

SUGARCANE ROOT GROWTH AND RELATIONSHIPS TO ABOVE-GROUND BIOMASS

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Abstract

Roots comprise the lesser known part of the soil-plant-atmosphere continuum and yet are essential to the supply of sufficient water and nutrients to ensure a successful crop. By understanding the growth and distribution of sugarcane roots, yields can be optimised through improved strategic decisions. This paper addresses root growth and relationships to above ground biomass. Issues discussed include the descending rate, distribution with depth and total length of roots, the effect of water availability, and the relationship between roots, total leaf number and green leaf area. The relationship between roots and total leaf number has never been published before and this paper offers a possible explanation for the change in the rate of green leaf appearance over time. All growth trends have been expressed in relation to cumulative heat units (base temperature = 10°C) which makes it possible to link data from different seasons and different parts of the world.

Introduction

In this section, current knowledge on root growth and distribution is summarised and serves as a basis for new information. Aspects discussed include sett roots, shoot roots, regeneration after harvest, carbon distribution, water availability, soil texture and root growth.

The sugarcane root system

The roots that emerge from the nodes after planting are sett roots, and are relatively thin and much branched. According to Weller (1930), only part of the total number of root primordia develop into roots while the remainder are kept in reserve and develop only if required. Glover (1967) reported that sett roots grow at a maximum rate of 24 mm/day and cease elongation when 150 to 250 mm long and only 11 days old. They turn dark, decompose rapidly and degrade within eight weeks of planting.

Sett roots serve the plant until the young shoots produce shoot roots which, compared with the sett roots, are relatively thick, white, succulent and less branched. Shoot roots also grow faster than sett roots and penetrate the soil at a steeper angle (Glover, 1967). The switch from dependence on sett roots to dependence on shoot roots usually occurs between the first and second month after planting and, by the end of the third month, the burden of supplying nutrients to the

plant rests entirely on the shoot roots (Lee, 1927; Glover, 1967).

Primary shoot roots become visible one week after planting, growing slowly initially but increasing the rate of growth later. The shoot roots produced later are finer and branch more freely than early primary shoot roots. The maximum growth rate of individual shoot roots in light soils is 75 mm/day for periods of one to two days, or 40 mm/day when their growth is averaged over a week. The average growth rate in heavy soils is 28 mm/day (Glover, 1967). Glover also found that the growth rate of individual roots before harvest was about 11 mm/day. Wood and Wood (1967) used the radioactive isotope, P^{32} , on a sandy soil to determine the depth of the rooting front and found that it reached 900 mm in 112 days, 1 500 mm in 161 days and 2 100 mm in 189 days. The final growth rate of the rooting front to a depth of 2 100 mm was around 11 mm/day.

The sugarcane root system is essential to the regeneration of the cane crop after harvest. Shoot roots cease to grow within three days after harvest and a flush of new roots appears from the basal nodes of the new shoots during the next month (Glover, 1968a). Estimates of the longevity of the old root system after harvest vary from about two to three weeks (Evans, 1964), eight weeks (Glover, 1968a) to several months (Clements, 1980; Hudson, 1963; Wood & Wood, 1967).

Carbon utilisation by the root system ranges from 8 to 26% for sugarcane aged 124 days, and depends on root temperature, air temperature and variety (Brodie *et al.*, 1965). Rostron (1974) established this value to be 17% for sugarcane variety NCo376 grown under irrigation, with 37 tons total dry biomass per hectare at the age of 224 days.

Baran *et al.* (1974) and Kingston (1978) showed that short irrigation intervals, which prevent the surface soil from drying, encourage a higher percentage of roots to develop near the soil surface. Extended irrigation intervals resulted in more extensive rooting at depth. Glover (1968b) noted that the active root system changed markedly as the soil went through wet and dry cycles. After a winter drought, the first spring rains caused root initials on some basal nodes to grow actively and to form a new superficial root system in the moist surface layer. Only when heavier summer rains recharged the soil profile did these roots extend downwards to re-establish the original root pattern in the soil.

Effects of water availability and soil texture on sugarcane root systems

Thompson and de Robillard (1968), with data obtained from a neutron probe, showed that water extraction in a sandy soil was effective to a depth of 1 800 mm when the crop was adequately irrigated, and to a depth of more than 2 100 mm under dryland conditions. In contrast, irrigated sugarcane on a clay soil did not remove water from depths greater than 900 mm, whereas dryland sugarcane exploited water to a depth of at least 1 200 mm.

Root systems growing in deep sands tend to be finer, more highly branched and deeper than those in heavier textured soils (Glover, 1968b). Primary roots observed through a rhizotron window in an undisturbed black cracking clay (Arcadia form) were few, relatively thick and well developed, with poorer secondary roots and subsequent branching compared with those in sandy soils. Roots of variety NCo376 were observed in an excavated sandy Hutton form soil to a depth of at least 4 000 mm (Anon, 1965).

Relationships between roots and aerial biomass

It has long been known that a relationship exists between above-ground biomass, root mass and root length (Blamey and Nathanson, 1977; Botha *et al.*, 1983; Brouwer and de Wit, 1969; Richner *et al.*, 1996). Glover (1970) noted that reduced aerial growth on a Hutton sand at the South African Sugar Association Experiment Station (SASEX) root laboratory was also reflected in the roots and suggested that a strong relationship exists between the aerial components and roots of sugarcane.

The above clearly illustrates that sugarcane root growth and development are well documented. However, there is a lack of quantitative information on the rates at which the rooting front penetrates soils, root distribution per depth interval, total root length over time and the relationship between roots and aerial biomass. Such information is important for crop models such as CANEGRO, where the below-ground biomass is also simulated and forms an integral part of the estimated soil water balance. The purpose of this paper is therefore to quantify these aspects of sugarcane roots for variety

NCo376, the variety that was used almost exclusively for the development of the CANEGRO crop model.

Materials and Methods

Data for this paper were collected from both pot and field trials for which characteristics are summarised in Table 1. Pot trials were used to quantify the relationship between roots and above-ground parameters, and the purpose of field trials were to study root distribution with soil depth.

To study the relationship between roots and aerial biomass, data were collected from two pot trials. The first pot trial was conducted in a glasshouse using pots with capacities of 6, 15 and 80 dm³. Measurements on plants were made destructively on 13 occasions utilising the smallest pots first. Simultaneously with the last four small pots (6 dm³), four medium size pots (15 dm³) were harvested. A similar procedure was followed for the change-over from the medium to the large size pots (80 dm³). The soil used was the topsoil of an oxisol (Hutton) (Anon, 1991) containing 6% clay and 3% silt. It was sterilised with methyl-bromide and packed to a density of 1.45 ton/m³ in all pots. The variety used was NCo376, which was pre-germinated from single eyed setts. One seedling was used per pot and no visual signs of stress were observed in the seedlings after transplanting. The trial was terminated 181 days after transplanting. Soil water content was measured by weighing selected pots weekly for the first month and twice weekly thereafter. On each occasion soil temperature, stalk height, stalk number and leaf number were recorded.

A further pot trial was conducted at the SASEX Central Field Station (CFS) at Umhlanga Rocks using a rainshelter that closed automatically at the onset of rain. To keep the trial as close as possible to field conditions, the pots were lowered into trenches lined with corrugated iron, to a depth where the soil surface of the pots and the surrounding field were at the same level. A total of 32 pots was used, half of which were filled with a Fernwood sand and the other half with a Hutton loamy sand, a Swartland sandy clay loam or a Swartland sandy clay. Each soil was divided into two groups, those kept

Table 1. General information for the sites from which data was collected.

Data set	Site	Trial type	Soil form*	Clay %	Silt %	Depth (mm)	Crop	Variety	Watering regime
1	Glasshouse	Pot	Hutton	6	3	480	Plant	NCo376	Irrigated
2 3 4	Root laboratory SASEX	Field	Hutton Cartref Shortlands	4 8 32	3 6 12	1 950 1 950 1 950	1st ratoon	NCo376	Irrigated
5 6 7 8	CFS rainshelter	Pot	Fernwood Hutton Swartland Swartland	5 10 20 36	4 6 12 16	500 500 500 500	Plant (x4) Plant Plant Plant (x2)	NCo376	Irrigated versus water stress
Only roots were sampled at the following sites:									
9	Root laboratory SASEX	Field	Hutton Cartref Shortlands	4 8 32	3 6 12	1 950 1 950 1 950	3rd ratoon	NCo376	Dryland
10	Modelling trial SASEX	Field	Arcadia	40	16	1 950	1st ratoon	NCo376	Dryland and irrigated

*Anon (1991)

well watered and those where the water was allowed to deplete. Thus, eight pots randomly placed in the trenches were used per treatment. Each pot had a depth of 600 mm and a volume of 87 dm³. Parameters measured included water use, leaf area and root length.

The root laboratory area consisted of three soils that were imported to the site in 1965 and repacked layer by layer to a total depth of two metres (see Table 1 for properties). The agronomy trial site was on a deep Arcadia form soil on dolerite parent material and had no drainage restrictions. The site had a slope of 20%. Parameters measured included root growth rate, rooting depth and root distribution in relation to soil depth.

Total root length index (L, km/m²) is often used in models to control the amount of roots produced by the crop as a function of time, which is normally expressed as days after planting (DAP). This time unit works well for annual crops where planting, flowering, and harvesting dates occur over relatively narrow time intervals.

Sugarcane is a semi-permanent crop in South Africa, and may be grown from three to more than 20 years before being ploughed out. Planting can take place in any month of the year although the optimum time is in spring, while harvesting is generally between April and December. DAP is therefore not a practical time unit for sugarcane, which is why cumulative heat units (CHU) were used instead. Van Antwerpen (1998) showed that root length index and leaf area index are closely related on a thermal time scale using a base temperature of 10°C for both parameters. Daily minimum and maximum air temperatures were used to calculate CHU using the following equation:

$$CHU = \sum_{i=1}^n (T_{max} + T_{min})/2 - T_b \quad (1)$$

where

- CHU = Cumulative heat units (°C)
 T_{max} = Daily maximum temperature (°C)
 T_{min} = Daily minimum temperature (°C)
 T_b = Base temperature at which leaf initiation ceases and which is 10°C for sugarcane (Inman-Bamber, 1994).

Results and Discussion

Descending rate of the rooting front

The data for irrigated cane, presented in Table 2, indicate that 87 days after variety NCo376 was planted at the root laboratory, roots reached a depth of 1 950 mm on the Hutton and Cartref sands, whereas on a Shortlands sandy clay loam a similar depth was reached only after 176 days. In both sands the average growth rate to reach a depth of 1 950 mm was 22,4 mm/day as opposed to an average of 11,1 mm/day in the Shortlands sandy clay loam. Based on these results, equations 2 and 3 were formulated for use in the estimation of rooting depth as a function of cumulative heat units for soils containing less than and more than 35% clay plus silt respectively. The number of data points to create equations 2 and 3 became three and four respectively, when no growth at zero heat units was added to each data set. The value of 35% clay plus silt was arbitrarily chosen and more data are needed to confirm these relationships.

$$\text{Sand RD} = 7,2572 + 1,6684 (\text{CHU}) \quad r^2 = 1,000 \quad n=3 \quad (2)$$

$$\text{Clay RD} = 2990,34 (\text{CHU}) / (1009,78 + \text{CHU}) \quad r^2 = 0,999 \quad n=4 \quad (3)$$

where

- Sand = soil containing <35% clay plus silt
 Clay = soil containing >35% clay plus silt
 CHU = cumulative heat units from germination (see equation 1)
 RD = rooting depth (mm).

Rate of root growth

From a glasshouse trial using 80 dm³ pots it was determined that the growth rate of roots was initially slow at 75 mm/°C day but, at a CHU value of about 1 500°C day, the rate had increased to 93 mm/°C day (see Figure 1). The rapid growth rate was maintained for a relatively short time of about 500°C day. Thus, after a CHU of 2 000°C day was reached, the growth rate slowed. Similar growth rates for unstressed cane were obtained from a second 80 dm³ pot trial conducted at the CFS rainshelter (Figure 2). See Table 3 for growth rates in various units.

Table 2. Comparison of the rates at which roots of variety NCo376 penetrated.

Soil form	Days after planting	CHU* (°C day)	Rooting depth (mm)	Mean root penetration rate (mm/day)	Mean root penetration rate (mm/°C day)
Hutton sand	49	704	1 200	24,5	1,70
	87	1 171	1 950	22,4	1,67
Cartref sand	49	704	1 200	24,5	1,70
	87	1 171	1 950	22,4	1,67
Shortlands sandy clay loam	49	704	1 200	24,5	1,70
	87	1 171	1 650	19,0	1,41
	176	1 945	1 950	11,1	1,00

*See equation 1

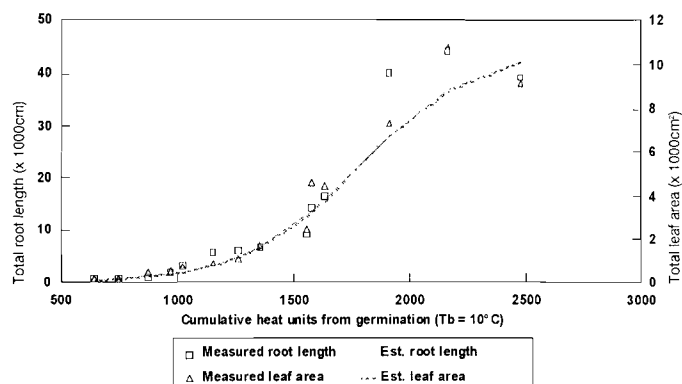


Figure 1. Total root length (Lt) and total leaf area (TLA) growth curves as a function of °C day. The estimated curves for these parameters were superimposed to illustrate their close relationship.

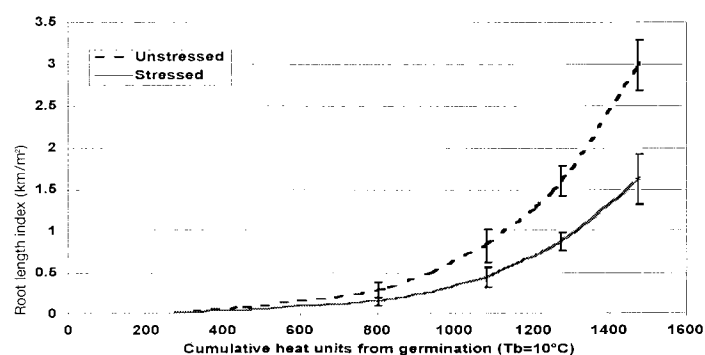


Figure 2. The effect of watering regime on the relationship between root length index and cumulative heat units for data collected from all soils used in the CFS pot trials (bars indicate standard error of the mean).

Table 3. Various time and root growth rate units of variety NCo376 grown in.

CHU (°C day)	MAP (approximate)	Root growth rates		
		mm/°C	mm/day	km/m ² /day
0 – 1500	3 (CHU = 1 500)	75	670	0,0186
1500 – 2000	5 (CHU = 2 000)	93	2878	0,0799

CHU = cumulative heat unit (see equation 1)

MAP = months after planting

km/m²/day = root length index per day

Effect of soil texture on root growth

Table 4 shows the effect of soil texture and water regime on root distribution. It is clear that when the percentage of roots per depth was compared for irrigated and dryland grown cane, with a reduction in plant available water, less roots were found in the surface soil layers and more in the deeper layers. This effect was less obvious for the soils with a high clay content. This is partly due to ample rain, which was only 276 mm less than the evaporative demand of 992 mm for the period ending when the roots were sampled.

Because of the inconclusive results for root development of cane grown under dryland conditions, only irrigated cane was considered to relate soil texture differences to quantities of root distribution per depth interval. Table 4 shows that the percentage root distribution was similar for irrigated cane

over a wide range of soil textures. It is envisaged that the effect of soil texture on root distribution will be far more pronounced under dryland conditions, with less roots expected near the surface and more in the deeper layers in dryland sandy soils than in dryland clay soils.

A difficult irrigation scheduling decision is the depth to be used when calculating total plant available water (mm) for a soil profile given the plant available water capacity (mm/m). The current recommendation is that a rooting depth value equal to the depth in which 85 to 95% of the roots are found should be used. Cumulative values from Table 4 indicated that this depth should be 1 200 mm regardless of water regime and soil texture. The only fraction that did not comply with this rule was the dryland sandy soil (Table 4), where 75% of the roots appeared within the first 1 200 mm of soil and 91% within a total depth of 1 350 mm.

Table 4. Percentage root distribution per depth interval for variety NCo376 grown with and without irrigation on clay and sandy soils.

Depth (mm)	Clay soil (32-40% clay)		Sandy soil (4-8% clay)		Sandy soil (GIB, 12% clay)	
	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland
450	59,94 (1,36)	57,11 (1,40)	55,33 (1,32)	31,45 (0,97)	57,90	37,89
750	10,38 (0,69)	12,73 (1,11)	18,13 (0,78)	21,71 (0,77)	15,20	31,68
1 200	11,92 (0,48)	14,79 (0,91)	11,70 (0,49)	21,44 (0,98)	7,01	18,01
1 650	12,40 (0,46)	11,49 (0,61)	11,37 (0,41)	16,47 (0,53)	9,36	9,31
1 950	5,38 (0,53)	3,89 (0,37)	3,47 (0,27)	8,24 (0,60)	10,52	3,10

Clay soil = Shortlands (32% clay and 12% silt) and Arcadia (40% clay and 16% silt), n = 8

Sandy soil = Hutton (4% clay and 3% silt) and Cartref (8% clay and 6% silt), n = 12

GIB = Hutton (12% clay and 10% silt) recalculated from Inman-Bamber (1986), n = 4

Values in brackets = Standard error of the mean

Effect of water stress on root growth

The objective of a second 80 dm³ pot trial conducted at the CFS rainshelter was to determine the effect of water stress on cane growth. Root length index was severely affected by a shortage of water, which was induced after the cane had reached a CHU of 750°C day. By the time the trial had reached a CHU of 1 500°C day the root length index of the stressed cane was about 50% of that obtained in the unstressed treatment (Figure 2).

Root distribution and water uptake

The data used to compare the relationship between water uptake and root distribution were obtained from cane growing under irrigation in three soils at the SASEX root laboratory. The mean percentage of soil water uptake was calculated for three periods, which were also the periods used to calculate the mean percentage of roots per soil depth.

Water uptake is proportional to rooting density (Taylor and Klepper, 1975). This was confirmed for the sugarcane variety NCo376 by Inman-Bamber (1986, p80). However, the relationship between these two parameters appeared to be linear for the Hutton and Cartref sands, while the percentage water uptake from the deeper soil layers in the Shortlands sandy clay loam was greater than the root percentages present in these deeper layers (Figure 3). When the data were examined in a 1:1 graph (not shown) it was clear that the amount of water depleted was less in the topsoil layers and more in the subsoil layers for all three soils compared with the percentage of roots present in these layers. This was possibly due to the fact that plant available water was depleted by about a third in the topsoil layers at the times selected to calculate the change in soil water content. This stage of water depletion was selected to minimise water losses from the profile through pathways other than uptake by the roots (drainage, evaporation).

Figure 3 also shows that the deeper and younger roots were

potentially more effective per unit root length in uptake of water when compared with roots in the surface layers. Taylor and Klepper (1975) first demonstrated this for maize and van Antwerpen *et al.* (1994) for sugarcane.

Relationships between roots and above ground biomass

Both total root length (Lt) and total leaf area (TLA) were found to correlate well with CHU, as reflected in equations 4 and 5. This implied that a good relationship could exist between Lt and TLA, and is shown in Figure 1. Equations 6 and 7 describe the relationship between these two parameters where, for the latter equation, these two parameters have both been converted to index units. The relationships with CHU in equations 4 and 5 are conventional sigmoidal growth curves, whereas the relationship between Lt and TLA in equations 6 and 7 was found to be linear. Equations 4 to 9 are given in Table 4.

It was observed from the glasshouse pot trial that the rate of leaf appearance slowed abruptly after reaching leaf 16 and a CHU of 1 500°C day (Figure 4). However, leaf appearance rates were comparable with those observed in field trials for variety NCo376 (Gosnell, 1968; Inman-Bamber, 1994). The inflexion in the curve at about 1 500°C day was also recorded by Inman-Bamber (1994), although for the 14th leaf of a first ratoon crop. Canopy development of ratoon crops is quicker than that in plant crops, which explains the difference in leaf number at the inflexion point (Thompson, 1988).

Total leaf number per stalk (TLS) as a function of CHU smaller and larger than 1 500°C day are given by equations 8 and 9 (Table 4) respectively. The inflexion point in the rate of leaf appearance in Figure 4 cannot be explained without looking at other growth trends of the sugarcane plant. One possible explanation for the sudden reduction in the rate of leaf appearance is that roots require a higher photosynthate allocation as they enter the fast growing phase (Figure 4). Both these changes occurred at about 1 500°C day.

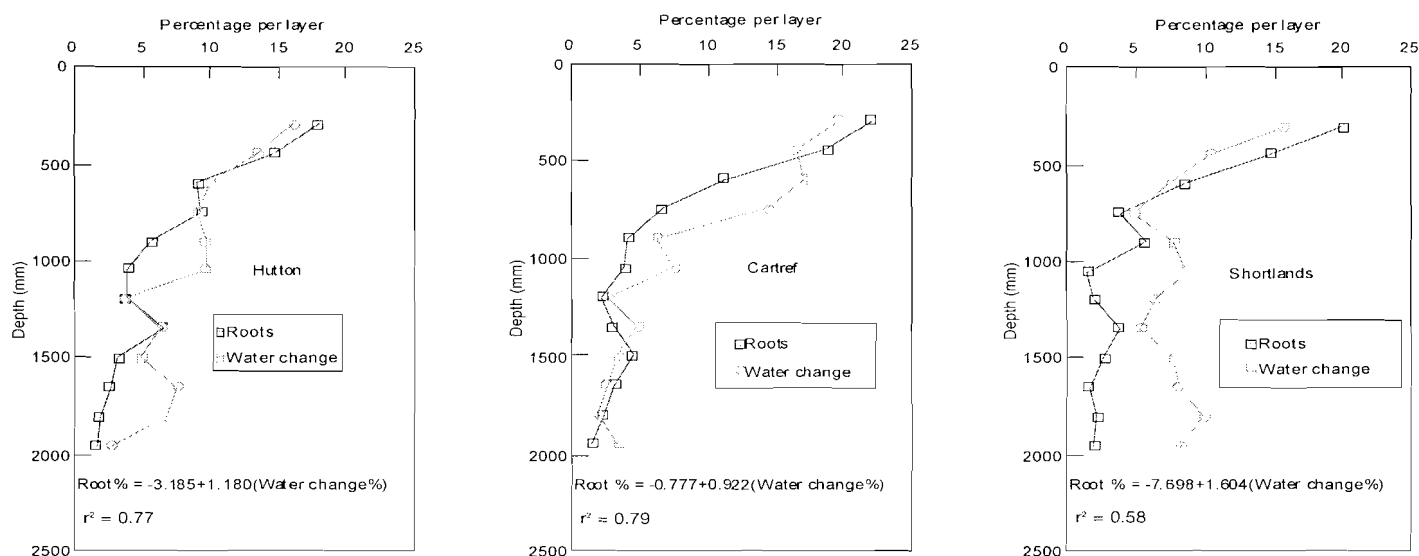
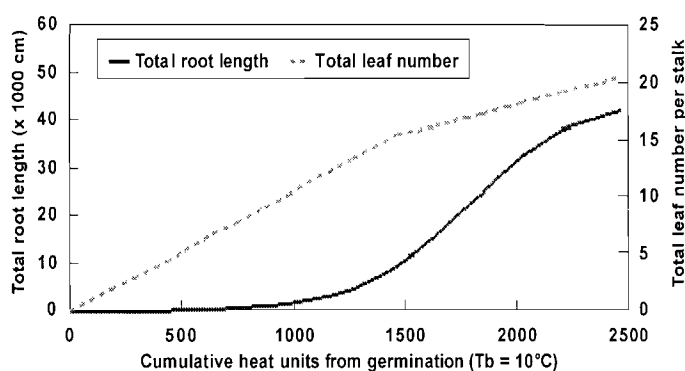


Figure 3. The relationship between percentage soil water change and percentage roots per layer for three different soils at the SASEX root laboratory.

Table 4. Equations relating various root and above-ground biomass parameters to each other and to cumulative heat units (CHU).

Equation	r ²	n	Units and conditions	Equ no.
$L_t = (45000/(1+1250 \text{ Exp}(-\text{CHU}/250)))$	0,94	12	L _t = cm CHU = see equ. 1	4
$\text{TLA} = (10750/(1+1250 \text{ Exp}(-\text{CHU}/250)))$	0,96	12	TLA = cm ²	5
$L_t = -530,73 + 4,7865(\text{TLA})$	0,97	12		6
$L = -0,3485 + 4,7865(\text{LAI})$	0,97	12	L = cm/cm ² LAI = cm ² /cm ²	7
$\text{TLS} = -0,00008 + 0,010614(\text{CHU})$	1,00	22	CHU < 1 500 °C day	8
$\text{TLS} = 7,5917 + 0,005296(\text{CHU})$	0,99	14	CHU > 1 500 °C day	9

**Figure 4.** The relationship between root length, leaf number and thermal time. Note the inflexion in both curves at about 1 500°C day.

Conclusions

From the data obtained was it possible to relate root growth to soil texture, soil water content and various above ground biomass parameters. The use of cumulative heat units as the time-related variable should simplify the use of the given equations for other climatic regions.

The effective rooting depth value required to calculate the total plant available soil water in a profile from the available plant water capacity was determined as 1 200 mm regardless of the watering regime and texture of the soil. Where the soil profile is shallower than 1 200 mm, the effective rooting depth should be taken as the depth at which the root impermeable layer occurs.

The effect of soil texture on the descending rate of roots was in accordance with that found in the literature. However, the equations given to estimate the descending rate should be useful in evaluating the estimated descending rate in crop models for soils with at least two textural ranges. More data are required in order to mathematically express the effect that soil texture had on the descending rate of roots.

Insufficient data were available to establish the effect of water stress on the critical heat quantity required to signal the increasing and decreasing rates of root elongation at

around 1 500°C day and 2 000°C day respectively. It is, however, possible that the availability of water will affect only the rate of development and not the reaction of the plant after the critical heat quantities have been accumulated.

To obtain an estimate of the amount of roots in a profile is laborious, and therefore equations relating easy measurable above-ground biomass parameters to root development could be useful. This paper shows that a good relationship exists between root length and leaf area and that roots enter their fast growing phase when leaves 14 to 16 have appeared on the main stalk, which occurs after the heat units have accumulated to 1 500°C day.

It is known that various parts of the sugarcane plant have different base temperatures beyond which growth will cease. Due to their inaccessibility this value has not been determined for the roots but, with the close relationship that exists between root length and leaf area, it is reasonable to assume that the base temperatures of these two parameters are similar.

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TRANSCRIPTIONAL AND TRANSLATIONAL EXPRESSION OF A WILD TYPE BACTERIAL TOXIN GENE SEQUENCE IN TRANSGENIC SUGARCANE

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Abstract

Genes from bacterial sources often confer characteristics that are seen as highly desirable for introduction into crops by genetic engineering. However, bacterial gene sequences are not always compatible with the molecular mechanisms operating in plants. In this work, expression of a truncated but otherwise unmodified bacterial toxin gene was examined in transgenic sugarcane clones. Molecular analysis of specific RNA transcripts and protein products, by Northern and Western blotting respectively, showed that RNA transcripts were characterised by significant premature polyadenylation triggered at specific points in the gene sequence, while protein levels were undetectable.

Introduction

In the genetic engineering of higher plants, the chosen transgene of interest is often bacterial in origin. Bacteria have evolved a diverse array of metabolic pathways and products not found in eukaryotic organisms, and the genes encoding those characteristics have the potential to add novelty to plant phenotypes. In addition, bacterial characters of interest are often single gene traits encoded by simple genes, suitable for cloning into small DNA vectors for delivery to the plant and subsequent integration into the plant genome. Examples of bacterial genes used widely in plant transformation are those encoding enzymes that effect herbicide resistance and those producing insecticidal proteins such as the endotoxins from strains of *Bacillus thuringiensis* (*B.t.*). A truncated native *B.t.* gene from *Bacillus thuringiensis* strain 234, isolated at Mount Edgecombe (Herrera *et al.*, 1994), has been used to produce a number of sugarcane transformants of varieties NCo310 and NCo376. The *B.t.* 234 toxin, of the CryIA(c) type, is particularly effective against the sugarcane stalk borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae). A subset of NCo310 plants confirmed as transgenic for the *B.t.* 234 gene has been the subject of gene expression studies at transcriptional (mRNA) and translational (protein) levels. Results of these expression analyses are reported here.

Materials and Methods

Sugarcane material

Various individual *B.t.* transformant plants and non-transgenic NCo310 were micropropagated to produce a number of clones of each type. These were maintained in the containment glasshouse at the South African Sugar Association Experiment Station (SASEX) at Mount Edgecombe using conventional pot fertilisation and automated watering regimes. Experimental material consisted of plants ranging from 500 mm to 2.5 m in height.

RNA extraction, RT-PCR and Northern analysis

Leaf tissue was the source of all RNA extracts used in this study. Third youngest leaves were randomly sampled from three NCo310 control plants and from three individual plants within each transformant line at each daily time point. Sampling was done at the same time each day (between 11h00 and 12 noon) and the same portion of the leaf removed in each case. Samples were pooled for each line, immediately frozen in liquid nitrogen and stored at -80°C. For reverse transcription-polymerase chain reactions (RT-PCR), DNA-free RNA was extracted using the SV Total RNA Isolation System (Promega), while for Northern analysis the RNeasy Extraction kit (Qiagen) was used to prepare larger amounts of total RNA. In each RT-PCR reaction, 1 µg RNA was used in a final volume of 50 µl in a single step procedure (Titan System, Boehringer Mannheim). RT-PCR products were analysed by electrophoresis in agarose gels (1%, w/v) and visualised by conventional ethidium bromide staining. RNA for Northern analysis (15 µg per sample) was fractionated through agarose (1.2%, w/v) in the presence of formaldehyde, and the resultant profiles checked by ethidium bromide staining for equality of loading and presence of undegraded ribosomal RNA bands before transfer to positively charged nylon membrane (Amersham) using the downward capillary blotting method of Chomczynski and Mackey (1994). Probe DNA was generated by PCR amplification from plasmid vector of a 1 850 bp fragment of the *B.t.* 234 gene using specific primers. *B.t.* gene amplification products were purified by agarose gel electrophoresis followed by excision of bands and column extraction (QIAquick, Qiagen). Isotopic labelling was by random