

**FINAL REPORT ON SRDC PROJECT CLW002  
(previously CSS02 & CSS2S)  
THE ROLE OF ROOT GROWTH AND ACTIVITY  
IN DETERMINING SUGARCANE PRODUCTIVITY**

by

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## SUMMARY

Research conducted in this project aimed to better understand the relationship between root and shoot growth, in areas such as how the size of the root system affects shoot growth, do particular root parameters have a controlling influence on shoot growth, how do soil characteristics affect root penetration rates, and how the root system develops through the life of a sugarcane crop. This was achieved through the application of a wide range of experimental techniques in both the glasshouse and field situation. The study of root systems in sugarcane is difficult - due to the size of the crop and the length of the cropping period. As a result there have been few previous studies on sugarcane root systems in Australia, and indeed around the world.

A number of techniques were either developed, or adapted, in this project research. A soil-less aeroponic culture technique was installed and refined at Tully Sugar Experiment Station. This allowed sugarcane roots to be examined on a daily basis and root measurements made, or root pruning to occur. This overcame the difficulty of dealing with the bulky, opaque soil medium. A tall pot system was adapted for sugarcane where sugarcane could be grown for an extended period in controlled conditions. This enabled plant water relations to be studied in association with modification to root growing conditions. Root image analysis techniques were further refined for sugarcane, allowing measurement of both whole glasshouse-grown root systems, or the quantification of root lengths in material from soil cores obtained in the field. A technique for growing sugarcane with a split root system was also adapted enabling the direct and indirect effects of water stress and root pruning in a soil culture to be examined, and the likely presence of root signals as a mechanism for control of shoot growth.

Studies using these techniques facilitated an examination of the relationship between roots and shoots under various experimental conditions - ranging from controlled conditions with no soil in the glasshouse, through other soil-based glasshouse trials, to the field situation. This gave depth to project results and a broader understanding of root-shoot relationships using a range of experimental observations.

Aeroponic and split root experiments suggested that a healthy root system normally functions at less than full capacity. Pruning root systems to 5% of their original size failed to reduce shoot growth under ideal growth conditions. This extra root capacity may allow the plant to cope with adverse environmental conditions, with the loss of some roots having minimal impact on shoot growth. The result suggests that if restrictions on root growth in normal cropping situations is reducing shoot growth, the efficiency of the field-grown root system must be very poor.

On the other hand, experiments also showed that root : shoot bio-mass ratios are strongly conserved in sugarcane. Pruning of either the shoot or roots led to a cessation in the growth of either the roots or shoot respectively, until the former ratio was again established. Research also showed that the value of the ratio varied between variety; some varieties developed a relatively bigger root mass than others.

Studies with soil treatments known to affect shoot growth, such as soil fumigation with methyl bromide and fertilisation with nitrogen, had a large effect on root system parameters. Fumigation almost doubled root length densities in both the glasshouse and field. The fumigant also appeared (by visual assessment) to improve root health. This suggests that growth responses to soil fumigation result at least in part from the greater utilisation of soil resources. Nitrogen reduced primary root extension but increased primary root number, diameter and total root length. This response may allow the sugarcane plant to concentrate the root system in soil with high concentrations of nutrients (in this case nitrogen). Pachymetra root rot had a big effect on root systems, principally reducing primary root length.

Root measurements showed that the ratio of primary : secondary : tertiary root lengths was strongly conserved in sugarcane. This characteristic may simplify field root system sampling using soil cores – only the larger roots need be measured and extrapolation of total root length made using the consistency of root length ratios. A modified strategy based on this observation would greatly reduce costs, particularly as the separation and identification of the origin of fine roots in cores, whether they are from weeds or sugarcane, is laborious and very time consuming.

Sequential sampling of the root system of a plant crop over a 12 month period established that root system development varies according to such characteristics as soil type and physical conditions. In the wet tropics, the root system had evenly developed through the row-interrow area days after planting. Up to 90 % of roots were above 90 cm depth 192 days after planting. In an irrigated situation in the dry tropics, close to 90 % of roots were above 60 cm depth due to the duplex soil profile. Soil compaction at the plough pan (30 cm) restricted early development but did not prevent colonisation of lower soil levels. Root length densities were found to be low in sugarcane relative to figures reported in the literature for other crops.

Tall pot studies showed that sugarcane leaf extension and transpiration is very sensitive to water stress, much more so than sorghum. This suggests that irrigation scheduling needs to ensure water stress is kept to a minimum to avoid restriction on shoot growth. The concept of plant available water could be used in further studies of root system efficiency.

Further sugarcane root research is needed. A method to quantify root health would enable better estimation of the effects of soil constraints on root function. Techniques developed in the research reported here may aid the study of root health and root functioning. Remote measures of root functioning would be ideal to avoid the expenditure required to sample root systems in the field and the associated difficulties. Further research on the factors governing the shoot-root relationship would be useful, particularly if the relationship could be modified to take advantage of the spare root capacity which appears to operate under ideal growth conditions. Before this can happen, better knowledge on the functioning ability of root systems under normal field conditions, and ways to improve this functioning ability, are needed. This is where the study of root systems complements the research currently being conducted in the Yield Decline Joint Venture, where the improvement of root growing conditions and root health are two major program emphases.

## **1.0 INTRODUCTION**

### **1.1 Background**

When the current project was initiated, poor root growth and activity were widely believed to be a major constraint to sugarcane productivity. However, there was no technology available to assess whether or not root growth and activity were constraining cane productivity. The project was established to examine the relationship between root growth and activity and sugarcane productivity. The strategic information to be generated was expected to provide the basis for estimating the potential value of overcoming poor root growth and/or activity by managing various soil constraints such as soil pathogens, poor soil structure, or low soil fertility.

Previous root research in sugarcane had been largely undertaken overseas, and generally some years ago. Evans (1936), working in Mauritius, excavated whole root systems and described root types. A foundation in our understanding of root morphology was laid. Other research added some detail about root morphology but due to technological constraints, could not adequately relate parameters such as root lengths, root distribution and activity to shoot growth. The difficulty in studying roots in an opaque, bulky medium (soil) has hindered the studies needed to understand the dynamics of the shoot - root relationship.

Bell (1938) was aware of problems with root growth in the Bundaberg area as early as the 1930s. In the 1960-80 period, it was noted that root systems were poor (particularly root health) in soils used for sugarcane monoculture in Queensland (Egan *et al.* 1984; Magarey 1986). Research conducted since then (Poor Root Syndrome, Yield Decline) consistently highlighted suboptimal root health within the Queensland sugar industry (Magarey and Bull 1994). Treatments that greatly improved root distribution and health were identified - including soil fumigation, general fungicides, and crop rotations - and these may provide useful tools in further studies. Treatments which improved root condition - health and functional integrity - almost always led to significant shoot growth improvements.

In SRDC-funded project BS56S, Reghenzani (1993) investigated sampling strategies required for describing in detail root system distribution in the field, at one point in time. The study, based on taking multiple soil cores from various positions around the sugarcane stool, showed that prohibitively large sample sizes were needed to achieve statistically acceptable data. The study also indicated that sugarcane ratoon crops had low root length densities (root length per unit volume of soil), compared to other crops.

This project arose from three independent preliminary research proposals submitted by BSES and CSIRO to SRDC. The three projects were subsequently aggregated within the Yield Decline Joint Venture.

Initially, the objectives of CLW002 were to:

- (i) quantify the relationship between root system characteristics and crop productivity;
- (ii) assess the methods for measuring root activity as indicators of root system constraints on crop productivity – with particular emphasis on methods that would allow remote assessment of root activity.

After the 18 month review of this project by SRDC, the objectives were expanded to:

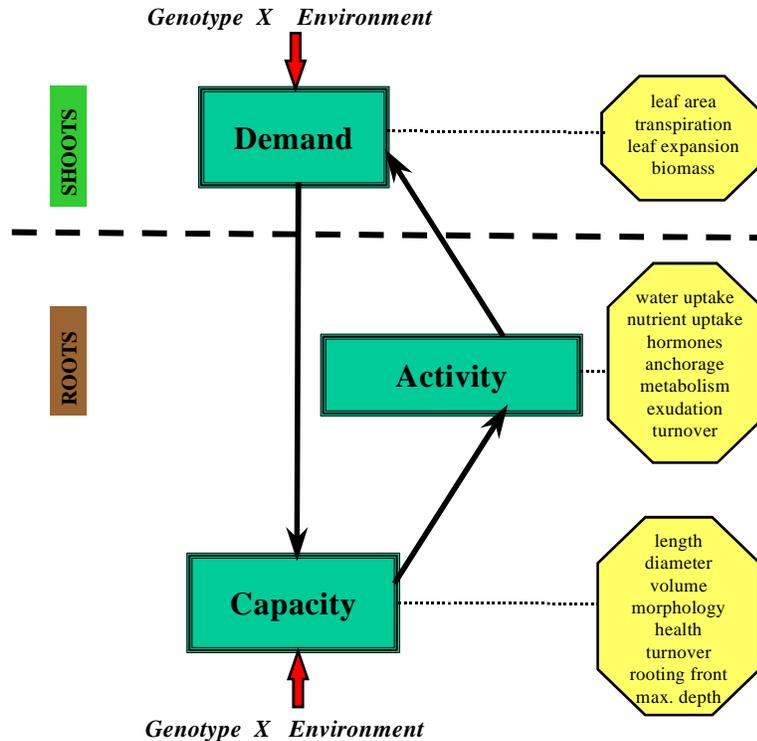
- (i) examine how shoot growth and root system activity is affected by changes in the size of the root system;
- (ii) determine how root characteristics are related to shoot growth and investigate the possibility of defining a ‘root health’ property;
- (iii) determine how soil properties affect root characteristics and the relationship between water uptake and shoot growth using measurements that allow extrapolation from the glasshouse to the field;
- (iv) determine how soil type and different soil properties affect the penetration rate of roots; and
- (v) understand how root systems develop throughout the life of a crop in relation to above-ground biomass accumulation and to compare root system distribution with root system activity.

## 1.2 Research framework

There are two properties of root systems that are critically important in determining sugarcane productivity– root system **capacity** and root system **activity**. The former – capacity – determines the soil resources that are potentially available to the plant, whilst the latter – activity – is a measure of the plant’s ability to acquire the available resources. Both properties are strongly influenced by plant genetics and their interactions with the environment and each is described by various root parameters (Figure 1). Ultimately, roots can constrain crop productivity in two ways – either by not making available the potential soil resources (sub-optimal capacity) or by not acquiring the resources made available (sub-optimal activity).

In order to know whether or not there are root constraints to crop productivity, it is necessary to have good information on root capacity and activity. In sugarcane, little was known about feedback mechanisms between roots and shoot growth.

**Figure 1: Conceptual relationship between root system and shoot growth**



### 1.3 Research activities

Within the above framework the project was subdivided into four activities to address the various aspects of the root supply/shoot growth relationship.

**Activity 1:** Research into basic properties of sugarcane root systems under controlled conditions with no soil constraints (aeroponics, J Reghenzani, D Grace). Data was gathered on the effect of genotype on root and shoot growth, on physiological responses of sugarcane in relation to root growth, and on the effect of modifications to root growth (root pruning) on shoot growth, simulating soil constraints. Results of Activity 1 are reported in Chapter 2.

**Activity 2:** The second activity expanded on Activity 1 by examining, in controlled, glasshouse, pot experiments, the effects on root and shoot growth of various soil constraints (eg yield decline, soil pathogens, nutrients) (R Magarey, D Grace). Plant factors considered included a range of root morphological parameters as well as the functioning ability of the roots (“root health”, root activity). Results of Activity 2 are reported in Chapter 3.

**Activity 3:** While the small pot, glasshouse experiments conducted within Activities 1 and 2 provided rapid results, there was a need to verify these relationships on a larger scale. This was achieved in glasshouse experiments with large pots and/or longer term trials in which the relationship between roots and shoots was examined (J Smith, R Nable, M Robertson, S Berthelsen), focussing on the physiology of the plant response to adverse soil conditions. Evidence for the involvement of feed-forward responses (eg root signals) in the regulation of sugarcane shoot growth were also sought. Results of Activity 3 are reported in Chapters 4 and 5.

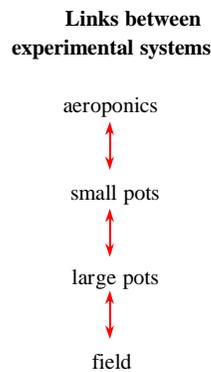
**Activity 4:** The final activity involved relating root and shoot growth in experiments in the field (R Nable, M Robertson, S Berthelsen) by examining the relationship between root and shoot development under different environmental conditions and in different soils.

**Method Development:** A further fundamental aspect of CLW002 was the development and application of tools to study roots and root systems. Method development occurred across all four activities, ranging from the design and testing of an aeroponics system, large pot study systems to the development of surrogate methods to determine root length densities of field collected samples with greater ease.

#### 1.4 Coordination and linkages within the project

Several lines of research were undertaken in CLW002, with all being linked and coordinated through the experimental systems and common experimental methods (Figure 2). The Project used experimental systems ranging from aeroponics, through pot to field experiments.

**Figure 2: Relationship between experimental systems used in the four project activities**

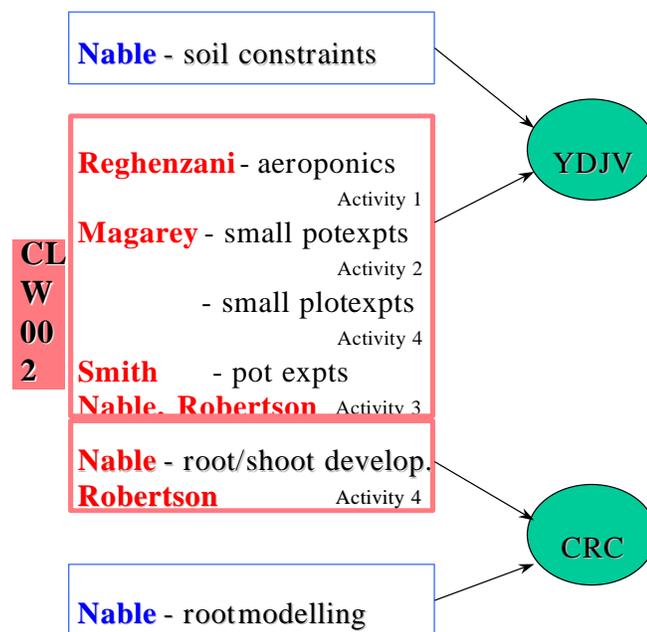


In this way the Project ensured that basic information being generated under controlled conditions was also assessed under field conditions and that patterns observed under one experimental system could be used to interpret those observed elsewhere.

Where ever possible, similar experimental methods were used in the various research activities of the Project. For example:

- soils from several field experiments (eg Yield Decline Joint Venture rotation trials) were also used in pot experiments;
- similar test treatments (eg sugarcane varieties with distinct root/shoot ratios; soil fumigation; specific soil properties) were used in several research activities;
- similar root characteristics were assessed and similar measurements of root and shoot growth, and root activity were used in all research activities.

**Figure 3: Internal project structure of CLW002**

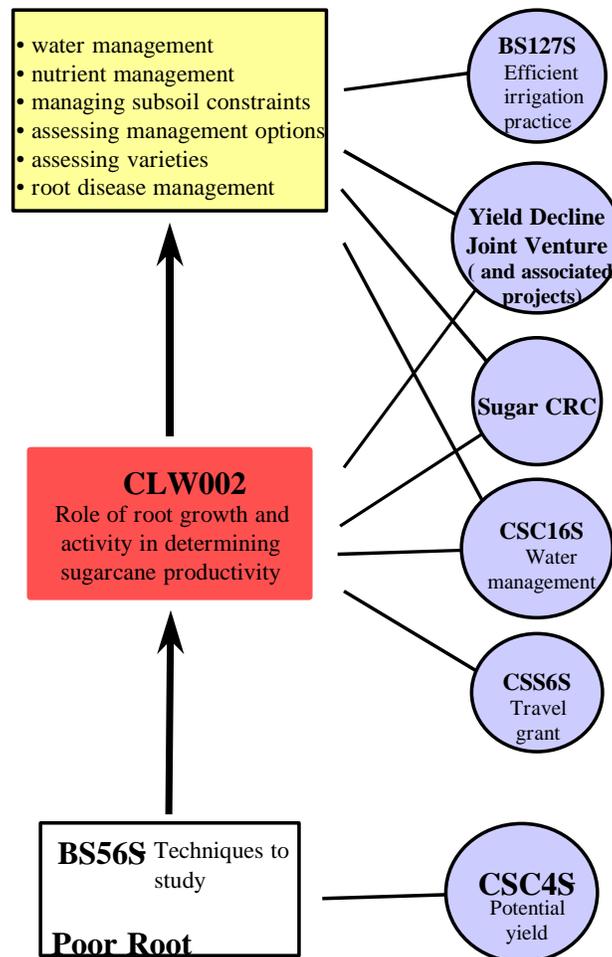


By taking the approach of using common methods and linking the experimental systems, the Project set out to maximise the value of results that were generated in the various research activities.

## 1.5 Coordination and linkages beyond the project

Coordination beyond CLW002 was achieved through linkages with related projects, and through CLW002 being part of the Sugarcane Yield Decline Joint Venture (SYDJV) and the Co-operative Research Centre for Sustainable Sugar Production (Sugar CRC). Figure 3 shows how the research objectives of CLW002 were placed within the SYDJV and the Sugar CRC. Figure 4 shows how research in CLW002 was linked to related research (past, present and future).

**Figure 4: Linkages between CLW002, the Yield Decline Joint Venture and the CRC for Sustainable Sugar Production**



## **2.0 AEROPONIC EXPERIMENTS: BASIC PROPERTIES OF SUGARCANE ROOT SYSTEMS AND EFFECTS OF ROOT PRUNING**

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### **2.1 Introduction**

Few detailed studies have been conducted into sugarcane root growth and activity, or into the relationship between roots and above ground productivity. Some reasons for the lack of research on sugarcane root systems include difficulties in observing or sampling root systems over time, and inability in determining the activity of the observed roots. Due to root system variability, large numbers of samples are required to describe full profile root distribution for crops (Upchurch 1987). For a fourth ratoon Q122 sugarcane crop, sample numbers for root description were found to exceed practical limits (Reghenzani 1993), and a sub-sampling strategy was suggested. While both approaches above provide an estimate of root system distribution or relative size, neither was entirely satisfactory. Cost was high, no data was provided on root system activity and limited information was provided on the relationship between roots and above ground productivity.

An effective and extensive sugarcane root system is required for the absorption of water and nutrients, and for the anchorage of plants in the soil. Despite much greater above ground biomass, sugarcane root length of almost 34,000 km/ha is much less than 60,000 - 100,000 km/ha commonly found for wheat (Reghenzani 1993). It has been suggested that large areas of Australian sugarcane are suffering loss of productivity directly attributed to debilitated root systems. While soil factors influencing root growth and health are being investigated, there is a need to establish the relationship of root systems with above ground growth. An aeroponic technique for growing, observing and manipulating sugarcane root systems was designed and constructed as a means of establishing the above relationship. Data on growth of three cultivars with different shoot:root ratios and the effect of root pruning are presented.

### **2.2 Materials and methods**

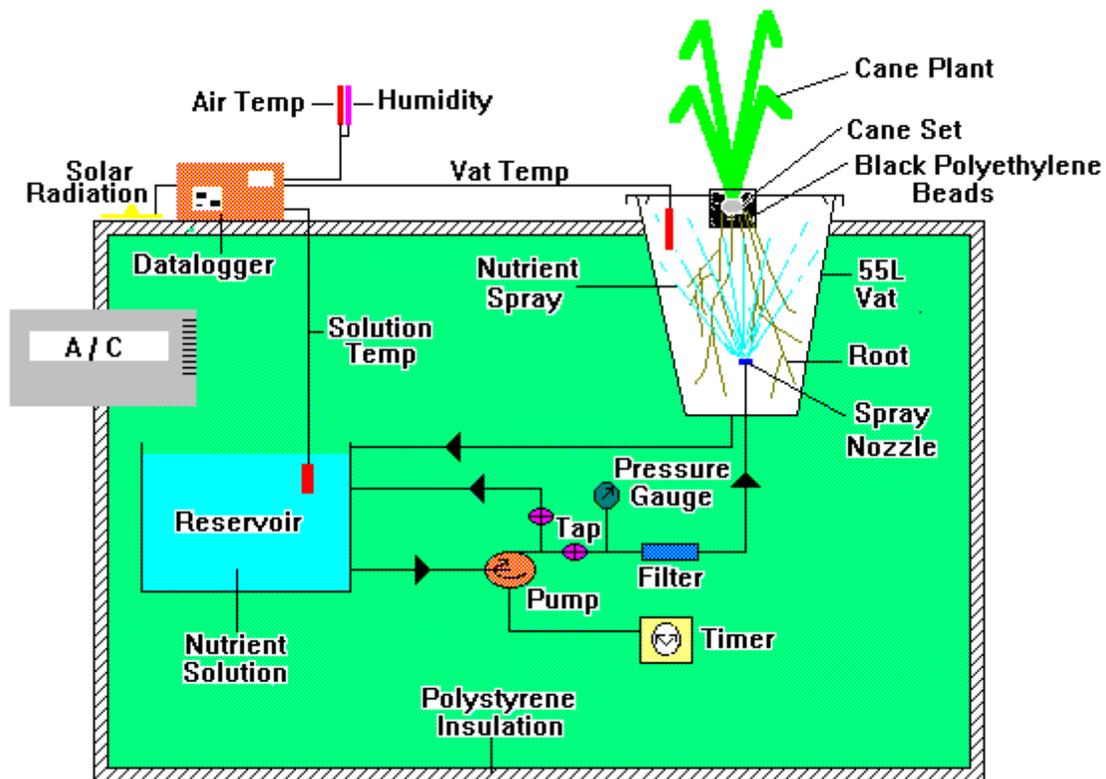
Aeroponics is defined as the culture of whole plants whose roots are suspended in and fed by nutrient solution spray. Weathers and Zobel (1992) have suggested aeroponics as the optimum soil-less culture system, because root temperature, nutrition, moisture and gaseous phase can be controlled. Previous aeroponic systems (Smucker and Erickson 1976; Zobel *et al.* 1976) were more complicated than the design reported in this paper.

The initial aeroponic facility constructed for this project (Figure 5) consisted of ten circular 55 L black, food-grade polyethylene vats, 555 mm in height and 490 mm in diameter. The number of vats was increased to 40 for the second experiment. Lids were painted white to reduce heat load and were modified by the addition of a second lip to eliminate light and prevent leakage. Holes (67 mm diameter) were drilled in each lid for plant access. Nutrient solution was sprayed onto roots through twin foggers each rated at 28 L/h (at 405 kPa), situated at the base of each vat. Nutrient solution drained from ten vats to a common graduated reservoir holding 40 L. A timer set to 15 min on, 15 min off, operated a 0.6 kW pump which supplied nutrient solution under pressure to the foggers. The system including

pump, reservoir tank and mist chambers were enclosed in an air-conditioned bench, similar to that used for glasshouse pot trials (Reghenzani 1984).

Single eye setts were germinated in 76 mm planter pots filled with black, high density polyethylene beads, under a 200  $\mu\text{m}$   $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  spray. Plants were graded on size and transferred to the aeroponic system two weeks after planting. As far as possible, plants of similar size were placed within each of the ten replicate groups. A commercial hydroponic twin pack powder (HydroLogic) supplied by Growth Technology, South Fremantle was used to make the nutrient solution. Elements and their nominal concentration (mg/L) when made up according to directions were: N(220); P(31); K(280); Ca(160); Mg(50); S(66); Fe(3); Mn(1); B(0.35); Zn(0.20); Cu(0.15); and Mo(0.05). When made up, the solution contained 2.5 g/L total dissolved solids, with an electrical conductivity of 2.25 mS/cm. Solution pH was adjusted to 6.0 using 1M KOH. The nutrient solution was changed every week unless it was necessary to replenish mid-week due to high plant water usage.

**Figure 5: Schematic layout of the aeroponic facility**



### **2.2.1 Preliminary trial**

Three sugarcane cultivars with a wide range of shoot:root ratios were chosen for the preliminary evaluation trial. The cultivars were Q78 (low ratio), Q138 (intermediate ratio) and Q162 (high ratio). Observations of root number, and length were made at two-week intervals until the twelfth week, when the trial was harvested. An additional observation was made at the eleventh week due to rapid plant growth. After harvest, plants were partitioned to shoots, stool, sett and shoot roots. Roots were scanned and classified as primary, secondary and tertiary roots, according to diameter. Dry weight was obtained for all components.

### **2.2.2 Root pruning experiment**

Four sugarcane cultivars were used in this experiment. In addition to the cultivars listed above, a fourth cultivar, Q114, was included in this experiment because of tolerance to *Pachymetra* root rot. One objective was to determine if there was some intrinsic character of the Q114 root system that may account for this property. Roots were pruned once per week according to the following schedule:

1. Control, no root removal;
2. 50% root removal, based on root length;
3. 50% root removal, based on root number;
4. 80% root removal, based on root length;
5. 80% root removal, based on root number;
6. 95% root removal, based on root length;
7. 95% root removal, based on root number;
8. removal of all root tips.

The weekly schedule of pruning was kept relative to the control. The root system of the control was inspected weekly and the appropriate measure calculated for each treatment. For instance if the control plant contained ten roots with average length of 200mm, then the roots on treatment two plants for that cultivar were pruned to 100mm and roots on treatment three plants were pruned by removing entire roots, leaving only five. Similar harvest data were recorded for this experiment as for the previous trial.

## **2.3 Results and discussion**

### **2.3.1 Preliminary trial**

The initial experiment was conducted to identify and solve problems with the system, to determine if cultivars reacted as they did in the field with respect to shoot:root ratio, to observe plant growth, and if this was restricted, to correct factors which may have caused the problem. Within the first week of transfer to the aeroponic system, Q78, known to be susceptible to iron deficiency, showed severe iron chlorosis. As a single foliar spray with 1% iron sulfate solution overcame the problem, an iron spray treatment was used routinely for future trials. There was some difficulty in differentiating between sett and shoot roots for the cultivar Q78, otherwise ease of observation of developing root systems was excellent.

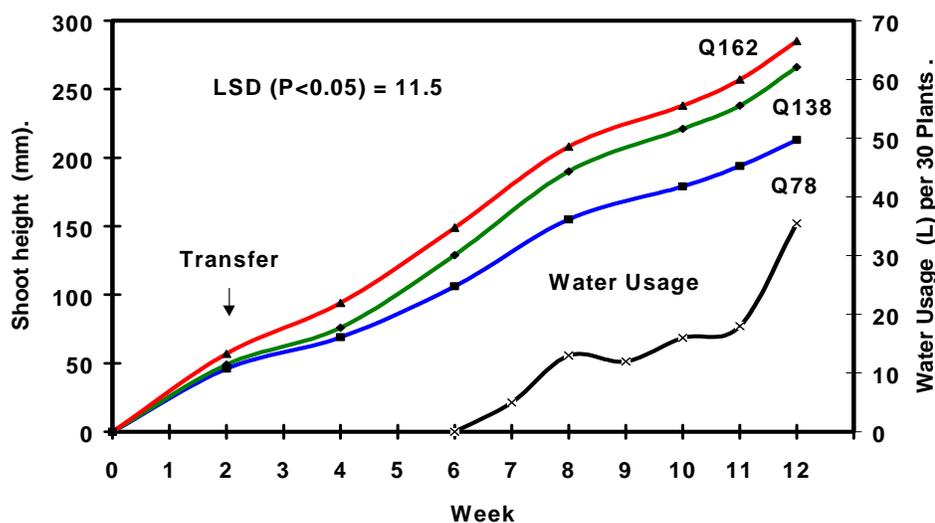
#### **2.3.1.1 Shoot:root ratio**

At the conclusion of the experiment, the three cultivars ranked according to shoot:root ratio in the same order as for previous pot and field trials ie Q162, Q138>Q78 ( $P<0.05$ ). Ratios were 3.39, 3.24 and 2.59 respectively. The above finding shows that in aeroponic culture, sugarcane cultivars reacted as expected. Maximum dry weight of shoots at an approximate shoot:root ratio of 3.5 is evidence of the inter-relationship between these two plant components.

### 2.3.1.2 Water use

Progressive water usage was monitored during the trial. There was no appreciable usage until week seven (Figure 6). Use over weeks 9 - 11 was depressed due to overcast conditions, while the increase in week 12 was consistent with fine, hot conditions, rapid growth and tillering of plants. As determined from the reservoir solution, both water and nutrient uptake can be used as progressive, non-destructive indicators of root system activity.

**Figure 6: Primary shoot height and water use for three sugarcane cultivars grown in aeroponic culture**



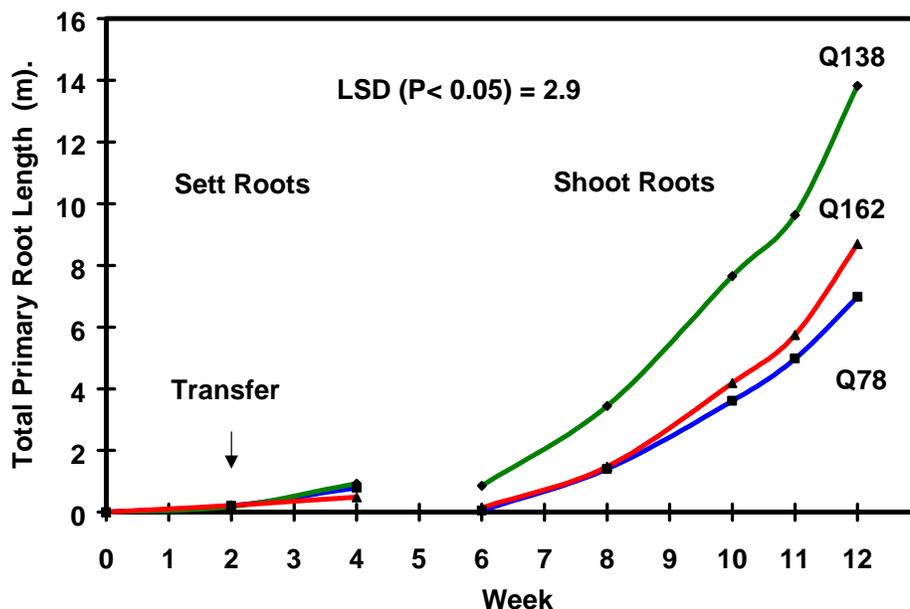
### 2.3.1.3 Primary shoot height

There was a near linear increase with time in shoot height for all three cultivars (Figure 6), indicating no restriction to growth, except from slight slowing on transfer from the germination facility and during the period of overcast conditions. Analysis of shoot height data indicated highly significant ( $P < 0.001$ ) effects due to cultivar (Q162 > Q138 > Q78) and time (week 12 > 11 > 10 > 8 > 6 > 7 > 4 > 2 > 0). Observed primary shoot growth compared well with plants in the field and differences between cultivars were consistent with known genetic characteristics.

### 2.3.1.4 Total primary root length

Only sett roots were apparent until week four (Figure 7). By week six, shoot roots emerged and their length then increased at an exponential rate. Analysis of progressive weekly data showed a very significant ( $P < 0.01$ ) root length difference due to cultivar, (Q138 > Q162 = Q78) and a highly significant ( $P < 0.001$ ) effect due to time (week 12 > 11 > 10 > 8 > 6 = 4 = 2 = 0). As for shoot growth, root length appeared to be increasing at a satisfactory rate, with significant cultivar differences due probably to differences in genetic potential.

**Figure 7: Total primary root length for three sugarcane cultivars grown in aeroponic culture**

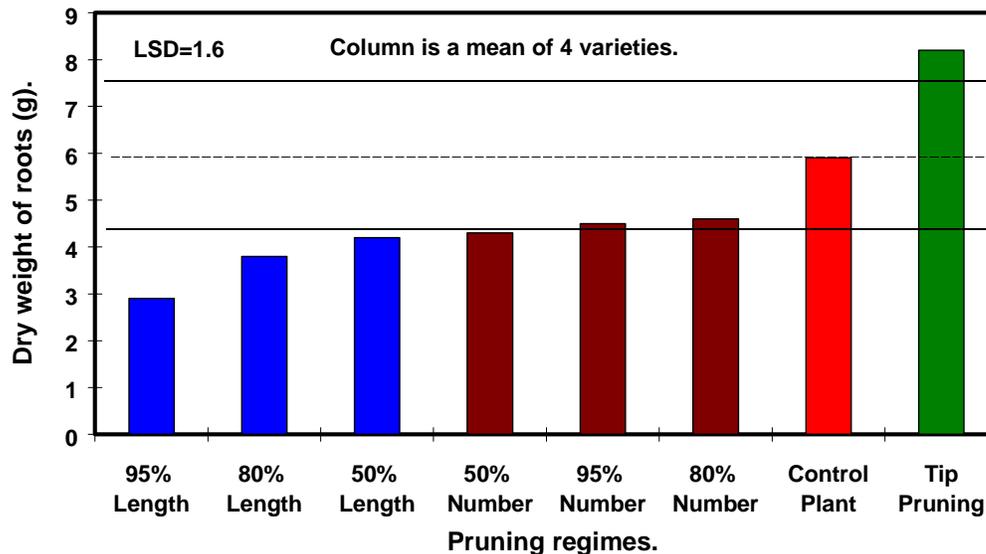


## 2.3.2 Root pruning experiment

### 2.3.2.1 Dry weight of roots

The effect of pruning regime on root dry weight at harvest is presented in Figure 8. Pruning based on root number resulted in a significant reduction in root dry weight compared to the control and was obviously more severe than pruning based on length. The most severe pruning regime, removal of 95% of entire roots, resulted in a 51% reduction ( $P < 0.05$ ) in root dry weight compared to control plants. Surprisingly, tip cutting, despite removing only a small amount of root material, encouraged prolific root growth, resulting in a 39% increase in dry weight of roots ( $P < 0.05$ ) (c.f. control).

**Figure 8: Dry weight of roots of sugarcane grown aeroponically**



for 12 weeks with various root pruning regimes

Despite large differences in degree of pruning, except for removal of 80 and 95% of entire roots, there were little measurable effects on root dry weight. This fact indicates substantial capacity for compensation by the sugarcane plant. Cultivars did not respond differently to imposed pruning treatments ie cultivar\*treatment interaction was not significant.

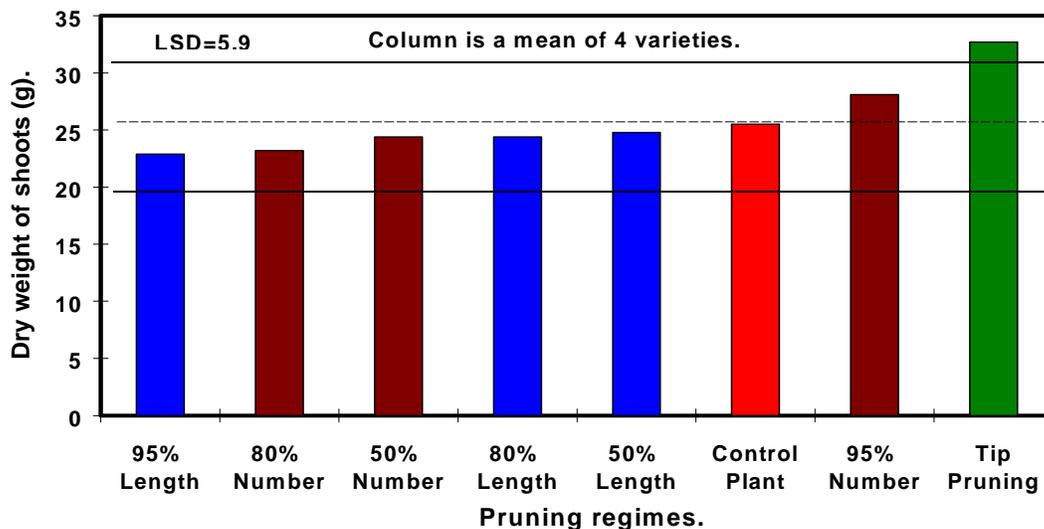
### 2.3.2.2 Dry weight of shoots

Cultivars responded similarly to imposed pruning treatments ie cultivar\*treatment interaction was not significant. All pruning treatments with the exception of tip removal did not significantly influence dry weight of shoots (Figure 9). This finding indicates that under ideal conditions within the aeroponic facility of optimum temperature, lack of pathogens, plentiful moisture and nutrient supply, sugarcane can cope with substantial root damage. The associated hypothesis is that in the field, where cane is suffering from yield loss due to a debilitated root system, roots must be functioning at something less than five percent

potential capacity. An increase of 28% ( $P < 0.05$ ) in shoot dry weight due to removal of small amounts of root tips was a surprising finding. If severe pruning of roots did not decrease shoot growth, why did increased root mass increase shoot growth?

This result could be indicative of the presence of root/shoot signals associated with the meristematic tissue of the root tip. A hypothesis is that the strong root/shoot relationship is a conservative survival mechanism. It is possible that shoots do not grow to the full potential of the root system, with growth restricted to accommodate possible future difficult conditions. Removal of the root tips may loosen this restriction. Another possibility is that the profusion of side branching and associated presence of vastly increased number of root tips, before removal at the next pruning, may increase auxin production and shoot growth. In either case, there are possible benefits to be obtained by understanding the relationship between shoots and roots and manipulating this relationship to gain increased productivity.

**Figure 9: Dry weight of shoots of sugarcane grown aeroponically for 12 weeks with various root pruning regimes**



### 2.3.2.3 Scanned total root length

There was a highly significant ( $P < 0.001$ ) relationship between scanned total root length and dry weight of tops:

$$\text{Dry weight of tops (g)} = 3.387\text{E-}05 * \text{Scanned total root length (mm)} + 16.05$$

$$R^2 = 0.54$$

This finding indicates that scanned total root length is more indicative of yield than dry weight of roots. The effect of pruning on measured root length presented in Table 1 shows that the strategy of removing entire roots is more severe than pruning based on length, consistent with the earlier comment, based on dry root weight. Despite removal of all but five percent of roots relative to the control treatment, in the most severe pruning regime, 40% of root length remained. This fact indicates the large potential for the plant to compensate even for severe root loss. The degree of compensation was sufficiently large to result in no significant difference between the control treatment and pruning to 50 and 80% by length.

**Table 1: Effect of pruning on scanned total root length**

<b>Pruning treatment</b>	<b>Scanned total root length (m) <sup>(1)</sup></b>		<b>Percent of control</b>
Tipping	504	a	136
Control	371	ab	-
80% by length	308	abc	83
50% by length	294	abc	79
95% by length	280	bc	75
50% by number	220	bc	59
80% by number	164	bc	44
95% by number	149	c	40

<sup>(1)</sup> Numbers followed by the same letter are not significantly different. (P<0.01)

## **2.4 Conclusions**

- The aeroponic culture method used in these experiments provided an excellent way to study sugarcane root systems in soil-less culture.
- Highly significant varietal differences in shoot and root growth, consistent with field responses, suggests that relationships established in these studies may be extrapolated more widely.
- Root pruning experiments suggest that the sugarcane plant does not fully utilise root systems when growing under ideal conditions. Productivity losses resulting from soil constraints in the field implies that root system efficiency is less than 5% or root length is less than 40% compared to a “healthy” root system.
- There is preliminary evidence to suggest root signals could be involved in root:shoot dynamics.

### **3.0 THE EFFECTS ON ROOT AND SHOOT GROWTH OF VARIOUS SOIL CONSTRAINTS**

Rob Magarey and David Grace, BSES, Tully.

#### **3.1 Introduction**

There are a number of different soil treatments which markedly affect sugarcane growth, including nitrogen fertilisation, soil fumigation with methyl bromide, and crop rotation. Detailed measurement of shoot growth has been made with some of these treatments, but few root growth measurements. Even under controlled conditions (eg glasshouse), most measurements have been of total root mass; little information on the effect of these treatments on specific root parameters has ever been gathered.

These treatments provide variation in both root and shoot parameters, and it was hoped that by measuring a range of root parameters, important ones governing shoot growth could be identified through correlative, and regression statistics. The purposes of the studies reported in this chapter were therefore two-fold:

1. to measure the effect of treatments on root growth;
2. to correlate key root parameters with shoot growth.

Most of the research was conducted under controlled conditions to enable the measurement of many root parameters (both in number of parameters, and speed). This allowed a number of experiments to be undertaken with a greater range of treatments. In one set of experiments, the relationship between glasshouse and field data was examined.

#### **3.2 Materials and methods**

##### **3.2.1 Glasshouse experiments**

Glasshouse experiments were undertaken under standard conditions, as detailed below.

Soils were sieved (0.5 cm aperture) to remove rocks, mixed thoroughly by hand, and weighed (1.40 kg dry weight) into 15 cm diameter terra cotta pots. Plants for experiments were pre-germinated from single-bud cuttings of sugarcane grown in University of California potting mix (Baker, 1957). When plants were 10-20 cm high they were transplanted into the terra cotta pots. Each pot contained one pregerminated plant which was fertilised with N, P and K, and with a basal dressing of trace elements (Hortico Trace Element Mixture at 1.65 g/pot, which contains 22% K, 2% Mg, 1% Fe, 1% Mn, 0.8% Cu, 0.8% Nz, 0.2% B, 0.1% Mo, 13% S). Plants were transferred to an airconditioned bench (Reghenzani, 1984) in a glasshouse at Tully Sugar Experiment Station and maintained for six weeks at 25-30° C. Pots were sub-irrigated using 2 cm deep clay saucers with water maintained in the saucers using an automatic drip irrigation system.

At harvest, roots were carefully washed free of soil and plant parameters measured.

### 3.2.2 Root measurements

#### *Image analysis equipment*

A flat bed scanner (Hewlett Packard HP: ScanJet 3c scanner) was used in association with Delta-T software (Delta-T Devices Ltd: Delta-T Scan) to measure a number of root parameters. Using a microscope graticule, the Delta-T software was calibrated to measure the different types of roots based on diameters of primary, secondary and tertiary roots. Mean root diameters and ranges for each root type are detailed in Table 2. Parameters measured included primary, secondary and tertiary root lengths, root diameter, and the derived variables total root length, and the ratio of primary:secondary:tertiary root lengths.

**Table 2: Mean root diameters and range for glasshouse grown sugarcane roots**

		Root diameter and range (mm)		
		Primary	Secondary	Tertiary
Glasshouse grown root systems	Mean	2.13	0.54	0.15
	Range	0.80-3.72	0.18-1.12	0.06-0.46
Delta-T Scan Intervals	Range	1.0-5.0	0.5-1.0	0.085*-0.50

\* Minimum diameter interval (mm) at 300 DPI resolution. Delta-T Scan.

#### *Other techniques*

In some experiments, total primary root length was measured by hand using a ruler. Root dry weight was calculated by cutting all roots from a plant, placing them in a paper bag and drying at 70°C until constant weight was reached. In order to remove any influence of soil attached to roots, the roots were separated from remnant soil using an air stream, and the remnant soil and bag reweighed; this weight was subtracted from the original weight to provide a “net root dry weight” figure.

Where *Pachymetra* root rot was present, the number of rotted and healthy primary roots per plant were counted, and the proportion of those rotted calculated. A percent rot figure is known to be related to the level of *Pachymetra* root rot inoculum present in the soil (Magarey, 1989).

Root diameters were measured using the root imaging equipment and also by hand-held vernier callipers (primary roots) in some experiments.

### **3.2.3 Experiments**

Experiments investigating the relationship between root parameters and shoot growth included the following treatments known to influence shoot growth: nitrogen, rotation crops, soil pasteurisation and *Pachymetra* root rot.

#### **3.2.3.1 Nitrogen**

##### **Experiment 1 (Glasshouse)**

A Tully series soil (Murtha, 1986) was obtained from the Tully Mill area, and prepared for a glasshouse experiment as described above. N was added to pot soils at doses of 0, 0.23, 0.046, 0.069, 0.092, 0.115 and 0.138g urea per pot representing field application of 0, 20, 40, 60, 80, 100, and 120 kg N/ha. The urea was applied in 100 ml of aqueous solution to the surface of pot soils.

##### **Experiment 2 (Glasshouse and Field)**

In a follow up experiment, the effect of N and fumigation on growth in both the glasshouse and field was compared. The field experiment was conducted on Tully Sugar Experiment Station between December 1995 and November 1996. Setts of the variety Q114 were pregerminated in a glasshouse and planted into field plots (4 metres x 3 metres). Uniform plants were selected and planted on a two-by-three grid pattern with one-metre spacing between plants. Fertiliser was applied to plots by hand before planting, with soil incorporation achieved using one pass of a rotary hoe. P was applied to all plots at 20 kg/ha (as Trifos) and K at 100 kg/ha as muriate of potash. Some plots were fumigated with methyl bromide at the same time.

Briefly, the fumigation process involved the following: black plastic was laid over the plot surface and buried around the edges to maintain a seal; methyl bromide was released in gaseous form under the plastic at 1,000 kg/ha. After two days, the plastic was removed and the soil aired to facilitate the venting of any remaining methyl bromide (Magarey and Croft, 1995).

The following treatments were applied: 0, 50, 100 and 200 kg/ha of nitrogen (N) with an additional treatment of fumigated soil with N applied at 100 kg N. Nitrogen was applied as urea. A completely randomised design was used with two replicates. The crop was grown for 12 months before yield measurements were made in December, 1996. This was achieved by hand-harvesting above-ground growth, with the weight of millable sugarcane recorded.

Root growth was investigated by taking soil cores to 70 cm depth using a 4 cm diameter soil corer inserted into the ground using an electric hammer. Cores were divided into sections (0-15, 15-30, 30-45, 45-70 cm) and roots within each soil core section washed out separately. Ten cores were taken from each plot, 30 cm from the centre of the stool. Root sampling was undertaken in November, 1996. Root lengths (Magarey and Grace, 1997) and root dry matter were recorded.

A glasshouse experiment was conducted in January-March 1996, using soil collected from the field site after treatments were applied. Soil sampling depth was 25 cm. Several samples were taken from plots to ensure appropriate representation. Other methods were as described above for glasshouse experiments.

### 3.2.3.2 Rotation

The Yield Decline Joint Venture, involving scientists from CSIRO, DPI, DNR and BSES, has been maintaining five rotation field experiments in different regions of Queensland. Locations include Tully, Ingham, the Burdekin, Mackay and Bundaberg (Garside *et al.* 1999). Similar crops have been rotated with sugarcane in each experiment, including pasture (*Brachiaria decumbens*, and pinto peanut), bare fallow, crop (soybean, peanuts, maize), and continuous sugarcane. Soils from these sites were used in glasshouse experiments to determine the effect of rotation on shoot and root growth. Details of the crops grown, and rotation periods, for each site are included in Table 3. Large treatment effects offered another opportunity to relate changes in root parameters to shoot growth.

Soils from each rotation trial were collected and prepared as described above for glasshouse experimentation. After six weeks growth in the glasshouse, soil was washed away from root systems, the shoots dried to constant weight and weighed, and the root system processed for root parameter measurement. Because of the large amount of root material, it was logistically impossible with the large number of treatments to measure all roots. Root systems were therefore pulled apart, and representative samples selected for measurement. Total figures were calculated using the known proportion of the sample taken for measurement (based on root number). Parameters measured included primary root number, primary root length, secondary root length, tertiary root length and primary root diameter. Derived variables included total root length, and ratios of primary:secondary:tertiary root lengths.

**Table 3: Details on the crops grown, and rotation periods, for rotation sites where soil was collected for glasshouse experiments**

Rotation Site	Rotation crops	Sampling dates	Rotation periods (months)
Tully	Soybean, maize, peanuts	June, '95	18
		Sept, '96	30
Herbert	Soybean, peanuts	May, '96	18
Burdekin	Soybean, peanuts	May, '96	24
Mackay	Soybean, peanuts	May, '96	18

### 3.2.3.3 Pasteurisation

Soil pasteurisation is known to greatly enhance root growth in a range of crops (Magarey and Bull, 1994; Magarey, 1996). Soil pasteurisation effects were investigated using soils from the rotation trials. Soil from each treatment was weighed into the terra cotta pots, and half of the total number of pots heated to 100°C for 90 mins in an autoclave (Atherton, Brisbane). After cooling, plants were transplanted into the pasteurised soils and grown for six weeks in the glasshouse along with the plants growing in untreated soils. Root parameters were measured and compared to data for root and shoot growth in untreated soils.

### 3.2.3.4 Pachymetra root rot

Pachymetra root rot is a well characterised root disease of sugarcane unique to Queensland (Magarey, 1991, Magarey and Croft, 1995; Croft and Magarey, 1984). Research with this disease offered the opportunity to investigate how a major root disease alters root parameters and how these root effects relate to shoot growth. Pachymetra root rot leads to a rot of the primary roots and to a debilitated root system. Poor root development also leads to a loss of stool anchorage. Some poorly grown crops of susceptible varieties exhibit widespread stool tipping, where in the absence of Pachymetra root rot, the root system easily maintains the anchorage necessary to prevent tipping.

Root system effects were investigated by including varieties of widely differing resistance to the disease, with and without Pachymetra root rot. Resistance is assessed on the basis of the proportion of primary roots destroyed by the pathogen. The varieties, and their resistance rating, are detailed in the following table (Table 4).

**Table 4: Varieties included in a pot experiment examining the effect of Pachymetra root rot on root and shoot growth.**

Variety	Q114	Q78	58N829	Q120	Q117	Q113	Q132	Q96	Q90
Resistance rating	1	1	2	3	4	4	5	6	8

1 = Resistant      9 = Susceptible

Standard glasshouse procedures (as described above) were used in these studies except that UC potting mix was substituted for the field soil.

#### *Inoculation procedure*

The inoculation procedure has been described in detail elsewhere (Croft, 1989; Magarey, 1986). Isolates of *Pachymetra chaunorhiza*, the pathogen causing Pachymetra root rot, were grown on corn meal agar (150 ml agar per aluminium foil plate) for 21 days at 28°C. After this period, when abundant oospore production had occurred, plates were macerated

in water using a kitchen blender, the spore concentration calculated, and potting mix inoculated at 63 spores/gram of soil. Equal volumes of disease-free potting mix were produced to provide a no-disease comparison.

The potting procedure, and growth conditions for the glasshouse experiment were the same as described previously. At harvest, root systems were washed free of potting mix, and assessed for *Pachymetra* root rot. Root parameters were measured using the flat bed scanner, and root and shoot dry weight recorded. The results were analysed using ANOVA and regression statistics, relating root disease variables with shoot weight etc.

### 3.2.4 Statistical analysis

Data were analysed using analysis of variance (Statistix IV, NH Analytical Software, Roseville, Minneapolis, USA). In addition, relationships were also investigated using correlative techniques and graphs. These were drawn using Sigmaplot software (SPSS, San Rafael, CA).

## 3.3 Results

### 3.3.1 Nitrogen

#### Experiment 1 (Glasshouse)

The effect of N on root and shoot parameters is detailed in Table 5. Greater doses of N led to a significant increase in shoot and root dry weight ( $P < 0.05$ ). The relationship between shoot weight and N was described by the equation, shoot weight =  $6.51 + 0.06 N$  ( $r^2 = 0.86$ ,  $P < 0.05$ ). Root parameters also varied with increasing N. Total primary root length increased substantially in response to applied N ( $r = 0.79$ ), while mean primary root length was negatively correlated with N dose ( $r = -0.35$ ). If the 0 N datum is omitted (and there were several very long roots in the 0 N treatment) there was an excellent negative correlation between mean primary root length and N ( $r = -0.93$ ).

**Table 5: The effect of nitrogen dose on root and shoot growth of sugarcane**

<b>Nitrogen treatment</b>	<b>Shoot weight</b>	<b>Root Weight</b>	<b>No. of primary roots</b>	<b>Mean primary root length (mm)</b>	<b>Total primary root length (mm)</b>	<b>Mean primary root diameter (mm)</b>
0 kg/ha	5.03	1.92	15	536	3691	1.14
20	8.73	3.18	21	816	7353	1.24
40	8.96	3.04	26	779	7986	1.23
60	11.24	3.82	29	763	9789	1.35
80	11.42	3.42	28	610	8920	1.34
100	13.53	3.75	28	552	8887	1.36
120	12.50	3.27	38	587	9384	1.24

## Experiment 2 (Field Trial)

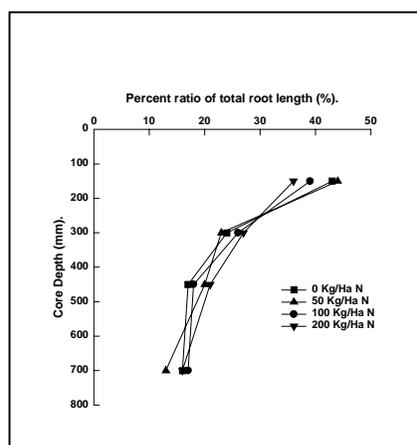
*Nitrogen* - Nitrogen increased shoot growth at low N levels, but there was no increase above 50 kg/ha applied N (Table 6).

**Table 6: The effect of nitrogen on plant parameters in the field**

Nitrogen dose (kg/ha)	Stalk no. (per plot)	Shoot wt (kg/plot)	Root wt. (g)	Prim. Root Length (m)	Sec. Root length (m)	Tert. Root length (m)	Total root length (m)
0	96.5	180.5	4.54	11.52	9.62	157.55	178.69
50	106.5	237.0	5.05	11.67	9.81	163.94	185.42
100	110.0	238.5	3.67	8.69	7.91	127.38	143.98
200	109.5	238.5	4.92	12.02	10.45	167.29	189.76

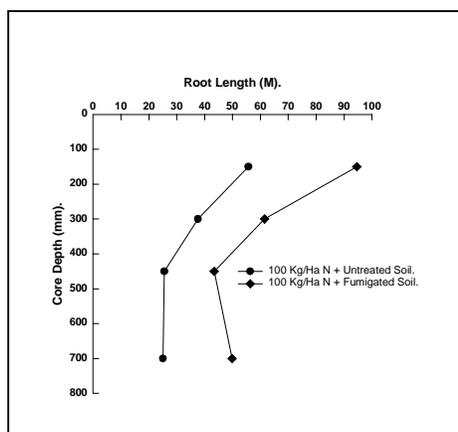
Root length varied considerably down the profile, being highest in the 0-15 cm range, and lowest at 45-70 cm depth as anticipated. Total root length down to 70 cm depth was of the order of 179-180 m within the 4 cm diameter cores. Nitrogen had no significant effect on root length (Figure 10).

**Figure 10: The proportion of root length down the profile as affected by nitrogen treatment**



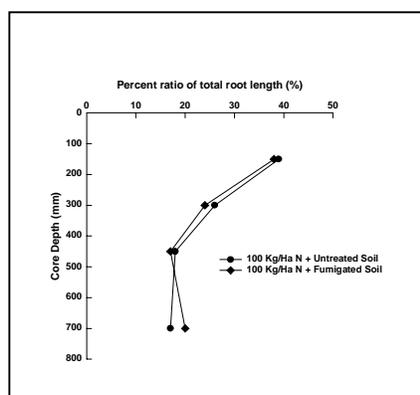
*Fumigation*: Fumigation led to a positive response in both stalk number, and shoot weight (Table 7). Root length was increased by 50-75% as was root weight within the soil cores (Figure 11).

**Figure 11: The effect of soil fumigation with methyl bromide on root length down the profile in a field trial on Tully Sugar Experiment Station**



When the proportion of root length down the profile was compared, fumigation had no measurable effect on root distribution (ie the proportion of the root system down the profile) within the zone measured (Figure 12).

**Figure 12: The effect of soil fumigation on the proportion of root length down the profile**



**Table 7: The effect of soil fumigation on plant parameters in the field**

Plant parameter	Stalk no. (per plot)	Shoot wt. (kg/plot)	Root wt (g)	Prim. Root length (m)	Sec. Root length (m)	Tert. Root length (m)	Total root length (m)
<i>Treatment</i>							
No fumigation	110.9	238.5	3.67	8.69	7.91	127.37	143.98
Fumigation	117.5	264.0	6.35	14.47	11.86	223.00	249.33

### Experiment 3 (Glasshouse Trial)

*Nitrogen:* Response to 200 kg applied N were large, with a 268% increase in both shoot and root weight compared to no applied N. Other responses generated included large increases in primary root number, primary root diameter, and total root length. The data are presented in Table 8. As expected, greater root growth responses to N application occurred when existing N levels were low. Nitrogen tended to increase all plant parameters, except mean primary root length. At low N, mean length was reduced, it increased at intermediate application dosages and decreased again at high dose.

**Table 8: The effect of nitrogen on plant parameters in a glasshouse experiment**

Nitrogen (kg/ha)	Shoot wt. (g)	Root wt. (g)	No. primary roots	Mean prim. Length (mm)	Prim. root diam. (mm)	Prim. root length (m)	Sec. root length (m)	Tert. root length (m)	Total root length (m)
0	3.00	0.91	12	339	1.14	3.34	4.00	46.49	53.82
50	4.56	1.62	17	443	1.26	5.16	6.93	82.15	94.23
100	6.71	2.55	22	461	1.24	8.20	10.68	126.68	145.56
200	11.05	3.35	29	416	1.43	9.90	12.21	137.50	159.62
+r-value	0.99	0.98	0.99		0.95	0.97	0.94	0.93	0.92

+ r-value is a correlation between the measured parameter and N dose.

*Fumigation:* Soil fumigation significantly increased shoot weight by 63%, root weight by 32%, and total root length by 23% (the latter was not significant). Fumigation also had large effects on primary root number, primary root diameter, and total root length (Table 9).

**Table 9: The effect of soil fumigation on plant parameters in a glasshouse experiment with 100 kg/ha (equivalent ) applied N**

Treatment	Shoot wt. (g)	Root wt (g)	No. prim. roots	Mean prim. length (mm)	Prim. root diam. (mm)	Prim. Root length (m)	Sec. Root length (m)	Tert. Root length (m)	Total root length (m)
No fumigation	6.71	2.55	22	461	1.24	8.20	10.68	126.68	145.56
Fumigation	10.95	3.36	34	419	1.39	9.46	12.57	158.24	180.27

The proportion of primary:secondary:tertiary root length was calculated for both glasshouse and field grown root systems and the data are presented in Table 10.

**Table 10: The effect of soil treatments on primary:secondary:tertiary root length ratios in both glasshouse and field experiments**

Treatment	Glasshouse			Field		
	Primary	Secondary	Tertiary	Primary	Secondary	Tertiary
Nitrogen (kg/ha)						
0	6	8	86	6	6	88
50	6	7	87	6	5	88
100	6	7	87	6	6	88
200	6	8	86	6	6	88
Fumigation						
No	6	7	87	6	6	88
Yes	5	7	88	6	5	89

### 3.3.2 Rotation

Rotation treatments generally had a significant influence on both shoot and root growth with variable effects on root parameters. Changes in root system parameters are detailed in Table 11.

**Table 11: Changes in root system parameters, and their correlation to shoot growth, in experiments with soils from the various rotation field experiments**

Rotation site	Significant ( $P < 0.05$ ) rotation effect		Root Parameter correlated to shoot growth	r-value
	Shoots	Roots		
Tully (1995)	Yes	Yes	Tertiary root length	0.49
(1996)	No	No	Total root length	0.48
			No. of primary roots	0.64
Herbert	Yes	Yes	Mean primary root length	0.46
			Total primary root length	0.45
Burdekin	Yes	Yes	Primary root number	0.48
Mackay	Yes	No	Total root length	0.42
			Tertiary root length	0.42

#### 3.3.2.1 Soil pasteurisation

Soil pasteurisation led to a much lighter colouring of root systems growing in treated soils. Root and shoot growth responses to soil pasteurisation are detailed in Tables 12 and 13 for each rotation glasshouse experiment.

**Table 12: Changes in root system parameters, and their correlation to shoot growth, in experiments with soils from rotation field experiments**

Rotation site	Significant (P<0.05) effect on shoot growth	Root Parameter correlated to shoot growth	r-value
Tully (1995)	Yes	Tertiary root length	0.75
(1996)	Yes	No. of primary roots	0.48
Herbert	No (P = 0.07)	Primary root length	0.44
Burdekin	Yes	Ratio P:S:T root lengths	0.66
Mackay	Yes	Total root length	0.75

**Table 13: The magnitude of responses to soil pasteurisation in soils from various rotation field experiments**

Rotation site	Parameter responding to pasteurisation	% increase (cf. control)
Tully 1995	Shoot weight (average all treatments)	64
	Total root length	86
	Primary root length	65
	Secondary root length	61
	Tertiary root length	91
Tully 1996	Shootweight (all treatments)	21
	Primary root length	50
	Tertiary root length	34
Herbert	Shoot weight (all treatments)	8
Burdekin	Shoot weight (all treatments)	54
Mackay	Shoot weight (all treatments)	107

### 3.3.3 Pachymetra Root Rot

In research with *Pachymetra* root rot, two aspects were considered:

- (1) the effect of *Pachymetra* root rot on shoot weight;
- (2) the influence of varietal resistance on the effect of *Pachymetra* root rot on the root system.

It is often assumed *Pachymetra* has little influence on root growth in resistant varieties; this hypothesis was tested.

A summary of the relationships between *Pachymetra* root rot and root parameters is detailed in Tables 14 and 15.

**Table 14: The relationship between root parameters, *Pachymetra* root rot and shoot weight in varieties of differing resistance to *Pachymetra* root rot**

Variety	Shoot/Root Parameter	Reduction (cf.control) caused by <i>Pachymetra</i> root rot (%)	Percent rotted roots (%)
<b>Q90</b>	Shoot weight	11	62
	Mean primary root length	63	
	Total primary root length	68	
	Root weight	57	
	Primary root number	15	
	Primary root diameter	5	
<b>Q114</b>	Shoot weight	17	19
	Number of primary roots	28	
	Total primary root length	15	
	Root weight	1 (increase)	
	Mean primary root length	15 (increase)	

**Table 15: Correlation of various root parameters (all treatments) with percent rotted primary roots, varietal resistance rating, and shoot weight**

Parameter	Root measurement	r-value
Percent rotted roots	Total primary root length	-0.81
	Mean primary root length	-0.71
Varietal resistance rating	Mean primary root length	-0.80
	Total primary root length	-0.65
Shoot weight	Total primary root length	0.56
Root weight	Mean primary root length	0.73

A summary table of the most highly correlated variables with shoot weight in each experiment is included below (Table 16).

**Table 16: Root parameters most highly correlated to shoot weight in nitrogen, rotation, pasteurisation, and Pachymetra root rot experiments**

Trial		Variable	r-value
<b>Nitrogen</b>	experiment 1	Primary root length	0.91
	experiment 2	Primary root number	0.99
<b>Rotation</b>	Tully 1	Tertiary root length	0.49
	Tully 2	Primary root number	0.64
	Herbert	Mean primary root length	0.46
	Burdekin	Primary root number	0.48
	Mackay	Total root length	0.42
<b>Pasteurisation</b>	Tully 1	Tertiary root length	0.75
	Tully 2	Primary root number	0.48
	Herbert	Primary root length	0.44
	Burdekin	Ratio of tertiary (vs primary, secondary root length)	0.66
	Mackay	Total root length	0.75
<b>Pachymetra root rot</b>		Primary root length	0.56

### 3.4 Discussion

Studies conducted in association with this research have shown that a number of soil-based treatments dramatically alter root system characteristics. Some of these changes are probably transient (for example nitrogen) though more research is needed to examine this. Nitrogen appeared to alter primary root characteristics the most in short term glasshouse experiments. Total primary root length, (increased), mean primary root length (decreased), and number of primary roots (increased) changed with increasing N application. Bosemark, (1954) found in cereals that nitrogen deficiency resulted in long, slender roots while the addition of N led to shorter sturdier root systems. This is similar to findings in the research reported here. It could be hypothesised that such a response would enable the plant to concentrate the root system in the soil containing maximum concentrations of N. In the field experiment, no difference between the proportion of the root system down the soil profile in the different N treatments after 12 months suggested that this effect did not occur in the field, or that it was transient in nature. Further testing of the hypothesis would require sequential root sampling at 1-2month periods during the course of crop growth.

Rotation treatments also significantly altered root growth. Responses varied considerably between sites and over years (Tully trials). General effects were an increase in root mass, total root length, primary root number, and mean primary root length. When shoot weight was correlated with root parameters, there was no consistently related root parameter. The mechanism for the response either varied between sites, or an important root parameter related to shoot growth was not measured.

In this study, the focus was on a 'macro' level; further studies should hone in at the 'micro' level, and could prove more successful in explaining the dynamics of shoot and root growth. Responses to crop rotation in field experiments, were also obtained in glasshouse pot experiments reported here. There is a need to further investigate the measurement of root system parameters, especially those related to root surface integrity, to monitor the effects of rotation, and other management strategies.

A similar effect was noted with soil pasteurisation. There does not appear to be a single most important root parameter (of those measured) which is strongly related to shoot weight. It should be noted that soil pasteurisation has an obvious effect on root system colour (not measured). In other experimentation, this has been associated with increased root hair density, less root surface discolouration, and better root surface integrity (Magarey and Bull, 1994; Magarey *et al*, 1995). The development of assays for these, or similar parameters, should be examined to determine if the assessment of these root qualities is more closely related to shoot growth. The efficient operation of roots no doubt relies on root surface integrity (as one important parameter), and disruption to this surface would be expected to reduce the efficiency of the absorption of nutrients and water.

Pachymetra root rot also had a major effect on root system characteristics principally reducing total and mean primary root length. Root weight was reduced much more than shoot weight in these short term experiments. In the resistant Q114, mean primary root length actually increased which reflects the ability of the variety to resist primary root infection. Total primary root length was reduced by Pachymetra in this variety however. When root system parameters were correlated (in all varieties), as expected percent rotted roots was negatively correlated with total primary root length, and mean primary root length. This appears to be the major effect of the root disease. Shoot growth across all varieties was most highly correlated with total primary root length, reflecting that resistance to the disease provided a yield advantage.

In these studies, there was a very strong and consistent relationship between primary, secondary and tertiary root growth. Despite large variation in total root lengths, and changes in root health, the proportion of primary : secondary : tertiary root length did not change. In previous research, yield decline soils supported root systems which appeared to lack fine roots. This research suggests that the growth of all root types was reduced equally. It appears that there is a very strong genetic influence on this root characteristic.

### **3.5 Conclusions**

- Soil-based treatments known to alter shoot growth have a large and varied effect on root growth (eg N fertilisation, crop rotation, soil fumigation).
- No single root parameter was consistently correlated with shoot growth in experiments where soil treatment led to increases in shoot and root growth.
- Measurements made in these experiments did not include root hair densities or root surface characteristics. Further experimentation should address this.

- A consistent ratio between primary, secondary and tertiary root lengths was found in root systems independent of soil treatment.

#### **4.0 THE RELATIONSHIP BETWEEN ROOT AND SHOOT GROWTH UNDER CONTROLLED CONDITIONS**

Jason Smith, CSIRO, Townsville.

##### **4.1 Introduction**

In the past, studies of the sugarcane root system have been largely descriptive (Lee, 1926; Evans, 1936; Srivastava and Ghosh, 1970), providing detailed descriptions of root system architecture under various soil conditions, but little information as to how crop productivity is related to the growth of the root system. Recent work has begun to look more closely at the relationship between parameters of root growth and above ground growth (Venkataramana and Naidu, 1982; Van Antwerpen *et al.*, 1993; Meinzer, 1991; Magarey and Grace; 1997), but there remains, relative to other species, little information concerning the effects of restricted root development on the growth of sugarcane.

Therefore, the aim of the series of pot studies presented in the following section was to provide a better understanding of how shoot growth of sugarcane is related to the growth of the root system, especially when the functional capacity and growth of the root system is reduced. Rather than looking at specific soil constraints, emphasis was placed on investigating the basic relationship between root and shoot growth, and the underlying physiological mechanisms, such that the findings would be applicable in a wider range of circumstances.

##### **4.2 Materials and methods**

All experiments were conducted at the CSIRO Davies Laboratory in Townsville between June, 1996 and March 1998. Statistical analysis of the data was carried out with GENSTAT ver 5.32 (Rothamsted Experimental Station, England). The ANOVA procedure was used for analysis of all treatment effects, with the exception of data presented in Figure 14, which was carried out using the backward elimination procedure of SAS ver 6.12.

##### ***Experiment 1***

###### ***a. Basic relationship between root and shoot growth***

Single plants of the varieties Q96, Q117 and Q124 were grown outside in 32 L pots filled with a 2.3:1 mix of sandy loam and peat (Kiwipeat<sup>®</sup>, Australasian Peat Limited). The pots were arranged in a fully randomised design, with three rows 1.2 m apart and 0.50 m between pot centres. Each pot was watered to excess three times a day with an automatic watering system, and fertilised monthly with 40 g of slow release fertiliser (Osmocote<sup>®</sup>, Scotts Australia Pty Ltd, N:P:K:S 19:3:8.3:4.9) and 200 ml of liquid fertiliser (Wuxal<sup>®</sup>, Schering Agrochemicals, 200 ml Concentrate:10 L water).

In the seven month period following planting on May 14, five destructive harvests were carried out to determine shoot weight (including the stool), leaf area and root mass. These measurements were made on four plants of each variety during the five progressive harvests, which were carried out 28, 53, 81, 133 and 200 days after planting. At each harvest the total green leaf area was recorded with an electronic planimeter. The weight of the above ground stem, root system, leaf and dead leaf of each plant was recorded after drying at 80°C.

*b. Root nitrogen content as an indicator of root system activity*

To assess the potential of using root nitrogen content as an indicator of root system activity, sub-samples of new root and mature root were collected at each harvest for total nitrogen analyses (Kjeldahl N *in* Rayment and Higginson, 1992). The new root material was obtained by cutting off 15-20 healthy new root tips about three centimetres from the end of the root (providing 0.1 g - 0.2 g DW). A similar amount of mature root was obtained by cutting three centimetre long pieces of dark, suberised root from close to the shoot. Both the new and mature root samples were rinsed in distilled water and dried at 70°C. After drying and weighing, the whole root system was cut up, mixed, and sub-sampled to allow total root system nitrogen content to be determined.

*Experiment 2. Response to partial root-zone drying*

Sugarcane plants of the variety Q96 were grown in 55 L pots containing 48 kg of a 3:2 sandy loam:peat (Kiwipeat<sup>®</sup>) potting mix. Each pot had a plastic divider down the centre to allow the root system to be grown into the two halves, with independent irrigation regimes. After three months growth under glasshouse conditions, during which time the plants were well supplied with water and nutrients, the experimental treatments were imposed.

The experiment, which ran for 32 days, was divided into three separate phases. In the first, or establishment, phase (days 1-9) plant growth rates were measured to establish initial growth rates under well watered conditions. In the second, or dry-down, phase (days 10-23) all pots were watered to field capacity then subject to three different treatments. For the first treatment, ten pots were maintained at field capacity, while in the second, irrigation was withheld on one side of 15 pots, so that one side would dry down while the other was maintained at field capacity. A further five pots were not watered again, and allowed to dry down for the 14 days.

At the end of the dry-down phase, recovery treatments were imposed (days 24-32), which separated the experiment into the final six treatments. Of the ten well-watered pots, five remained well watered (WWW), while the other five had all the roots in one side of the pot

excised from the plant (WWC). Any new roots that grew on this side were removed on a daily basis. Five of the partially drought stressed plants had the roots in the dry half of the soil excised from the plant (WDC), while a further five had the dry side of the pot re-watered (WDW). The remaining five were maintained with half the pot un-watered (WDD). The fully drought stressed plants had both sides of the pot re-watered to field capacity (DDW).

Plant water status was monitored by recording leaf water potential of the last fully expanded leaf at midday using a pressure chamber (PMS Instrument Company, Corvallis, Oregon, USA). Due to the large size of sugarcane leaves, and to minimise the amount of destructive sampling required, measurements were made on leaf tips rather than the whole leaf. These samples were obtained by cutting the leaf blade through to the mid-rib, approximately 30cm from the terminal end of the leaf, then stripping the tip from the midrib to provide a sample with a width of 8-10mm for insertion in the pressure bomb. Although using leaf tips results in slightly lower leaf water potential values due to the gradient along the leaf, the use of samples with partly exposed vascular structure still gives a reliable measure of leaf water status in sugarcane (Saliendra *et al.*, 1990). The time between sampling and starting chamber pressurisation was kept below 20 seconds to minimise water loss from the sample.

Stomatal conductance was determined using a Mk3 Automatic Porometer (Delta-T Devices, Cambridge, England). Measurements were made mid-morning on the lower surface of the last fully expanded leaf at approximately 75% of its length. Where possible, measurements were made in conditions of full sunlight, and on unshaded leaves.

Plant elongation rate (*PER*) was recorded by measuring the height, from a fixed point at the base of the plant, to the top of each expanding leaf. Several leaves were concurrently monitored to ensure the data obtained were always from leaves in the linear expansion phase. Stem length was measured from the same point at the base of the plant to the last visible dewlap at the top of the sheath of the most recently expanded leaf. By subtracting stem growth from the total change in plant height, leaf elongation rates could be determined. The measurement of stem elongation by measuring the height to the last visible dewlap is a standard method, (based on the fact that the sheath of the last fully expanded leaf has stopped growing and any further vertical displacement is due to elongation of the stem (Van Dillewijn, 1952)).

Re-watering of the wet side of the partial drought stress treatment and the fully watered controls was done at mid-afternoon during each day of the dry-down phase. Daily water use determined by weighing, and in the partial drought stress treatment, using 20 cm TDR probes (TRACE<sup>®</sup>, Soilmoisture Equipment Corporation, Santa Barbara, USA). During the recovery phase all treatments were watered to excess.

### ***Experiment 3. Response to defoliation, root pruning and partial drought stress***

Sugarcane plants (variety Q96) were planted in germination trays, and after three weeks, three 'seedlings' were transferred to each of 40, 55 L pots. During the following two months, in which the plants were grown under glasshouse conditions, each pot was watered freely and supplied with 100 g of 3-4 month slow release fertiliser (Osmocote<sup>®</sup>, Scotts Australia Pty Ltd, N:P:K:S 19:3:8.3:4.9) and 200 ml of liquid fertiliser (Wuxal<sup>®</sup>, Schering Agrochemicals, 200 ml Concentrate:10 L water).

Twelve weeks after planting the pots were assigned to four different treatments. In the first treatment (root pruning), half the root system was removed by excising all the roots on one side of the plant. In the second treatment (shoot pruning) all the leaves up to, and including, the last fully expanded leaf were removed. This reduced the shoot weight by 40% and the leaf area by 64%. In the third treatment, 50% of the root-zone was allowed to dry down in the same manner as in the previous experiment. In the final treatment (control), the plants remained intact. All pots, with the exception of the drying side of the partial root-zone drying treatment, were watered to field capacity at approximately 5:00 pm each day for the duration of the experiment.

Destructive harvests were undertaken 0, 5, 11, 19 and 26 days after the treatments were imposed to follow the response of plants to imposed changes in the root:shoot ratio. Plant growth and leaf water potential measurements were made during the experiment in the same manner as described in the previous section.

#### ***Experiment 4. Response to root volume restriction***

Sugarcane plants (variety Q117) were grown in a glasshouse in 0.35, 0.94, 2.4, 5.2 and 12.9 L pots which were made from 28 cm long sections of 3.8, 6.3, 10.4, 15.4 and 24.2 cm diameter PVC pipe. The base of each pot was covered with nylon fabric with a pore size of 75  $\mu\text{m}$  (Nytel<sup>®</sup>, Industrial Filters, Brisbane), that was freely permeable to water, but stopped root growth through the bottom of the pot. To prevent the pots falling over as the plants grew, all of the PVC pots were placed inside large 32 L black plastic pots and packed in with the same potting mix that was used inside the smaller pots (2:2:1 mixture of sand, river gravel and peat screened through a 2 mm sieve). In addition to the pots with closed bases, two further treatments were imposed in which plants were grown in the 2.4 and 12.9 L pot sizes without bases, such that the roots were free to grow through the bottom of the pot and into the soil of the larger 32 L pot.

All watering was done with an automatic watering system, which was initially set to water each plant for 30 minutes, three times a day. As the size of the plants increased the irrigation regime was changed so that the plants were watered for 5 minutes every half hour. Nutrients (Wuxal<sup>®</sup> and Osmocote<sup>®</sup>) were supplied to excess. Shoot growth parameters, leaf water status and stomatal conductance were recorded during the experiment, and at 138 DAP (days after planting), the plants were harvested to determine root weight, shoot weight and leaf area.

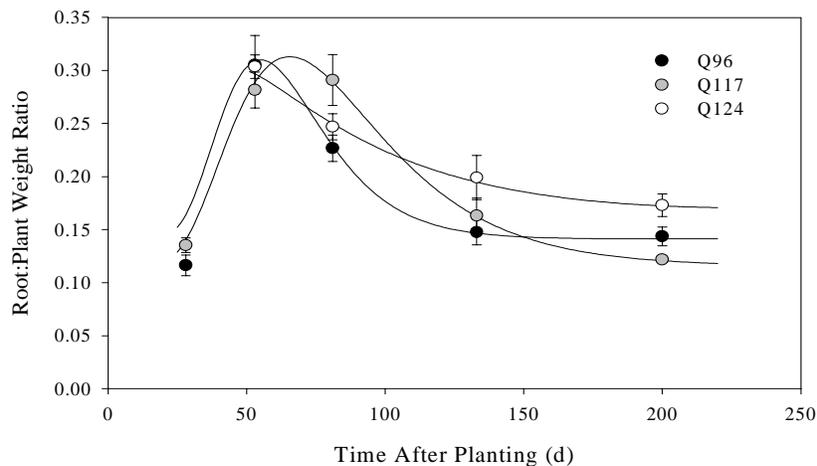
### 4.3 Results and discussion<sup>1</sup>

#### *Experiment 1*

##### *a. Basic relationship between root and shoot growth*

The root and shoot growth of three varieties of sugarcane was monitored over a seven month period, and it was found that after an initial increase, the root:shoot ratio of all three varieties decreased with plant age (Figure 13). The initial increase in the root:shoot ratio may be specific to sett grown sugarcane, as the reserves of mineral nutrients and carbohydrates within the sett could allow a degree of independence between shoot and root growth rates. However, the subsequent decrease in the root:shoot ratio with plant age is consistent with the behaviour of most other plant species (Wilson, 1988).

**Figure 13: Root dry weight, expressed as a ratio of total plant dry weight, of three varieties of pot grown sugarcane over a 200 day period. Error bars  $\pm$  SE mean (n=4).**

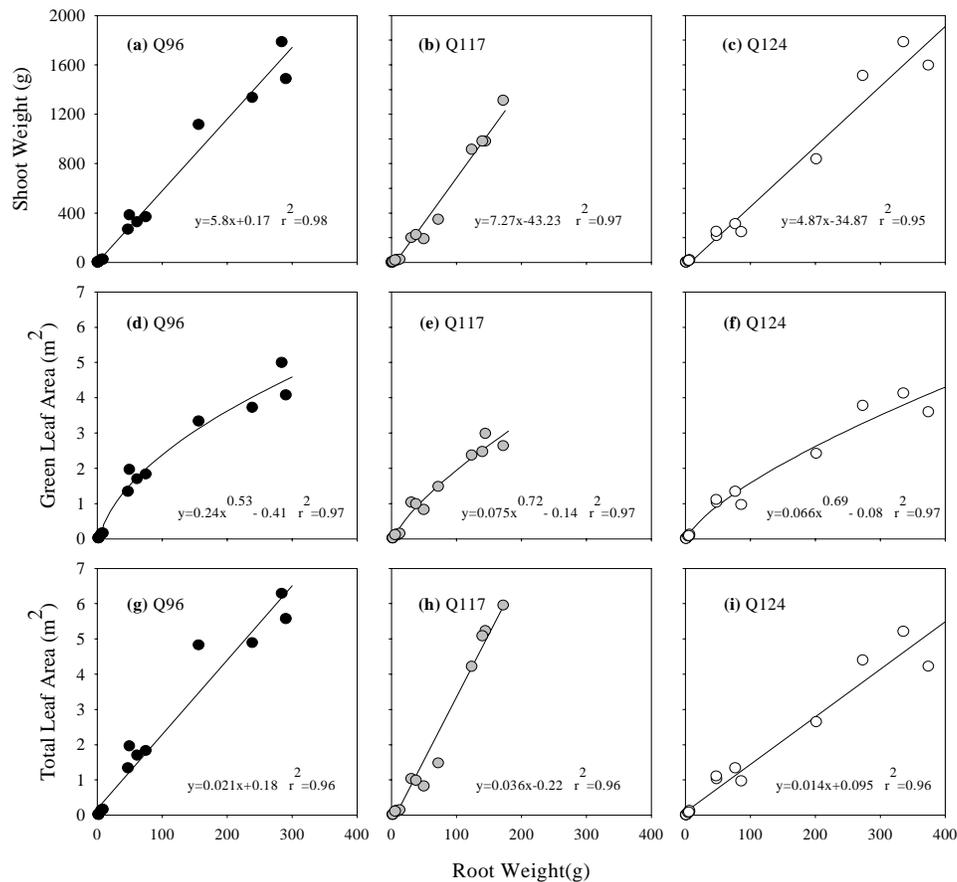


As a more functional assessment of the relationship between root growth and shoot growth, the development of leaf area was also monitored. It was found that the amount of root per unit green leaf area increased with age, but when the area of senesced leaves, as estimated from their dry weight, was included, there was also a linear relationship between leaf area and root weight. One explanation for this apparent increase in root weight relative to leaf area, is that the roots became thicker and heavier as the plants aged. However, studies carried out as part of this project have shown that the ratio of fine:medium:coarse roots remains remarkably stable across different soil conditions, varieties and time (Magarey and Grace, 1997, R Nable, personal communication), suggesting that changes in root morphology were not the reason for the increased root weight. Another explanation, and one that may account for the linear

<sup>1</sup> The data presented in this report show the main findings of the experiments only. For additional results, and a more detailed discussion of experimental findings, see Smith (1998).

relationship with root weight after the senesced leaf area was added, is that as the plants aged, an increasing proportion of the root system was made up of dead roots. As it was not possible to separate live from dead roots, an absolute increase in root length per unit leaf area cannot be ruled out.

**Figure 14: Root weight of three varieties of pot grown sugarcane, as recorded at five harvests 28, 53, 81, 133 and 200 days after planting, graphed against total shoot dry weight (a-c), green leaf area (d-f) and total leaf area including senesced leaves (g-i). Graphs (a) to (c) are on the same scale, as are graphs (d) to (i).**



Significant differences were seen between the root:shoot ratios and root weight:leaf area ratios of all three varieties (Figure 14). Q124 had the highest proportion of roots relative to above ground biomass and green leaf area, while Q96 had a higher root:shoot ratio than Q117, but a lower root weight:leaf area ratio. Nour and Weibel (1978) found that drought resistant varieties of sorghum generally had a higher root:shoot ratio than less tolerant varieties, suggesting that a larger root system may be an advantage under conditions where water shortage is likely to occur. Although there are many other characteristics that may influence the performance of plants when water supply is limited, the root:shoot ratios of the three varieties of sugarcane do appear to be consistent with the environments in which they are predominantly grown. Q124, with the highest root:shoot ratio, is generally grown under

non-irrigated conditions, while Q96 is grown almost exclusively under full irrigation. Q117 appears to contradict this observation, as it had a lower root:shoot ratio than Q96, but is grown under a wider range of environments. However, Q117 had less leaf area per unit root weight than Q96, which is likely to be more important in terms of water use than shoot weight.

**b. Root nitrogen content as an indicator of root system activity**

An alternative method to using root weight as measure of root system function, which was developed to overcome the difficulties in distinguishing live from dead roots, is to use the protein nitrogen content of the root system as an index of root system activity (Helal, 1991; Helal *et al.*, 1996). This method is based on the fact cellular proteins break down more rapidly than structural components of the roots, and by measuring the total nitrogen content of the root system an indication of the amount of living root can be obtained. In the current experiment, the root system of the three sugarcane varieties showed a similar decline in nitrogen content as the maize plants studied by Helal *et al.* (1996). However, such a decline in nitrogen content would be expected, even in plants growing under ideal conditions, as the proportion of mature roots increased relative to the number of new roots. Therefore, in the present study, this method was extended to include measurements of the nitrogen content of old and new root fractions, such that the amount of new root within the root system could be calculated.

**Table 17: Nitrogen content of different root fractions (total root system, root tip and old roots) expressed as a percentage of root dry weight**

Variety	Root Sample	Harvest				Mean
		2	3	4	5	
Q96	Root tips	3.19	3.82	2.26	2.02	2.82
	Old root	0.99	1.41	0.60	0.32	0.83
	Total	1.86	1.71	-	0.43	1.33
Q117	Root tips	3.47	3.95	2.01	2.40	2.96
	Old root	1.24	1.24	0.70	0.49	0.92
	Total	1.94	1.89	-	0.64	1.49
Q124	Root tips	2.78	3.94	2.36	2.37	2.86
	Old root	1.06	1.28	0.52	0.32	0.80
	Total	2.11	1.85	-	0.49	1.48

The nitrogen content of both the old and new root fraction declined with plant age (Table 17). The fall in the nitrogen content of the old root fraction would be expected, as in the initial harvests these roots were still relatively young, and did not show the same degree of suberisation as the roots in later harvests. The reason for the decline in the nitrogen content of the new root fraction is less clear, but may be related to structural differences of the smaller roots taken in the early harvests, than to differences in the cellular nitrogen content. Despite this, the nitrogen content of the new root fraction stabilised at around 2.2% four months after planting, and it could be expected that the nitrogen content of the old root fraction would also stabilise as the roots fully matured. If this were the case, and these values could be assumed to be constant in crops older than four-five months, then measuring the

nitrogen content of a representative portion of the total root system can not only provide an indication of the activity of the root system, but also enable the amount of new and old root system to be calculated. The following example, using the data for the root nitrogen content of Q96 from the final harvest, shows the calculations required to determine these values.

$rw_{tot}$  = Total root system weight.  $N_{tot}$  = N content of total root system ( $g\ g^{-1}\ DM$ ).  
 $rw_{new}$  = Weight of new roots.  $N_{new}$  = N content of new roots ( $g\ g^{-1}\ DM$ ).  
 $rw_{old}$  = Weight of old roots.  $N_{old}$  = N content of old roots ( $g\ g^{-1}\ DM$ ).

$$\therefore (rw_{tot} \times N_{tot}) = (rw_{new} \times N_{new}) + (rw_{old} \times N_{old}) \quad (4.1)$$

let  $rw_{old} = rw_{tot} - rw_{new}$

$$\therefore (rw_{tot} \times N_{tot}) = (rw_{new} \times N_{new}) + (N_{old}(rw_{tot} - rw_{new})) \quad (4.2)$$

$$\therefore rw_{new} = ((N_{old} \times rw_{tot}) - (N_{tot} \times rw_{tot})) / (N_{old} - N_{new}) \quad (4.3)$$

In equation (4.3), let  $rw_{tot} = 100$

$$\therefore rw_{new} = ((0.0032 \times 100) - (0.0043 \times 100)) / (0.0032 - 0.0202) \quad (4.4)$$

$$\therefore rw_{new} = 6.47$$

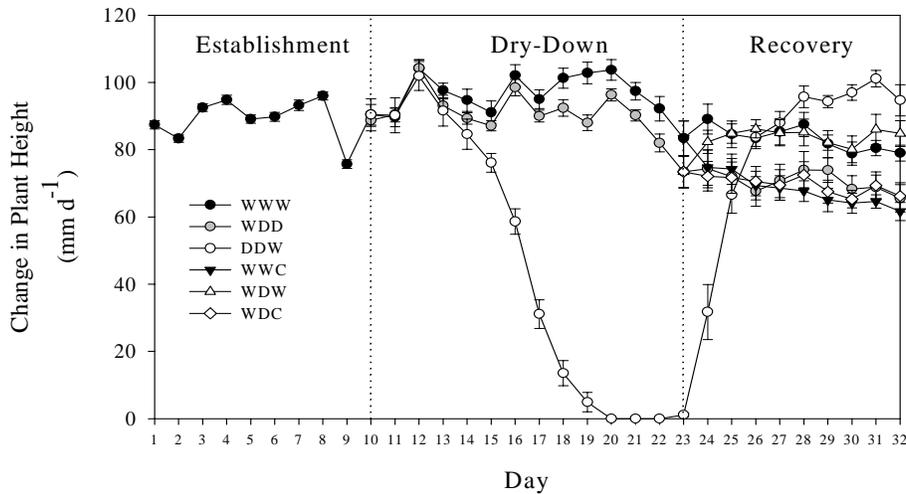
From equation (4.4), 6.47% of the root system of Q96 was new at the final harvest, while by the same calculation, Q117 and Q124 had 7.85 and 8.36% new root respectively. The fact that significant differences were found between the nitrogen content of the old and new root fractions, and that differences were seen between varieties suggests that this method may provide a relatively simple means of assessing root system activity. In the present study no treatments were applied that might change the activity of the root system, and as such these results do not say much in their own right. However, it is interesting that the three varieties ranked in the same order with respect to the amount of new roots and to the root weight:leaf area ratio. Under field conditions, where several treatments have been imposed, and where root samples can be taken from plants of the same variety and age, this method could be used to assess relative differences in root system activity. It could also be

a useful technique in pot trials, or split-root system experiments, where the change in the amount of functional root in response to adverse soil conditions may be better measured using the root nitrogen content than root weight.

**Experiment 2. Response to partial root-zone drying**

Drought stressing half the root system in vertically divided pots reduced the mean *PER* by around 10% (Figure 15), and the stomatal conductance of the last fully expanded leaf by up to 30% (Figure 16).

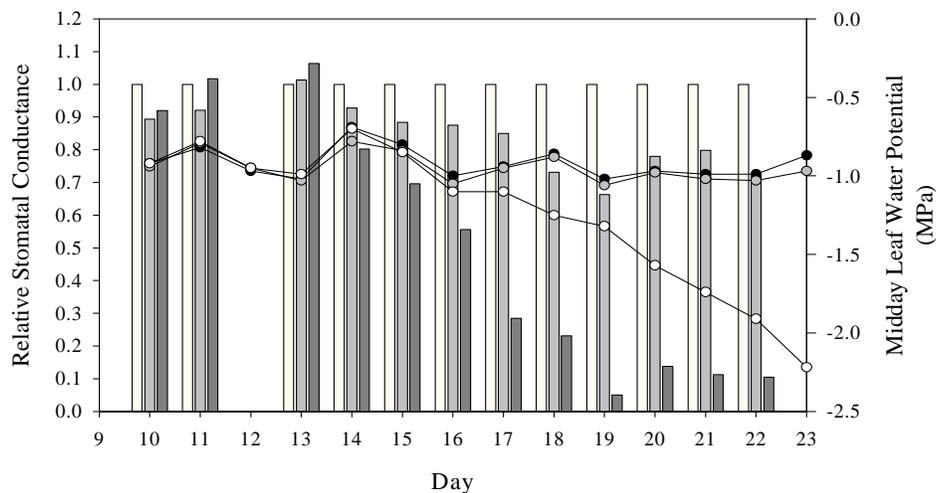
**Figure 15: Daily plant growth showing combined stem and leaf elongation over the three phases of the experiment. In this and subsequent Figures, treatments are (WWW) fully watered controls, (WWC) fully watered with the half root system then excised, (WDD) one side of pot dry, (WDC) roots in dry side excised, (WDW) roots in dry side re-watered and (DDW) fully drought stressed then re-watered. Error bars  $\pm$  SE mean.**



During the same period the leaf water potentials of these plants were slightly lower, but not significantly different from the well watered controls. When the whole root system was allowed to dry down, leaf expansion and stomatal conductance began to fall several days before there was any significant difference in leaf water potential between the drought stressed and well watered plants. Treatments imposed at the start of the recovery phase caused significant changes in *PER*, but these were not accompanied by similar changes in leaf water potential, suggesting that plant water status as measured by leaf water potential may not have been the reason for the observed differences in growth.

Initially this experiment was designed to investigate the involvement of ‘root signals’ in regulating the shoot growth response of sugarcane to adverse soil conditions, and the results are similar those from other studies which have shown that the initial plant response to drought is more closely related to the conditions of the soil than water status of the shoot (Blackman and Davies, 1985; Passioura, 1988; Davies and Zhang, 1991). However, despite the fact that partial root-zone drying had no statistically significant effect on plant water status, there was a small, but consistent, difference between the leaf water potentials of the partially drought stressed and control plants. This suggests that hydraulic effects, as opposed to chemical signalling from the drying roots, may have been at least partly responsible for the reduction in shoot growth.

**Figure 16: Stomatal conductance of plants with half their roots exposed to drying soil (■-WDD) and those with all roots exposed to drying soil (■-DDW), relative to the stomatal conductance of fully watered controls (■-WWW). Leaf water potential of each treatment is shown for comparison.**



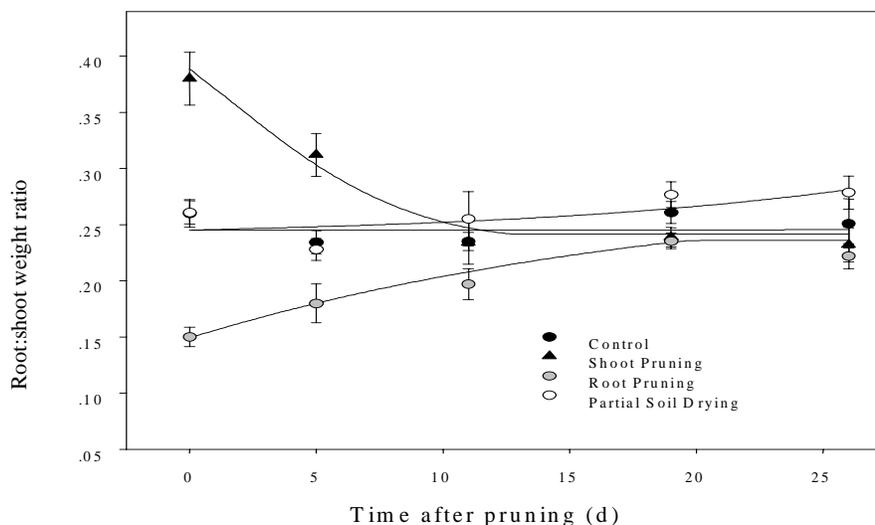
Irrespective of the underlying mechanism, this study has shown that partial root system stress can cause a significant reduction in shoot growth of sugarcane. However, compared to the number of roots involved, the reduction in growth was relatively small. This finding would suggest that under field conditions, adverse soil conditions in part of the root-zone will not necessarily have a detrimental effect on plant growth. However, in the present study, the roots that were not exposed to drying soil were supplied with near optimal levels of water and nutrients, and the soil was free from any other physical or biological constraints that may have reduced the effectiveness of remaining roots. Therefore, under field conditions, the effect of partial root system stress on the growth of sugarcane will not only depend on the proportion of roots involved in the stress, but also on the soil conditions in the remaining root-zone and the ability of those roots to compensate.

Despite the relatively small magnitude of the response to partial root system stress, the results of this study have demonstrated that shoot growth and physiology is closely related to the status of the root system. Furthermore, the fact that losing half of the root system only caused a 10% reduction in growth and water use, indicates that the roots of sugarcane normally function below their potential capacity. If inhibitory root-signals were partly, or entirely, responsible for the reduction in growth and transpiration that was observed in the dry-down phase, then the lack of a response to the recovery treatments suggests that their role in adapting the plant to the onset of root system stress is likely to be short term. In the longer term, the drop in growth that occurred in the well watered plants after half the root system was excised, and the fact that the growth fell to the same level as the plants with half their root system in drying soil, or plants that had their drying roots excised, suggests that the response of the shoot was related to the amount of functional root system.

### *Experiment 3. Response to defoliation, root pruning and partial drought stress*

Brouwer (1963) stated that the growth response of the plant to disturbances in the equilibrium between the roots and shoot is to re-establish it as soon as possible. The results of the present study demonstrate that the behaviour of sugarcane is consistent with this, as removing either part of the shoot or part of the root system, caused the relative growth rates of the roots and shoot to change to re-establish the root:shoot ratio (Figure 17).

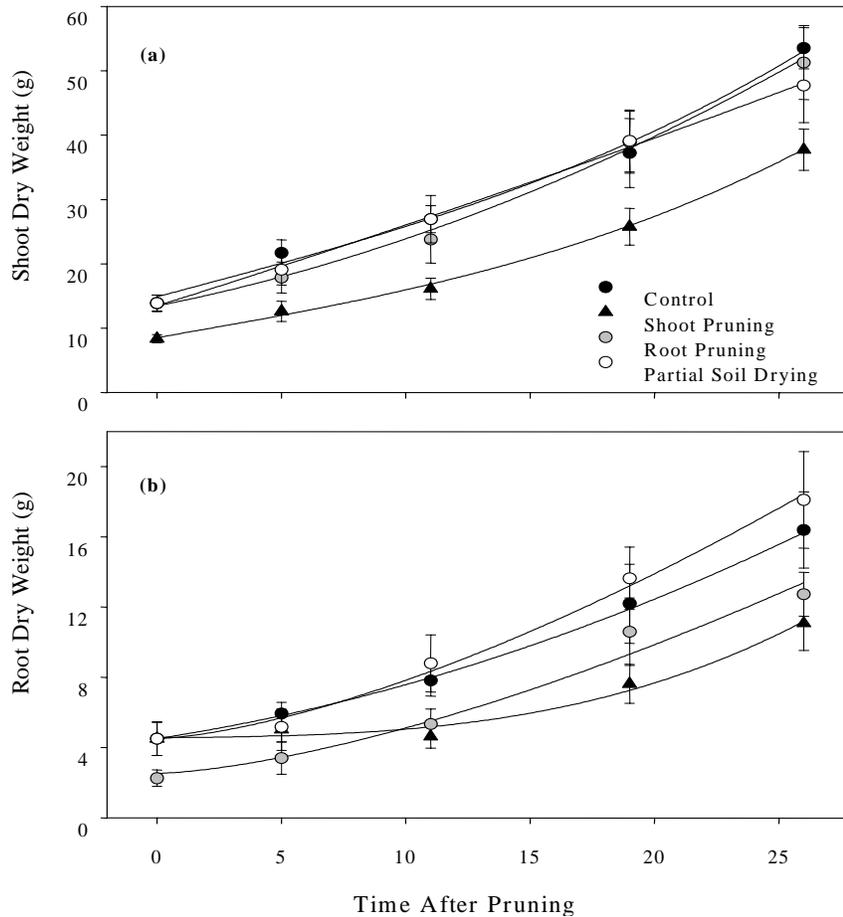
**Figure 17: Recovery of the root:shoots weight ratio after the removal of 50% of the root biomass, or 40% of the shoot biomass, as determined with destructive harvests made 0, 5, 11, 19 and 26 days after the treatments were imposed. Open symbols show the response to partial root-zone drying. Error bars  $\pm$  SE mean.**



With the defoliation treatment, the growth of the root system completely stopped (Figure 18), leading to a recovery of the root:shoot ratio within 11 days. Previous studies have shown a similar reduction in the root growth of other species after part of the shoot is removed, although the response was dependant on the severity of the defoliation treatment (eg Evans, 1971; Hodgkinson and Bass Becking, 1977). Evans (1971) found that defoliating ryegrass

2.5, 5 and 7.5 cm above ground level reduced the rate of root elongation by approximately 98, 23 and 51% respectively, while the root elongation rate of wallaby grass was similarly dependant on the level of defoliation (Hodgkinson and Baas Becking, 1977). In a later study by Evans (1972), it was found that the reduction in growth after defoliation was accompanied by a decrease in the soluble carbohydrate concentration in the roots. Adding glucose and sucrose to the rooting medium largely restored the growth of the roots, suggesting that a shortage of carbohydrates was responsible for the reduction in root elongation after defoliation.

**Figure 18: Increase in (a) shoot dry weight, and (b) root dry weight of sugarcane plants after removing 40% of the shoot biomass and 50% of the root biomass. Open symbols represent plants which were grown in pots with one half of the root system exposed to drying soil. Error bars  $\pm$  SE mean (n=6).**



Sugarcane appeared to be less sensitive to the loss of part of the root system, than to the loss of part of the shoot. Root pruning caused a maximum reduction in the plant elongation rate (*PER*) of approximately 14%, which is consistent with the response of the plants to the root pruning treatments imposed in the previous split-root system experiment (Table 18). However, once again this growth reduction was not proportional to the amount of roots removed, supporting the suggestion that the root system of sugarcane normally functions below its potential capacity, and that if part of the root system is lost, the remaining roots can increase their uptake of water and nutrients to compensate. The fact that the root-pruned plants had a similar plant water status as the controls, and did not use significantly less water supports this observation (Data not shown).

**Table 18: Mean daily change in plant height between harvests, expressed as a percentage of the growth of the control treatment. Initial relative growth rates refer to the six day period prior to the treatments being imposed. n = the number of pots (each containing three plants) which remained after each harvest.**

Treatment	Initial* n = 10	Growth period			
		H1 to H2 n = 8	H2 to H3 n = 6	H3 to H4 n = 4	H4 to H5 n = 2
Control	100	100 <sup>ac</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100
Shoot pruning	103.7	92.6 <sup>a</sup>	90.9 <sup>bc</sup>	99.5 <sup>a</sup>	112.1
Root pruning	99.8	87.2 <sup>b</sup>	85.7 <sup>b</sup>	96.5 <sup>a</sup>	97.4
Partial Drought	102.3	103.8 <sup>ac</sup>	96.5 <sup>ac</sup>	86.1 <sup>b</sup>	79.4

\*No significant differences between treatments

Andrews and Newman (1968) observed that the relationship between transpiration and the amount of roots does not appear to be linear, and that the effect of root pruning on transpiration becomes relatively greater as a greater proportion of roots are removed. In their experiment, removing 60% of the root system of wheat reduced transpiration by 25-30%, but had little effect on the growth of the roots or shoot. In the present study, removing 50% of the root system had no effect on transpiration, but did cause a reduction in *PER*. However, the observations of these and other authors (Brouwer, 1963) suggest the root systems of plants do have spare capacity, and that an estimate of 30-40% for sugarcane, at least under ideal conditions, is consistent with other plant species.

As an alternative to root pruning as a means of reducing the functional capacity of the root system, half of the root system of plants in a further treatment were allowed to dry down in the same manner as described in the previous experiment. The *PER* rate was reduced in a similar manner, although due to a lower evaporative demand and smaller leaf area of plants in the present study, the soil in the un-watered side of the pot took much longer to dry down (data not shown). Towards the end of the experiment it was found that the calibration of the TDR probes slightly over-estimated the amount of water in the soil, and as a result the wet side of the pots had also partly dried down. Therefore, the reduced growth rate of these plants between the third and fifth harvests may be greater than would have otherwise

occurred. However, the growth response was similar, and the absolute increase in root growth in the wet side of the pots suggests a growth response towards the re-establishment of an equilibrium between above and below ground growth.

The aim of this experiment was to determine whether the root:shoot ratio of sugarcane is a conservative trait. The rapid recovery of the root:shoot ratio after defoliation treatments suggests this is the case, but the response of the plant to root pruning treatments is less conclusive. Shoot growth was reduced when half of the root system was removed, suggesting that the response of the plant to such treatments was to re-establish the original balance between root and shoot growth. However, this response alone would have been insufficient to restore the root:shoot ratio during the experimental period. Under field conditions it may be that the size of the shoot and root system are always out of balance to some small degree, as the response of the shoot to the loss of part of the root system suggests that the plant may not react fast enough to maintain the ideal root:shoot ratio under constantly changing environmental conditions. However, the fact that the root system normally functions below its potential capacity, may ensure that the functional capacity of the roots remains in balance with the shoot, even if the biomass is not.

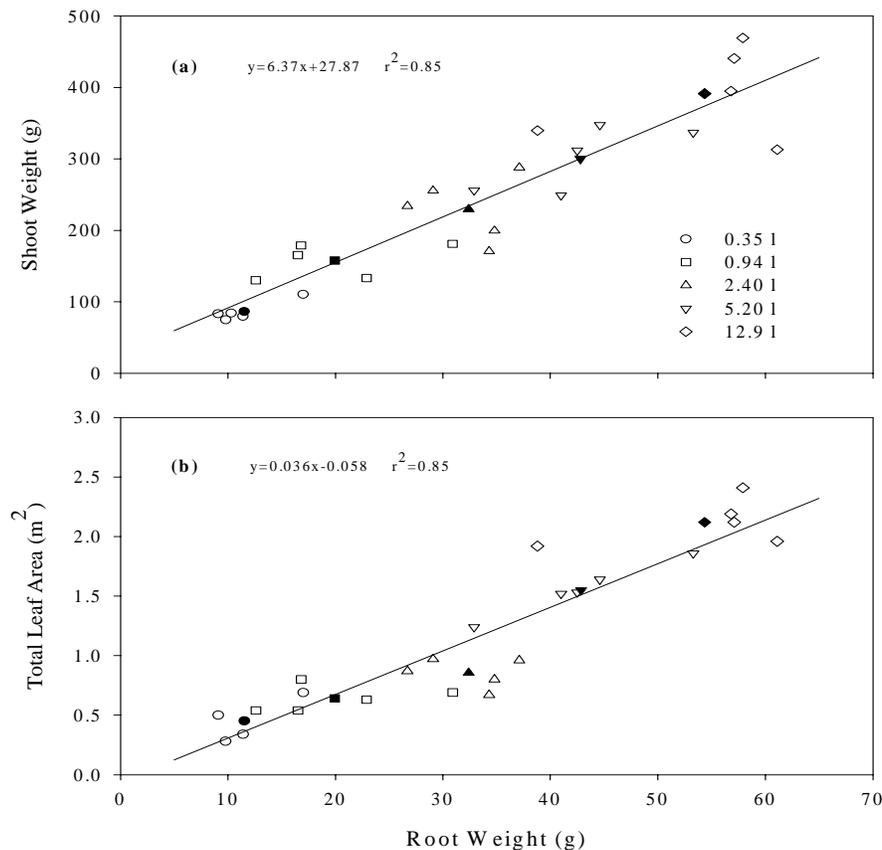
#### ***Experiment 4. Response to root volume restriction***

Confining the root system to a limited volume of soil caused a significant reduction in the above ground growth of sugarcane, with total plant dry weights increasing by more than 400%, as the soil volume increased from 0.35 L to 12.9 L. Despite these differences in plant size, root restriction stress had a minimal effect on biomass partitioning between the roots and shoot, and between the stem and leaves. As a result, there was no significant difference between the root:shoot weight ratio, and the root:leaf area ratio, across the five pot size treatments (Figure 19). Restricting the rooting volume slowed both the rate of leaf appearance and the rate of stem elongation, and also reduced the length and width of mature leaves. However, these effects became less evident as the soil volume increased, and only caused a significant reduction in the leaf area and shoot weight of the two smaller pot sizes. In the larger pot sizes there were no significant differences between the final leaf area on the main stem (Figure 20), and only slight differences between the rates of leaf appearance. Instead, the difference in leaf area and shoot weight between treatments was largely determined by the number of main tillers (Table 19), which increased from 1 to 2.8 between treatment extremes.

One explanation for the effect of restricted rooting volume on the growth of the sugarcane plants, is that the root system was unable to meet the demand of the shoot for water when confined to a small volume of soil. However, the effect of water stress has largely been ruled out of studies of root restriction stress in the past, and in the current experiment measurements of leaf water potential made at three hourly intervals on the day before harvest showed no difference between the water status of plants in the five different pot sizes. Similarly, although plant nutrient concentrations were not measured during the experiment, it is possible that nutrient stress contributed to the reduction in plant growth. However, the plants were well supplied with nutrients, and once they became established after transplanting, showed no visible symptoms of nutrient stress.

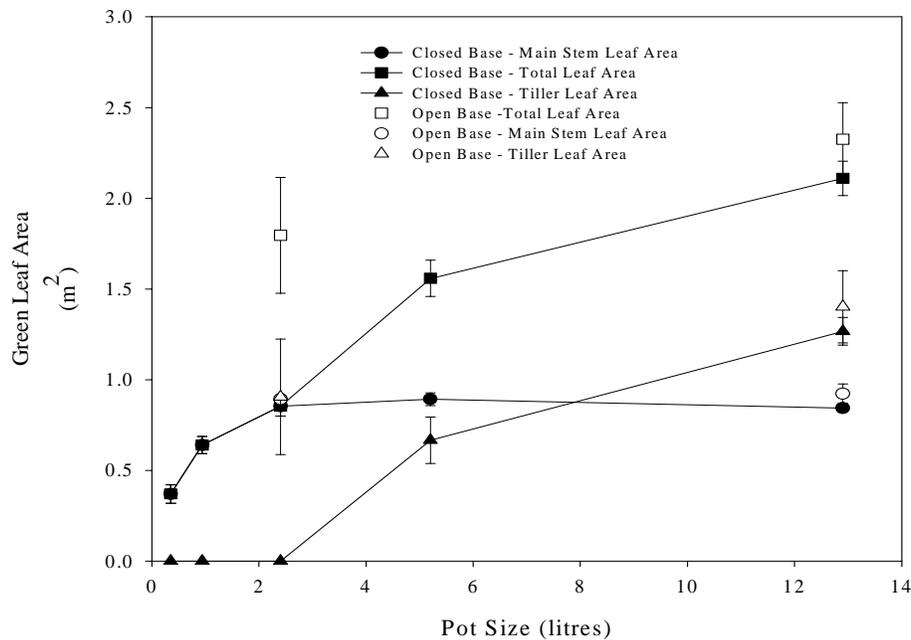
The response of the shoot to root restriction stress is similar to the response to high strength soil, and it has been suggested that in monocotyledonous species at least, the effect of limited soil volume on plant growth is caused by the high mechanical impedance experienced by the roots (Cook *et al.*, 1996). The results of the present study appear to be consistent with this hypothesis, as the rate of leaf appearance, leaf expansion and stomatal conductance, decreased (data not shown) with decreasing pot size in the same manner as increasing soil strength affected the shoots of young wheat plants in the study reported by Masle and Passioura (1987). However, despite these apparent similarities, the slowed rate of leaf appearance and leaf development did not account for the differences in shoot weight and leaf area between the larger pots sizes. As was previously mentioned, the differences in leaf area and shoot weight between these treatments were largely determined by the number of tillers.

**Figure 19: Root dry weight of sugarcane plants grown in 0.35, 0.94, 2.4, 5.2 and 12.9 L pots graphed against shoot dry weight (a), and total green leaf area (b). Regression lines fitted to individual plant data (open symbols), with treatment means shown for clarity only (solid symbols).**



Peterson *et al.* (1984) reported a similar reduction in the tiller number of young wheat plants in response to root restriction stress, with number of tillers reduced from 3.4 to 2.3 when the pot size decreased from 3.4 to 0.17 L. The authors ruled out the effects of water, nutrients and shading in their experiment, suggesting that root restriction stress did have a more direct impact on tiller development. Visual observations during previous experiments have indicated that new tillers develop their own root systems relatively quickly. Therefore, it is possible that the high density of roots in the surface layers of the pots increased the soil strength to a point where the tillers were unable to develop their own root system, which in turn slowed the growth of the tiller. Another explanation for the reduction in tillering, and one which is supported by the slowed rate of leaf appearance, is that root restriction stress slowed the rate of development, and subsequently slowed the rate of tiller appearance.

**Figure 20: Total green leaf area, and the leaf area on the main stem and tillers. Closed symbols represent the five pot sizes with mesh bases, and the open symbols for the corresponding sizes without mesh bases. Error bars  $\pm$  SE mean (n=5).**



However, in the larger pot sizes there were no differences between the growth rate and development of leaf area on the main stem, suggesting that in the earlier stages of the experiment, tillers would have developed at the same rate and have experienced similar lighting conditions. Also, the difference in tiller development between the 2.4 L open and closed based pots, when the main stem of both treatments developed at a similar rate, and where the surface layers of pots of both treatments were tightly packed with roots, suggests that tiller development may have been more directly influenced by the effects of root restriction stress.

Previous studies have shown that at a given stage of development, and under constant environmental conditions, plants maintain a predictable, and genetically pre-determined, root:shoot ratio. This raises the possibility that plants actively maintain the root:shoot ratio, and that shoot growth was reduced to maintain a balance between the above ground biomass and the amount of roots permitted by the volume of the pot. Previous experiments showed that sugarcane growth can be sustained at near normal levels with 50% of the root system removed. However, the subsequent recovery after the treatment was imposed, demonstrated that the root:shoot ratio of sugarcane will not remain out of balance if the plants are allowed to recover. It has also been demonstrated that sugarcane can maintain near normal levels of shoot growth under repeated root pruning regimes in an aeroponic system (Reghenzani and Grace, 1996; see section 3). However, as root and shoot growth largely remained in proportion under root restriction stress, it appears that the sugarcane shoot will not normally grow out of balance with the root system.

**Table 19: Main tiller number and total tiller number of sugarcane plants grown in 0.35, 0.94, 2.4, 5.2 and 12.9 litre pots. Tiller numbers on plants grown in the same diameter pots as the 2.4 and 12.9 litre, but without mesh bases, are also shown. The roots of plants in the two pots with open bases were able to explore a larger soil volume than indicated by the pot size. However in this table, and in subsequent figures, they are defined as 2.40 L Open Base and 12.9 L Open Base.**

Pot Size	Main Tiller Number LSD = 1.01 (P<0.01)	Total Tiller Number LSD = 1.87 (P<0.01)
0.35L	1.0 <sup>a</sup>	1.4 <sup>a</sup>
0.94L	1.0 <sup>a</sup>	2.2 <sup>a</sup>
2.40L	1.0 <sup>a</sup>	3.2 <sup>a</sup>
5.20L	1.6 <sup>ac</sup>	5.8 <sup>b</sup>
12.9L	2.6 <sup>bc</sup>	5.2 <sup>b</sup>
2.40L Open Base	2.6 <sup>bc</sup>	5.2 <sup>b</sup>
12.9L Open Base	2.8 <sup>bc</sup>	5.2 <sup>b</sup>

The mechanism governing the balance between root and shoot growth remains unclear. However it is possible that a reduced supply of cytokinins from the roots may be involved, as the concentrations of these hormones, which are known to promote shoot growth and stomatal opening, have been observed to decrease under root restriction stress in other plant species. Supporting this hypothesis are the results of Carmi and Heuer (1981), which show that the application of cytokinins can partially overcome the effects of root restriction on bean plants.

Increases in abscisic acid (ABA), which inhibits leaf growth and stomatal opening, have also been observed under root restriction stress (Ternes *et al.*, 1994). Similar increases in ABA are seen in plants growing in compacted soil, and it is possible that the reduced growth and stomatal conductance of the plants in the smaller pot sizes was caused by ABA produced by the roots as they experienced increasingly high soil strength conditions.

In conclusion, the results of this study suggest that sugarcane shoot growth will remain in balance with the root system when root development is restricted through limitations in the available soil volume. The reduction in shoot growth did not appear to be related to nutrient or water supply, and it is possible that shoot growth was reduced in response to the high mechanical impedance experienced by the roots. However, it is not clear to what extent the reduction in tillering, which accounted for most of the differences between treatments, can be attributed to the direct effects of root restriction stress, or more indirectly to the experimental design. Despite these questions, one of the main implications of this study is the potentially confounding effect of soil volume in pot experiments where shoot growth characteristics are studied. In experiments where the roots of plants are allowed to grow through the bottom of pots, or where different shaped pots are used, there may be less restriction to growth than seen in the current experiment. However, the results of the present study indicate that at least 10 L of soil would be required for experiments running for three to four months under glasshouse conditions, if the effects of root restriction stress are to be avoided.

It is more difficult to interpret the results of this study in terms of crop performance under field conditions, as it is unlikely that root growth would be restricted so severely by any normal soil constraints, or at the same time be so well supplied with water and nutrients. However, the results would imply that if the growth or size of the root system is reduced by soil constraints such as high mechanical impedance, or high strength subsoils, then shoot growth will also be slowed such that a balance between shoot and root weight is maintained. Under field conditions, soil constraints which limit the extent of root exploration are likely to leave the plant more vulnerable to water and nutrient stress, and as a result, higher application rates, or better management of these resources, may improve plant growth if these factors become limiting. However, in the current experiment the plants were supplied with luxury amounts of water and nutrients, indicating that increased application rates of water and fertiliser will not overcome the effects of soil constraints that limit the size of the root system.

#### **4.4 Conclusions**

- In the short term at least, the roots of sugarcane may be capable of functioning at a higher level of activity than would be recorded under 'normal' conditions. Therefore, the root system may have spare capacity under optimal soil conditions, and the loss of functional root may not necessarily cause a reduction in growth if conditions are otherwise sufficiently favourable for the remaining part of the root system to compensate.
- Under typical field conditions, in contrast to pot or aeroponic studies, plants have to deal with localised water and nutrient depletion, and pressure from other biological, chemical and physical soil constraints. This may explain why sugarcane seems to have 'more roots than are required' in pot studies where water and nutrients are continually replenished. It would also suggest that the root system probably functions close to, or at, potential capacity under normal field conditions, and may have little ability to compensate for a further decline in soil conditions.
- The root:shoot ratio of sugarcane, as defined by environmental conditions and developmental stage, appears to be a conservative trait. This is most likely a

consequence of the need to maintain a balance between the functional capacities of the root system and shoot, and is consistent with existing models of root:shoot relations.

- Given the conservative nature of the root:shoot ratio, soil constraints that reduce the physical size of the root system will cause a proportional reduction in above ground growth, even if the plant is amply supplied with water and nutrients. Similarly, the relative growth rates of the roots and shoot will always trend towards maintaining a balance between above and below ground growth, even if less roots could theoretically meet the demand of the shoot.
- Chemical signalling from the roots may be involved in the response of sugarcane to adverse soil conditions, but the magnitude of the response is relatively small. Therefore, adverse soil conditions in part of the root zone are unlikely to have any more effect on above ground growth than would otherwise be expected by the loss of functional roots.
- The use of root nitrogen content to estimate the size of different root fractions has potential to provide a method for the more detailed assessment of root system activity. Further development would be necessary, especially with regard to the stability of the root nitrogen content of root fractions under varying environmental conditions (for instance varying plant nutrition), calibration against actual root length data, and how these measurements relate to above ground growth. However, even allowing for these considerations, this method is relatively straightforward, and could be carried out with standard soil / root sampling equipment and analytical procedures.
- Although not directly addressed by this study, another possible means by which the activity, or effectiveness, of the root system could be assessed is through measurements of hormone concentrations in the shoot. The fact that changes in the soil environment are accompanied by changes in plant hormone concentrations, suggests that such a method may have some merit. However, at the present, there appears to be insufficient information concerning the active forms of different hormones, interactions between hormone groups, compartmentalisation and the importance of different hormone pools, interactions with other internal and external environmental conditions, or simply their modes of action, for any useful conclusions to be drawn about the relationship between hormone levels and the activity of the root system.

## **5.0 THE RELATIONSHIP BETWEEN ROOT FUNCTION AND TRANSPIRATION UNDER CONTROLLED GLASSHOUSE CONDITIONS**

Ross Nable, Suzanne Berthelsen, CSIRO, Townsville.

### **5.1 Introduction**

One of the most common forms of stress that can affect the performance of the root and shoot systems is soil drying and insufficient soil moisture to sustain transpiration. The intensive nature of sugarcane cropping systems has compounded this by leading to a reduced availability of plant water through increased soil strength and poor physical structure, both leading to decreased root activity and distribution. The ensuing poor performance of the root system under such conditions is believed to be one of the reasons for the reduced productivity of soils continuously cropped to cane.

Building upon the more basic investigations between root and shoot growth presented in the previous Chapter, the main objective of the work carried out in this component of the research was to derive quantitative relationships between root function (water uptake in a drying soil) and shoot growth of sugarcane. Furthermore, the secondary aim was to develop and to evaluate an experimental approach using large pots (approximately 40 L) that could be used routinely to assess the effects of soil properties on root function and shoot growth and could bridge the scale between small pots and field experiments. Experiments to derive the relationships were repeated in different soils and at different times of the year to examine whether the relationships would vary with soil type and transpirational demand. Sorghum was used as a comparison species, as there were published values for the quantitative relationships of interest and like sugarcane, it is a tropical C4 grass.

### **5.2 Materials and methods**

Three shadehouse experiments were conducted at the Davies Lab, Townsville.

One-eye setts that had been sprouted for 14 d were transplanted into 42 L pots (24.5 cm diameter x 90 cm high polyvinyl chloride pipe). Ten plants were transplanted into each pot, being thinned to 5 similar-sized plants per pot after a further 14 d. Sorghum seed were sown directly into the 42 L pots. Approximately 20 seeds were sown into each pot, being thinned to 10 similar sized plants per pot after 7 d.

To minimise soil evaporation, the soil surface in each pot was covered with approximately 2 cm layer of sand, on top of which was placed approximately 2 cm coarse gravel. Two control pots (without plants) showed very little (< 0.4 L) evaporative loss in any experiment compared to total water loss (5.6 – 8.9 L).

In the first sorghum experiment (so1), columns were packed with alluvial sandy, clay loam (melanic Tenosol (Isbell, 1996); Ustropept, US Taxonomy). In the first sugarcane experiment (su1), columns were packed with a commercial sandy, clay loam. Whilst in the second sorghum, and second and third sugarcane experiments (so2, su2 and su3, respectively) the pots were forced directly into a red earth (red Kandosol (Isbell, 1996)) and filled with intact cores. All soils received a luxury supply of all nutrients (commercial fertiliser). Over a

several-day period, water was added to the pots until drainage had reached negligible levels and therefore the soils had reached the drained upper limit. To create suction and thereby limit the possibility of waterlogging, all pots were placed on moistened, finely graded sand. Gauze was used to prevent roots growing out the bottom of pots.

Environmental conditions in the shadehouse were not fully controlled. Thus, as experiments were conducted at different times of the year, environmental conditions (including transpirational demand) varied between experiments (Table 20).

A dry-down treatment was commenced when roots were observed at the bottom of all pots. Thus rooting depth was assumed to be the same as pot height. Within individual experiments, there was little variation (2-3 d) between pots in the time taken for roots to reach the bottom. Similarly, there was little variation between species in the time taken for roots to reach the bottom – for sorghum, approximately 30 d and for sugarcane, approximately 60 d.

There were two treatments in each experiment – dry-down and well-watered (see later for details), with seven replicates of each.

The heights of plants and leaf number at the commencement of the dry-down treatment are shown in Table 20. It can be seen that plants from the same species were of similar size in each experiment, at the commencement of the dry-down treatment.

All pots were weighed daily at approximately 4.00 pm with a device based on that of Hatfield *et al.* (1989). Transpiration was calculated as the difference in mass from the previous day. Each dry-down pot was paired with a well-watered pot to which water lost through evaporation was added back. The precision of the weighing system was - 0.1 kg. Plants were harvested and oven-dried at the end of each experiment. Biomass gain from plant growth (approx. 27 – 55 g) over the dry-down period was small relative to the mass of water lost (approx. 7 – 9 kg). Therefore, plant growth introduced little error into the transpiration measurements.

To assess shoot extension, incremental increases in the distance from the pot surface to the tip of a growing leaf (held vertically) were measured regularly (usually daily) as the dry-down period progressed. Young, actively extending leaves were selected for measurement.

All experiments were terminated when no further water loss and no shoot extension was detected in the dry-down treatments. The time taken to reach this termination point varied considerably between experiments (Figure 21). The final pot mass was used to calculate the lower limit and hence plant available water (PAW). The fraction of PAW (FPAW) remaining in the dry-down pots was calculated daily for each pot using the following equation:

$$\text{FPAW} = \frac{\text{Pot mass on a specific day} - \text{Pot mass at lower limit}}{\text{Pot mass at drained upper limit} - \text{Pot mass at lower limit}}$$

Daily leaf extension and transpiration data were subjected to a double normalisation to remove (i) the effects of daily fluctuations in transpirational demand and (ii) the variation amongst plants in leaf size (Ray and Sinclair, 1997). Firstly, data from dry-down pots were divided by those from the control pots. Secondly, these relative data for each pot were divided by the average shoot extension and transpiration data for that pot over the initial

period before the dry-down treatment commenced. These double normalisation values were called normalised shoot extension ratio (NSER) and normalised transpiration ratio (NTR).

The fraction of plant available water at which shoot extension or transpiration begins to decrease is noted as  $PAW_t$ . To determine  $PAW_t$ , piecewise linear regression procedures were utilised. As double normalisation was used to calculate NSER and NTR, the initial plateau in these values at the early stages of soil drying were constrained to equal 1.0 (Ray and Sinclair, 1997). The downsloping regressions were not forced through zero as the best-fit regressions intercepted very close to zero.  $PAW_t$  is then defined by the intersection of the two regression lines.

### 5.3 Results and discussion

Ratcliff *et al.* (1983) showed that PAW varied widely with soil texture classes. Similarly, in the present experiments, the three different soils used (Table 20) produced different PAW (Table 21). Also, as indicated by Ratcliff *et al.* (1983), PAW for the same soil did not vary much for different species. Thus, the red earth had similar PAW when used with either sorghum (so1) or sugarcane (su2 and su3) (Table 21).

**Table 20: Experimental conditions of the tall pot experiments**

<b>Experiment</b>	<b>so1</b>	<b>so2</b>	<b>su1</b>	<b>su2</b>	<b>su3</b>
Species	sorghum	sorghum	sugarcane	sugarcane	sugarcane
Date	Nov – Jan, 1994/5	Nov – Jan, 1995/6	Feb – Apr 1995	Apr – Jun 1995	Nov – Jan, 1995/6
Temperature range - °C (min – max)	23 - 38	21 - 37	24 - 40	21 - 36	21 - 37
Soil type	alluvial sandy, clay loam	red earth	sandy, clay loam	red earth	red earth
Plant height – cm (onset of dry-down)	50	50	90	90	90
Leaf number (onset of dry-down)	6	5	5	6	6

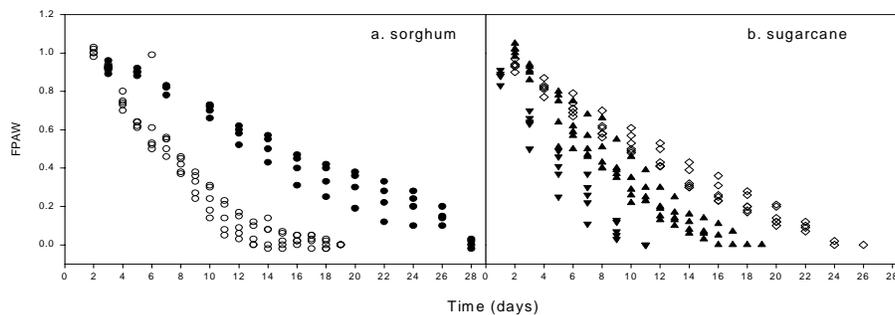
Also in the present experiments, different experimental conditions resulted in different times taken for the pots to dry down and hence different rates of drying (Figure 21). For sorghum and sugarcane, the time taken to use 90% of the available soil water varied from 10 to 26, and 9 to 22 days, respectively. Most importantly, the combined effects of different soils and different environmental conditions appear to have created different rates of water stress development in each experiment.

Despite these differences in the rates of water stress development, response curves and  $PAW_t$  for either shoot extension or transpiration were not significantly different ( $P < 0.05$ ) within each species for the different experiments (Figures 22,23). Seemingly, therefore,  $PAW_t$  may be more stable than implied by Sadras and Milroy (1996), who suggested that differences in the above experimental factors, such as the rate of soil drying and  $PAW$ , could explain variation in reported  $PAW_t$  for individual species. Indeed, Sinclair and Ludlow (1986) concluded that the response of transpiration to soil drying was not affected by the rate of soil drying and would apply under both glasshouse and field conditions. Whether or not such consistency occurs in sugarcane between glasshouse- and field-derived  $PAW_t$  remains to be verified.

**Table 21: PAW of the soils used in the 5 dry-down experiments, expressed as either total volume or volumetric water content (vwc)**

Experiment	so1	so2	su1	su2	su3
PAW – total vol. (cm <sup>3</sup> )	8,900	6,400	8,400	6,200	5,600
PAW – vwc (cm <sup>3</sup> cm <sup>-3</sup> )	0.20	0.14	0.19	0.14	0.13

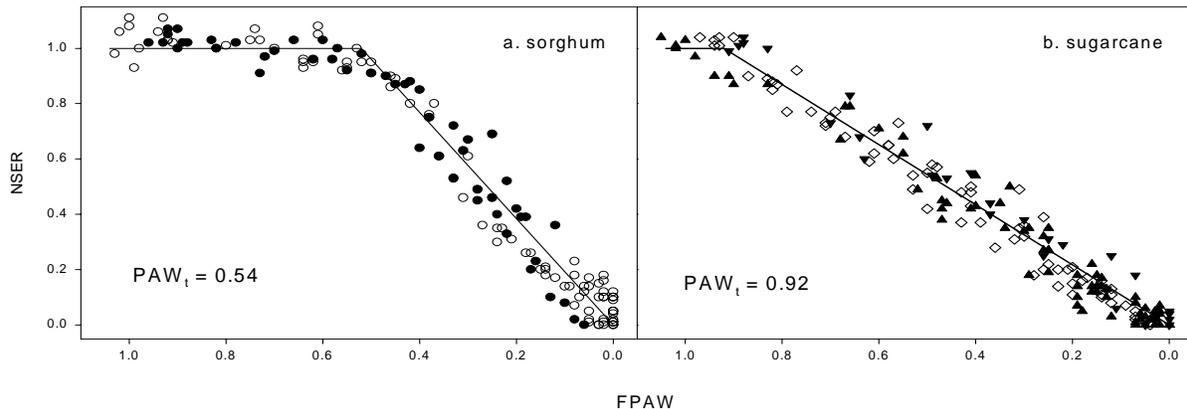
**Figure 21: Changes in the fraction of plant available water (FPAW) during drying cycles with (a.) sorghum and (b.) sugarcane. Symbols represent different experiments with each species: ● so1 and (O)so2; and (◊) su1, (▼) su2 and (▲) su3.**



The  $PAW_t$  for sorghum shoot extension (0.54; Figure 22) and plant transpiration (0.47; Figure 23) were consistent with those published previously for sorghum, from both glasshouse and field experiments (leaf extension - 0.25 to 0.50 and plant transpiration - 0.22 to 0.53 (Wright and Smith, 1983; Rosenthal *et al.*, 1987; Hammer and Muchow, 1990)).

The present data show that in both sugarcane and sorghum shoot extension is more sensitive (ie has a higher  $PAW_t$ ) to soil drying than transpiration (Figure 22, 23). Similar differences in sensitivity have been reported previously for sugarcane (Hudson, 1968; Inman-Bamber and de Jager, 1986a,b) and for other species (Sadras and Milroy, 1996), leading to generalised models for the response of these processes to soil water. Other workers have found that in sorghum, leaf extension is more sensitive than transpiration. For example,  $PAW_t$  for sorghum leaf extension and transpiration, respectively, were reported by Rosenthal *et al.* (1987) to be 0.44 and 0.28 and by Hammer and Muchow (1990) to be 0.25 and 0.20.

**Figure 22: Normalised shoot extension ratio (NSER) as a function of the fraction of plant available water (FPAW) remaining on each day of a drying cycle for each pot of (a.) sorghum and (b.) sugarcane. Symbols represent the same experiments as in Figure 21. The lines are the regressions obtained by piecewise linear regression procedures, with the horizontal regression constrained to equal 1.0. The regressions for the slanted lines were: sorghum  $y = 0.00326 + 1.8448x$  ( $R^2 = 0.97$ ); and sugarcane  $y = -0.0164 + 1.1053x$  ( $R^2 = 0.97$ ).**

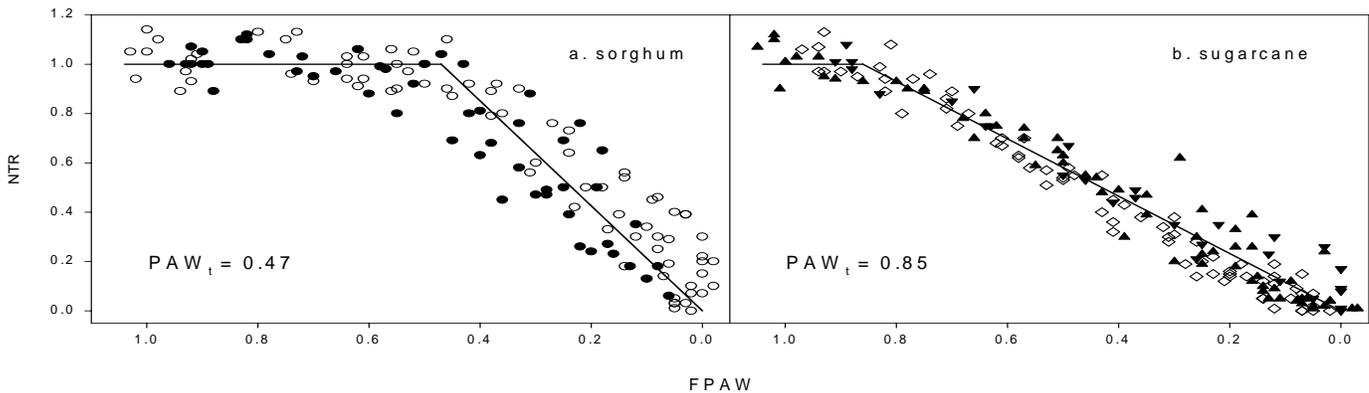


The present data also indicate that sugarcane shoot extension and transpiration is extremely sensitive to soil drying, considerably more sensitive than sorghum. There was almost no threshold for shoot extension (0.92) and a high threshold for transpiration (0.85). A similar level of sensitivity was seen in sugarcane by Ray and Sinclair (pers. comm.) from a small pot experiment, and this was much more sensitive than maize.

The high sensitivity of sugarcane to soil drying may have implications for water requirements and irrigation scheduling in the field. Such a sensitive response implies that a high frequency of irrigation is necessary to maximise leaf expansion, yet this is in conflict with common commercial practice in irrigated sugarcane systems. It may be that short-term water stress effects on leaf extension are reversible and hence do not translate into yield losses. Roberts *et al.* (1990) showed that “the activity of water stressed plants after re-wetting has often shown higher rates than treatments remaining fully-irrigated throughout and may contribute to compensatory growth between treatments”. Inman-Bamber and de Jager (1986a) found that leaf extension rates took about three to four days to recover to rates exceeding those of unstressed plants. Whether the enhanced extension is due to higher leaf turgor through osmotic adjustment or due to accumulated assimilates is unclear. What remains to be determined is the level of water deficit required to sustain a non-reversible effect on leaf area expansion.

**Figure 23: Normalised transpiration ratio (NTR) as a function of the fraction of plant available water (FPAW) remaining on each day of a drying cycle for each pot of (a.) sorghum and (b.) sugarcane. Symbols represent the same experiments as in Figure 21. The lines are the regressions obtained by piecewise linear regression procedures, with the horizontal regression constrained to equal 1.0. The regressions for the slanted lines were: sorghum  $y = -0.0022 - 2.1434x$  ( $R^2 = 0.96$ ); and sugarcane  $y = -0.0240 - 1.1962x$  ( $R^2 = 0.97$ ).**

As well as the direct manner in which water supply triggers this highly sensitive response in



sugarcane it may be that other soil factors indirectly produce a similar response by their effect on root function. For example, poor root health or other adverse physico-chemical properties could reduce water uptake by plants, leading to decreased shoot growth, even when water supply appears to be unlimited. Adverse soil properties may also directly affect root function and hence shoot growth, without water uptake being affected. As Nable and Webb (1993) showed in wheat, even with a luxury supply of water, zinc deficiency in only part of the root zone led to reduced yield, even though the shoots showed no signs of zinc deficiency or water deficit.

The pot technique described in this section offers considerable potential for comparing the effect of soil treatments on root functioning ability. For instance, how does soil fumigation affect the ability of root systems to extract available soil moisture? Does crop rotation influence root function in a similar way? Some of these questions could be answered in further experiments with this system.

#### **5.4 Conclusions**

- It is possible to establish a unique relationship for sugar cane between the fraction of plant available water (PAW) and measures of shoot activity such as shoot extension ratio and transpiration ratio, provided the data is normalised to eliminate the effects of soil type.
- The results presented indicate that sugarcane shoot extension and transpiration are extremely sensitive to soil drying, considerably more sensitive than sorghum. There was almost no threshold for shoot extension ( $PAW_t = 0.92$ ) and a reduction of transpiration also initiates at fairly high ratios of plant available water ( $PAW_t = 0.85$ ).
- In addition to providing unique information on  $PAW_t$  for sugarcane, the current experiments saw the development of an experimental system for sugarcane that used sufficiently large soil volumes (42 L) for plants to grow for several months, thereby filling the gap between small pot and field experiments.
- By using PAW and  $PAW_t$  as a physiological framework (which requires the depth of rooting to be defined), the system provides a basis for examining the effects of soil properties on root activity, with the derived data being useful in crop growth simulation models.

### **6.0 THE RELATIONSHIP BETWEEN ROOT AND SHOOT DEVELOPMENT IN THE FIELD UNDER DIFFERENT ENVIRONMENTAL CONDITIONS AND IN DIFFERENT SOILS**

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#### **6.1 Introduction**

Evans (1936), working in Mauritius, described root types following excavation of whole root systems, and laid a foundation in our understanding of root morphology. Other research added some detail about root morphology but due to technological constraints, could not adequately relate parameters such as root lengths, root distribution and activity to shoot growth.

Work as early as the 1930s in the Bundaberg area identified problems with poor root growth (Bell 1938). Research conducted since then (Egan *et al.* 1984; Magarey 1986; Magarey and Bull 1994) has consistently highlighted sub-optimal root health in soils used for sugarcane monoculture in Queensland. Treatments that greatly improved root distribution, health and functional integrity were identified - including soil fumigation, general fungicides, and crop rotations - and these almost always led to significant shoot growth improvements. However, investigations into understanding the effects of these treatments on root growth per se, and the dynamics of the shoot - root relationship, are difficult as roots grow in soil, - an opaque, bulky medium.

Devising a sampling strategy to study root growth in sugarcane is further hindered by the high spatial variability encountered due to the peculiarities of sugarcane growth and agronomy. Sugarcane is propagated vegetatively from stem cuttings, grown in widely spaced rows (1.5 m), has a long crop growth duration (12-16 months) with very large biomass production.

Reghenzani (1993) investigated sampling strategies required for describing sugarcane root system distribution in detail in the field, at one point in time. The study, based on taking multiple soil cores from various positions around the sugarcane stool, showed that prohibitively large sample sizes were needed to achieve statistically acceptable data. The study also indicated that sugarcane ratoon crops had low root length densities, compared to other crops.

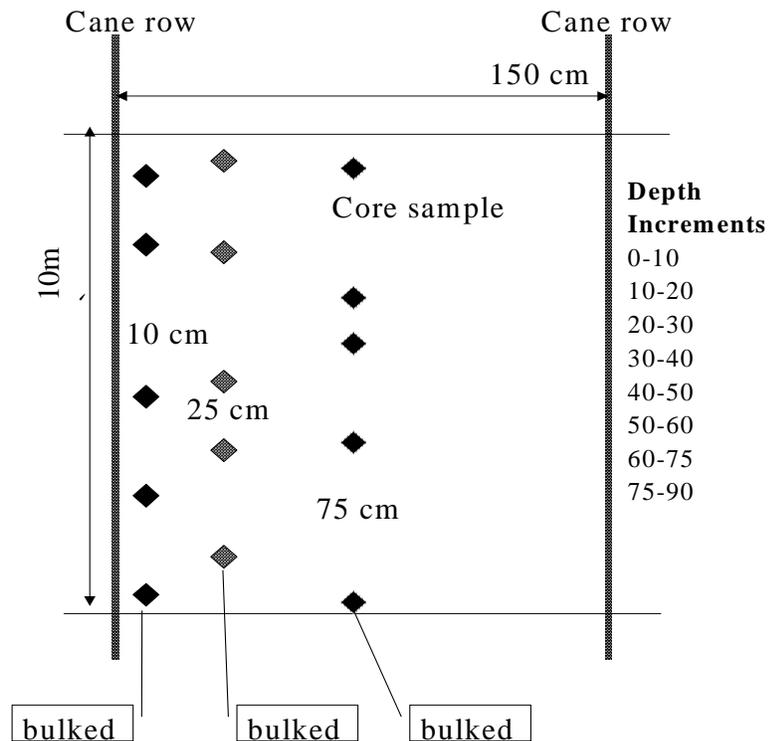
The interdependence of shoot and root growth is particularly important. The purpose of this study was to understand how root systems develop throughout the life of the crop and relate this to the above-ground biomass accumulation. This information could be used to decide on the most appropriate time to sample for roots relative to crop growth. In addition, a sampling strategy, based on that of Reghenzani (1993), was tested in its ability to provide a means of measuring root growth and/or root parameters that could be used to assess treatment effects.

## **6.2 Materials and methods**

Sugarcane plant crops were sampled at trial sites in the wet tropics and the semi-arid tropics, near Tully and Ayr, respectively. The Tully trial was on the Sugarcane Yield Decline Joint Venture Rotation Trial site, at 17°53' S latitude and 145°58' E longitude. The variety was Q138 and the soil was a granite gravel (Yellow Kandosol, Isbell, 1996). The Kalamia trial site was in Field 11 at the Kalamia Estate, at 19°32' S latitude and 147°24' E. longitude. The variety was Q96 and the soil was a clay/loam over sand (Anthroposol, Isbell, 1996). The Tully trial was rain-fed and the Kalamia trial fully irrigated. Fertiliser and irrigation rates followed standard recommendations for the districts.

At Tully, samples were collected from three replicate plots, of dimensions 6m (4 drills) \* 6 m, at 65, 91, 127, 162, 192, 220, 260 and 295 days after planting (DAP). At Kalamia, samples were collected from four replicate plots, of dimensions 9m (6 drills) \* 50 m, at 79, 110, 140, 170 and 252 days after planting. At both sites, root samples were collected by soil coring at each sampling time. Steel tubes of 50 mm diameter were pushed vertically with a jack-hammer into the soil to a depth of 90 cm and then removed with a ladder jack. After removal from the tubes, the soil cores were divided into the following depth increments: 0-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-75, and 75-90 cm. The sampling strategy is shown in Figure 24. Samples were collected on only one side of the row as Reghenzani (1993) found equal root distribution on both sides. They were collected at three positions across the inter-row: 10, 25, and 75 cm from the row. Samples were not collected in the row, for even though most root weight and length would be expected immediately beside and under the stool, Reghenzani (1993) found most variability occurred at this position.

**Figure 24: Diagrammatic representation of the sampling strategy used for collecting root samples at field trials**



In order to minimise variation along the row, five cores were collected at each of the three inter-row positions. At Kalamia five cores were collected at random locations along ten linear meters of cane row (Figure 24), while at Tully the cores were collected randomly from nine linear meters (three rows \* 3 m). The various individual depth increments from these five cores were combined into one sample. After collection, samples were stored at 4°C until processing. Additional cores were collected for bulk density determinations of each depth increment at the beginning of the experiment.

At Tully, at the same time as root samples were collected, yield parameters and leaf area were measured according to the methods of Muchow *et al.* (1993). Briefly, along the same length of row from which root samples were collected, all stalks were counted, cut and total fresh weight measured in the field. A subsample of 15 stalks was retained to determine stalk fresh weight, stalk dry weight, and leaf area.

Roots were carefully extracted from soil samples using a root washing system based on that of Smucker *et al.* (1982). Recovered roots were then spread evenly in a single layer onto A4 sheets of blotting paper and photocopied, to provide a “hard copy” for storage and potential future use. The resulting images were analysed as described by Magarey and Grace (1997). Briefly, a flat-bed scanner (Hewlett Packard ScanJet IIP) was used in association with image analysis software (Delta-T Scan) to measure the diameter and length of root fragments and to derive total root length. By using the mass and bulk density data of each depth increment, the root length was expressed as root length density,  $l_v$  ( $\text{cm cm}^{-3}$ ). After photocopying, root samples from Tully were dried at 70°C and mass determined.

To compare above-ground biomass accumulation to root growth it was necessary to convert the root length to  $\text{m root/m}^2$  ground surface area. For each of the three sampling positions across the inter-row, the total root length for each core (to 90 cm) was converted to length per surface area ( $\text{m m}^{-2}$ ) based on the 5 cm diameter of the sampling tube. To convert this to root length per surface area of crop, a weighting factor of 1:1.86:1.43 was used for the three positions. The weighting factor assumed that the ‘10 cm’ position represented 0 - 17.5 cm across the row, ‘25 cm’ represented 17.5 - 50 cm, and ‘75 cm’ represented 50 - 75 cm.

### 6.3 Results and discussion

At Tully 65 DAP, and at Kalamia 79 DAP, roots were found to a depth of 90 cm in all three positions across the inter-row (Figures 25 and 26), indicating minimum rates of downward movement of the rooting front of  $1.4 \text{ cm d}^{-1}$  and  $1.2 \text{ cm d}^{-1}$  at each site respectively. Whilst these rates are somewhat less than for other tropical  $\text{C}_4$  grasses, earlier sampling may have produced higher and more comparable rates.

At the first harvest, highest  $l_v$  at both sites were found close to the row in the surface layers of the soil profile. However, with subsequent harvests  $l_v$  increased both across the row and with depth. By approximately 192 DAP at Tully and 140 DAP at Kalamia maximum  $l_v$  were reached at all depths and distances from the row, with the final pattern of root distribution established. At both sites, maximum  $l_v$  were in the range 1.0 to 1.5 cm cm<sup>3</sup>, which is low compared to reported values for other grasses (eg 1-12 for wheat and barley; 1-10 for rice; 1-10 for sorghum and maize, for review see Van Noordwijk and Brouwer 1991), but similar to that reported for sugarcane by Reghenzani (1993). It should be noted, however, that values for  $l_v$  are heavily dependent upon the methods used to extract, process and measure roots from soil samples. In very few publications are there sufficient details presented to allow meaningful comparisons of methods. Thus, it is suggested that the apparently large differences between  $l_v$  of sugarcane and of other related species may in fact not be as meaningful as first appears.

At Tully, the pattern of distribution was the classical inverted cone (Figure 25), no doubt reflecting the relatively uniform soil texture throughout the profile. Whilst there was clearly reduced  $l_v$  at 30–40 cm depth, which coincided with a compacted layer (M Braunack, BSES Tully, pers comm), roots penetrated this compacted zone and  $l_v$  increased again below it.

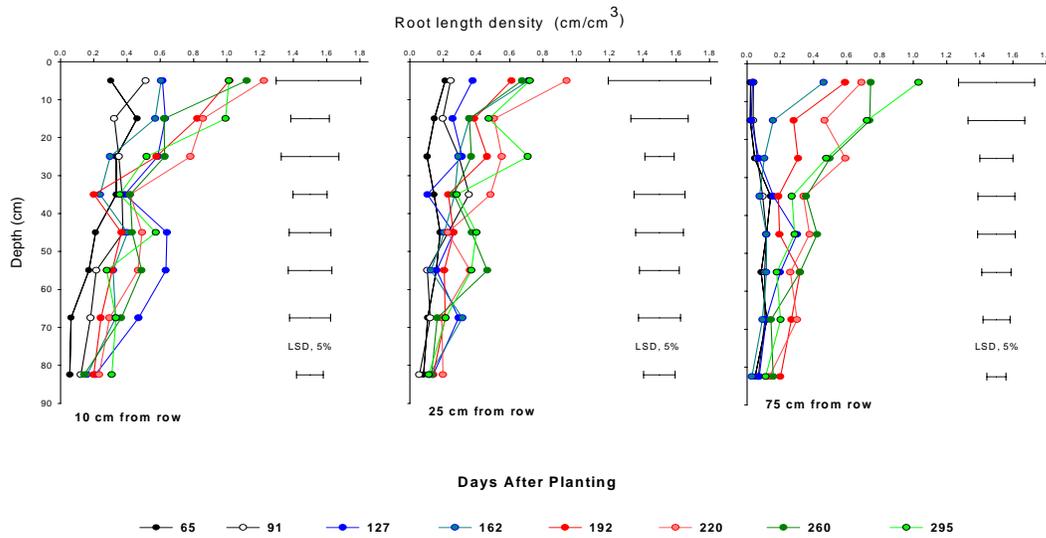
The relative distribution of roots down the profile at Tully (Table 22), revealed that from approximately 127 DAP the proportion of roots found in any depth increment remained quite stable at 10 and 25 cm from the row. It was only from approximately 192 DAP that the proportions remained stable at 75 cm from the row. Once stabilised, the proportion of roots found at any depth increment was remarkably similar across the row, with approximately 60 and 90 percent of the roots being in the top 40 and 90 cm of the profile, respectively.

At Kalamia, the pattern of distribution was very different to that at Tully, with almost all of the roots being confined above 50 cm (Figure 26). That is, above the depth in the profile where the strong texture contrast occurred. Unlike Tully, where roots grew past the compacted layer and proliferated below, at Kalamia few roots grew into the sandy subsoil and there was very little proliferation in it. The high soil strength of the subsoil caused the roots to proliferate across the surface of the sandy subsoil, with some samples revealing a tangled mat of roots reminiscent of those found at the bottom of enclosed pots.

**Table 22: The cumulative percentage of total profile root length found in each depth increment throughout the growing season of a plant crop at Tully.**

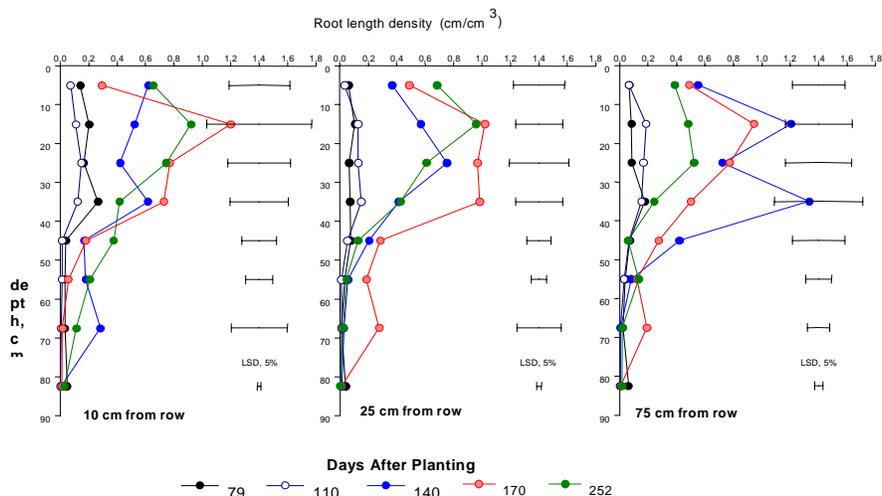
Distance from row (cm)	Depth (cm)	Days after planting								mean	SE
		65	91	127	162	192	220	260	295		
10	0-10	14.6	24.1	13.4	17.6	25.3	28.7	23.3	20.3	<b>20.9</b>	<b>1.9</b>
	10-20	37.1	35.7	26.8	34.9	45.3	44.8	37.5	40.8	<b>37.9</b>	<b>2.1</b>
	20-30	53.4	48.4	38.7	44.4	59.2	59.1	51.5	51.5	<b>50.8</b>	<b>2.5</b>
	30-40	69.6	62.2	48.1	53.0	64.7	67.0	61.8	59.4	<b>60.7</b>	<b>2.5</b>
	40-50	79.9	75.4	62.3	65.9	73.3	75.9	71.3	71.2	<b>71.9</b>	<b>2.0</b>
	50-60	88.1	82.3	75.3	75.1	80.8	83.9	81.5	76.4	<b>80.4</b>	<b>1.6</b>
	60-75	92.8	92.5	91.6	90.6	90.8	92.6	94.3	87.4	<b>91.6</b>	<b>0.7</b>
	75-90	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	<b>100.0</b>	<b>0.0</b>
25	0-10	17.3	16.6	19.0	25.5	22.2	25.7	21.9	18.5	<b>20.8</b>	<b>1.3</b>
	10-20	29.1	27.8	29.9	38.2	36.6	39.6	34.0	31.4	<b>33.3</b>	<b>1.6</b>
	20-30	37.4	44.6	44.2	49.8	53.7	55.2	45.8	53.1	<b>48.0</b>	<b>2.1</b>
	30-40	49.4	64.8	49.0	60.5	62.3	68.8	55.1	61.4	<b>58.9</b>	<b>2.5</b>
	40-50	64.0	78.4	61.0	68.7	71.8	75.4	68.5	73.3	<b>70.2</b>	<b>2.0</b>
	50-60	77.1	84.1	68.5	73.7	79.3	85.3	85.0	84.6	<b>79.7</b>	<b>2.2</b>
	60-75	90.2	94.4	87.8	91.7	90.7	91.7	92.0	93.7	<b>91.5</b>	<b>0.7</b>
	75-90	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	<b>100.0</b>	<b>0.0</b>
75	0-10	3.1	6.4	3.1	37.2	25.6	22.4	23.1	30.6	<b>18.9</b>	<b>4.6</b>
	10-20	6.0	11.6	5.5	49.8	36.2	36.2	43.8	51.0	<b>30.0</b>	<b>6.8</b>
	20-30	13.1	19.8	12.0	58.4	48.3	52.9	57.2	64.7	<b>40.8</b>	<b>7.8</b>
	30-40	34.5	32.9	26.1	64.6	55.3	62.7	66.7	72.0	<b>51.8</b>	<b>6.3</b>
	40-50	51.7	49.3	54.0	74.7	63.0	75.3	78.8	80.5	<b>65.9</b>	<b>4.6</b>
	50-60	64.4	63.6	73.1	83.9	74.5	82.6	87.6	85.4	<b>76.9</b>	<b>3.3</b>
	60-75	90.0	86.4	89.4	95.6	88.4	93.9	92.7	94.1	<b>91.3</b>	<b>1.1</b>
	75-90	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	<b>100.0</b>	<b>0.0</b>

**Figure 25: Root distribution throughout the growing season of a plant crop at Tully**



The relative distribution of roots down the profile at Kalamia (Table 23), revealed that from approximately 110-140 DAP the proportion of roots found in any depth increment remained quite stable at all three positions across the row. Once stabilised, the proportion of roots found at any depth increment was remarkably similar across the row, with approximately 60 and 90 percent of the roots being in the top 30 and 50 cm of the profile, respectively. Thus, as noted earlier, the roots at Kalamia were confined to a much shallower depth than at Tully.

**Figure 26: Root distribution throughout the growing season of a plant crop at Kalamia**



**Table 23: The cumulative percentage of total profile root length found in each depth increment throughout the growing season of a plant crop at Kalamia.**

Distance row (cm)	Depth (cm)	Days after planting					mean	s.e.
		79	110	140	170	252		
10	0-10	13.7	15.6	21.8	13.3	18.7	<b>16.6</b>	<b>1.6</b>
	10-20	36.9	37.5	40.3	48.7	46.2	<b>41.9</b>	<b>2.4</b>
	20-30	55.9	67.4	54.9	71.6	69.6	<b>63.9</b>	<b>3.5</b>
	30-40	83.6	93.6	73.6	91.7	80.3	<b>84.6</b>	<b>3.7</b>
	40-50	87.7	96.1	79.4	97.1	89.4	<b>89.9</b>	<b>3.2</b>
	50-60	91.1	98.5	85.6	98.8	94.4	<b>93.7</b>	<b>2.5</b>
	60-75	95.5	99.9	99.7	99.5	98.9	<b>98.7</b>	<b>0.8</b>
	75-90	100.0	100.0	100.0	100.0	100.0	<b>100.0</b>	<b>0.0</b>
25	0-10	15.4	6.6	15.7	11.9	23.3	<b>14.6</b>	<b>2.7</b>
	10-20	41.0	29.7	39.5	35.9	58.8	<b>41.0</b>	<b>4.9</b>
	20-30	56.3	54.6	72.0	56.8	78.9	<b>63.7</b>	<b>4.9</b>
	30-40	71.3	83.1	87.7	79.2	93.0	<b>82.8</b>	<b>3.7</b>
	40-50	87.2	93.9	95.4	86.4	97.4	<b>92.1</b>	<b>2.2</b>
	50-60	92.4	95.7	97.6	90.8	99.1	<b>95.1</b>	<b>1.5</b>
	60-75	96.5	98.7	99.4	99.7	99.9	<b>98.8</b>	<b>0.6</b>
	75-90	100.0	100.0	100.0	100.0	100.0	<b>100.0</b>	<b>0.0</b>
75	0-10	8.3	9.2	12.2	12.5	20.6	<b>12.6</b>	<b>2.2</b>
	10-20	24.2	33.4	37.6	38.1	43.5	<b>35.3</b>	<b>3.2</b>
	20-30	43.1	58.3	54.2	61.3	72.8	<b>57.9</b>	<b>4.8</b>
	30-40	69.2	84.3	86.8	77.2	86.8	<b>80.9</b>	<b>3.4</b>
	40-50	81.0	94.0	97.5	87.1	90.0	<b>89.9</b>	<b>2.9</b>
	50-60	86.7	98.2	99.5	91.3	97.5	<b>94.7</b>	<b>2.4</b>
	60-75	90.9	99.3	99.8	99.7	98.8	<b>97.7</b>	<b>1.7</b>
	75-90	100.0	100.0	100.0	100.0	100.0	<b>100.0</b>	<b>0.0</b>

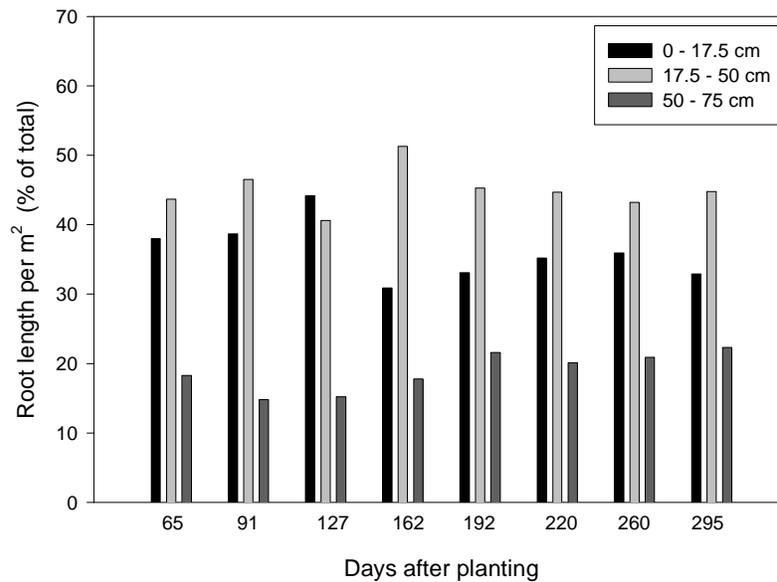
Another variable of interest is the total root length, on an area basis, below various portions of the inter-row. This variable is calculated using the scaling-up factor referred to above, which when used to calculate for the three areas represented by the three coring positions, yielded the data presented in Figures 27 and 28. They show that at Tully, throughout the sampling period, there were similar proportions of roots below the three areas across row. There were approximately 40 percent of the roots below both the 0-17.5 cm and 17.5-50 cm from the row and approximately 20 percent below 50-75 cm from the row. At Kalamia, there was considerably more variation throughout the season, though as might have been expected from the relative distribution data above, the proportions found below the areas across the row were more even than at Tully.

It has been suggested (Van Noordwijk and Brouwer, 1991) that specific root lengths (m root length per g root dry weight) are reasonably constant within a species or variety. Furthermore, average values of specific root lengths can be used to convert older root weight data (eg G Ham, unpublished data) or simulated root weight data to root length densities. Both are

particularly important in simulation models which calculate water and nutrient uptake on a root length basis (Van Noordwijk and Brouwer, 1991). For Tully, the mean specific root length for all samples collected was  $26.7 \text{ m root g root DW}^{-1}$  (s.e. 0.83). This value is considerably lower (by up to a factor of 10) than those reported for other species (for review see Van Noordwijk and Brouwer, 1991). However, as argued above for  $l_v$ , values of specific root length are heavily dependent upon the methods used to extract, process and measure roots from soil samples. Comparing reported values may not, therefore, be very informative.

As well as developing an understanding of the development and distribution of the root system, a major objective of the current experiment was to examine interdependence of shoot and root growth. In particular, there was great interest to see if the close relationship observed in the previous experiments in aeroponics, and small and large pots of soil, would be observed in the field.

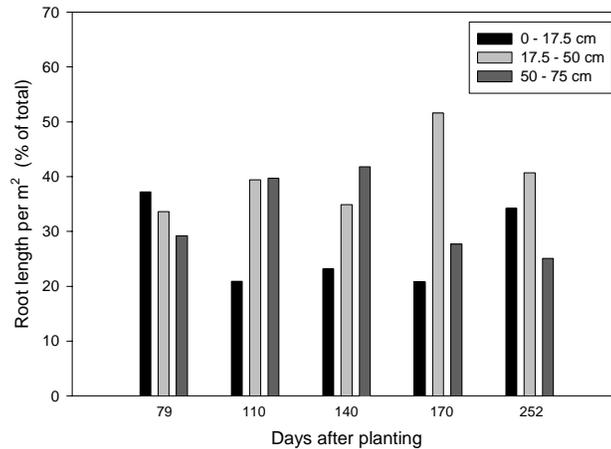
**Figure 27: The percentage of total root length found at each of three sampling positions across the row throughout the growing season of a plant crop at Tully**



The change in the root:shoot ratio of the plant crop at Tully (Figure 29) shows a decline throughout the growing season, a behaviour observed with most other plant species (Wilson, 1988). However, the data in Figure 30 confirms the close relationship between roots and shoots throughout most of the sampling period. Indeed, both leaf area index (LAI) and total shoot fresh weight were closely correlated with root length, using either a sigmoidal fit,  $R^2 = 0.95$  and  $0.94$ , respectively (Figure 31) or a linear fit,  $R^2 = 0.92$  and  $0.92$ , respectively (not shown on graph, Figure 31). The choice of curve ultimately depends on an understanding of the biology of the system, which at this stage is not well developed, although the proceeding

discussion would favour a sigmoidal relationship. Similar close relationships between root length ( $\text{m m}^{-2}$ ) and LAI have also been shown in pearl millet and groundnut (Squires, 1990).

**Figure 28: The percentage of total root length found at each of three sampling positions across the row throughout the growing season of a plant crop at Kalamia**

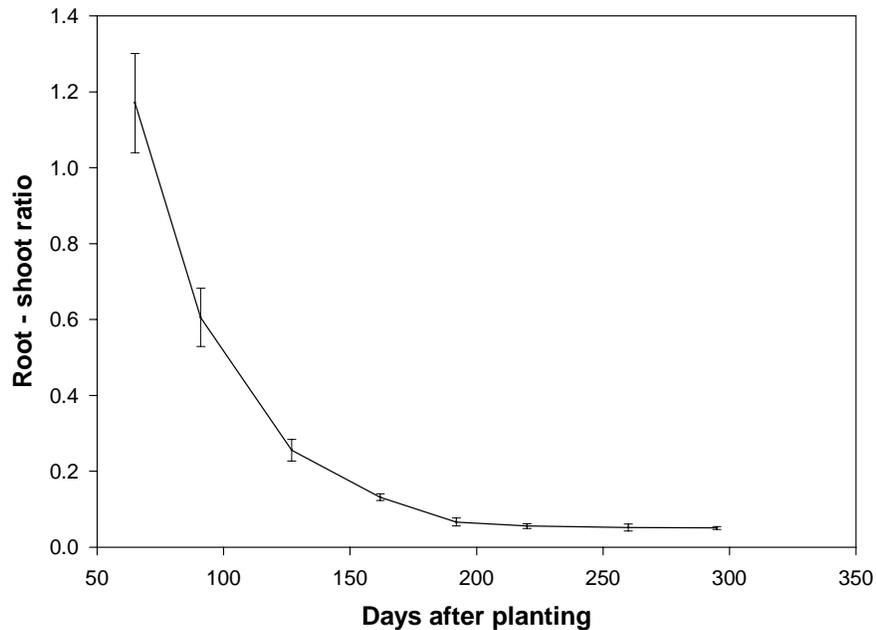


It is very apparent from several experiments conducted in this project that there is a very close relationship between sugarcane root and shoot growth. A simple conclusion would be that large crops are associated with large root systems. Though, as Gregory (1994) argues, this should not lead to the conclusion that there is a strict balance between root and shoot growth based upon size. Rather, there is more likely to be a functional balance between roots and shoots, based primarily upon supply of assimilates from the shoots and the supply of water and nutrients from the roots. Depending upon the environmental conditions that are operating, the growth balance between roots and shoots (eg the root-shoot dry matter ratio) will change (within a genetically determined range). It is suggested, however, that the functional balance between roots and shoots will be much more conservatively maintained under different environmental conditions. All of which makes distinguishing cause from effect, when trying to understand what's limiting crop growth, a very difficult task indeed.

Whilst the data shown in Figure 25 and Figure 26 provide a unique insight into the dynamics of a sugarcane root system's growth and development, they were very time-consuming and resource-expensive to acquire. It is estimated that to produce the data for each of these figures, approximately one person-year was required to collect soil samples from the three positions across the inter-row (10 cm, 25 cm and 75 cm), extract, sort and scan roots, and to analyse the scanned images. That is, approximately one week for ten combined depth increment samples. Obviously, this time investment is prohibitive and likely to be a severe

impediment to gathering similar data in the future. Yet such data could be very important in studies aimed at assessing the effects of soil properties on crop productivity and in environmental studies tracing the movement of agricultural chemicals through soils. Clearly, a simplified, less resource-expensive procedure is required to assess root systems.

**Figure 29: Changes in root:shoot ratio throughout the growing season of a plant crop at Tully**

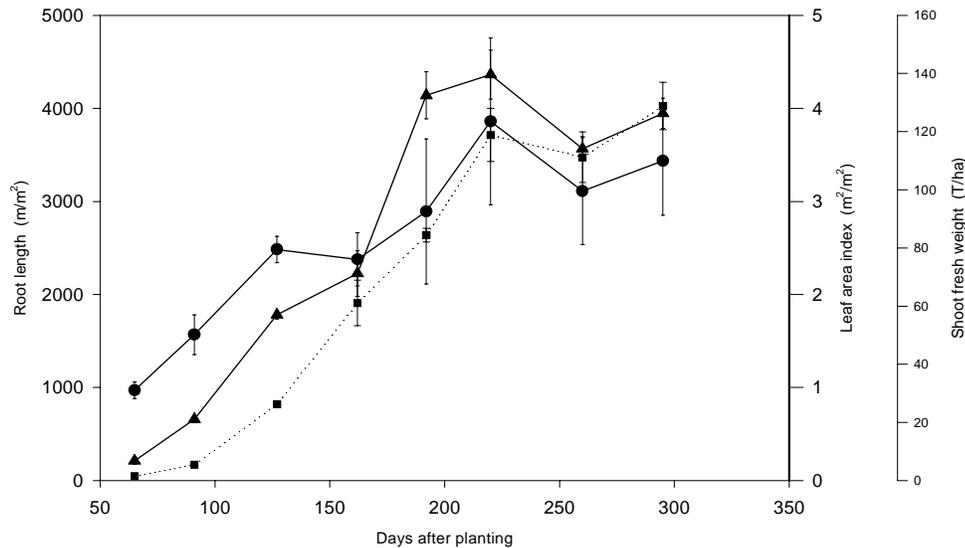


The most time-consuming step in the procedure outlined above was separating and sorting the roots prior to scanning, particularly with the depth increments close to the surface that contained most organic matter (trash). This step accounted for approximately 60 percent of the total procedure and is clearly the step in which big gains could be made. To reduce the time taken for this step, we further analysed the data to assess the possibility of using the observation by Magarey and Grace (1997) that there were consistent relationships between the three root classes of sugarcane roots (ie primary, secondary and tertiary roots).

As in Magarey and Grace (1997), our data showed that at the two experimental sites there was a consistent ratio amongst what we termed coarse, medium and fine roots throughout most of the root zone and over much of the growing season (Tables 24 and 25). Moreover, the ratio from these field trials (approximately 8:11:81) is remarkably similar to those from the earlier study (approximately 9:5:86) which included several pot experiments and various soil treatments. Though the ratio at Tully was considerably different at 75-90 cm, it should be remembered that at least 80 percent of the total root length is found in the upper 40-50 cm

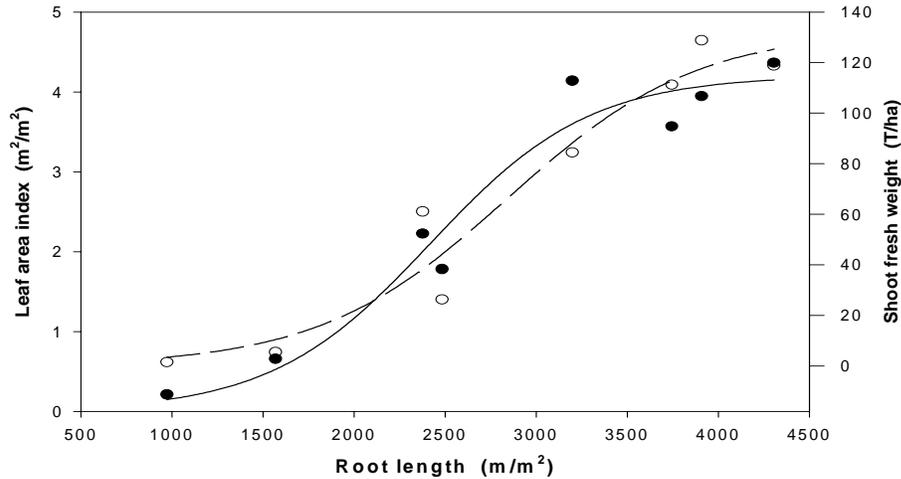
of most soil profiles. Thus, deviations from the ratio at depth, even large deviations, have little impact on the average ratio of the entire soil profile or therefore the total root length that is derived from it.

**Figure 30: Changes in root length (●), leaf area index (▲) and shoot fresh weight (■) throughout the growing season of a plant crop at Tully.**



As the ratio of these root class lengths remains quite stable, we examined the relationships between the length of roots of several specified diameters and the total root length. Figures 32 and 33 show such relationships plotted for three root diameter classes from Tully and Kalamia, respectively. It is very clear that even when only coarse roots ( $> 0.5$  mm diameter) are selected, they are highly related to total root length ( $R^2 = 0.756$  and  $0.932$  for Tully and Kalamia, respectively). When roots of smaller diameters are included ( $> 0.34$  mm and  $> 0.255$  mm) the relationships improve further, so that the length of the coarse + medium roots (ie roots with diameters  $> 0.255$  mm) is an extremely good predictor of total root length at both sites. Moreover, even when data are combined from both experimental sites (Figure 34) and so include different varieties and two irrigation treatments, the length of these two root classes was a very good predictor of total root length, even though they represented  $< 20$  percent of the total length.

**Figure 31: The relationship (sigmoidal fit) between root length, leaf area index (●) and shoot fresh weight (○) of a plant crop at Tully.**



There is another advantage to focusing on only the larger roots, which stems from their appearance. Namely, that larger, older sugarcane roots are characteristically dark-coloured and shrivelled due to lignification and disintegration of part of their cortex (Van Dillewijn, 1952). Accordingly, we've been able to easily distinguish sugarcane from other species in samples, as none of the other species that have been encountered had these two sugarcane root characteristics. However, this is only true for larger roots, as fine sugarcane roots, like those from the other species, are whitish and not obviously shrivelled. Clearly, being able to visually distinguish sugarcane from other species could be very important in situations where other species, commonly weeds, are present.

From field experiments with sugarcane conducted in southern Queensland, to assess the effects of ameliorants on subsoil acidity, root lengths and diameters were measured using the same methods described above. Quite encouragingly, they found ratios amongst the root diameter classes that were very similar to those in Tables 24 and 25:

fine – 81-84%; medium – 12-14%; and coarse – 4-6%

**Table 24: The effect of crop age and depth of soil on the percentage of total root length found in three morphological classes of a sugarcane plant crop (Q138) from Tully.**

Depth increment (cm)	Root class	% of total root length								mean (s.e.)
		Time (days after planting)								
		65	91	127	162	192	220	260	295	
0-10	fine	87	81	76	83	82	80	83	79	<b>81 (1)</b>

	medium coarse	9 4	12 7	15 9	11 6	12 6	13 8	12 5	14 7	<b>12 (2)</b> <b>7 (1)</b>
20-30	fine	82	81	82	87	79	82	76	79	<b>81 (1)</b>
	medium	12	13	12	9	14	12	15	13	<b>13 (2)</b>
	coarse	5	7	7	3	7	6	9	8	<b>6 (1)</b>
75-90	fine	46	59	61	69	58	58	52	66	<b>58 (3)</b>
	medium	29	24	22	19	23	23	22	20	<b>23 (3)</b>
	coarse	26	17	18	12	19	19	26	14	<b>19 (2)</b>

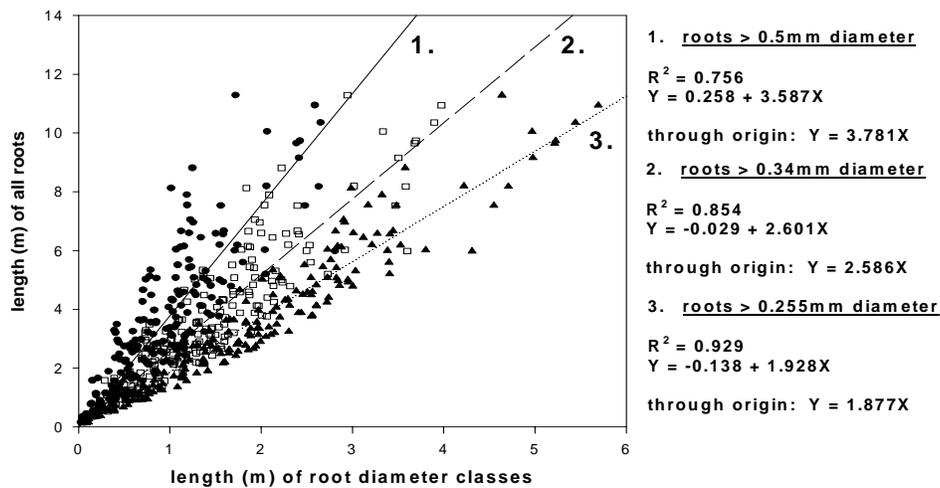
**Table 25: The effect of crop age and depth of soil on the percentage of total root length found in three morphological classes of a sugarcane plant crop (Q96) from Kalamia Estate, Ayr.**

Depth increment (cm)	Root class	% of total root length					mean (s.e.)
		Time (days after planting )					
		79	110	140	170	252	
0-10	fine	91	80	80	86	81	<b>84 (2)</b>
	medium	5	15	13	10	12	<b>11 (2)</b>
	coarse	4	5	7	4	7	<b>5 (1)</b>
20-30	fine	88	65	83	84	77	<b>80 (4)</b>
	medium	8	14	11	11	14	<b>11 (1)</b>
	coarse	4	21	6	5	9	<b>9 (3)</b>
75-90	fine	94	58	80	88	88	<b>81 (6)</b>
	medium	5	7	15	10	12	<b>10 (2)</b>
	coarse	1	35	6	3	0	<b>9 (7)</b>

Notwithstanding the consistent data so far, this simplified approach for assessing root length distribution of field-grown sugarcane may need to be assessed more widely before it can be recommended for widespread use. It may be, for example, that separate relationships need to be developed for different soils, varieties, for plant crops and ratoons, and so on. In general, however, the simplified approach offers the possibility to greatly reduce the effort required to acquire root length distribution data.

Previous sugarcane research has produced information on root mass distribution and its relation to water extraction (G Ham, unpublished data). It would be very useful to be able to convert root mass to root length, especially for use in model simulations. The data in Figure 35 show that for the Tully site, there is a good relationship between root weight and root length ( $R^2 = 0.68$ ), which reflects the specific root length data reported above. Thus, not only could existing root mass data be converted with some confidence, but the possibility also exists for only root mass to be measured and for the specific root length factor to be used to convert these to root lengths. Obviously, root length data derived by this means is unlikely to be as accurate as root length data measured directly. However, as with so much of root research, accuracy and research effort must be balanced against each other. Root mass is easier to measure, and may be the better parameter to use when accuracy is not critical.

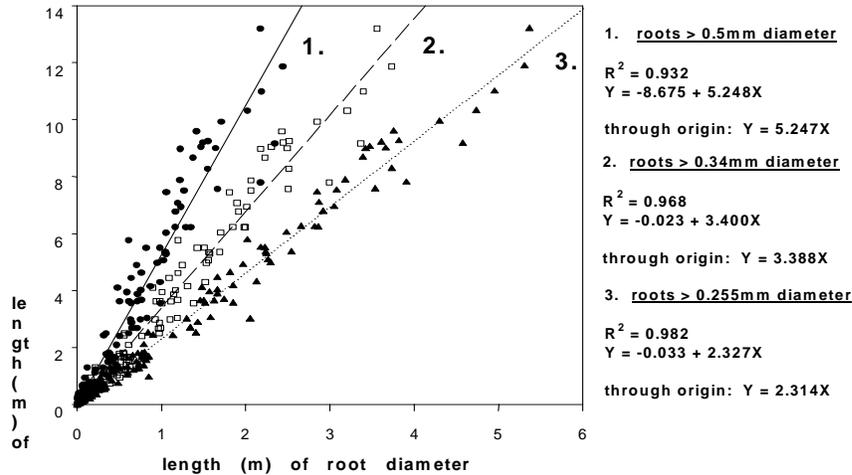
**Figure 32: The relationship between total root length and the length of specific root diameter classes of a plant crop from Tully.**



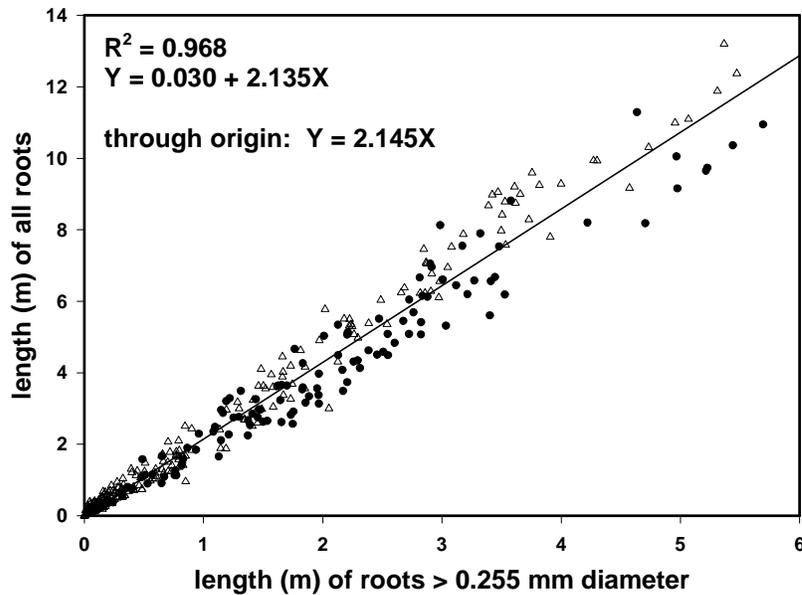
The strong and consistent relationship between roots of different diameters offers a basis for greatly reducing the effort required to gain quantitative data on root length distribution, especially from field studies. In our experience, however, even focusing on only large diameter roots does not overcome all problems. For example, in samples (particularly samples in the top 30 cm of the soil profile) from trials where there were weeds and

retained trash, the task of separating and measuring sugarcane roots was almost impossible. Careful site preparation and maintenance may still be necessary for several months prior to root sampling.

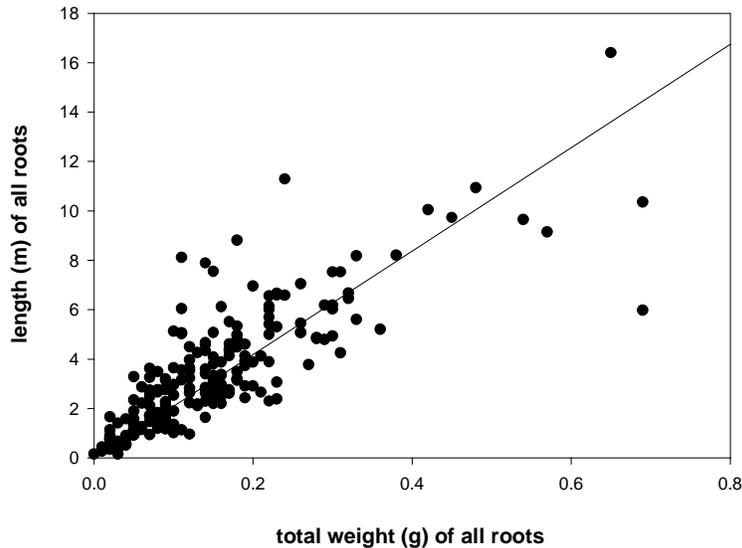
**Figure 33: The relationship between total root length and the length of specific root diameter classes of a plant crop from Kalamia**



**Figure 34: The relationship between total root length and the length of a specific root diameter class of a plant crops from Tully (●) and Kalamia (Δ).**



**Figure 35: The relationship between total root length and root weight of a plant crop from Tully**



## 6.4 Conclusions

- Maximum root length densities were relatively low, in the order of 1.0 to 1.5 cm cm<sup>3</sup>. The reasons for this may be associated with the sampling technique rather than actual cane characteristics, so that the root length density data presented is likely to be more robust in relative terms rather than absolute terms.
- Downward movement of the rooting front is relatively rapid in unconstrained soil conditions (ie without textural contrast, compaction layers etc), with rates of 1.2 to 1.4 cm d<sup>-1</sup> being only slightly lower than those of other C4 grasses.
- Relative distribution of roots at depth tends to be similar irrespective of distance from rows from 110-140 days after planting onwards, indicating that cane has a high capacity to fully explore the soil volume at depth.
- A close relationship between shoots:roots was also observed in the field, confirming the results from glasshouse pot studies. This implies that the pot studies relate reasonably well to the field situation.

- Cane tends to maintain a conservative balance between shoots and roots, making it difficult to detect effects of soil constraints on root growth/activity and shoot/productivity response.
- A reasonably constant ratio of fine:medium:coarse roots was observed across the sites studied and over various growth stages, corroborating similar findings from pot studies. If this ratio indeed remains constant across different sites and climatic regions, it may form the basis for a greatly simplified sampling and root quantification procedure based on solely determining the coarse root lengths and inferring total root length density using the amount of coarse roots as an estimator. This approach has the potential to greatly diminish the cost of root sampling. However, verification across other soil types and cane varieties is still required.

## **7.0 OUTCOMES AND FUTURE RESEARCH NEEDS**

### **7.1 Outcomes**

Project CLW002 and associated research have provided basic insights into sugarcane root systems, several tools for future root research, and a strategy for investigating root system distribution in the field. Outcomes against revised project objectives can be summarised as follows:

#### *Outcomes against objectives:*

- 1. To examine how shoot growth and root system activity is affected by changes in the size of the root system.**
  - Research using aeroponic facilities showed that the pruning of root systems to only a fraction of their original size did not diminish growth of shoots, when the remaining roots were in good health. The apparent limitation of shoot growth by poor root development in the field suggests that root health, and functional ability, in the field is markedly reduced.
  - A similar relationship was suggested by split-root experiments where the excision of half of the root system did not affect shoot size. An in-built over-capacity of root systems seems to occur in sugarcane, and indeed in other crops also.
  - In contrast, other glasshouse experiments investigating the plant response to changes in root system size showed that there is a strong response mechanism to maintain a consistent shoot: root ratio. Shoot pruning leads to reduced root growth until the ratio is brought back into balance, while root pruning will slow shoot growth until the ratio is again conserved. Root volume restrictions similarly led to restricted shoot growth, with a conserved shoot:root ratio.

- Shoot:root ratios were shown to be variety specific. Some variation in the ratio was found between varieties, and this was consistent between experiments.
- A number of soil treatments increasing shoot growth, such as soil fumigation, nitrogen fertilisation and soil pasteurisation, all increased root growth dramatically.

**2. To determine how root characteristics are related to shoot growth and to investigate the possibility of defining a ‘root health’ property.**

- Glasshouse experiments examining the relationship between various root parameters and shoot growth, did not find a close relationship between parameters such as total root length, length of fine roots, mean primary root length (or any other one) and shoot growth. Of those measured, total root length was as closely related to shoot growth as any other parameter.
- Previously it was thought that yield decline reduced the proportion of fine (tertiary) roots in the root system; this research showed that all three root types (primary, secondary and tertiary) were affected equally by yield decline.
- Research established that the proportion of primary : secondary : tertiary root length remained constant in root systems growing under a range of soil conditions, and when subjected to a range of treatments. This was consistent in both glasshouse and field experiments. There appears to be a strong genetic determination of root length ratios.
- The application of nitrogen tended to reduce primary root length, but increased primary root number. This is a similar finding to that obtained in wheat where it was attributed to increased auxin production. This may be a mechanism to maintain active roots at sites where nutrients are available.
- No single root health property was identified. Research suggested that root surface properties, such as root hair densities, or root epidermal cell integrity, could be parameters that may be used to assay for root health. The production of peroxidase (an enzyme produced by roots in response to injury) was examined in preliminary experiments; there appeared to be some relationship between peroxidase activity and “unhealthy” roots- as might be expected from the reaction to root injury. Further research is needed to explore the relationship between peroxidase, root health and shoot growth.

**3. To determine how soil properties affect root characteristics and the relationship between water uptake and shoot growth using measurements that allow extrapolation from the glasshouse to the field.**

- A large pot glasshouse system was adapted for use with sugarcane, allowing shoot growth response to water stress to be determined for sugarcane over a time period sufficient to be related to field conditions. This system could be used in the future to examine the ability of root systems affected by different soil treatments (and

properties) to utilise soil water, there-by providing data on root functioning ability as affected by treatment.

- Experiments with this system showed that sugarcane shoot growth (shoot extension and transpiration) is much more sensitive to water stress than sorghum. This has implications for irrigation scheduling in commercial fields.

**4. To determine how soil type and different soil properties affect the penetration rate of roots.**

- Experiments at two field sites over a season enabled root development to be studied in contrasting environments – the wet tropics (rain-fed) and the dry tropics (fully irrigated). Soil compaction at the irrigated site, and a texture contrast, led to restricted root penetration rates and root system development. In unconstrained soil conditions, root penetration rates were relatively rapid with rates of 1.2- 1.4 cm / day.
- Penetration rates were slightly lower than in other C4 grasses.

**5. To understand how root systems develop throughout the life of a crop in relation to above-ground biomass accumulation, and to compare root system distribution with root system activity.**

- Root system development was followed at two sites (as mentioned in point 4 above). Flushes in root growth appeared to correspond to the development of the sett and shoot root systems. Within 100 days after planting, roots had reached a depth of 90 cm across the row-interrow profile; by 192 days, there was a consistent proportion of roots distributed down the profile in both the row and inter-row. At the site in the wet tropics, 90% of the roots were above 90 cm depth, while at the irrigated site, soil compaction and a texture contrast led to most roots being above the 60 cm depth increment.
- The root:shoot ratio declined in the growing crop, but stabilised 200 days after planting.
- Total shoot fresh weight was closely correlated to total root length.
- Root length densities were found to be low, relative to those in other crops.

***Research tools:***

- *Field sampling:* Using the knowledge that there is a constant relationship between primary, secondary and tertiary root lengths, considerable time for processing samples could be saved by measuring root length of the more easily managed larger roots and extrapolating this data to estimate total root lengths.

- *Tall pots:* A pot technique which better simulates the field situation in relation to cropping period, and root distribution, has been adapted to sugarcane. The system lends itself to the study of such parameters as root activity.
- *Large split pots:* This system has been employed successfully to examine the feedback between roots and shoots. As with the tall pots, they contain sufficient soil for plants to grow for several months. They are ideal for assessing the effects of a heterogeneous soil environment on crop growth.
- *Aeroponics facility:* A soil-less system for studying root-shoot dynamics has been built and operated successfully. Basic physiological responses between shoots and roots can be investigated with this system.
- *Computer scanning and image analysis:* A system based upon flatbed scanning and image analysis software has been adapted for use in sugarcane.

Other important tools to which research in this project has made a contribution are:

- *Crop growth models:* Crop growth models like APSIM-Sugar offer a powerful tool to assess the effects on crop production of changing root system parameters. Further knowledge of shoot-root relationships are needed to drive this model. It is vital that strong links are maintained between root research and crop growth modelling.
- *Genotypic variation:* Differences between sugarcane varieties offer both a research tool to explore root physiology as well as a means to develop strategies to manage constraints to root growth.

## 7.2 Future Research Needs

### *Predicting the root zone from easily available soil data*

Knowledge of the resource base is essential for sustained sugar production. Obtaining information on above-ground resources (radiation, rainfall, temperature, leaf area), is now routine, but information on below-ground (soil) resources (water, nutrients) is much more difficult to gain. A major obstacle to collecting information on soil resources is the difficulty in determining the size of the root zone and the soil resources contained within that root zone.

Establishing the size of the root system in different environments is not easy using conventional technology, such as soil coring and image analysis techniques for quantifying root lengths. Research reported here indicates that measuring the larger roots only, and calculating total root lengths on the basis of consistent root length ratios (primary:secondary:tertiary roots) may simplify and speed root system measurements. However, it will still be too costly in most situations to fully assess root distribution with sufficient accuracy. Further research is necessary to develop alternative systems; a remote measure would be ideal.

The industry would **benefit** in two principle ways from being able to use easily available data to predict the size of the root zone. Firstly, costs in determining root system size would be

reduced since the time consuming techniques currently required would no longer be necessary. Secondly, ready access to this information would enable more researchers, and farmers, to obtain accurate estimates of water and nutrient availability, leading to improved advice, and consequently better irrigation and fertiliser application efficiency.

### ***Root “health”***

A root “health” (functional integrity) assay was an important tool not finalised in this project. There is a need to develop an assay for root “health” to gain an indication as to whether poor shoot growth is a result of poor root health (and lack of root ability to take up soil resources) or a result from a lack of soil resources. Such an assay would provide a valuable tool for assessing the effects of soil constraints on root system activity. In the longer term, a remote assay for root activity (perhaps based on a physiological response in the leaves) would be ideal for use in field studies.

The ability to assess root “health” would be of considerable **benefit** to the sugar industry, principally by highlighting the type of limitations which reduce productivity. Our current knowledge of crop growth limitations makes it difficult to decide if growth limitations are a result of sub-optimal soil resources (eg, nutritional deficiencies), or sub-optimal root performance. An indication of which factors predominate would be useful in setting directions for further research into improved productivity.

Root “health” has been identified as a high priority issue for the SYDJV.

### ***Identifying specific soil constraints to the size of root zone, and functioning ability of roots***

Identifying specific soil constraints that are affecting the size of the root zone, and root functioning ability, is a major issue for the sugar industry, both in terms of productivity and environmental management. There are of course many potential biological and physico-chemical constraints that could be operating in cane fields; the challenge is to identify the constraints and to develop practical management options. Likely constraints to root capacity and activity include: waterlogging, water deficit, inadequate supply of nutrients (eg calcium), chemical toxicities (eg aluminium), sodicity/salinity, diseases, pests, and soil compaction. Priority for research will vary depending on the type and magnitude of the constraint.

Perhaps the most important question is how can the constraints be identified and their impact assessed? Following is a proposed strategy, based upon the understanding, that there is a rapid feedback between roots and shoots, and that it is preferable to identify a constraint operating at the crop scale before detailed research is undertaken at the plant scale. The proposed strategy is currently being used in the SYDJV.

### Root Research Strategy

#### **Step 1 Identify root constraint to crop production**

**CROP SCALE**

- Does crop production meet the potential yield predicted from climatic conditions?
- Is a root constraint limiting crop production? – field data and simulations

#### **Step 2 Identify and investigate specific root constraints**

**CROP then PLANT SCALE**

- What are the potential factors causing the root constraint? – biological and physico-chemical factors; spatial information - correlations
- How do specific factors affect root distribution and activity? – detailed research if necessary

#### **Step 3 Develop management strategies**

**PLANT then CROP SCALE**

- How can these factors be managed? – direct management, breeding

How **benefits** to the industry will be realised will depend upon the specific constraints being investigated. For some constraints – eg calcium nutrition – benefits may be realised in the short-term through better fertiliser management. Benefits from overcoming other constraints – eg waterlogging – may not be realised for longer, as management strategies will be considerably more difficult, perhaps involving plant breeding. It may not be possible to manage some constraints.

### **Turnover/Exudation**

Root turnover – defined here as the death, decomposition, and regrowth of roots plays several important roles in crop productivity. Firstly, input from roots (especially carbon and nitrogen) due to turnover is recognised as an important contributor to the soil resource and hence to soil processes (eg nutrient cycling, decomposition). The importance of fine root input, in particular, has been highlighted. Secondly, as roots are a sink for assimilates, the rate of their turnover will influence the resources available for above-ground growth. Thirdly, the rate of turnover will determine the relative age of roots within a root system, and hence will influence root system activity.

Root turnover will occur both during a crop season and after harvest. Unfortunately, there is virtually no information on the former and only a little, contradictory information on the latter. Factors contributing to turn-over include soil disturbance, root injury from pathogens, changes in above ground conditions, water stress and other types of environmental stresses. Root turnover leads to changes in the efficiency of use of photosynthates and hence to crop growth effects. Despite its unquestioned importance, very little is known about sugarcane root turnover.

Two research tools have been successfully utilised to examine root turnover and exudation – minirhizotrons and  $^{15}\text{N}$  foliar labelling. Neither technique is currently being used in sugarcane research in Australia, though each could contribute in various areas of research.

An issue that is closely related to root exudation (and to decomposing root material in the soil) is **allelopathy** – growth inhibition to sugarcane and to other organisms due to the presence of toxic substances from sugarcane. Known to be the cause of “soil sickness” in a number of production systems, allelopathy could play an important role in sugarcane yield decline. Indeed experimental evidence from Magarey and Bull (1994b) showed that extracts (with micro-organisms removed) from “yield decline” soils inhibited the growth of sugarcane plants in soil to which it was applied.

Clearly, the direct contribution that roots make to the soil resource and to soil health needs greater understanding.

**Benefits** to the industry will be realised by (i) identifying and managing factors affecting turnover/exudation and thereby limiting sugarcane productivity; and (ii) through use in crop growth models to obtain information on nutrient cycling, organic matter, and carbon sinks in the soil to assist with management decisions.

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## **10.0 PUBLICATIONS ARISING FROM THE PROJECT**

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