate-nitrogen in soil extracts. *Queensland Journal of Agricultural and Animal Sciences* **33**, 161-166.

Bonnett GD, Salter B, Albertson PL (2001) Biology of suckers: late-formed shoots in sugarcane. *Annals of Applied Biology* **138**, 17-26.

Borden RJ (1945) The effect of nitrogen fertilization upon the yield and composition of sugar cane. *The Hawaiian Planters' Record* **49**, 259-312.

Borden RJ (1948) Nitrogen effects upon the yield and composition of sugar cane. *The Hawaiian Planters' Record* **52**, 1-51.

Borthwick H (1972) The biological significance of phytochrome. In 'Phytochrome. Proceedings of a symposium held at Eretria Greece September 1971.' (Eds K Mitrakos and W Shropshire Jr.) pp 25-44. (Academic Press: London)

Brotherton GA (1980) The influence of extraneous matter on C.C.S. *Proceedings of the Australian Society of Sugar Cane Technologists* **2**, 7-12.

Bull TA, Glasziou KT (1963) The evolutionary significance of sugar accumulation in *Saccharum*. *Australian Journal of Biological Science* **16**, 737-742.

Bull TA, Bull JK (2000) High density planting as an economic production strategy: (a) overview and potential benefits. *Proceedings of the Australian Society of Sugar Cane Technologists* **22**, 9-15.

Bull TA, McLeod R (2000) High density planting as an economic production system: (d) Econmoic assessment and industry implications. *Proceedings of the Australian Society of Sugar Cane Technologists* **22**, 119-125.

Casal JJ (1988) Light quality effects on the appearance of tillers of different order in wheat (*Triticum aestivum*). *Annals of Applied Biology* **112,** 167-173.

Casal JJ, Smith H (1988) The loci of perception for phytochrome control of internode growth in light-grown mustard; Promotion by low phytochrome photoequilibria in the internode is enhanced by blue light perceived by the leaves. *Planta* **176,** 277-282.

Casal JJ, Deregibus VA, Sanchez RA (1985) Variations in tiller dynamics and morphology in *Lolium multiflorum* Lam. Vegetative and reproductive plants as affected by differences in red/far-red irradiation. *Annals of Botany* **56,** 553-559.

Casal JJ, Sanchez RA, Deregibus VA (1987a) Tillering response of *Lolium multiflorum* plants to change of red/far-red ratio typical of sparse canopies. *Journal of Experimental Botany* **38**, 1432-1439.

Casal JJ, Sanchez RA, Deregibus VA (1987b) The effect of light quality on shoot extension growth in three species of grasses. Annals of Botany **59**, 1-7.

Casu R, Dimmock C, Thomas M, Bower N, Knight D, Grof C, McIntyre L, Jackson P, Jordan D, Whan V, Drenth J, Tao Y, Manners J (2001) Genetic and expression profiling in sugarcane. *Proceedings of the International Society of Sugar Cane Technologists* **24**, 542-546

Chapman LS, Larsen PL, Jackson J (2001) Trash conservation increases cane yield in the Mackay district. *Proceedings of the Australian Society of Sugar Cane Technologists* **23**, 176-184.

Chen JG, Cheng SH, Cao W, Zhou X (1998) Involvement of endogenous plant hormones in the effect of mixed nitrogen sources on growth and tillering of wheat. *Journal of plant nutrition* **21**, 87-97.

Child R, Morgan DC, Smith H (1981) Control of development in *Chenopodium album* L. by shadelight. The effect of light on quality (red:far-red ratio) on morphogenesis. *New Phytologist* **89**, 545-555.

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction. *Analytical Biochemistry* **162**, 156-159.

Chow WS, Goodchild DJ, Miller C, Anderson JM (1990) The influence of high levels of brief or prolonged supplementary far-red illumination during growth on the photosynthetic characteristics, composition and morphology of *Pisum sativum* chloroplasts. *Plant Cell and Environment* **13**, 135-145.

Chundawat BS, Patel NL (1992) Studies on chemical desuckering in banana. *Indian Journal of Horticulture* **49**, 218-221.

Clark GB, Memon AR, Tong CG, Thompson GA, Roux SJ (1993) Phytochrome regulates GTP-binding protein activity in the envelope of pea nuclei. *The Plant Journal* **4**, 399-402.

Clarke WB, Player MR, Weiss GH (1988) Effect of extraneous matter on millers and growers costs. *Proceedings of the Australian Society of Sugar Cane Technologists* **10**, 39-46.

Clements HF (1980) Sugarcane crop logging and crop control: Principles and practices. pp. 241-244. (Pitman Publishing: London)

Crook TD, Pope GM, Staunton SP, Norris CP (1999) A survey of field CCS versus mill CCS. *Proceedings of the Australian Society of Sugar Cane Technologists* **21**, 33-37.

Daniels J, Roach BT (1987) A taxonomic listing of Saccharum and related genera. *Sugar Cane* Spring supplement, 16-20.

Davis MH, Simmons SR (1994) Tillering response of barley to shifts in light quality caused by neighbouring plants. *Crop Science* **34,** 1604-1610.

Deinum B, Sulastri RD, Zeinab MHJ, Maassen A (1996) Effects of light intensity on growth, anatomy and forage quality of two tropical grasses (*Brachiaria brizantha* and *Panicum maximum* var. *trichoglume*). *Netherlands Journal of Agricultural Science* **44**, 111-124.

Deregibus VA, Sanchez RA, Casal JJ, Trlica MJ (1985) Tillering responses to enrichment of red light beneath the canopy in a humid natural grassland. *Journal of Applied Ecology* **22,** 199-206.

Deren CW, Raid RN (1997) Yield components of sugarcane subjected to flood at planting. *Journal of the American Society of Sugar Cane Technologists* **17**, 28-36.

Donald CM (1968) Breeding of crop ideotypes. *Euphytica* **17**, 385-403.

Dwiveldi UN, Khan BM, Rawal SK, Mascarenhas AF (1984) Biochemical aspects of shoot differentiation in sugarcane callus: I. Nitrogen assimilating enzymes. *Journal of Plant Physiology* **117**, 7-15.

Eavis BW, Cumberbatch J (1977) Sugar cane growth in response to mulch and fertilizer on saline-alkali subsoils. *Agronomy Journal* **69**, 839-842.

Ebrahim MK, Zingsheim O, El-Shourbagy MN, Moore PH, Komor E (1998) Growth and sugar storage in sugarcane grown at temperatures below and above optimum. *Journal of Plant Physiology* **153**, 593-602.

Ellen J, van Oene H (1989) Effects of light intensity on yield components, carbohydrate economy and cell-wall constituents in spring barley (*Hordeum distichum* L.). *Netherlands Journal of Agricultural Science* **37**, 83-95.

Escalada RG, Plucknett DL (1975) Ratoon cropping of Sorghum: II. Effect of daylength and temperature on tillering and plant development. *Agronomy Journal* **67**, 479-484.

Eussen JHH (1981) The effect of light intensity on some growth characteristics of alang-alang (*Imperata cylindrica* (L.) Beauv. Var. *Major*). *Biotrop Bulletin* **18**, 3-12.

Evans LT, Wardlow IF, Williams CN (1964) Environmental control of growth. In 'Grasses and grasslands'. (Ed. C. Barnard.) (Macmillan & Co. Ltd.: Melbourne.)

Everson CS, Everson TM, Tainton NM (1988) Effects of intensity and height of shading on the tiller initiation of six grass species from the highland sourveld of Natal. *South African Journal of Botany* **54**, 315-318.

Fejer SO (1960) Effects of gibberellic acid, indole-acetic acid, coumarin, and perloline on perennial ryegrass. *New Zealand Journal of Agricultural Research* **3,** 734-743.

Galston AW (1947) The effect of 2,3,5-tri-iodobenzoic acid on the growth and flowering of soybeans. *American Journal of Botany* **34**, 356-360.

Garcia del Moral LF, Ramos JM, Recalde L (1984) Tillering dynamics of winter barley as influenced by cultivar and nitrogen fertilizer: A field study. *Crop Science* **24**, 179-181.

Gardner JL (1942) Studies in tillering. *Ecology* **23**, 162-174.

Garrison R, Briggs WR (1972) Internodal growth in localized darkness. *Botanical Gazette* **133**, 270-276.

Garside AL, Noble AD, Berthelsen JE, Richards CL (1998) Fallow histories: effect on nitrogen contribution, growth and yield of plant and ratoon crops of sugarcane. *Proceedings of the Australian Society of Sugar Cane Technologists* **20**, 104-111.

Gifford RM, Marshall C (1973) Photosynthesis and assimilate distribution in *Lolium multiflorum* Lam. Following differential tiller defoliation. *Australian Journal of Biological Science* **26,** 517-526.

Glasziou KT, Bull TA, Hatch MD, Whiteman PC (1965) Physiology of sugar-cane. VII. Effects of temperature, photoperiod duration, and diurnal and seasonal temperature changes on growth and ripening. *Australian Journal of Biological Science* **18**, 53-66.

Gosnell JM (1971) Some effects of a water-table level on the growth of sugarcane. *Proceedings of the International Society of Sugar Cane Technologists* **14**, 841-849.

Grassl CO (1974) The origin of sugarcane. *Sugarcane Breeders Newsletter* **34**, 10-18.

Harrison MA, Kaufman PB (1980) Hormonal regulation of lateral bud (tiller) release in oats (*Avena sativa* L.). *Plant Physiology* **66**, 1123-1127.

Harrison MA, Kaufman PB (1982) Does ethylene play a role in release of lateral buds (tillers) from apical dominance in oats? *Plant Physiology* **70**, 811-814.

Hartt CE, Kortschak HP, Forbes AJ, Burr GO (1963) Translocation of 14C in sugarcane. *Plant Physiology* **38,** 305-318.

Hes JW (1954) The influence of suckers on the yield of sugarcane. *The Sugar Journal* **16**, 25-31.

Holmes MG, Wagner E (1980) A re-evaluation of phytochrome involvement in time measurements in plants. *Journal of Theoretical Biology* **83**, 225-265.

Hsiao AI, McIntyre GI (1984) Evidence of competition for water as a factor in the mechanism of root bud inhibition in milkweed (*Asclepias syraca*). *Canadian Journal of Botany* **62,** 379-384.

Hughes RM, Muchow RC (2000) Variation in sucrose concentration with crop age in primary, sucker and dead stalks in New South Wales environments. *Proceedings of the Australian Society of Sugar Cane Technologists* **22**, 200-205.

Hurney AP, Berding N (2000) Impact of suckering and lodging on productivity of cultivars in the wet tropics. *Proceedings of the Australian Society of Sugar Cane Technologists* **22**, 328-333.

Isbell RF, Edwards DG (1988) Soils and their management in the Australian wet tropics. In 'Proceedings of the National Soils Conference 1998: review papers'. (Ed. Loveday J) pp. 152-180. (Australian Society of Soil Science Inc.)

Isbell VR, Morgan PW (1982) Manipulation of apical dominance in sorghum with growth regulators. *Crop Science* **22**, 30-35.

Ivin PC, Doyle CD (1989) Some measurements of the effect of tops and trash on cane quality. *Proceedings of the Australian Society of Sugar Cane Technologists* **11**, 1-7.

Jackson P, Bonnett G, Chudleigh P, Hogarth M, Wood A (2000) The relative importance of cane yield and traits affecting CCS in sugarcane varieties. *Proceedings of the Australian Society of Sugar Cane Technologists* **22**, 23-29.

Jannoo N, Grivet L, Seguin M, Paulet F, Domaingue R, Rao PS, Dookun A, D'Hont A, Glaszmann JC (1999) Molecular investigation of the genetic base of sugarcane cultivars. *Theoretical and Applied Genetics* **99**, 171-184.

Kamada I, Yamauchi S, Youssefian S, Sano H (1992) Transgenic tobacco plants expressing *rgp1*, a gene encoding a *ras*-related GTP-binding protein from rice, show distinct morphological characteristics. *The Plant Journal* **2**, 799-807.

Kasperbauer MJ, Karlen DL (1994) Plant spacing and reflected far-red light effects on phytochrome-regulated photosynthate allocation in corn seedlings. *Crop Science* **34,** 1564-1569.

Kasperbauer MJ, Tso TC, Sorokin TP (1970) Effects of end-of-day red and far-red radiation on free sugars, organic acids and amino acids in tobacco. *Phytochemistry* **9,** 2091-2095.

Keating BA, Catchpoole VR, Bridge BJ, Bristow KL (1994) Assessing nitrate losses below sugarcane crops. In 'Proceedings of the Workshop on Measurement and Management of Nitrogen Losses for groundwater protection in agricultural production systems'. (Ed. Bond WJ) pp. 37-42. (Institution of Engineers: Canberra)

Kirby EJM, Faris DG (1972) The effect of plant density on tiller growth and morphology in barley. *Journal of Agricultural Science Cambridge* **78,** 281-288.

Labanauskas CK, Dungan GH (1956) Inter-relationship of tillers and main stems in oats. *Agronomy Journal* **48,** 265-268.

Lafarge TA, Hammer GL (2002) Tillering in grain sorghum over a wide range of population densities: Modelling dynamics of tiller fertility. *Annals of Botany* **90**, 99-110.

Langer RHM (1963) Tillering in herbage grasses. *Herbage Abstracts* **33**, 141-148.

Lawes RA, Wegener MK, Basford KE, Lawn RJ (2000) Commercial cane sugar trends in the Tully sugar district. *Australian Journal of Experimental Agriculture* **40**, 969-973.

Lawn RJ (1980) The potential contribution of physiological research to Pigeonpea improvement. *International workshop on Pigeonpea* **1**, 151-164.

Leopold AC (1949) The control of tillering in grasses by auxin. *American Journal of Botany* **36**, 437-440.

Leslie JK, Wilson GL (1996) Productivity trends in sugarcane in the wet tropics. (Technical Report 1/96. SRDC, Brisbane.)

Linedale AL, Ridge DR (1996) A successful campaign to minimise harvesting losses within the Queensland sugar industry. *Proceedings of the Australian Society of Sugar Cane Technologists* **18**, 1-5.

Ludlow MM, Ferraris R, Chapman L (1990) Variation in the net photosynthetic rates of sugar cane leaves and differences in the ratio of red:far-red light beneath the canopy among varieties with different ratooning capacities. *Proceedings of the Australian Society of Sugar Cane Technologists* **12,** 105-110.

Mahmoud ZM, Osman AM (1981) Tillering of wheat as influenced by nitrogen and seed rate in the Sudan. *Journal of Agricultural Science* **97**, 619-627.

Marshall C (1967) The use of radioisotopes to investigate organisation in plants, with special reference to the grass plant. In 'Isotopes in Plant Nutrition and Physiology'. pp. 203-216. (International Atomic Energy Agency: Vienna)

Marshall C, Sagar GR (1968a) The distribution of assimilates in *Lolium multiflorum* Lam. Following differential defoliation. *Annals of Botany* **32**, 715-719.

Marshall C, Sagar GR (1968b) The interdependence of tillers in *Lolium multiflorum* Lam. – a quantitative assessment. *Journal of Experimental Botany* **19**, 785-794.

Martin JP (1938) Sugar cane diseases in Hawaii. Experiment Stations Hawaiian Sugar Planters Association pp. 295.

Martin JP, Eckart RC (1933) The effect of various intensities of light on the growth of the H 109 variety of sugar cane. *The Hawaiin Planters' Record* **37**, 53-66.

McDonald L, Morgan T, Kingston G (2000) Chemical ripeners: an opportunity for the Australian sugar industry. *Proceedings of the Australian Society of Sugar Cane Technologists* **22**, 290-295.

McIntyre GI (1972) Developmental studies on *Euphorbia esula.* The influence of the nitrogen supply on the correlative inhibition of root bud activity. *Canadian Journal of Botany* **50,** 949-956.

McIntyre GI (1987) Studies on the growth and development of *Agropyron repens*: interacting effects of humidity, calcium, and nitrogen on the growth of the rhizome apex and lateral buds. *Canadian Journal of Botany* **65,** 1427-1432.

McIntyre GI (2001) Control of plant development by limiting factors: a nutritional perspective. *Physiologia Plantarum* **113**, 165-175.

McIntyre GI, Hunter JH (1975). Some effects of the nitrogen supply on growth and development of *Cirsium arvensae. Canadian Journal Botany* **53,** 3012-3021.

Mondal MF (1993) Effect of number of sucker per hill on growth and yield on banana in a ratoon crop. *Pakistan Journal of Scientific and Industrial Research* **36**, 267-269.

Mongelard JC, Mimura L (1971) Growth studies on the sugarcane plant. I. Effects of temperature. *Crop Science* **11**, 795-800.

Montaldi ER (1974) Effects of sucrose and other substances on leaf form of *Cynodon dactylon* (L.) Pers. *Revista de la Facultad de Agronomia, Universidad Nacional de la Plata* **50**, 61-74.

Morgan DC, Smith H (1981) Control of development in *Chenopodium a*lbum L. by shadelight: effect of light quality (total fluence rate) and light quality (red:far-red ratio). *New Phytologist* **88,** 239-248.

Morgan PW, Gausman HW (1966) Effects of ethylene on auxin transport. *Plant Physiology* **41**, 45-52

Morgan PW, Miller FR, Quinby JR (1977) Manipulation of sorghum growth and development with gibberellic acid. *Agronomy Journal* **69**, 789-793.

Murtha GG (1986) Soils of the Tully – Innisfail area, North Queensland. (Divisional Report No. 82, CSIRO Division of Soils).

Nelson DW (1983) Determination of ammonium in KCl extracts of soils by the salicylate method. *Communications in Soil Science and Plant Analysis* **14**, 1051-1062.

Ng Kee Kwong KF, Paul JP, Deville J (1999) Drip-fertigation – A means for reducing fertilizer nitrogen to sugarcane. *Experimental Agriculture* **35**, 31-37.

Nigam SN, McIntyre GI (1977) Apical dominance in the rhizome of *Agropyron repens*. The relation of amino acid composition to bud activity. *Canadian Journal of Botany* **55,** 2001-2010.

Olmstead CE (1941) Growth and development in range grasses. I. Early development of *Bouteloua curtipendula* in relation to water supply. *Botanical Gazette* **102**, 499-519.

Papageorgiou J, Bartholomew HC, Doherty WOS (1997) HPAE-PAD: A rapid and precise method for sugar analysis. *Proceedings of the Australian Society of Sugar Cane Technologists* **19**, 79-386.

Phillips IDJ (1975) Apical dominance. *Annual Review of Plant Physiology* **26**, 341-367.

Pope, G.M. (1997). Mulgrave CCS declines to record low levels in 1993 and 1995. *Proceedings of the Australian Society of Sugar Cane Technologists* **19**, 30-37.

Qureshi FA, McIntyre GI (1979) Apical dominance in the rhizome of *Agropyron repens*: the influence of nitrogen and humidity on the translocation of 14C-labeeled assimilates. *Canadian Journal of Botany* **57,** 1229-1235.

Rands RD, Dopp E (1938) Pythium root rot of sugarcane. *United States Department of Agriculture Technical Bulletin* **666**, 1-95.

Roach BT (1989) Origin and improvement of the genetic base of sugarcane. *Proceedings of the Australian Society of Sugarcane Technologists* **11**, 34-47.

Robertson FA, Thorburn PJ (2000) Trash management - consequences for soil carbon and nitrogen. *Proceedings of the Australian Society of Sugar Cane Technologists* **22**, 225-229.

Robinson JC (1987) Poor desuckering reduces banana yield. *Information Bulletin: Citrus and Sub-Tropical Fruit Research Institute (South Africa)* **181,** 8-9.

Sagar GR, Marshall C (1966) The grass plant as an integrated unit - some studies of assimilate distribution in *Lolium multiflorum* Lam. In 'Proceedings of the 9th International Grassland Congress'. pp. 493-497.

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning : a laboratory manual (Cold Spring Harbor Laboratory Press: New York).

Scurfield G (1959) The effect of gibberellic acid on the early growth of species of *Phalaris*. *Australian Journal of Science* **21,** 49-49.

Shrivastava AK, Kumar A (1984) Effects of split application of nitrogen on tillering in sugarcane. *Indian Journal of Plant Physiology* **27**, 328-333.

Shrivastava AK, Kumar A, Singh GB (1992) Initiation and emergence of tillers in sugarcane. *Indian Sugar* **42**, 605-612.

Silberbush M, Lips SH (1991) Potassium, nitrogen, ammonium/nitrate ratio, and sodium chloride effects on wheat growth. II. Tillering and grain yield. *Journal of Plant Nutrition* **14**, 765-773.

Singh G, Chapman SC, Jackson PA, Lawn RJ (1999) Yield accumulation in sugarcane under wet tropical conditions - effect of lodging and crop age. *Proceedings of the Australian Society of Sugar Cane Technologists* **21**, 241-245.

Singh G, Chapman SC, Jackson PA, Lawn RJ (2000) Lodging - a major constraint to high yield and CCS in the wet and dry tropics. *Proceedings of the Australian Society of Sugar Cane Technologists* **22**, 315-321.

Singh US (1977) Nitrogen and sugarcane X. Correlation between leaf nitrogen and tillering. *Indian Sugar* **27**, 449-450.

Singh US (1978a) Nitrogen and sugarcane. VII-Analysis of cane yield in relation to the availability of soil nitrogen. *Indian Sugar* **28**, 181-184.

Singh US (1978b) Nitrogen and sugarcane. XVI-Available soil nitrogen during different phases and crop growth. *Indian Sugar* **28**, 121-124.

Skalova H, Krahulec F (1992) The response of three *Festuca rubra* clones to changes in light quality and plant density. *Functional Ecology* **6,** 282-290.

Skinner JC (1967) Grading varieties for selection. *Proceedings of the International Society of Sugar Cane Technologists* **12**, 938-049.

Skinner RH, Simmons SR (1993) Modulation of leaf elongation, tiller appearance and tiller senescence in spring barley by far-red light. *Plant Cell and Environment* **16,** 555-562.

Smirnoff N (1996) The function and metabolism of ascorbic acid in plants. *Annals of Botany* **78**, 661-669.

Smith H (1982) Light quality, photoperception, and plant strategy. *Annual Review of Plant Physiology* **33**, 481-518.

Smith H (1990) Signal perception, differential expression within multigene families and the molecular basis of phenotypic plasticity. *Plant, Cell and Environment* **13**, 585-594.

Smith NJ, McGuire PJ, Mackson J, Hickling RC (1984) Green cane harvesting – A review with particular reference to the Mulgrave mill area. *Proceedings of the Australian Society of Sugar Cane Technologists* **4**, 21-27.

Soopramanien GC (2000) Sugarcane morphology, anatomy and physiology. In 'A guide to sugarcane diseases'. (Eds Rott P, Bailey RA, Comstock JC, Croft BJ, Saumtally AS) pp. 13-20. (Cirad Publication Service: Montpellier)

Spiertz JH, Ellen J (1972) The effects of light intensity on some morphological and physiological aspects of the crop perennial ryegrass (*Lolium perenne* L. var. '*Cropper*') and its effect on seed production. *Netherlands Journal of Agricultural Science* **20**, 232-246.

Stanford G (1963) Sugarcane quality and nitrogen fertilization. *The Hawaiin Planters' Record* **56**, 289-333.

Steel RGD, Torrie JH (1980) Principles and procedures of statistics. A biometrical approach. pp. 172-200. (McGraw-Hill International Book Company: New York)

Suge H, Iwamura H (1993) Effects of cytokinin and anticytokinin on the tillering of barley. *Japanese Journal of Crop Science* **63**, 595-600.

Templeton WC, Mott GO, Bula R (1961) Some effects of temperature and light on growth and flowering of tall fescue, *Festuca arundinacea* Schreb. *Crop Science* **1**, 216-219.

Tucker DJ (1976) Effects of far-red light on the hormonal control of side shoot growth in the tomato. *Annals of Botany* **40,** 1033-1042.

Turner DW (1972) Banana plant growth. 1. Gross morphology. *Australian Journal of Experimental Agriculture and Animal Husbandry* **12,** 209-214.

van Dillewijn C (1952) Botany of Sugarcane. (Chronica Botanica: Waltham,USA.)

Verret JA, McLennan RH (1927) The effect of sunlight on cane growth. *The Hawaiin Planters' Record* **31**, 116-121.

von Arnim A, Deng XW (1996) Light control of seedling development. *Annual Review of Plant Physiology and Plant Molecular Biology* **47**, 215-243.

Wattal PN, Asana RD (1974) Responses of tall and dwarf varieties of wheat to light intensity. *Indian Journal of Agricultural Science* **44**, 707-711.

Willms WD (1988) Response of rough fescue (*Festuca scabrella*) to light, water, temperature, and litter removal, under controlled conditions. *Canadian Journal of Botany* **66**, 429-434.

Wilson D, Buckley M (2001) Instructions for using tRMA: tools for R Microarray Analysis (Version 1.5.1). (CSIRO Mathematical and Information Sciences, CSIRO Australia: Canberra).

Wilson GL, Leslie JK (1997) Productivity trends in sugarcane in the wet tropics. *Proceedings of the Australian Society of Sugarcane Technologists* **19**, 21-29.

Woodward EJ, Marshall C (1988) Effects of plant growth regulators and nutrient supply on tiller bud outgrowth in barley (*Hordeum distichum* L.). *Annals of Botany* **61**, 347-354.

Zaino S, Reggiani R, Bertani A (1990) Preliminary evidence for involvement of GTP-binding protein(s) in auxin signal transduction in rice (*Oryza sativa* L.) coleoptile. *Journal of Plant Physiology* **136**, 653-658.

Appendices

Appendix 4.1 Solutions required for RNA extraction and hybridisation

Solutions:

Denaturing solution: 4 M guanidinium isothiocyanate, 25 mM sodium citrate. Place 50 ml of 250 mM sodium citrate in a beaker. Add 236.3 g guanidinium isothiocyanante. Add water and make up to 500 ml. Dispense into 100 ml aliquots and keep at 4 °C in the dark.

5.7 M cesium chloride: Dissolve 100 g CsCl in water and make up to 104 ml. Treat with DEPC and store at room temperature.

DEPC treatment of solutions: Add 0.1 % v/v diethylpyrocarbonate to the solution (except those containing Tris). Incubate over-night at 37 °C. Autoclave prior to use.

20 x SSC: Dissolve 175.3 g of NaCl, 27.6 g of $NaH_2PO_4.H_2O$ and 7.4 g EDTA in 800 ml of H_2O. Adjust the pH to 7.4 with NaOH (~6.5 ml of a 10 N solution). Adjust the volume to 1 litre with H_2O. Dispense into aliquots. Sterilise by autoclaving (Sambrook *et al.* 1989).

Appendix 6.1 Determination of soil nitrate-N from 2M KCl extracts (adapted from Best 1976)

Reagents

- *Copper solution*: Dissolve 2 g $CuSO_4.5H_2O$ in 500 ml distilled water
- *Working copper solution*: Dilute 3 ml to 500 ml
- *Hydrazine sulphate*: Dissolve 0.3 g in 500 ml of distilled water, stable for 1 month
- *Buffer solution*: Dissolve 11 g sodium tetraborate and 1.25 g NaOH in 450 ml of distilled water. Make up to 500 ml.
- *Colour reagent*: Add 50 ml conc. HCl to 400 ml distilled water. Dissolve 5 g of sulphanilamide in this dilute acid. Add 0.25 g of N-1-nanaphthyldiaamine dihydrochloride, and when dissolved, make up to 500 ml.

Standards

Stock nitrate (100 ppm NO_3-N): Dissolve 0.3609 g oven dried potassium nitrate in about 400 ml distilled water, add 75 g KCl, and make up to 500 ml.

Working standards: Pipette 0, 1, 2, 3 and 4 ml of stock nitrate solution into 100 ml vol. Flasks to give 0, 1, 2, 3 and 4 ppm N. Dilute with 2 M KCl.

Method

- Add 1.5 ml of working copper solution to a 15 ml falcon tube
- Add 0.75 ml of sample (or standard) and swirl

- Add 1 ml of hydrazine and swirl
- Add 1.5 ml of buffer and swirl
- Place in a 37 °C water bath for 15 min
- Add 1.5 ml of colour reagent
- Allow at least 25 minutes for colour development, then read at 520 nm using a 1 cm cell. Colour stable for at least 12 hours.

Appendix 6.2 Determination of soil ammonium-N from 2M KCl extracts (adapted from Nelson 1983)

Reagents

- *Disodium ethylenediaminetetraacetic acid solution (6% w/v)*: Dissolve 6 g of Na_2EDTA in 80 ml of deionised water and make up to 100 ml.
- *Sodium salicylate – sodium nitroprusside reagent*: Dissolve 7.813 g $NaC_7H_5O_3$ and 125 mg of $Na_2Fe(CN)_5NO.5H_2O$ in 80 ml of deionised water and make up to 100 ml.
- *Buffer-sodium hypochlorite reagent*: Dissolve 2.96 g NaOH and 9.96 g $Na_2HPO4.7H_2O$ in 60 ml of deionised water. Add 10 ml sodium hypochlorite solution (ca. 5% NaOCl). Adjust to pH 13 with NaOH and dilute to 100 ml with deionised water.

Standards

Stock ammonium: Dissolve 0.2358 g ammonium sulphate [$(NH_4)^2SO_4$; previously dried at 100 °C for 4 h] in 2 M KCl solution and make up to 1.0 L in a volumetric flask. 1 ml contains 50 µg NH_4^+.

Working standards: Pipette 0, 2, 4, 6 and 8 ml of stock ammonium solution into 100 ml vol. flasks to give 0, 1, 2, 3 and 4 ppm N. Dilute with 2 M KCl

Method

- Pipette 1 ml unknown (or standard) into a 15 ml Falcon tube

- Add 0.2 ml EDTA solution and vortex
- Add 0.8 ml sodium salicylate-sodium nitroprusside reagent, 2.6 ml H_2O and 0.4 ml buffer-sodium hypochlorite reagent, shake.
- Place in a water bath (37 °C) for 30 minutes to develop colour.
- Allow to cool to room temperature and measure absorbance at 667 nm using a 1 cm cell.

Appendix 7.1 Calculation of LSD from a split plot design (Steel and Torrie, 1980)

$$LSD = t \times SED$$

$$SED = \sqrt{\frac{2[(b-1)E^b + E^a]}{rb}}$$

$$t = \frac{(b-1)E^b t^b + E^a t^a}{(b-1)E^b + E^a}$$

where: r was the number of replicates of the treatment

b was the number of replicates of time

E^a was the error mean square for between subjects

E^b was the error mean square for within subjects

t^a was the t value with degrees of freedom used to calculate Ea

t^b was the t value with degrees of freedom used to calculate Eb