MODERATION OF GROWTH AND SUCROSE FLUX IN SUGARCANE BY TEMPERATURE

By

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DECLARATION

I hereby declare that the research work reported in this thesis is the result of my own original work except where acknowledged. I also declare that the results of this work have not otherwise been submitted in any form for any degree or diploma to any university.

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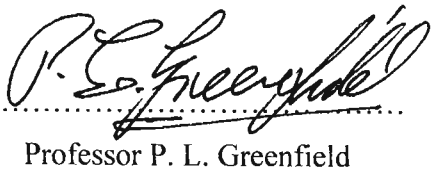
D. Ngomane

I, Peter Lorimer Greenfield, supervised the above candidate in the conduct of his dissertation study.



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ABSTRACT

Sugarcane plants (cultivar NCo376) were studied to assess the effects of temperature and season (spring and autumn equinox) on the morphological and physiological response of stalk components. Plants were grown from single-eyed setts for ca. five months and then placed into three temperature controlled glasshouses (22/12°C (C), 27/17°C (W) and 32/22°C (H) day/night temperatures). The plants were sampled twice weekly over a one month period, and internodes 4, 6 and 10 of the primary haulms of each plant sampled for growth and sugar analysis.

During spring, the leaf emergence rates were 0.0303, 0.1095 and 0.1682 leaves d^{-1} at temperatures C, W and H, respectively; and 0.0327, 0.0824 and 0.113 leaves d^{-1} in autumn. The phyllochron intervals were 114°Cd in spring and 147°Cd in autumn. Highest green leaf blade area of the primary haulms was achieved at H (438.0 and 511.7 cm^2 in spring and autumn, respectively). The stalk extension rates were 1.22, 1.02, 0.38 cm d^{-1} (spring) and 1.35, 0.98, 0.45 cm d^{-1} (autumn), respectively, in descending order of temperature. Total biomass and stalk biomass per plant were not affected by temperature, despite the differences in stem elongation. Internodes of plants at C were shorter but thicker and heavier than the comparable internodes of plants at W and H.

In autumn, the mature internode sucrose concentrations were 35.5, 29.2 and 25.5% at C, W and H, respectively; corresponding to mean RS% of 5.7, 9.8 and 13.3%, and fibre % of 58.8, 61.1 and 61.3%, at the respective ascending order of temperature. Sucrose % in the mature internodes in spring were 27.8, 20.9 and 19.9% at C, W and H, respectively; corresponding to RS% of 5.9, 9.76 and 10.9% and fibre % of 66.3, 69.4 and 69.2% at the respective ascending order of temperature. Temperature effect on the concentration of the stalk components of the immature internodes was in general not significant. Sucrose partitioning coefficients in the mature internodes were 0.25, 0.21 and 0.20 in spring and 0.50, 0.32 and 0.21 in autumn (at C, W and H, respectively). Data that resulted from this study, which is isolated to temperature and cultivar NCo376 can be used in models of sugarcane that simulate leaf appearance and senescence, assimilate partitioning between

leaf and stalk and assimilate partitioning between the stalk components namely sucrose, reducing sugars and fibre.

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GENERAL INTRODUCTION

Sugarcane (*Saccharum* spp.) is an important tropical agricultural cash crop that is cultivated for its sucrose, which is accumulated in substantive quantities in the stalk of the plant (Grivet and Arruda, 2002). It belongs to the genus *Saccharum*, an important member of the Andropogoneae tribe, belonging to the grass family Poaceae (Corderio *et al.*, 2003). Modern cultivated sugarcane cultivars are complex hybrids synthesized from *S. officinarum, S. barberi, S. sinense and S. spontaneum* (NanSheng *et al.*, 2005). Early cultivars were made from crosses of the wild, vigorous and disease resistant *S. spontaneum* and the high sucrose containing *S. officinarum* (Berding and Roach, 1987), and modern cultivars are derived from those early interspecific genotypes, followed by cyclical intercrossing and selection (Grivet and Arruda, 2002). Sugarcane is grown in more that 90 countries in the world, with Brazil and India taking the lead; and the crop contributes about 65 % of the raw sugar produced worldwide, the rest coming from sugar beet (Zambrano *et al.*, 2003).

The sugar industry of South Africa is important, in terms of the national economy of the country. It contributes substantially to the country's agricultural and industrial investments, foreign exchange earnings and employment. Annual sugar sales from the Southern African Customs Union (SACU) and the world markets total up to R6 billion, and an estimated R2.38 billion to the county's foreign exchange earnings. The industry employs about 85 000 people, and direct and indirect employment is estimated at 350 000 in South Africa. Over one million people are dependent on the sugar industry (anonymous, 2005). Local sugar yield is affected, from season to season, by weather changes. A record crop of 2.729 million tons of sugar extracted from 23 million tons of sugarcane, harvested from an estimated 328 000 hectares, was achieved in South Africa in the 2002/2003 season (Davis, 2003). In the same season, a record cane-to-sugar ratio of 8.32 (Singels *et al.*, 2003) and a record recoverable value (RV) percentage of 12.54% of sucrose and a mean of 70 t cane ha^{-1} was achieved (Davis, 2003). On top of all the agronomic inputs, in terms of genotype selection, soil treatment, pest and disease control, and harvesting and handling of the crop, sugar yield is largely a function of climate. The

2002/2003 season had exceptionally good weather with less winter rains, which were well distributed over the season, and that contributed to the exceptionally high productive season (Singels *et al.*, 2003).

Over the years, several events that challenge the stability of the sugarcane industry of South Africa have occurred. The international price of sugar is undergoing a steady decline of 2% pa, and with the need of one percent improvement of wages pa (in real terms), it implies that the sugar industry has to improve its productivity by at least 3 % pa or face a dramatic decline (Murray, 2000). Also, the pricing of sugar in both the local and export market according to the US$ based reference pricing system, that relies on the South African minister for finance, the US treasury and the SA Rand-to-US$ exchange rate, has contributed to the sugar value decline (Murray, 2000). The continuous build-up of surplus sugar in the world, and allowing Brazil to compete in new markets has further increased the tension in the sugar industry (Ryan, 2000). The environmental factors have continuously affected the performance of the sugarcane genotypes that are bred (Gilbert *et al.*, In Press). Among others, temperature and rainfall during the harvesting season are responsible for yield variation from year to year (Singels *et al.*, 2003). The sugarcane growers have always suffered the downside of the cane consignment payment system, which is based on the recoverable value of their cane, and it does not exploit the quality pattern that is distinct across regions and across the harvesting season, characterized by poor quality at start and end of the harvesting season, which result from the seasonal climatic pattern (Guilleman *et al.*, 2003). It is therefore imperative to embark on research programs to provide for mechanisms for improving productivity and to gather more information that relates the climate to key physiological processes of the sugarcane plant, and use the information for production improvement (Murray, 2000).

Sugarcane has distinctive evolutional advantages, which make it superior under a range of environmental conditions when compared with other plant species. Being a C4 plant and originating from the tropics, sugarcane has a lower CO_2 compensation point and is able to fix carbon at relatively high temperatures and lower atmospheric CO_2 concentration. This is due to the high affinity of phosphoenolpyruvate (PEP) carboxylase

that is not present in the C3 plants (Taiz and Zeiger, 1991). Unlike the C3 plants, which saturate at about 50% of full sunlight, the C4 plants do not show light saturation, allowing them to be able to harvest as much solar energy as possible and convert it to chemical energy (Coombs *et al.*, 1985). With the ability of C4 plants to concentrate carbon dioxide at the site of carboxylation by ribulose bisphophate carboxylase/oxygenase (RUBISCO), and thereby decreasing the competitive inhibition by oxygen, they are able to suppress C2 pathway activity, significantly decreasing the rate of fixed carbon loss from photorespiration under conditions of stress, a phenomenon which does not occur in C3 plants (Taiz and Zeiger, 1991).

Sugarcane growth and development is measured in phytomeric units consisting of a leaf primodium associated with a subtending node and an intercalary meristem that develops to form the internode (Moore, 1995). Under favourable growth conditions (high temperature, sunlight and moisture, and adequate nutrients) the time interval between successive phytomeric units can be about 5 days, whereas under unfavourable growth conditions over 20 days can elapse between successive phytomeric units (Moore, 1995). If conditions are not supportive of growth, but not inhibiting to leaf photosynthesis, the photoassimilates produced are stored as sucrose in the vacuoles of internodal parenchyma storage cells (Legendre, 1975). Young leaves are initially sinks (from emergence until leaf collar emergence), a period during which the leaf is expanding. After leaf collar appearance, the leaf ceases to expand and it switches from being a sink to becoming a source, and it is only then that the subtended internode begins to elongate (Moore, 1995).

The sugarcane morphological characters and their ability to store sucrose have been studied. High sucrose-storing cultivars are characterized by thick girthed haulms with high fresh weight, high haulm moisture content and low fibre content, as opposed to low sucrose-storing cultivars, which have thin and fibrous haulms with a low fresh weight (Zhu *et al.*, 1997). Inman-Bamber *et al.* (2002) also related sucrose content of NCo 376 with some morphological features of the plant. A significant positive correlation was found between sucrose content and total leaf number, a measure of plant physiological age, and with haulm (stalk) yield. Sucrose content was negatively correlated with green

leaf number per shoot and with leaf area index (LAI). Green leaf number per shoot was affected by soil water status and was reduced under conditions of water stress, coupled with a rise in sucrose percentage from reduced stalk extension (Inman-Bamber and De Jager, 1986).

Sugarcane crop productivity is principally affected by three climatic factors, namely radiation, temperature and rainfall (Muchow *et al.*, 1994). Of the three, rainfall surplus or deficit can be alleviated, although this correction (either by irrigation or drainage systems) is often not economic (Rostron, 1971). In addition to the environmental factors, other factors that affect sugarcane growth and sucrose yield include genotypic differences, crop age at harvest (Inman-Bamber, 1996; Rostron, 1972), agronomic practices such as weeding (Manana *et al.*, 1996), irrigation (Mazibuko *et al.*, 2002), pest and disease control (Mazodze and Conlong, 2003), etc. When moisture and nutrients are not limiting, radiation and temperature remain the important determinants of biomass production in sugarcane (Muchow *et al.*, 1997; Park *et al.*, 2005). Estimating productivity in terms of the efficiency of conversion of incident short wave length radiation to dry matter (Chang *et al.*, 1963; Thompson, 1978), and in terms of accumulated growing degrees days (GDD) (Yang *et al.*, 2004) is therefore often used. A study by Allen (1976) indicated that the yield of irrigated sugarcane is positively related to both solar radiation and GDD. Temperature and radiation are linked effects (Muchow *et al.*, 1994), and in the field it is not possible to separate their individual contributions to productivity (Rostron, 1971; 1972).

Dry matter partitioning is an important controlling component of sugarcane growth. The value of cane consignments delivered to the mill is calculated from stalk mass components, particularly the mass partitioned to sucrose (Inman-Bamber *et al.*, 2002). Ebrahim *et al.* (1998a) highlighted that in sugarcane crop production practice, sugar yield results from two plant components, namely plant biomass production and the concentration of sucrose in the stalk. Sucrose yield is therefore dependant in part on the partitioning of dry matter to the stalks and on partitioning of stored sucrose to the stalk storage parenchyma cells. The desire of every sugarcane producer is, therefore, to

maximize stored chemical energy in the form of sucrose in the storage cells of the internodes of malleable stalks of the plant at harvest (Roberts *et al.*, 1985). This is especially so because storage sugar is the component of total biomass for which the crop is harvested. Sucrose accumulated in the stalk of sugarcane is therefore the most important component of the crop (Lingle and Smith, 1991). A high concentration of sucrose improves the juice quality as it increases sucrose recovery in the sugar mill (Lingle and Irvine, 1994), an important factor of the currently used system of cane consignment payment. The yield of sucrose can be increased by (i) increasing the net amount of dry matter produced by the whole plant (if sucrose % cane is held constant), (ii) increasing the proportion of stalk to roots and leaves (harvest index), and (iii) increasing the sucrose % dry matter of the stalk (if yield of stalk is held constant), or (iv) by the combination of the three (Rostron, 1971). Sucrose percentage in sugarcane stalks has been shown to be affected by radiation, temperature and plant water status (Inman-Bamber *et al.*, 2002; Hartt, 1965a). The effects of these environmental factors on plant growth are observed to change during the growing season and their effects on sucrose yield are observed at harvest (Irvine, 1983).

In his presidential address speech at the 74th annual congress of SASTA in 2000, Murray (2000) mentioned that the agricultural sector of the sugar industry has room for productivity improvement, and therefore, should target the development and implementation of new technology, as a counter to the negative effects of the environment and the extended milling periods on the quality of cane. The basic and fundamental step in the efforts to improve productivity is generating and accumulating as much knowledge as possible, from physiology studies, about the key physiological processes in sugarcane and their interaction with the environment and with farm practices. This information can then be used directly for crop management or indirectly for crop simulation models (Lisson *et al.*, 2005). Quantitative understanding of plant-environment relationship is the foundation for the decisions made during the cropping period from genotype and site selections, crop management, infrastructure investment and marketing (Park *et al.*, 2005). Hence, the objectives of this study was to quantify the isolated effect of temperature on the growth parameters of sugarcane and on assimilate

partitioning to the stalk components, namely sucrose, non-sucrose and fibre in spring and autumn; data of which will be useful in filling up some knowledge gaps in the currently used sugarcane simulation models. Data on the temperature response of the phenological processes (leaf appearance and senescence, leaf area and stalk extension rate), assimilation (total biomass, leaf and stalk mass) and assimilate partitioning between stalk and leaf material and between the stalk components will be used in refining cane models.

1. LITERATURE REVIEW

1.1 Effect of solar radiation on sugarcane leaf photosynthesis

Generally, plants produce dry matter from carbon dioxide, water and nutrients, in the presence of solar irradiance by the process known as photosynthesis (Roberts *et al.*, 1985). The amount of crop biomass produced under favourable moisture, temperature and nutrient conditions is dependent on the amount of usable light available and the proportion of the light intercepted by photosynthetic structures (mainly the leaves) and converted to biomass (Rostron, 1971; Glover, 1972a; Singels *et al.*, 2005b). Sugarcane is native to the tropical climate and exhibit climate responses that reflect adaptation to the tropical climate, which are characterized by high radiant flux and temperature.

Glover (1972b) defined net assimilation as the difference between gross carbon assimilation and respiration. Net biomass gain (or net productivity) $\mathbf{P_n}$ is determined by four factors: (i) quantity of incident light **Q**; (ii) proportion of the light intercepted by photosynthetic organs β; (iii) the efficiency of converting the intercepted light energy into chemical energy (biomass) ε; and (iv) the respiratory losses **R**; which can be linked together by the following equation:

$$\mathbf{P_n = Q\beta\varepsilon - R} \text{ (Roberts } \textit{et al.}\text{, 1985)}..... (1)$$

It implies, therefore, that net productivity can be increased by maximizing the component of gross assimilation $\mathbf{Q\beta\varepsilon}$, which is composed of functions of light, and minimizing the respiration component **R**. For most of the currently used cultivars, radiation use efficiency (RUE) is limited by incident and intercepted radiation (Muchow *et al.*, 1994). In the study of Muchow *et al.* (1994) a maximum value of the fraction of intercepted radiation of 0.93 was achieved from a non-water stressed crop, and plants attained a maximum growth rate of 41 g m^{-2} d^{-1} over a 40-day period. This value was compared with a growth rate of 54 g m^{-2} d^{-1} for the C4 species *Pennisetum typhoides* (Begg, 1965). Using RUE of 1.75 g MJ^{-1} implies that incident solar radiation of 33 MJ m^{-2} d^{-1} would be required to attain the growth rate value of 54 g m^{-2} d^{-1} in sugarcane. Such rates of radiation are not common in most sugarcane growing regions (Muchow *et al.*, 1994). The proportion of light intercepted by photosynthetic organs can be improved genetically by

modifying crop canopy structure (Irvine, 1975). The efficiency of converting intercepted light into dry matter is regulated by temperature, and thus not possible to control in a field situation. Rostron (1971) reported that sugarcane converts about 50% of the 12-18% of available incident visible light energy into growth. As a C4 species, sugarcane does not show saturation to light (Berry and Björkman, 1980), sugarcane leaf photosynthesis increases with stronger light (MuQuing *et al.*, 1998). In South Africa, radiation effects are observed from sucrose yield of crops grown in different latitudes and altitudes (Inman-Bamber, 1994).

Radiation use efficiency of sugarcane is greater than that of most other C4 plants, despite low single leaf photosynthesis (Inman-Bamber, 1994). According to Muchow *et al.* (1994), crop biomass (**BM**; g m^{-2}) can be expressed as a function of intercepted radiation (S_i; MJ m^{-2}) and the radiation use efficiency (**RUE**; 1.75 g MJ^{-1}) by a linear equation:

BM = 1.75±0.005*Si* + 125±109 (Muchow *et al.*, 1994).....(2);

and that S_i is determined by the crop duration, leaf area development, and by radiation extinction coefficient. A study by Muchow and Carberry (1989) indicated that maize leaf area development is influenced by temperature, while its RUE is influenced by specific leaf nitrogen content (Muchow and Davis, 1988). Glover (1974) reported that the rates of apparent photosynthesis of whole plants of sugarcane under favourable moisture conditions are linearly related to the rates of solar radiation incident on the horizontal surface up to intensities of ca. 840 W m^{-2}. Very high correlation between the apparent photosynthesis and incident radiation was observed, and that the correlation was higher (r^2 = 0.91) on older plants (17-18 months) than on smaller plants (r^2 = 0.71 for 3 months old plants). However, the study by Muchow *et al.* (1997) indicated that the linearity of the function of apparent photosynthesis of whole plants and accumulated radiation does not hold beyond 12 months of growth.

Muchow *et al.* (1997) made a finding relating the utilization of intercepted radiation and the time of growth of a high yielding sugarcane crop. It was observed that the efficiency

of radiation utilization for growth was less after the first 12 months than during the first 12 months. On a study conducted at Kunia in Hawaii, the H73-6110 cultivar intercepted more radiation during early growth, up to 0.78 at 120 days after planting (DAP). It reached 0.94 at 366 DAP, and thereafter began to drop. Seasonal RUE was shown to drop from 1.52 g MJ^{-1} at 354 DAP to 1.24 g MJ^{-1} at 445 DAP. These results are in agreement with the results of Muchow *et al.* (1994) who reported that the LAI for cultivar Q96 declined from a peak value of 6.8 at 297 DAP to 3.9 at 445 DAP, resulting to a decline of the fraction of intercepted radiation from 0.93 at 297 DAP to 0.79 at 445 DAP. Measuring whole plant photosynthesis, Glover (1974) reported that the linear relationship between the amount of CO_2 assimilated per unit area of green leaves and accumulated radiation holds for plants between 3 and 18 months old.

The drop in intercepted radiation, hence in RUE, was explained by Glover (1974) to be a result of loss of stalks from 9.2 to 7.7m^{-2} post 366 DAP. Das (1936) reported that the number of stalks that have growing tops begin to drop post 12 months of plant growth. Robertson *et al.* (1996a) observed differences in early growth between plant crops of two cultivars (Q117 and Q138), and since there were no differences between leaf area per stalk of the two cultivars it was concluded that differences were due to stalk population differences and consequently in whole plant LAI. Inman-Bamber (1994) associated stalk mortality with the shading of younger shoots by older shoots. Rostron (1972) observed that the population of harvested cane stalks dropped with increasing age between 32 and 52 weeks of age, and associated the drop in growth rate of plants with increasing age as caused by lodging and production of non-millable shoots. It was concluded that the final yield of crops growing beyond 12 months was not related to direct utilization of radiation but to stalk death, resulting in reduced biomass per unit area and increased biomass per stalk. Numerous works on radiation use and biomass accumulation indicate that there may be differences in biomass accumulation between cultivars but these differences are not reflected in the final stalk and sucrose yields at harvest post 12 months (Evenson *et al.*, 1997; Muchow *et al.*, 1994; Muchow *et al.*, 1997; Robertson *et al.*, 1996a); implying that maximizing early radiation interception and biomass accumulation will not result in

higher yields at harvest in areas where biomass production reaches maximum long before harvest (Robertson *et al.*, 1996b).

It is apparent that the effect of increased radiation may not be readily observed when other factors are limiting. Allison *et al.* (1997) reported that the effect of increased photosynthetically active radiation (PAR) on leaf photosynthesis of sugarcane is readily observed on cane leaves that have high levels of specific leaf nitrogen (SLN) content. The rate of photosynthesis of the youngest fully emerged leaves increased almost linearly with increase in SLN (ranging between <1 to 1.7 g m^{-2}); and the rate of increase was higher at greater PAR (ranging between 80 to 1600 μmol m^{-2} s^{-1}). Obviously, the time taken by plants to reach the point of maximum RUE is influenced by temperature, nutrient and water stresses. Inman-Bamber (1991) reported that the time to reach maximum radiation use was shorter for plants grown at higher temperatures than at low temperatures. Water stress causes leaf rolling on cane leaves and thereby lowering net accumulated radiation (Inman-Bamber and De Jager, 1986; Glover, 1972a).

The development of leaf area is critical in the establishment of full leaf canopy for maximum radiation interception to achieve high crop productivity (Sinclair *et al.*, 2004). Glover (1974) showed that the slope of an apparent photosynthesis-radiation graph doubles by doubling the number of plants per unit area. Interception of radiation by sugarcane leaves is a function of the geometry of crop canopy (Irvine, 1975). Maximum radiation interception is associated with erect rather than horizontal leaves in the upper canopy (Duncan, 1971). However, this thought is not widely accepted by many researchers (Ariyanayagam *et al.*, 1974; Irvine, 1975; McCree and Keener, 1974; Russell, 1972). Irvine (1975) suggested that cultivars with many horizontal leaves should be grown to achieve early canopy closure and early yield accumulation, and that this is especially for temperate regions which have only a few months of growing period.

Sugarcane crops in South Africa are currently harvested at an earlier stage than is optimum for physiological considerations of sucrose productivity in order to minimize the risk of damage by the stalk borer *Eldana saccharina*. This has resulted in a general

increase in the proportion of land in the industry that remains without a complete canopy of leaves (Inman-Bamber, 1994). The effect of this is decreased radiation intercepted by the leaves, and hence a considerable loss of productivity for the industry in most years (Inman-Bamber, 1991). Inman-Bamber (1994) suggested using a reduced row spacing to speed-up canopy closure, and the use of cultivars with rapidly developing canopy as measures to reduce the negative effects of harvesting sugarcane at an earlier stage. Singels and Smit (2002) observed that canopy closure had a linear relationship with row spacing and that there was an increase of 26% of canopy closure for every 1.0m reduction of row spacing.

1.2 Effect of temperature on leaf photosynthesis of sugarcane

Physiological processes in sugarcane, from germination (Miah *et al.*, 2003; Singh, 1988), leaf emergence (Inman-Bamber, 1994; Ono and Nakanishi, 1983), shoot emergence, stalk extension (Liu *et al.*, 1998), leaf growth and canopy development (Keating *et al.*, 1999; Sinclair *et al.*, 2004), to translocation and partitioning of assimilates (Keating *et al.*, 1999) are driven by temperature. This makes temperature an important variable in sugarcane growth and development. Sugarcane has the C4 photosynthetic apparatus (Goatly *et al.*, 1975), and has optimum photosynthetic rates at higher temperatures and radiation than C3 plants (Berry and Björkman, 1980). The optimum temperature for sugarcane leaf photosynthesis is between 25 and 35°C (Ebrahim *et al.*, 1998a). Photosynthesis is only markedly inhibited by temperatures above 40°C and below 15°C (MuQuing *et al.*, 1998). Extremely low temperatures (5°C) adversely affect photosystem II (PS II), and in turn, decrease the relative efficiency of energy capture by PS II and the quantum yield (MuQuing *et al.*, 1999).

A common feature of tropical plants, sugarcane being one, is that they are sensitive to chilling temperatures (Berry and Björkman, 1980). Terzaghi *et al.* (1989) found that membrane lipids of tropical plants undergo lateral phase separation at 10°C, or even above 10°C, whereas their temperate counterparts begin lateral phase separation at much lower temperatures. Cultivation of sugarcane is limited by chilling temperatures of 15°C in most subtropical areas (Ebrahim *et al.*, 1998b). Studies show that temperature affects

photosynthesis by affecting the photosynthetic parameters of the leaves, namely chlorophyll content, Hill reaction, chloroplast membrane organization, chlorophyll fluorescence parameters associated with PS II and electron transport activity (Ebrahim *et al.*, 1998b). In turn this disrupts the entire metabolic and physiological processes concerning photosynthesis. Temperatures below 10-12°C lower the Calvin Benson cycle and cause physical dissociation of PS II reaction unit and PS I (the light harvesting unit) (Lyons, 1973). Temperatures higher than 45°C physically disrupt the thylakoid membrane lipids (Kaniuga and Michalski, 1978).

On a study to investigate the effect of cool temperatures on photosynthesis and stomatal conductance in field grown sugarcane, Grantz (1989) observed that cool winter nights and cool early morning temperatures (ca. 14°C) and ca. 20°C in summer were low enough to inhibit photosynthesis and stomatal conductance measured on the following day, indicating that cool nights may have residual effects on leaf photosynthesis on the following day. Studies have also shown that non-optimum germination temperatures have residual effects on stalk number, stalk weight and sugar yield per stool of sugarcane (Gascho *et al.*, 1973; Irvine, 1983). Gascho *et al.* (1973) observed a range between 4.3 kg and 9 kg (total sugar per stool 37 months after planting) for plants germinated between 10°C and 31°C.

1.3 Effect of temperature on the morphological growth of sugarcane

The effect of temperature on the morphological growth of sugarcane is observed from the marked differences in the rate of canopy development of crops with different ratoon/planting dates. Summer (December) started crops develop their canopy more rapidly than winter (June) started crops (Singels *et al.*, 2005b). The results of Singels *et al.* (2005b) showed that it took ca. 100 days for a December started crop of NCo 376 to achieve 80% of intercepted radiation, whereas it took 180 days for a June started crop, and that the seasonal mean intercepted radiation for the June started crop was 80% of that of the December started crop. In their study, Singels *et al.* (2005b) observed that the important parameters which defined crop canopy such as shoot profile of leaf size and shoot density were significantly affected by crop start date, which subsequently

influenced the percentage of radiation intercepted by the crops, and ultimately the tonnage and sucrose yield at harvest.

Optimum air temperature for the growth of sugarcane (25-35°C) is similar to that which is optimum for photosynthesis (Ebrahim, *et al.*, 1998a). There is no effective growth below 12-15°C (Sartorius, 1929). Inman-Bamber (1994), working with two South African cultivars (NCo 376 and N12), reported the base temperatures for leaf and tiller appearance as 10°C and 16°C, respectively. Ebrahim *et al.* (1998a) showed that low temperature (15°C) produced slowly growing plants with significantly shorter and fewer internodes relative to plants grown at 27°C (optimum) and 45°C (supra-optimum). There was no significant difference in internode numbers found between plants grown at 27°C and 45°C, although the internodes of plants grown at 45°C were one third the length of those of plants grown at 27°C. Levels of invertase activity (acid and neutral invertases), significant in young and actively growing internodes, were highest at 27°C and 40-50% lower in the internodes of plants grown at 15°C and 45°C; and this was said to account for the highest growth rate of plants grown at 27°C.

A study by Ebrahim *et al.* (1998b), who grew sugarcane at 15°C (below optimum), 27°C (optimum and control) and 45°C (above optimum), showed that high temperatures accelerated the rate of leaf senescence on sugarcane, and plants maintained fewer green leaves. Plants grown at 15°C had only a few dry leaves, but the rate of leaf emergence was strongly suppressed. Green leaf area per plant was highest at 27°C and lowest at 15°C. Green leaf area was higher at 45°C than at 15°C, indicating that the high rate of senescence at the higher temperature was compensated by a high rate of leaf appearance. A striking finding in Ebrahim *et al.* (1998b) study was that plants grown at 27°C had the lowest green leaf blade area per shoot dry weight, indicating that a relatively smaller leaf canopy at optimum temperature was able to support the stalk growth to a greater extent than larger leaf canopies at inadequate temperatures. In addition to air temperature, root temperature is an important factor for leaf development. In another study by Mongelard and Mimura (1972), some important morphological parameters of sugarcane, such as shoot height increase, total increase in spindle elongation, and mean increase in dry

weight of whole plants showed a linear relationship with a range of root temperature from 12.5 to 33.5°C. Because low temperature reduces stalk growth rate (Mongelard and Mimura, 1972), cane grown in cool areas, such as the Natal midlands tends to be grown for extended periods. This subjects plants to many adverse environmental effects and prolongs the period for disease infection.

1.4 Effect of radiation on growth: daylength and sugarcane flowering

Flowering is an economically important environmentally-controlled physiological process in sugarcane, which impinges on crop yield and quality (Berding and Hurney, 2005). Photoperiod is an important requirement for flowering of sugarcane (Rao, 1977). Floral initiation will only occur when the daylength is about 12.5 hours, and in South Africa, this occurs between the 5th of and the 17th of March, but otherwise the seasonal dates of floral induction may vary with the geographical regions and latitude (Rao, 1977). In addition to the photoperiodic requirement, there is also a requirement of appropriate plant age (number of mature internodes), warm night temperature (above ca. 21°C), ambient RH, and physical and nutritional status of the plant (no moisture or physiological stress) (Cifuentes, 1975). The induction of flowering in sugarcane causes the apical meristem of shoots to stop growing. Bunch-tops and side shoots may form from a prolonged period of drought following floral induction (Gosnell and Long, 1973).

Shortly after flowering, flowered shoots may accumulate sucrose in their immature internodes at faster rate than non-flowered shoots (Gosnell and Long, 1973). Gosnell and Long (1973) also observed that for the first three months after flowering, sugarcane parameters such as fibre content, juice purity, sucrose % DM, and RV % were significantly higher for flowered cane than non-flowered cane; and that the opposite was observed for moisture content and reducing sugars (RS), especially at the upper part of the stalks. Pithiness of stalks was severe on flowered stalks. However, the results of Rao (1977) showed a considerable contrast to the results of Gosnell and Long (1973). In the study of Rao (1977), cane yield reduction due to flowering was 56.6% and 33.8% on the plant crop and on the ratoon crop, respectively; and sugar loss was 69.1% and 35.4% for

the plant crop and ratoon crop, respectively. The differences in the results of the two studies could be attributed to the period after flowering on sampled plants.

1.5 Sucrose and the cropping season

Partitioning of photoassimilates in plants occurs between the harvestable and non-harvestable organs. In sugarcane the products of photosynthesis are used for growth, maintenance and storage as sugars in the stalk (Rostron, 1971). The growth rate of sugarcane is the main factor that influences sugar storage and content in the stem. During conditions favouring rapid growth, carbohydrates produced from photosynthesis are largely used for vegetative growth and only little is stored as sucrose (Truen, 1972).

Sugarcane is one of few crops that have evolved a unique system involving the storage of sucrose. The crop synthesizes, translocates and stores sucrose to a greater extent than any other crop on earth (Alexander, 1973; Moore, 1995). There exists competition for photoassimilates between growth and storage in sugarcane, making them reciprocally related (Sacher *et al.*, 1963a). Sucrose content in sugarcane stalks changes over the seasons throughout the year. In South Africa the lowest sucrose % cane (fresh weight basis) occurs after the wet and warm summer season, when the crop is growing rapidly; whereas highest sucrose % cane occurs after the dry, cool season, usually towards the end of winter (Glover, 1971), there being a 4 to 6 week lag in sucrose % cane response to the environmental conditions. Working with NCo 376, Inman-Bamber *et al.* (2002) observed that sucrose content of mature internodes of sugarcane stalks was lowest in autumn ($0.45 gg^{-1}$ DM), highest in spring ($0.56 gg^{-1}$ DM) and decreased during summer. This observation clearly indicates that a significant amount of stored sucrose is hydrolyzed into energy-supplying sugars and remobilized to support growth during the warm and moist summer conditions, which are favourable for growth. This observation also showed that better growing conditions (higher moisture and temperature) do not support partitioning to sucrose storage but encourage more partitioning to enhance growth of roots, leaves and stem. Clearly one of the most important criteria for enhancement of sucrose yield is to manipulate the partitioning of assimilates, either genetically or through physiological manipulation moderated through growth regulants (ripeners).

Inman-Bamber (1996) reviewed data from more than 400 released cultivar trials in South Africa, identifying variation in sucrose % cane in paired comparisons from –2% to +35% (on dry mass basis) relative to that of NCo 376. Some cultivars derived the relative benefit of higher sucrose % from increased dry mass %, some from higher sucrose % cane, and most gained benefit from the combination of both factors. In other words in most cultivars that inherently produced more sucrose than NCo 376, they allocated dry mass more efficiently (increased partition) to the stem. Inman-Bamber (1996) went on to show that in mature internodes sucrose mass was dependent on sucrose % dry mass, and that the latter component as well as % dry mass in these internodes was more affected by crop age than season. Mature internodes rapidly accumulated additional sucrose (up to 55% DM) in winter, or lost a little (less than 5%) in summer. Sucrose % DM increased more rapidly, and was conserved better in younger than older internodes. He concluded that in NCo 376, seasonal variation in sucrose % arises because of fluctuations in the proportion of expanding to expanded stalk tissue, and to a lesser extent due to seasonal variations in DM %. Also, that stored sucrose was not readily remobilised, and that reductions in sucrose % cane should not necessarily be regarded as a reduction in sucrose yield (total accumulated sucrose).

Following this study, Inman-Bamber (2002) reported that maturation in sugarcane could be described in two phases, one of which is when the sucrose concentration (DM) of basal internodes is increasing, and the other in which sucrose concentration has reached maximum. Relating to the second phase, increased accumulation of sucrose concentration of whole stalks was dependant on the ripening of the upper immature internodes. In other words, once the crop is through the first phase, the seasonal accumulation of whole stalk accumulation is largely due to partitioning of sucrose in the upper immature internodes. This is mediated by factors such as water and nutrient stress, and temperature. Singels *et al.* (2000) quantified the partitioning of assimilates to sucrose ranging from 0.14 for stalk mass of 3 t ha^{-1} to 0.33 for stalk mass of 9 t ha^{-1} in a well watered sugarcane crop, to values between 0.22 and 0.44 in a water-stressed crop. Water stress increased the

partitioning of assimilates to sucrose storage, explaining how sucrose yield is less affected with the progression of stress than is biological yield (t cane ha^{-1}).

In NCo 376, a ceiling value of sucrose concentration of about 0.55 gg^{-1} DM was observed for individual stalks weighing more than 150g (Inman-Bamber, 2002). This value compares with the 0.57 to 0.59 gg^{-1} values reported by Berding (1997) for the Australian sugarcane environment for whole crops, and 0.48 gg^{-1} in South African and Australian crops (Robertson *et al.*, 1996a). Inman-Bamber *et al.* (1998) suggested an upper value of sucrose concentration of 0.6 gg^{-1}; there is considerable room for improvement in sucrose productivity in this respect. Inman-Bamber (2002) went on to show that sucrose concentration of the basal internodes was lowest in autumn and highest in spring, and as the mass of sucrose followed the same pattern, he concluded that the changes in sucrose concentration were due to changes in mass per internode rather than dilution by other solutes and cell wall constituents. Export of sucrose would then augment the dilution concept.

1.6 Sucrose traffic on the sugar freeway of sugarcane

It is widely accepted that sucrose is the sugar that is translocated in sugarcane and in many other plants (Hatch and Glasziou, 1964; Rae *et al.*, 2005). Hartt *et al.* (1963) followed the pathway of assimilates when translocated from the leaves to various parts of the sugarcane plant. They reported that the major pathway followed by labelled photosynthates from the leaf is down the parallel veins to the midrib and sheath. However a significant amount of the labelled photosynthate was observed to pass directly down the lamina and into the sheath, bypassing the midrib. The appearance of labelled sucrose in the midrib was explained by Alexander (1973) as due to the gradual union of the midrib with the veins along the flow course from the leaf tip to the leaf collar (dewlap). Movement of photoassimilates in the leaves begins immediately after their synthesis. Radioactivity was detected some 55cm away from a leaf exposed to CO_2 within 5 minutes. Hatch and Glasziou (1963) observed a much faster rate of assimilate movement. The outer green part of the central part of the sheath conducts more photoassimilates than the inner white part. The presence of bundles closer to the outer epidermis than to the

inner epidermis accounts for this observation (Hartt *et al.*, 1963). Assimilates enter the stem from the sheath nearly in a horizontal direction (direction of the dewlap) to the centre of the stem, and then take a perpendicularly downward direction for as many as eight internodes, before some of the assimilates reverse direction upwards (Artschwager, 1925). Some of the ascending assimilates may reach spindle leaves, immature storage tissue and meristematic area before the descending assimilates reach the roots (Hartt *et al.*, 1963). It was also shown in the results of the study by Hartt *et al.*, (1963) that produced assimilates firstly get accumulated in the nodal section (a region with high concentration of conducting tissue) rather than in the internodes. Some assimilates were also observed to move out of the roots into the nutrient solution. A rather striking finding by Hartt *et al.* (1963) was the translocation of assimilates from a leaf of one stalk to other stalks within a stool. In one experiment it was found that radioactive ^{14}C incorporated into leaf blade number 3 of one stalk was detected as far as in leaf blades number 2 of 15 other stalks within the same stool.

1.7 Enzyme activity and sucrose metabolism

A number of enzymes operate in the sugarcane plant during its development (Glasziou, 1961; Sacher *et al.*, 1963a). Complexity arises through the activity of these different enzymes in the same overall reaction but in opposite directions (Ebrahim *et al.*, 1998a). The concentration of various sugars in the sugarcane stalk is influenced by the balance of the activity of these enzymes (Zhu *et al.*, 1997). The primary enzymes of sucrose metabolism are the invertase (EC 3.2.1.26), which cleaves sucrose into glucose and fructose; sucrose synthase (EC 2.4.1.13), which can either cleave sucrose into UDP-glucose and fructose or catalyse sucrose synthesis from glucose and fructose; and sucrose phosphate synthase (EC 2.4.1.14), which synthesises sucrose-6-phosphate, which in turn, is dephosphorylated into sucrose by sucrose-phosphate phosphatase (Lingle and Smith, 1991). The sucrose pool is thus under continuous rapid cycle of synthesis and cleavage, depending on conditions affecting the enzymes involved in the cycle (Whittacker and Botha, 1997). The activity of the enzymes varies with season during sugarcane's ontogeny, thus making the metabolism of sugar and internodal contents vary with internode development (Hatch and Glasziou, 1963).

Three invertase enzymes operate in the metabolism of sucrose in sugarcane, namely soluble acid invertase (SAI), neutral invertase (NI) and cell wall acid invertase (CWAI) enzymes (Albertson *et al.*, 2001). The CWAI in sugarcane internodes has not had much attention from researchers (Lingle, 2004). The activity of this enzyme seems to increase with internode age in the same way as sucrose content. It is thought that CWAI contributes to sucrose storage (Lingle, 2004). Immature internodes of sugarcane contain high levels of SAI (optimum activity between pH 5 and 5.5); whereas mature internodes contain high levels of NI (optimum activity at pH 7) (Hatch and Glasziou, 1963). In their studies (Sacher *et al.*, 1963b; Hatch and Glasziou, 1963; Gayler and Glasziou, 1972) reported that these two invertases (SAI and NI) have a key role in regulating sucrose translocation in conducting tissues, and hence its subsequent utilization for growth or storage. High positive correlation between internode extension and SAI was found, whereas a high positive correlation between movement of sugar into storage bodies and NI was found. It was concluded that the NI regulates the translocation of sugars from vascular to storage tissue of mature internodes. However, the results of the study by Zhu *et al.* (1997) and Veith and Komor (1993) did not support this idea.

Low sucrose-storing cultivars of sugarcane retain relatively high levels of SAI in their mature internodes, whereas high sucrose-storing cultivars contain relatively high levels of NI in their mature tissue and no SAI (Hatch and Glasziou, 1963). The early idea that NI and SAI played the major role in controlling sugar flux in mature storage tissue of sugarcane stalks was counteracted by Zhu *et al.* (1997) and by Veith and Komor (1993). The activity of SAI should be low before sucrose can accumulate, but there are other factors that affect accumulation (Zhu *et al.*, 1997). Zhu *et al.* (1997) showed that SAI enzyme activity varied with internode age, and genotype; and that the SAI activity was highest in the most immature internodes of low sucrose-storing cultivars and lowest in the high sucrose-storing cultivars. SAI activity values of 350 and <50 μmol min^{-1} g^{-1} were found for low sucrose-storing cultivar Mol5829 and for high sucrose storing cultivar LA Purple, respectively. SAI activity was reported to prevent most, but not all sucrose accumulation. Low SAI in high sucrose-storing cultivars was not sufficient to account for

sucrose accumulation. In the same study by Zhu *et al.* (1997), it was shown that there was no correlation between NI, SS, SPS and sucrose concentration of individual internodes of the high sucrose-storing cultivar LA Purple; whereas a nonlinear correlation (r^2=0.70; P<0.002) between sucrose concentration and the activity of SAI and between sucrose concentration and the difference of SAI and SPS activities (r^2=0.71; P<0.002) was found, indicating that both the SAI activity and the difference between the SAI and SPS activities account for sucrose flux in sugarcane internodes. However, analysis of mean values of enzyme activities of individual internodes per stalk showed that the nonlinear correlation between sucrose concentration and SAI activity was low (r^2=0.52; P<0.003); whereas the correlation between sucrose concentration and the difference between SPS and SAI activity was high and positive (r^2=0.86; P<0.001). Hence sucrose accumulation and storage on the whole stalk basis was said to be accounted for by the balance between SAI activity and SPS activity more than it is accounted for by the SAI activity alone (Glasziou and Gayler, 1972). Similar results were found by Lingle (1999).

A study by Lingle (2004), to investigate the effect of transient temperature change on sucrose metabolism in sugarcane internodes, showed that there was no significant temperature effect on sucrose concentration for any internode. A slight increase in sucrose concentration in the most immature internodes as a result of a chilling effect was observed, but this slight increase was not associated with sucrose metabolism enzymes (SAI, NI, SS, SPS and CWAI), since there was no significant change in the activity of these enzymes due to low temperature. Hence she rejected the hypothesis that short time exposure of plants to low temperatures affects sucrose metabolism in sugarcane. She associated the slight increase in sucrose concentration in response to transient chilling effect with low-temperature suppression of sucrose cleavage enzymes.

1.8 Effect of solar radiation on assimilate translocation in sugarcane

In yet another study, Hartt (1965b) investigated the effect of light on the translocation of photoassimilates. From the results of this study, it was shown that light affected two aspects of assimilate translocation in sugarcane. One is the polarity (direction) of

assimilate movement and the other is the percentage of translocates. Basipetal translocation of assimilates was favoured by light, whereas acropetal translocation was favoured by the dark. Total or overall translocation (acropetal plus basipetal) was greater in the light than in the dark (Hartt, 1965b). The light dependent basipetal movement of assimilates was described by Glasziou and Gayler (1972) as a "push" system that is capable of movement against an apparent gradient. In contrast, the "pull" system (acropetal movement) depends on a pressure flow and it occurs in the dark.

1.9 Effect of temperature on assimilate translocation in sugarcane

Low temperatures inhibit sucrose translocation from the leaves to the stalk of sugarcane. This was evident from the observation that higher sucrose concentrations were observed in the leaves of plants grown at 15°C than at 27°C (Ebrahim *et al.* 1998a). Similar observation was made by Mongelard and Mimura (1972). On the other hand, high temperatures were not found to inhibit assimilate translocation in the leaves and stalks. In fact, high temperatures (up to 45°C) were found to be optimum for sugar translocation in sugarcane plants (Ebrahim *et al.*, 1999). However, high temperatures are associated with high leaf respiration (Ebrahim *et al.* 1998a; Glover, 1972b). This was evident from low sucrose concentration both in the leaves and stalk segments of plants grown at 45°C (Ebrahim *et al.* 1998a). This implies that substantial amounts of carbohydrates are lost at high temperatures through respiration, leaving little for translocation to the stalks. It was clearly shown in the Ebrahim *et al.* (1998a) study that leaf respiration rate was 3 to 5 times higher at 27°C and 45°C than at 15°C. Glover (1973) showed that on a unit weight basis leaves account for the larger amount of carbohydrates lost through respiration than stalks at a similar temperature only at the early age of the crop. As the biomass of stalks increases with time, respiration rate of stalks exceeds that of leaves, and highest levels of carbohydrates are lost through stalk respiration. Glover (1972b) estimated the rate of dark respiratory losses **R** (in mg CH_2O min^{-1} kg^{-1} dry weight) of rooted living stalks by the following equation:

$$\mathbf{R = 0.2495T - 3.046} \text{ (Glover, 1972b)} \ldots\ldots\ldots (3)$$

where T is the mean night temperature in °C. This suggests that sugarcane grown in areas with warmer mean night temperatures are bound to lose higher levels of stored sugars from their stems than cane grown in areas with cooler mean night temperatures. However, chilling night temperatures may pose serious subsequent defects on the photosynthetic parameters of the plants (Ebrahim *et al.*, 1998b).

The amounts and rate of assimilate translocation in sugarcane are affected by air and root temperature, radiation, soil water and nutrient deficiency (particularly N, P and K) (Hartt, 1965a). The results of a study by Hartt (1965a) indicated that the optimum temperature for photoassimilate translocation in sugarcane might be higher than 30°C. Wendler *et al.* (1990) reported a 45°C optimum temperature for sugar translocation in sugarcane. Temperature coefficients between 1.1 and 1.5 for assimilate translocation from the leaf blade, between 1.01 and 1.7 for translocation down the stem and between 3.9 and 16.2 for translocation up the stem, were obtained by Hartt (1965a). This observation suggested that the movement of assimilates from the leaves and down the stem involves physical or physio-chemical processes; whereas translocation up the stem is regulated by chemical processes particularly at the stem apex (Jansen and Taylor, 1961).

Low temperatures inhibit both photosynthesis and translocation of photoassimilates from the leaves to the stalk of plants. Ebrahim *et al.* (1999), working with suspension cells, investigated the effect of temperature on sugar transport systems for interpretation of sugarcane growth at sub-optimum temperatures. [It should be noted that valid information was obtained using suspension cells mainly because of similarities of suspension cells and storage parenchyma cells of sugarcane are observed; these include the preferred uptake of hexoses over sucrose even in the presence of excess sucrose in the apoplastic space (Komor *et al.*, 1981); and the synthesis-hydrolysis cycle (Wendler *et al.*, 1990)]. It was observed that low temperature strongly inhibited the hexose uptake systems within the sink cells; and that higher temperatures were not harmful. The optimum temperature for hexose uptake was 45°C. In this study it was also shown that the major uptake system in young rapidly growing internodes of sugarcane stalks, which have a strong affinity for reducing sugars (glucose and fructose), was observed in the

suspension cells. Glucose uptake by suspension cells was observed between 15°C and 60°C. The rate of uptake (μmol min^{-1} g_{DW}^{-1}) increased from approximately 0 μmol min^{-1} g_{DW}^{-1} at 15°C and gradually increased to about 1 μmol min^{-1} g_{DW}^{-1}at 35°C. A sharp increase from 1 μmol min^{-1} g_{DW}^{-1} to ca. 2 μmol min^{-1} g_{DW}^{-1} was observed between 35°C and 45°C, and then began to drop sharply to zero between 50°C and 60°C.

Ebrahim *et al.* (1999) went on to investigate the storage of sugars and metabolism of sucrose in the suspension cells under different temperature regimes. They observed that sucrose disappeared rapidly from the medium at 27°C and 45°C, and hexose levels increased. However, the rapid hydrolysis of sucrose at 45°C did not result to high levels of hexoses, as was the case at 27°C. This was explained by the observed fast hexose uptake rates at 45°C (as discussed above) and the high respiration rates observed by Ebrahim *et al.* (1998a) at 45°C. Sucrose hydrolysis was very low at 15°C and there was no significant increase in hexose levels. These observations clearly explain the idea of cold ripening of sugarcane. Similar observations were made for *Cucurbita* tissues, except that maximum uptake occurred at 30°C and the temperature range of uptake was between 0 and 55°C (Webb, 1967). At the whole plant level, cold temperature (low water content and nutrient deficiency) suppress stalk extension and increase sucrose content from reduced meristematic demand for photoassimilates (Inman-Bamber and De Jager, 1988).

1.10 Sucrose metabolism and the source-to-sink movement

Sucrose is the major sugar form that is produced in the leaves of sugarcane, translocated in the phloem, and it can either be stored in the vacuoles of storage parenchyma cells of the stalk (Moore, 1995) or be used up by the growing sites of the plant (shoot and root apices) (Rae *et al.*, 2005). The direction of assimilate translocation along the phloem is not determined by the relative positions of the source and sink in the plant, and it is not defined by gravity (Taiz and Zeiger, 1991) but by concentration gradients (Taiz and Zeiger, 1991). The other theory of translocation is by diffusion, and it is not accepted due to observed rates of translocation being far more rapid than that which could be manually attained through diffusion alone. The rate of assimilate diffusion is estimated at 1m 8 $years^{-1}$, compared with 1m h^{-1} for the pressure-flow theory (Taiz and Zeiger, 1991). In

sugarcane, the primary source of assimilates is the mature leaves, and the primary sinks are the stalks, apical meristems and other non-photosynthetic organs, such as the roots. Some organs can be both sources and sinks simultaneously. For example, the immature leaves are a source because they photosynthesize and at the same time they are recipients of assimilates for their expansion (Taiz and Zeiger, 1991). In the source tissues, sucrose is actively loaded into the companion cell-phloem sieve element complex and causes a steep drop in water potential. As a consequence osmotic influx of water into the phloem occurs, thereby increasing turgor pressure, which promotes movement away from the source (Rae *et al.*, 2005). At the sink tissues, phloem unloading causes a drop in osmotic pressure in the sieve element of sink tissues and a rise in water potential of the phloem. The resulting water potential gradient between the phloem and xylem causes water to leave the phloem back to the xylem, thereby reducing the turgor pressure of the phloem sieve element of the sink tissue (Rae *et al.*, 2005; Taiz and Zeiger, 1991).

Sucrose accounts for up to 75 % (DW) of the soluble solutes in the bottom internodes (from internode No. 10 and older, counting from the top of the stalk), and can reach over 90 % at the end of the crop cycle (Moore, 1995; Hartt *et al.*, 1963). According to Glasziou and Gayler (1972), sucrose is transported through the phloem without breakdown and re-synthesis. Hatch and Glasziou (1963) showed that post-phloem sucrose was broken down into fructose and glucose by invertases and re-synthesised to sucrose in the storage tissue. The results of Hawker and Hatch (1965) showed that all of the translocated sucrose was hydrolyzed before storage on mature cane; and Sacher *et al.* (1963b) showed that more than 70 % of translocated sucrose was hydrolyzed in immature cane internodes. Reviewing the results of the two studies, Moore (1995) concluded that the hydrolysis of sucrose before storage in the sugarcane storage cells is obligatory.

However, results of some studies show evidence of sucrose uptake uncleaved (Lingle, 1989; Thom and Maretzki, 1992), indicating that there could be two uptake systems on the plasma membrane of storage cells, one for sucrose and the other for the hexoses (Moore, 1995). It is speculated that tissue treatment prior to uptake studies caused the contrast between results of earlier the studies that support sucrose apoplastic cleavage and

those of the later studies that support sucrose uptake without cleavage. In the earlier experiments, tissue was washed in water for one hour (Moore, 1995), whereas in the later studies tissue was washed in mannitol for one hour before the uptake study (Lingle, 1989; Thom and Maretzki, 1992). It is thought that the water treatment increased turgor in the apoplast, thermodynamically preventing sugar movement from the apoplast to the storage compartment; whereas mannitol which did not raise the turgor pressure, encouraged direct movement of sucrose from the apoplast to storage compartment of the storage cells without cleavage. Another speculation is that high turgor could have de-activated carriers of sucrose across cell membranes (Moore, 1995).

A model produced by Glasziou and Gayler (1972) showed that post phloem sucrose underwent a cycle of cleavage and re-synthesis between the apoplastic space, cytoplasmic space and the vacuole of the storage cells. There are at least three possible ways in which sucrose is thought to be passed through apoplastic space [the apoplastic space was defined by Briggs and Robertson (1957) as the volume of tissue that rapidly comes to diffusion equilibrium with the medium to which the tissue is placed]. It includes the aqueous phase of the cell wall (Glasziou and Gayler, 1972). Firstly, sucrose is thought to be transported through the plasmodesmata that connect the conducting phloem sieve element and the endodermis cell that surrounds the phloem. Secondly, it is thought that sucrose from the sieve element is transported through the plasmodesmata (direct cell-to-cell connection) to the storage parenchyma cells first and then leaks from the parenchyma cells to the apoplastic space (Oparka and Prior, 1988). Thirdly, it is thought that sucrose enters the apoplastic space from both unloading from the phloem and leakage storage parenchyma cells (Moore, 1995). Leakage of sucrose from storage cells to the apoplastic space is important for regulation of osmotic potential of the storage cells (Hawker, 1965), which can be lower than -2MPa on mature cane parenchyma cells (Moore, 1995; Moore and Cosgrove, 1991).

Sucrose that enters the sugarcane apoplastic space from the phloem sieve element is hydrolyzed to glucose and fructose by the invertases (Sacher *et al.*, 1963b). Sucrose uptake by storage cells is dependent on its hydrolysis by the invertases in the apoplastic

space before being transported to the storage compartment (Glasziou and Gayler, 1972). Hexose carriers transport glucose and fructose from the apoplastic space to the metabolic compartment (cytoplasmic compartment) of the storage parenchyma cells, where hexose interconversion and phosphorylation, and formation of uridine diphosphate glucose (UDPG) from glucose take place (Bowen and Hunter, 1972). There are a host of enzymes in the metabolic compartment that operate between the incoming hexoses and sucrose phosphate. Sucrose phosphate is thought to be de-phosphorylated into sucrose in the vacuolar membrane on its transit to the vacuole. Hydrolysis of sucrose phosphate was not shown to occur in the cytoplasmic compartment before transit to the vacuole nor was it shown to occur at the vacuole after transit (Glasziou and Gayler, 1972). Depending on sink demand at the meristematic sites, which is mediated by temperature, moisture and nutrient levels, vacuolar sucrose can be hydrolyzed back to the hexoses and be driven off the storage cells to supply energy at the growth sites.

Although there has been tremendous progress over the past years in developing high sucrose storing cultivars, climatic conditions still control the performance of these cultivars, and this has been a persistent problem to plant breeders and crop physiologists. Since sugarcane is grown in different regions of the world, with a range of climatic factors, and with climatic variation within regions from year to year, it is important to understand the environmental effects and their interaction with the key physiological processes of sugarcane. Such understanding is especially important in choosing correct cultivars for specific soil types and for implementing correct crop management, infrastructure and marketing decisions. It is also fundamental in developing accurate simulation models, an important tool that has found use in the sugar industry of South Africa for the past 13 years now. Such understanding is also important in breeding programs to base primary targets when choosing genes for cultivars suitable for specific climatic conditions.

2 MATERIALS AND METHODS

2.1 Glasshouse description and environmental control

Plants were grown in three 4.5 x 4.0m glasshouses (Fig. 2.1.1); one run at 22°C day temperature and 12°C night temperature (22/12°C) (C), the second glasshouse was run at 27°C day temperature and 17°C night temperature (27/17°C) (W), and the third glasshouse at 32°C day and 22°C night temperature (32/22°C) (H). Twenty potted plants were fitted into each glasshouse. Temperature and relative humidity were moderated in the glasshouses. Temperature was controlled by electronic sensors in the air conditioning return air in ducts. Cooling of air was achieved by blowing cool air through a refrigeration system and heating of air was achieved by blowing air over heating elements into the glasshouses. Control of temperature to within ±1°C was achieved using hot gas bypass system on the air conditioning plant. Relative humidity was monitored by humidity and temperature sensors and regulated by the calculation of vapour pressure deficit relative to the saturation point (100% RH).

Figure 2.1.1: The glasshouse structure where plants were grown under controlled temperature and relative humidity. Solar radiation received was natural and it was measured electronically.

2.2 Environmental data collection

Temperature, solar radiation and relative humidity (RH) were measured electronically. Temperature and RH were measured in each growth room by a Visala (Origen) sensor and the signal passed to a Campbell Scientific CR10X processor and incident solar radiation was measured using LICOR PAR quantum sensors with the signal likewise passed to the CR10X. A mean of two readings of all environmental sensors from continuous measurements was recorded every 15 minutes and this data was logged from two complete temperature and RH instruments adjacent to each other (about 1m apart). The quantum sensors were mounted above the canopy of the plants and they were adjusted upwards as the plants grew upwards.

2.3 Pre-trial production of plant materials

Single bud stem cuttings (setts) of *S. officinarum* cv. NCo 376, 7.5cm long, containing a vegetative bud 2.5cm to the upper end and 5cm to the bottom end were cut from ca. three nodes from the middle of each seedcane stalk, dipped in a fungicide (2.5mL ERIA in 5L of water) for 30 minutes, and planted in plastic seedling trays (260x230x110mm) containing sterile vermiculite (Fig. 2.3.1). Trays were kept in a greenhouse at 25°C and watered daily. The seedlings were established at the South African Sugar Research Institute (SASRI) at Mount Edgecombe for different periods for each of the three studies, and transferred to the University of KwaZulu-Natal in Pietermaritzburg for transplanting. Pre-trial plant managements for the three trials were not similar. The first trial was regarded as a pilot trial, and the results of this trial were not included in this thesis.

Figure 2.3.1: Seedlings growing in vermiculite in seedling trays, about 4 weeks old, ready to be transplanted.

2.4 Sampling procedure

For each trial, ten destructive samplings were conducted periodically through one month (Table 2.4.1). Sampling was random within a temperature room. Two plants per temperature treatment were harvested on each sampling date between 8H00 and 9H00. Time between sampling of individual plants was minimized in order to prevent diurnal effects. Dry leaves per stalk were carefully counted before harvesting. Haulms (shoots) of each plant were cut at ground level using a pair of secateurs and transferred to a cold room (5°C) before being processed in order to minimize post harvest weight loss through respiration. Cut haulms were separated into primary haulm and tillers. Each haulm was defoliated and the green leaves were separated into green leaf blades, sheaths and furled leaves. Leaves were designated dead if the whole of their area had become necrotic. Necrotic tissues on otherwise green leaves were removed and added to the dead leaf mass. The leaves were then placed in perforated paper bags, oven dried at 70 °C for 48 hours and reweighed after drying. Stem length of each haulm was measured from its base to the tip of the apical meristem using steel tape. The internodes of each stalk were counted. Internodes number 4, 6, and 10 (counting from top) of every primary shoot were

separated from each stalk and their individual diameter and length measured using vernier calipers and a steel rule, respectively. These three internodes (4, 6 and 10) were taken further for chemical analysis (Chapter 2.8). The selected internode tissue and tissue from the rest of the stalks were oven dried at 70°C for 48 hours and then weighed.

Non-destructive repeated measurement was conducted on two plants per temperature treatment, which were the last plants to be destructively sampled. Stalk extension rate, stalk profile of leaf area, leaf appearance and senescence, leaf photosynthesis and fluorescence were measured on these plants twice per week.

Table 2.4.1: Sampling dates of three trials (equinox 20 March and 22 September)

TRIAL 1	Autumn 2004	TRIAL 2	Spring 2004	TRIAL 3	Autumn 2005
SAMPLE No.	DATE	SAMPLE No.	DATE	SAMPLE No.	DATE
1	16-Mar-04	1	01-Sep-04	1	15-Mar-05
2	19-Mar-04	2	03-Sep-04	2	18-Mar-05
3	23-Mar-04	3	07-Sep-04	3	22-Mar-05
4	29-Mar-04	4	10-Sep-04	4	25-Mar-05
5	02-Apr-04	5	14-Sep-04	5	29-Mar-05
6	06-Apr-04	6	17-Sep-04	6	01-Apr-05
7	09-Apr-04	7	21-Sep-04	7	05-Apr-05
8	13-Apr-04	8	24-Sep-04	8	08-Apr-05
9	16-Apr-04	9	28-Sep-04	9	12-Apr-05
10	20-Apr-04	10	01-Oct-04	10	15-Apr-05

2.5 Leaf activity measurement (SASRI)

In another study by the SASRI staff, leaf photosynthesis and fluorescence were measured twice per week using an infra red gas analyzer (IRGA) and a plant efficiency analyzer (PEA), respectively. The details of this section will not be discussed in this thesis.

2.6 Total radiation and radiation interception (SASRI)

Total incident radiation was measured and the data used by the SASRI staff in a supplementary study. The details of this section will not be discussed in this thesis.

2.7 Growth analysis

Stalk extension rate

Measurement of stalk extension rate was carried out throughout the temperature treatment period on stalks of two plants that were destructively sampled last in each of the three temperature controlled glasshouses. Stalk extension rate was measured both manually by repeatedly measuring the height of stalks using a tape, and electronically using auxanometers. Manual measurements were carried out twice per week by measuring stalks of plants from a reference point at the base of plants to the top visible dewlap (TVD) of each shoot. Electronic measurement was carried out using auxanometers connected to a data logger (Fig. 2.7.1) recording stalk height every 30 minutes.

Figure 2.7.1: Illustration of auxanometers attached to leaf sheaths. (a) Transducers clipped to separate shoots, (b) Downloading data from the logger to the computer.

Leaf area

The blade area of individual leaves was calculated by multiplying the length by the width of fully expanded leaves, and by a shape factor to account for the non-rectangular shape of the leaves. The shape factor was assumed as 0.71 (Singels *et al.*, 2005b). Leaves were assumed to have fully expanded once the leaf collar emerged from the leaf sheaths. Measurement of leaf length and width was performed on shoots of two plants that were harvested last from each temperature, on which the repeated measurements were done. The leaves were measured before and after temperature differentiation, using a steel rule.

Internode length and diameter

Length and diameter of Internodes number 4, 6 and 10 (counting from the top) of shoots were measured post harvest using steel rule and vernier callipers.

2.8 Stalk chemical analysis

Extraction of sugars

Extraction and analysis of sugars was conducted as described by Smith (1981). Sugars were extracted from dried, milled tissue of internodes 4, 6, and 10 (counting from the top of shoot) of the primary haulms using 80% v/v ethanol. Internode tissue was milled to pass through a 40 mesh. A 200mg sample of milled tissue was carefully weighed and placed into a 125mL Erlenmeyer flask, followed by 50mL of 80% ethanol. The flasks were sealed with parafilm and mechanically shaken on an electric shaker for one hour. The extract was then filtered through Whatman No. 40 paper into a 250mL beaker. The residue and filter paper were washed three times with 80% ethanol. Beakers with filtrate were then placed on a warm hot-plate in a fume cupboard to evaporate the ethanol. Distilled water was occasionally added into the beakers to replace the evaporated ethanol and to prevent complete drying of the sample. The disappearance of the characteristic odour of ethanol from the beakers indicated complete evaporation of ethanol. At this stage the aqueous solution was filtered through Whatman No. 40 paper into a 100mL volumetric flask, washing the residue and filter paper three times. The solution was then brought to volume with distilled water, to make the stock solution for sugar analysis.

Sugars in extracts were determined by titration procedures. Preparations of solutions used for the sugar analysis were as follows:

Reagent "50"

The Reagent "50" was prepared by dissolving 25g of anhydrous Na_2CO_3 and 25g of $C_4H_4KNaO_6.4H_2O$, in 600mL of distilled water. Then a 75mL of a 10% $CuSO_4.5H_2O$ solution was pipetted below the surface of the solution of Na_2CO_3 and $C_4H_4KNaO_6.4H_2O$. A 20g of $NaHCO_3$, 1g of KI and a 200mL of KIO_3 solution containing exactly 3.567g of KIO_3 per litre was added. The solution was mixed thoroughly and rinsed in a 1L volumetric flask.

Potassium iodide-potassium oxalate (KI-Kox) solution

The solution of Potassium iodide-potassium oxalate was prepared by dissolving 2.5g of KI and 2.5g $COOC.COOK.H_2O$ in 100mL distilled water.

1.0N Sulphuric acid

A solution of 1.0N H_2SO_4 was prepared by diluting 27mL of concentrated sulphuric acid in 1L distilled water.

Starch indicator

An indicator solution of starch was prepared by stirring 1g of soluble starch into 15mL of cold distilled water, which was then added to 100mL of boiling water with a 1g HBO_3 added to it at boiling. The mixture was allowed to further boil for about one minute and then cooled slowly.

0.02N Sodium thiosulphate

A 0.1N stock solution of $Na_2S_2O_3.5H_2O$ was prepared by dissolving 25g of $Na_2S_2O_3.5H_20$ and 1g of NaOH in distilled water in a 1L volumetric flask. This solution was made to stand overnight, and then it was diluted to 0.02N by adding 100mL of the stock solution to a 500mL volumetric flask and making it up to volume.

Standard sugar

A standard sugar solution was prepared by dissolving 1g exactly of oven dried glucose in a 1L saturated solution of benzoic acid.

Analysis was in two parts. The one was for the determination of reducing sugars and the other for total sugars in the tissue samples. Analysis of sugars in cane tissue using the titration methods takes advantage of the reducing power of the reducing sugars present in tissue. It was assumed that glucose and fructose were the principal reducing sugars in the cane tissue and that other reducing sugars were present in insignificant amounts and sucrose was the major non-reducing sugar in the sugarcane tissue samples.

Reducing sugars

Three, 5, and 8mL aliquots of sugar solutions from internodes 10, 6 and 4, respectively (count from the top of shoot) were placed in 25 x 200 mm test tubes, and diluted such that the final volume of the solutions was 10mL (Table 2.8.1). A glucose standard solution (3mg) was also tested. Ten mL of reagent "50" was added to the test tubes containing aliquots of the sugar solutions and to the test tube containing 10mL of distilled water for a blank titration. The test tubes were then heated for 15 minutes in a boiling water bath and cooled rapidly in a cool running water bath. When tubes had cooled down to below 30°C, 2mL of KI-Kox solution was added to each tube, followed by 10mL of 1.0N sulphuric acid. Test tubes were then rotated on a vortex mixer to stir the solution and to dissolve all copper II oxide. Five drops of starch indicator (approx. 0.25mL) were added and the solution was titrated with 0.02N sodium thiosulphate solution on a magnetic stirrer. The end point was marked by the change of colour of the solution from blue to light green.

Table 2.8.1: Volumes of sample aliquots, water and reagent "50".

	Vol. solution (mL)	Dist. water (mL)	Reagent "50" (mL)	Final volume (mL)
Internode 10	3	7	10	20
Internode 6	5	5	10	20
Internode 4	8	2	10	20
STD solution	3	7	10	20
Blank	0	10	10	20

Sugar content from internode tissue in milligrams was calculated from the following equation:

mg sugars in sample =

$$\frac{\text{(Molarlity of STD sugar x Vol. STD sugar) x (Vol. Blank Titr. – Vol. unknown sugar solution)}}{\text{(Vol. Blank Titr. – Vol. STD sugar Titr.)}}$$

where molarlity of the standard sugar is in milligrams per litre. Percent sugars were calculated from the following equation:

% sugars = mg sugar x dilution factor x 100 % ÷ sample weight (mg)

where the dilution factor is 100 ÷ Vol. of sample aliquot.

Sucrose

It was not possible to determine sucrose content directly using the titration procedure due to its inability to react (i.e., sucrose lacks the reducing power). However, hydrolysis of sucrose molecules gives its monomeric constituents, glucose and fructose, which are reducing agents. One millilitre of 1.0N sulphuric acid was added to test tubes containing sugar solutions before the addition of Reagent "50", mixed thoroughly on a vortex, and heated for 15 minutes in a boiling water bath. Tubes were then cooled rapidly in cold running water bath to below 30°C. Solutions were then neutralized with 1.0mL of 1.0N of sodium hydroxide. The solutions were then tested for reducing power as described in section 2.8.2. Total sugar content was determined from analysis of samples with hydrolyzed sucrose. Sucrose content was calculated from the difference between the results of analysis with hydrolyzed sucrose and the results of analysis without hydrolysis of sucrose. An outline of the analysis of sugars is shown in Fig. 2.8.1.

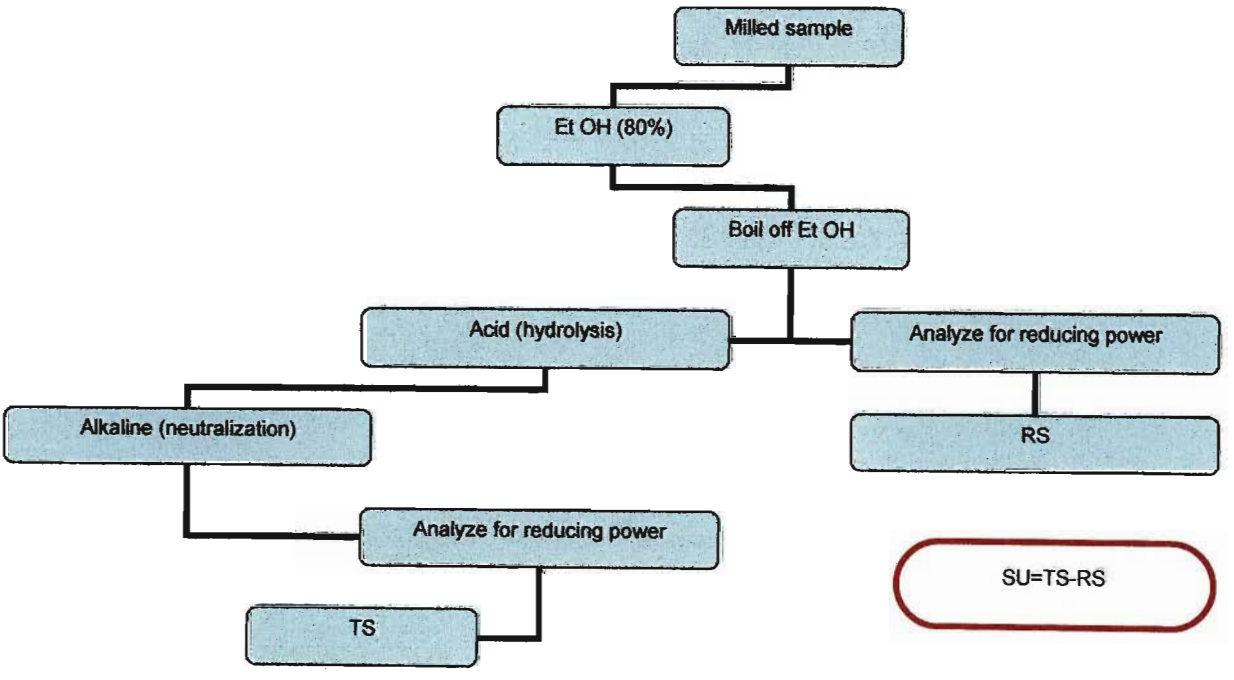


Figure 2.8.1: Schematic representation of procedure for sugar analysis. Et OH = Ethanol, TS = Total sugars, RS = Reducing sugars.

Fibre

Fibre percent was calculated from subtracting % TS from 100%.

Statistical analysis of data

Data was analysed for differences between treatments using the GenStat 7th edition statistical package using repeated measurement analysis over the 10 sampling dates. The level of significance was set at 5%.

2.9 Trial 1

Setts for trial 1 were derived from seedcane obtained from Kearsney Farm, and were germinated on the 2nd of October 2003 and allowed to grow in trays for approximately one month, after which the seedlings were transplanted, one each, into 80L pots containing soil with the characteristics shown in Table 2.9.1. Plants were grown in a shadehouse until the 2nd of March 2004, when they were transferred to controlled temperature and RH in three glasshouses set to the C temperature and 60%, respectively, with natural radiation. Twenty potted plants were placed in each glasshouse, spaced 80cm x 90cm apart. Plants were adapted to the glasshouse conditions for two weeks before the start of trial period on the 16th of March at which time the temperature treatments were imposed. Plants were top dressed twice before the sampling period. Two weeks after transplanting 50 kg P ha^{-1} and 100 kg N ha^{-1} was top dressed using single superphosphate (10.5% P) and urea (45.5% N), respectively; and two weeks prior to temperature treatment commencement with 50 kg N ha^{-1}. Plants were watered manually using a hose, both in the shade house and in the glasshouses until a minute amount of drainage was observed from the base of the pots. For this trial all shoots of each plant were allowed to grow through to the sampling period and the mean of all shoots was used in the plant morphological growth analysis.

2.10 Trial 2

Each seedling was potted first into a 14.7L black plastic bag (0.25m diameter and 0.3m length) containing soil with the characteristics shown in Table 2.9.1, and all plants were placed in one glasshouse. The potted plants were watered once daily using a hosepipe. Night temperatures in this glasshouse ranged between 12.5 and 18.5°C and day temperatures ranged between 24 and 32.5°C. Extremely high temperatures were controlled by the wet wall system and extremely cold temperatures were controlled by an electrical heater system. On the 19th of April 2004, 50kg P ha^{-1} and 100kg N ha^{-1} was top dressed using single superphosphate (10.5% P) and (45.5% N), respectively. On the 19th of May 2004 plants were re-potted into 80L bins containing the same soil as described above, and placed in three glasshouses, allocating 20 plants per glasshouse. All

glasshouses were run at the W temperature and 60 % RH with natural sunlight. From time to time, plants were interchanged between and within the glasshouses in order to eliminate environmental variability between and within the glasshouses for a duration of the 14 weeks conditioning period. For this trial, smaller shoots were removed 5 days before temperature treatment commencement, followed by continuous snipping off of any new emerging shoots throughout the sampling period. Because the number of smaller shoots removed per plant was not the same from plant to plant, the number of shoots per plant carried over to the sampling period varied between plants. Plants were top dressed again four days before temperature differentiation with 50kg N ha^{-1} using urea. Temperature treatments as per Trial 1 were commenced on the 1st of September 2004. Every week, plants were watered to dripping and after dripping had ceased the weight of plant, pot, soil and water were determined using a scale balance. On the following day, the pot with plant, soil and remaining water was reweighed and the difference calculated. For that week, water equivalent to the weight difference was added in each pot daily.

Table 2.9.1: Physical and chemical analysis of potted soil, in which sugarcane for the three trials was grown. Soil analysis was conducted at the KwaZulu-Natal Department of Agricultural Laboratories, Cedara.

Soil character	Trial 1	Trial 2 and 3
Sample density (g/mL)	1.16	1.04
P (mg/L)	10	7
K (mg/L)	242	213
Ca (mg/L)	1925	18.5
Mg (mg/L)	593	503
Exch. Acidity ($cmol_c$/L)	0.05	0.11
Tot. cations ($cmol_c$/L)	15.16	14.03
Acid saturation (%)	0	1
pH (KCL)	5.54	5.87
Zn (mg/L)	14.7	11.6
Mn (mg/L)	9	3
Cu (mg/L)	5.3	4.8
NIRS clay (%)	37	59

2.11 Trial 3

Seedlings were germinated on the 6th of October 2004 from setts derived from seedcane obtained from the SASRI farm at Mount Edgecombe. Seedlings, one each, were transplanted directly into 80L pots on the 30th and 31st of October 2004 and placed in a shade house (50%) to grow for 120 days before they were transferred to the glasshouses for temperature treatment. Plants were moved to the glasshouses on the 2nd of March 2005, and were given two weeks to acclimatize to the glasshouse environment as per Trial 2. The same soil used for trial 2 was used for trial 3. Prior to transplanting, the soil was thoroughly mixed in a cement mixer with 50kg P ha^{-1}, 100kg N ha^{-1} and 50kg K ha^{-1} from single superphosphate (10.5% P), urea (45.5% N) and KCl (50% K), respectively. The number of shoots per plants was monitored and only five shoots (primary haulm plus four other tillers per plant) were allowed to grow out in each pot. Shoots emerging after the fifth shoot were snipped off using secateurs. Plants were top dressed two weeks before temperature differentiation with 50 kg N ha^{-1} using urea. Watering of plants during the sampling period was similar to that described for Trial 2.

3 RESULTS AND DISCUSSION

3.1 General growth and glasshouse performance

It is common in sugarcane trials to have a high variability within treatments (Mongelard and Mimura (1971; 1972). Observed variability within a temperature treatment was high in this study, especially in the spring equinox crop (Trial 2). This was due to a number of reasons. Firstly, in as much as great care was taken to select uniform setts (single bud setts) at planting, somaclonal variation between the setts was not avoidable, which subsequently caused differences in the vigour of the resulting individual plants (Sweby, *et al.*, 1994). Secondly, it was not possible to select setts from seedcane haulms of uniform physiological age, and also to select the setts from a uniform position on the seedcane haulms. Thirdly, there was a high variability in growth characters between the primary haulms of different plants, whereas the primary haulms were used for the measurement of plant growth, in terms of leaf number, stalk extension, rate of leaf elongation, leaf area and sugar content. Also, in these experiments the one month duration at which plants were treated with the different temperatures was too short. Only the parameters that respond to transient temperature treatment, such as leaf photosynthesis and stalk extension rate (SER) would be apparent over this period. All the above contributed significantly to the variation that was observed in sugarcane trials and therefore reduced the chances of detecting significant differences between treatments.

3.2 Phenology

3.2.1 Leaf appearance

The primary haulms of the two plants which comprised the final sample from each temperature treatment, and on which the repeated measurement of leaf number, stalk height and stalk profile of leaf area were performed, were assumed to have a uniform physiological age. Therefore, it was reasonable to compare the number of leaves per shoot for the three temperature treatments using the mean leaf number of the primary haulms of the two plants per temperature treatment rather than the mean of all the shoots, which all had different physiological ages.

The rate of leaf appearance per shoot in both the spring and autumn equinox crops was lower at C than at W and H temperatures (Figs. 3.2.1.1 and 3.2.1.3). In spring, the mean rate of leaf appearance on the primary haulms at C was 0.0303 leaves d^{-1}. The highest mean rate of leaf appearance occurred at H (0.1682 leaves d^{-1}). At the intermediate temperature W, leaves emerged at a rate of 0.1095 leaves d^{-1}. A trend more similar to that of the mean leaf emergence on the primary shoots over time was observed for the mean leaf emergence on all other tillers of each plant, and this was common on both trials (Figs. 3.2.1.2 and 3.2.1.4). The corresponding rates of leaf emergence per plant (plant of five shoots) for the three temperatures were 0.741, 0.529 and 0.193 leaves d^{-1} $plant1^{-1}$, at C, W and H, respectively, in spring.

In autumn the mean rates of leaf appearance on the primary haulms at the three temperatures were 0.113, 0.0824 and 0.0327 leaves d^{-1} at H, W and C, respectively (Fig. 3.2.1.3); and for the entire stools, they were 0.5451, 0.4741 and 0.1527 leaves d^{-1}, respectively (Fig. 3.2.1.4). Since some shoots were snipped off from plants in both trials, the values of leaf appearance per plant in both trials were not an accurate representation of the rate of leaf appearance per plant in an undisturbed situation.

From the leaf appearance rate equations, it can be deduced that in spring it took 33.0, 9.1 and 5.9 days between the emergence of subsequent leaves at the three levels of ascending temperature, respectively. These values corresponded to 30.6, 12.1 and 8.8 days in autumn. Similar results were found from a temperature study by Ebrahim *et al.* (1998a), who compared the leaf emergence rate of plants grown at 15, 27 and 45°C, and observed that the rate of internode accumulation per shoot (equivalent to leaf emergence rate) was lower at 15°C and higher at 27 and 45°C. Inman-Bamber (1991) also showed that the rate of PAR interception by leaves of winter started crops were much lower than for the crops that were started in the summer months; and this was explained mainly by the slow emergence rate of leaves during the cold winter months (Singels *et al.*, 2005b).

The minimum thermal unit requirement of the plant, beyond which a leaf can emerge (phyllochron) explain the lower rate of leaf emergence at C. Plants at C accumulated

thermal units at a rate of 7°Cd d^{-1}compared with plants at W and H, which accumulated thermal units at 12 and 17°Cd d^{-1}.

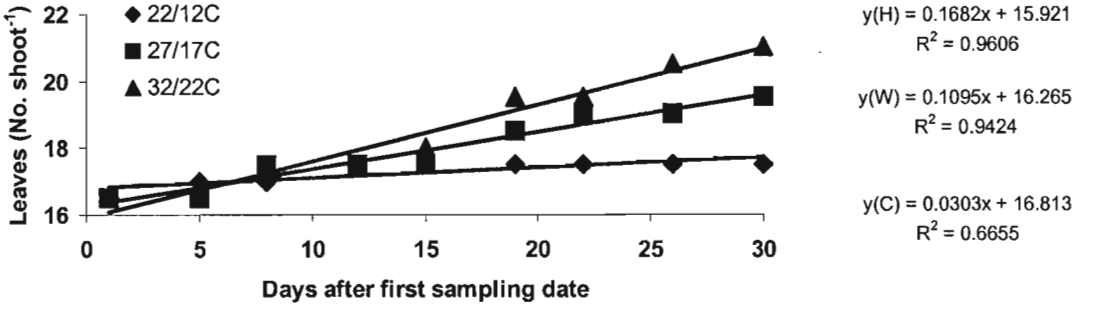


Figure 3.2.1.1: Total number of leaves (green and senesced) per shoot with progression of time of sugarcane plants grown during the spring equinox at three temperatures. The number of leaves per shoot was obtained from the mean leaf number of the primary haulms of each repeatedly measured plant per temperature treatment. On the fitted line equations in (b) hot (32/22°C), warm (27/17°C) and cool (22/12°C) temperatures are denoted by C, W and H, respectively.

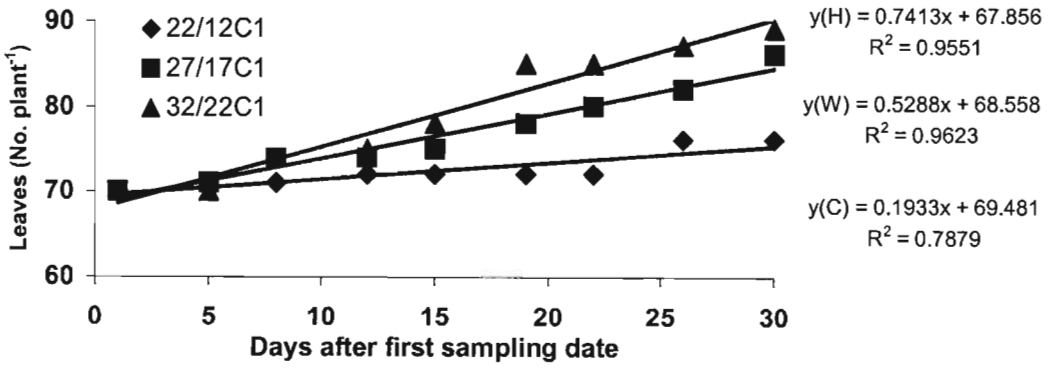


Figure 3.2.1.2: Total number of leaves (green and senesced) per plant with progression of time of sugarcane plants grown over the spring equinox at three temperatures. Leaf count was on five shoots per plant; the numbers of leaves per plant on the first day of temperature differentiation for the three temperature treatments were adjusted to a common value.

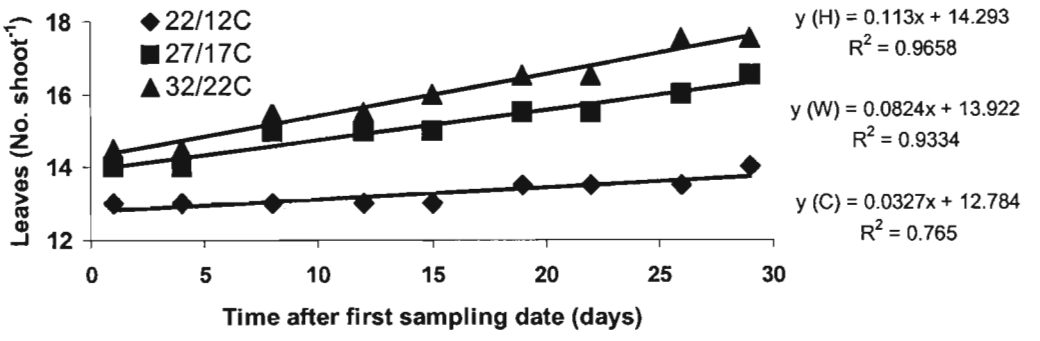


Figure 3.2.1.3: Total number of leaves (green and senesced) per shoot with progression of time of sugarcane plants grown over the autumn equinox at three temperatures. The number of leaves per shoot was obtained from the mean leaf number of the primary haulms of each repeatedly measured plant per temperature treatment.

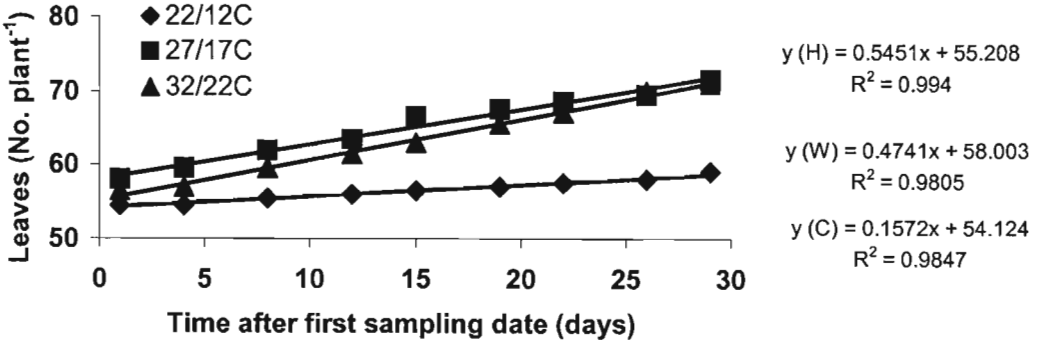


Figure 3.2.1.4: Total number of leaves (green and senesced) per plant with progression of time of sugarcane plants grown over the autumn equinox at three temperatures. Leaf count was on five shoots per plant.

Cumulative total leaf number per degree day

There was an apparent close association between the number of leaves per shoot and accumulated degrees days (°C d) using a base temperature (T_b) of 10°C (Inman-Bamber, 1994; Singels *et al.*, 2005b), irrespective of the temperature at which the plants were grown (Fig. 3.2.1.5). This association was defined by a linear fitted line with a predictor equation for the number of leaves per shoot over a range of thermal time between 0 and 510°C d accumulated after temperature differentiation. The rate of leaf accumulation was 0.0088 and 0.0068 leaves shoot^{-1} °C d^{-1} in spring and autumn, respectively. This indicated that in the spring equinox crop it took 114°Cd for a leaf to emerge from the primary haulm of each plant grown in either of the three temperature regimes, whereas in the autumn equinox crop it took 147°Cd; that is, if temperature is the only factor that defines a thermal unit. These values of phyllochron units corresponded well with values found by Inman-Bamber (1994) who reported a phyllochron interval of 109°C d (0.0092 leaves shoot^{-1} °Cd^{-1}) for leaves up to leaf 14 and 169°Cd (0.0059 leaves shoot^{-1} °Cd^{-1}) for leaves that emerged after leaf 14 for NCo376 (the same cultivar used in this current study). In that same experiment, phyllochron interval for cultivar N12 was found to be 118°Cd (0.0085 leaves shoot^{-1} °C d^{-1}) up to leaf 14 and 200°C d (0.005 leaves shoot^{-1} °C d^{-1}) after leaf 14. Similar results were found by Sinclair *et al.* (2004) who reported a range of phyllochron interval from 87 to 118°C d (0.011-0.0085 leaves shoot^{-1} °C d^{-1}) for cultivar CP80-1743 grown during the spring conditions of Florida, USA. It is more appropriate to make comparison of the phyllochron intervals obtained in this study with the phyllochron intervals of the later leaves reported from the results of previous studies since the mean number of primary haulm leaves in this study was 16 for the spring crop and 14 for the autumn crop before the temperature treatment was employed.

It is thought that larger leaves require an extended number of thermal units to develop than smaller leaves (Bonnets, 1998). This may account for the longer phyllochron in autumn (147°Cd) than in spring (114°Cd), as leaf blade area per leaf rank was higher in autumn than spring (Section 3.2.3).

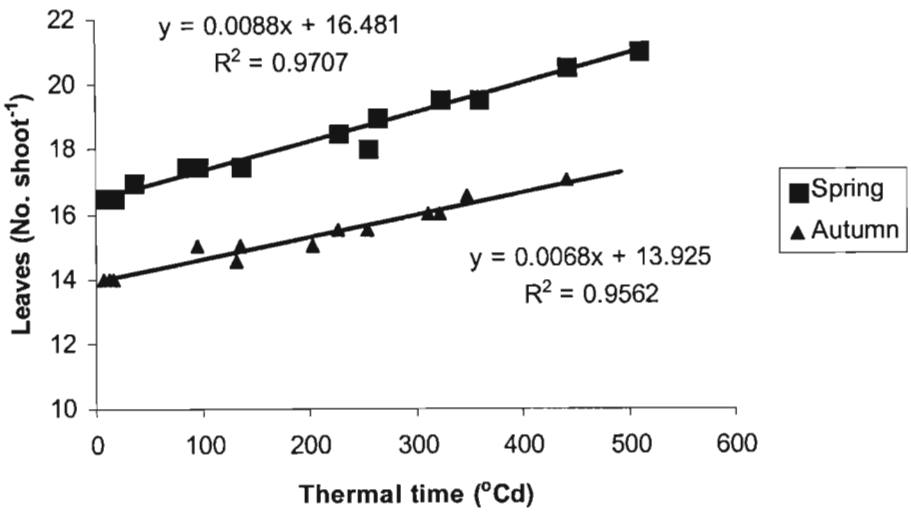


Figure 3.2.1.5: Cumulative number of leaves with accumulated thermal time of sugarcane plants grown over the spring and autumn equinoxes, (T_b=10°C). Data points are representatives of total number of leaves per shoot accumulated by plants from all the three temperatures over the one month period of temperature treatment.

Cumulative green leaf number per degree day

There was a slightly negative slope (-0.00004) observed on the graph of cumulative green leaves with thermal time in spring (Fig. 3.2.1.6a), indicating that for this trial the rate of leaf senescence was slightly higher than the rate of leaf appearance. In autumn, the slope of the fitted line for the number of green leaves during the course of the trial was slightly positive (0.00004) (Fig. 3.2.1.6b), indicating that the rate of leaf emergence was slightly higher than the rate of leaf senescence. It was also clear that the number of leaves that stay green per plant did not depend on the number of thermal units accumulated (R^2=0.01% in both trials).

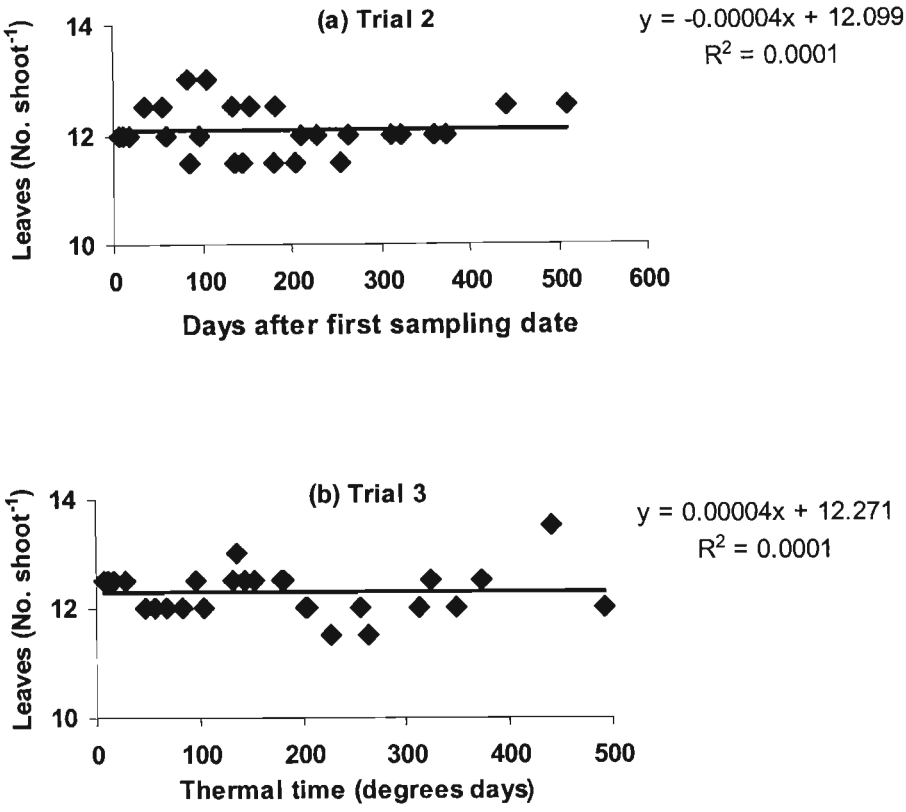


Figure 3.2.1.6: Cumulative number of green leaves with thermal time for sugarcane plants grown over the spring equinox (a) and autumn equinox (b).

3.2.2 Leaf senescence

The senesced leaves of the primary haulms of the repeatedly measured plants were used to compare the rates of leaf senescence between plants in the three temperature regimes. The rate of senescence of the bottom leaves was higher at the higher levels of temperature (W and H) and lower in the cool temperature (C). These results are in agreement with the results of Ebrahim *et al.* (1998a), who observed that leaf senescence rate was higher at supra-optimum temperature (45°C) and lower at sub-optimum temperature (15°C). For spring, the rates of leaf senescence at the three temperatures were 0.14, 0.11 and 0.03 leaves d^{-1} at H, W and C, respectively (Fig. 3.2.2.1). In autumn the rate of leaf senescence

in the three temperatures were 0.10, 0.11 and 0.04 leaves d^{-1} at H, W and C, respectively (Fig. 3.2.2.2).

A linear fitted line between the accumulated number of senesced leaves and thermal time indicated that the leaves of the primary haulm senesced at a rate of 0.0083 leaves $°Cd^{-1}$ in spring (Fig. 3.2.2.3) and 0.0078 leaves $°Cd^{-1}$ in autumn (Fig. 3.2.2.3). In other words, it took ca.120°C d for one leaf at the bottom of the shoot to senesce for the plants grown over the spring equinox, and ca. 128°Cd for the plants grown over the autumn equinox.

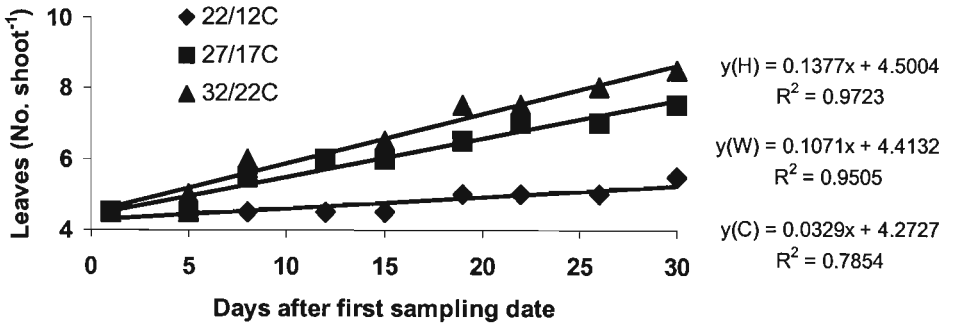


Figure 3.2.2.1: Number of dead leaves per shoot with progression of time of sugarcane plants grown over the spring equinox at three temperatures. Each data point is representing the mean number of dead leaves for two primary haulms. Leaves were designated dead when more than half of their blade area had become necrotic.

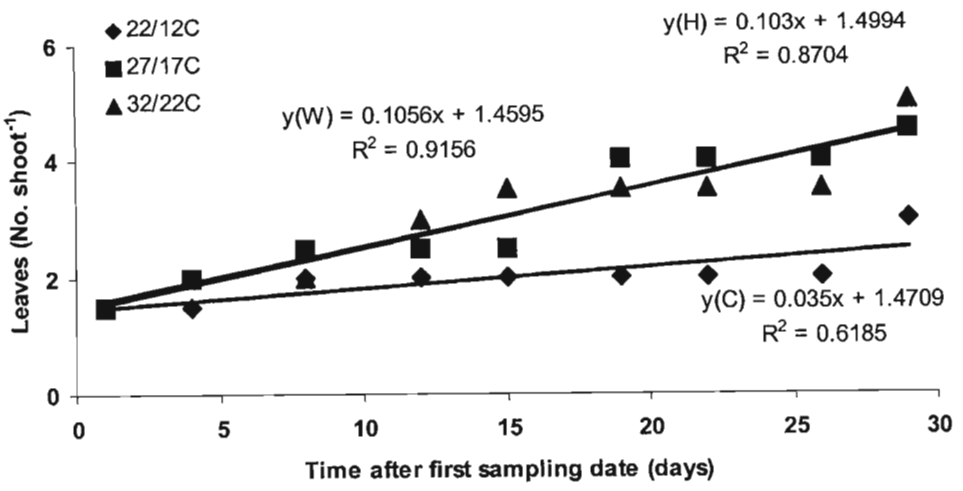


Figure 3.2.2.2: Number of dead leaves per shoot with progression of time of sugarcane plants grown over the autumn equinox at three temperatures. Each data point is representing the mean number of dead leaves for two primary haulms.

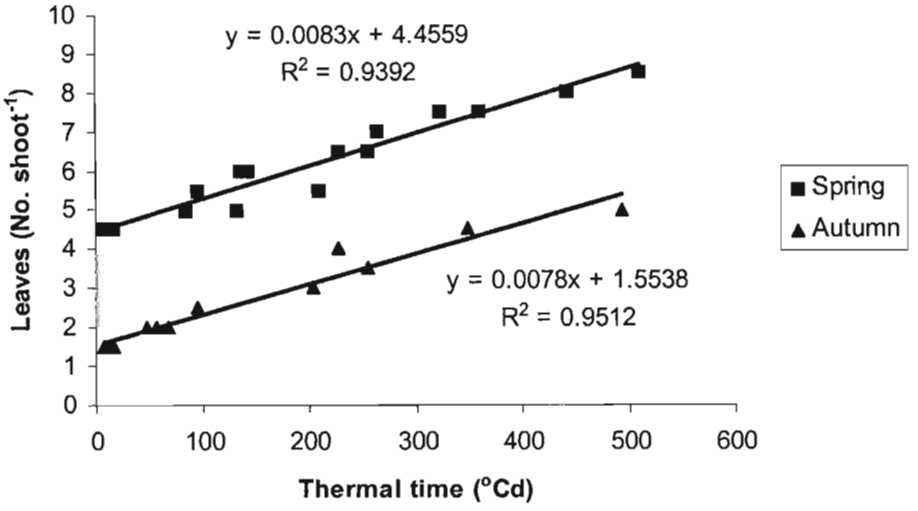


Figure 3.2.2.3: Number of dead leaves over thermal time for sugarcane plants grown over the spring equinox (a) and over the autumn equinox (b). The thermal time was determined by subtracting the base temperature (assumed 10 degrees days) from the mean temperature and then multiplying that quantity by the number of days after temperature differentiation. Leaves were designated dead when more than half of their leaf blade had become necrotic.

As observed by Inman-Bamber (1994), there was a close link between leaf emergence and leaf senescence. This association was more pronounced when the rates of leaf emergence and leaf senescence were expressed in terms of thermal time (physiological age) than time in normal days (chronological age). In this study, the rate of leaf senescence on a thermal time basis was notably similar to the rate of leaf emergence in both trials. In spring the rate of leaf emergence was 0.0088 leaves °C d^{-1}, comparing well with the rate of leaf senescence of 0.0083 leaves °C d^{-1}. In autumn the rate of leaf emergence (0.0068 leaves dd^{-1}) also compared well with the rate of leaf senescence (0.0078 leaves °C d^{-1}). This indicated that at a particular maturity stage of the sugarcane plant the rate of leaf appearance at the shoot top dictated the rate of leaf senescence at the bottom of the shoot through a shading effect. In his study Inman-Bamber (1994) correlated leaf emergence and senescence for NCo 376 and N12 and found a correlation coefficient of R^2=0.96 for both cultivars. This indicated that the prediction of the number of leaves that emerged or senesced per shoot was more appropriate from rates of leaf emergence/senescence on thermal time basis (°Cd) than normal time (days).

The close association of leaf emergence rate and the senescence rate of the lower leaves is supported by the idea that there is a vertical gradient of irradiance and the ratio of red light to far red light (R:Fr) down the crop canopy, which results from gradual extinction (down the shoot) of incident light due to absorption by chlorophyll (Ballaré and Casal, 2000); and as more leaves are added at the top of the shoot, the gradient increases and the bottom leaves become more exposed to low levels of radiation as well as R:Fr ratio. Rousseaux *et al.* (1996), working with sunflower, showed that the combined effect of this effect was an accelerated rate of leaf senescence. It was not surprising, therefore, that the rate of senescence of the bottom leaves was higher at W and H where new leaves emerged at a higher rate than at C.

The higher number of thermal units taken for one leaf to senesce in autumn (128°Cd) than in spring (120°Cd) may be explained by the fewer leaves, hence a lower shading effect during autumn than spring. Also, tiller density was lower in autumn, as smaller shoots

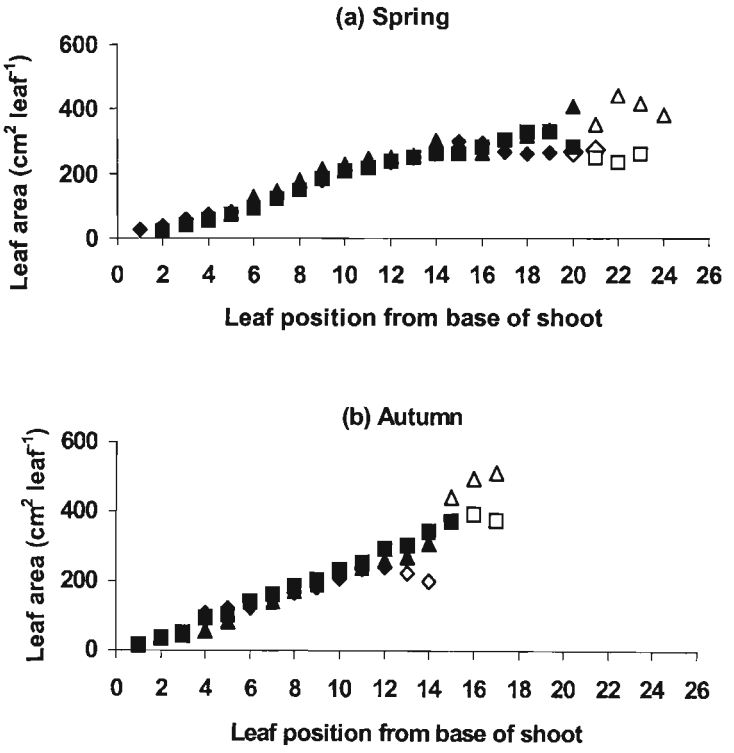
were pruned to five shoots per stool compared to the spring crop, and this further reduced the shading effect on the autumn crop than in spring.

3.2.3 Leaf area

The observed primary haulm profiles of leaf area varied with temperature in both trials. The cool temperature C delayed leaf development (leaf elongation and expansion), whereas temperatures W and H favoured leaf development. It should be noted that the number of leaves of the haulms of the plants selected for the repeated measurements were different between the three glasshouses, and this explains the noticeable differences in the leaf positions of the last leaves on plants in the three glasshouses at the onset of temperature differentiation. Leaves that appeared after temperature differentiation showed differences in their final leaf sizes (Fig. 3.2.3.1). In spring, leaf area at H reached a maximum (438 cm^2) on leaf No. 21 and then the area of the subsequent leaves started to decrease (Fig. 3.2.3.1a). Meanwhile, leaf area decreased for the leaves that emerged after the cold temperature treatment (C).

The observed primary haulm profile of leaf area in autumn was similar to that of spring (Fig. 3.2.3.1b), except that temperature differentiation occurred when the number of leaves per shoot was fewer for plants in autumn than that in spring. Subsequent tillers had the same trend as the primary haulms (Figs. 3.2.3.2 and 3.2.3.3). However, their leaf area was higher for the same leaf position than the primary haulms (Fig. 3.2.3.4). This was due to the fact that the shoots of different order, which are differently positioned in the stool, tend to receive different radiation regimes (Singels *et al.*, 2005b). It is believed that under shade conditions, supplemental Fr light increases the rate of leaf elongation without affecting the phyllochron interval (Skinner and Simmons, 1993). The larger leaves that develop early on higher order shoots tend to become a larger source of assimilates to subsequent leaves and further increase their rate of elongation (Singels *et al.*, 2005b). The differences in leaf area of similar positioned leaves of similar order shoots before temperature treatment was probably due to physiological age differences between haulms labeled with the same tiller number.

The results of this study accorded with the results of the study by Singels *et al.* (2005b) who observed that the expansion of leaves that developed during the winter season, when temperature and radiation were low, was less than that of the leaves that developed in the summer season when temperature and radiation were high. Ebrahim *et al.* (1998a) also observed that low temperature (15°C) significantly inhibited leaf area development as



compared with the optimum temperature (27°C).

Figure 3.2.3.1: Stalk profile of leaf area for the primary haulms of plants in the three temperature regimes for sugarcane plants grown over the spring equinox (a) and autumn equinox (b). ♦ Leaf area at 22/12°C before temperature differentiation; ◊ Leaf area at 22/12°C after temperature differentiation; ■ Leaf area at 27/17°C before temperature differentiation; □ Leaf area at 27/17 after temperature differentiation; ▲ Leaf area at 32/22°C before temperature differentiation; Δ Leaf area at 32/22°C after temperature differentiation.

A similar observation was made for the autumn crop, except that there was no data beyond leaf 17 to clearly show the exact leaf number that would have attained the maximum leaf area. Also, the maximum blade area (511.7 cm^2) (assuming that maximum leaf area was at leaf 17) attained at H was at a lower leaf rank but still higher than the maximum area per leaf blade attained by the primary haulm of spring. This difference could be explained by the environmental conditions and management practices during pre-trial establishment of plants for the two trials. The spring crop was established during the winter months when solar radiation and temperature were lower; whereas the autumn crop was established during the summer months when radiation and temperature were higher. Since the same temperature treatments resulted in different maximum leaf blade area for the two trials, it indicated that leaf size is not controlled by temperature and radiation alone. Singles *et al.* (2005b) reported that in addition to the influence of temperature and radiation on final leaf size, there was also the effect of the effective source size that is available to produce these leaves. The leaves of the autumn plants that emerged after temperature differentiation were supported by a larger source size (larger whole stem green leaf area) than for spring; and hence a higher maximum leaf blade area was achieved in autumn than in spring.

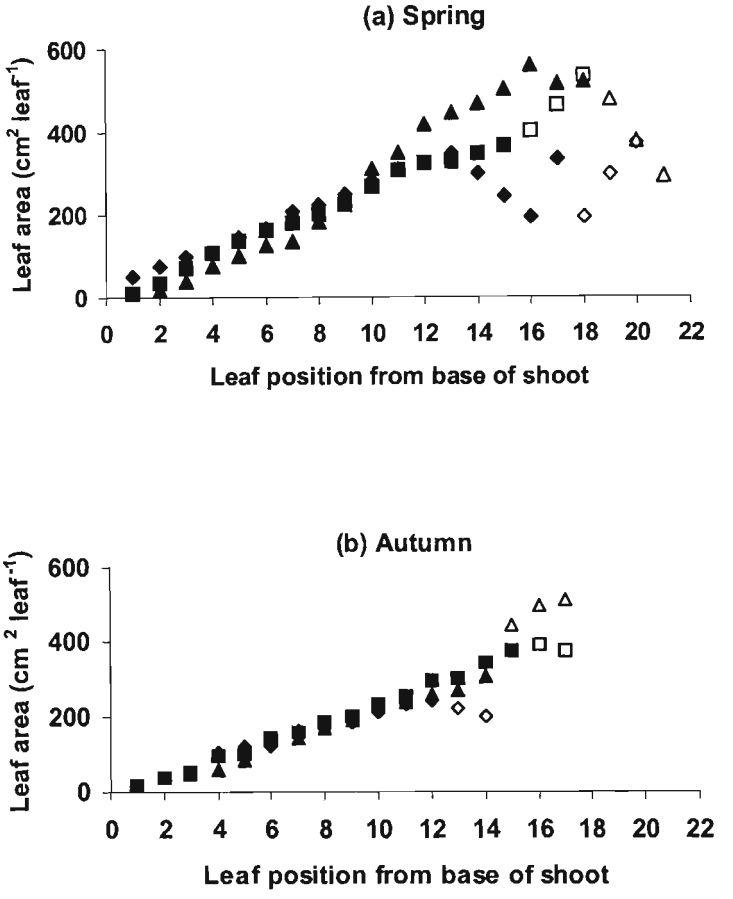


Figure 3.2.3.2: Stalk profile of leaf area for tiller number 1 for plants grown over the spring equinox (a) and autumn equinox (b). ♦ Leaf area at 22/12°C before temperature differentiation; ◊ Leaf area at 22/12°C after temperature differentiation; ■ Leaf area at 27/17°C before temperature differentiation; □ Leaf area at 27/17 after temperature differentiation; ▲ Leaf area at 32/22°C before temperature differentiation; Δ Leaf area at 32/22°C after temperature differentiation.

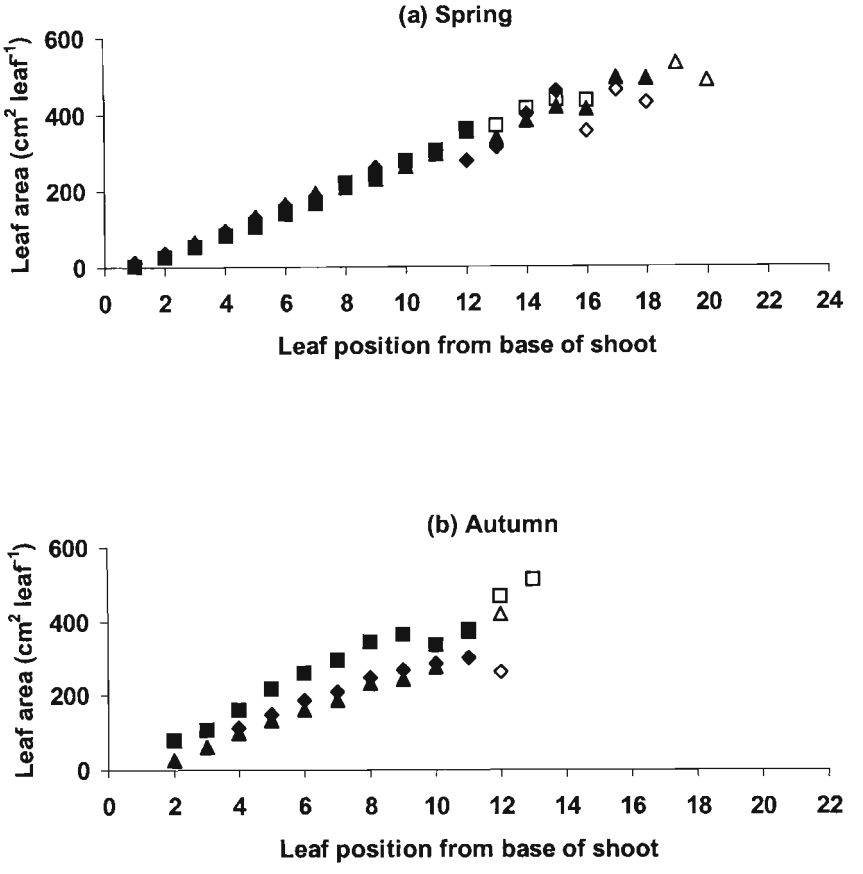


Figure 3.2.3.3: Stalk profile of leaf area for tiller number 2 of plants grown over the spring equinox (a) and autumn equinox (b). ♦ Leaf area at 22/12°C before temperature differentiation; ◊ Leaf area at 22/12°C after temperature differentiation; ■ Leaf area at 27/17°C before temperature differentiation; □ Leaf area at 27/17 after temperature differentiation; ▲ Leaf area at 32/22°C before temperature differentiation; Δ Leaf area at 32/22°C after temperature differentiation.

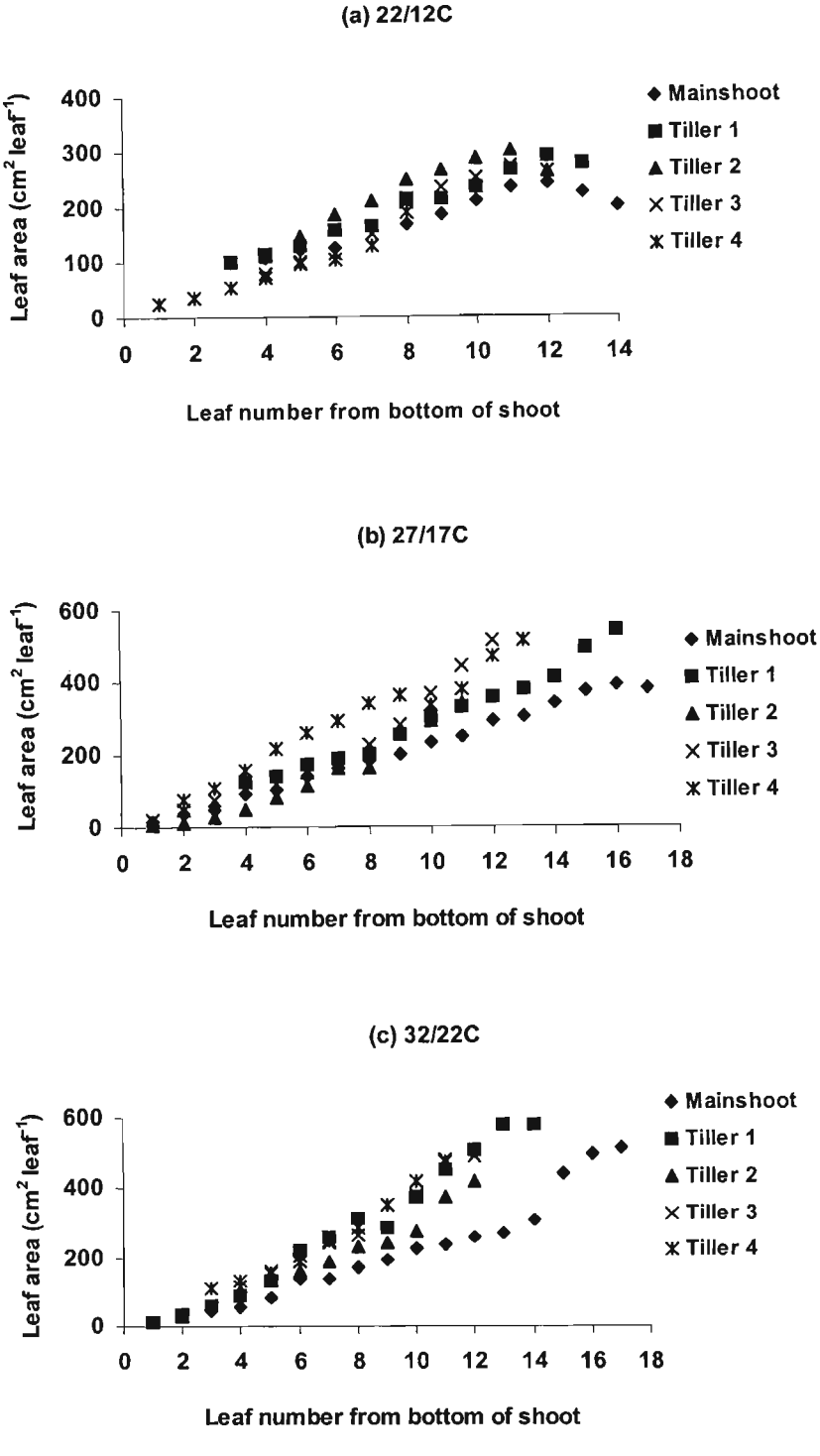


Figure 3.2.3.4: Blade area of leaves of the primary haulm and four other tillers of plants grown at 22/12°C (a), 27/17°C (b) and 32/22°C (c) over the autumn equinox, as a function of leaf position. Tillers were ranked according to their order of appearance starting from the first one to appear.

3.2.4 Stalk height

Stalk extension was inhibited by the C temperature and was higher at H in both trials. In spring the stalk extension rate (SER) of the primary haulms in the three temperatures were 1.22, 1.02 and 0.38cm d^{-1} at H, W and C, respectively, (Fig. 3.2.4.1a); and in autumn the SERs were 1.35, 0.98 and 0.45cm d^{-1}, at H, W and C, respectively (Fig. 3.2.4b).

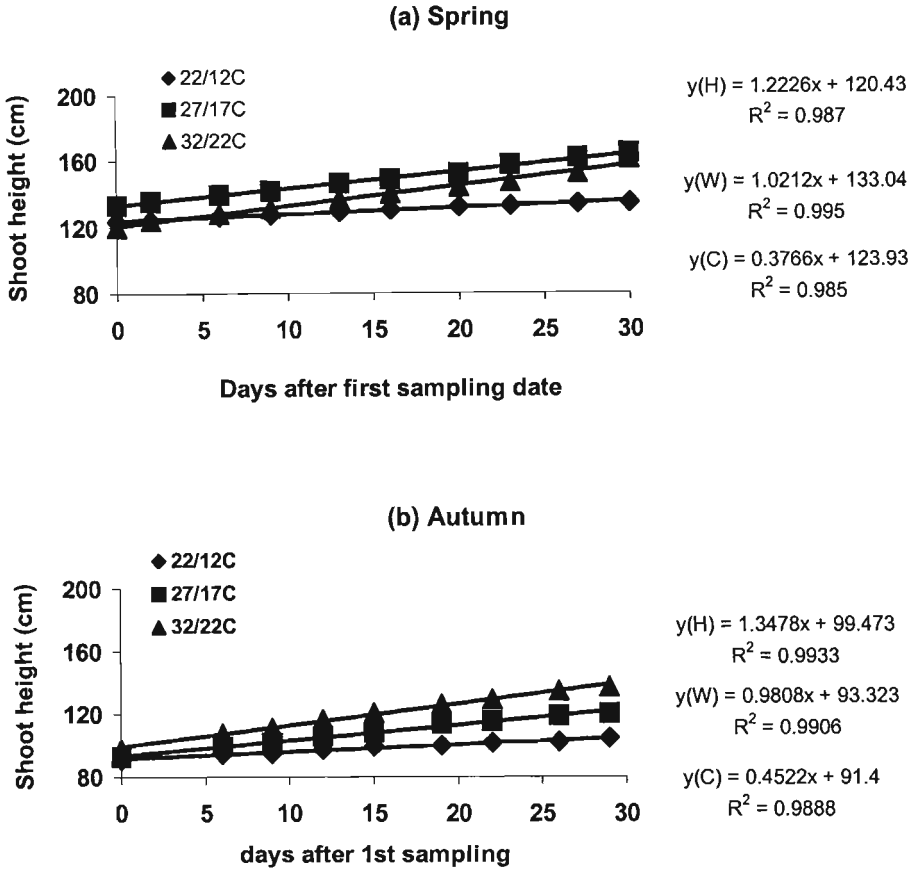


Figure 3.2.4.1: Stalk extension over the sampling period of plants grown in three temperatures over the spring equinox (a) and autumn equinox (b). Each data point represents the mean of the height of the primary haulms of two plants that were sampled last in each temperature.

Stem length is a function of internode number and the length of individual internodes (Berghage, 1998). It was not surprising therefore that stem extension was lower at the

cool temperature (C), where both the internode appearance and extension rates were inhibited than at the higher (W and H) temperatures. It is known that stem extension results from cell division and extension (Erwin *et al.*, 1994; Sachs, 1965), which are affected by plant growth regulants (Salisbury and Ross, 1985). Mongelard and Mimura (1972) associated sugarcane stalk extension with gibberellic acid (GA). GA stimulates cell division at the basal meristematic cells (Sachs, 1965), which results from the stimulation of cells in the G1 phase to enter the S phase (Liu and Loy, 1976). The growth of the increased number of cells increases stem extension (Salisbury and Ross, 1985). In sugarcane stem GA has an effect of increasing the invertase enzymes, which in turn hydrolyze sucrose into glucose and fructose (Glasziou, 1969), which contribute to cell wall formation and cause cell water potential to decrease (become more negative). This has an effect of causing rapid entrance of water into cells, causing the cells to expand; hence the higher SER that was observed at W and H. According to Mongelard and Mimura (1972) GA synthesis and its translocation to the stem tissues are inhibited by temperatures lower than 18°C. Considering this, it is likely that the observed inhibition of stalk extension at C resulted from either the inhibition of GA synthesis from its sites of biosynthesis and its translocation to its sites of action or its action.

Stem extension is also said to be a function of the difference between day and night temperatures (DIF) (Berghage, 1998). Studies show that a positive DIF accelerates the rate of internode appearance and extension in many plant species including *Cucumis sativus* L. (Xiong *et al.*, 2002; Grimstad and Frimanslund, 1993), *Campanulla isophylla* M. (Torre and Moe, 1998), *Nepholepis exaltata* (Erwin *et al.*, 1993), *Solanum tuberosum* L. (Kozai *et al.*, 1995), *Lycopersicon esculentum* (Grimstad, 1993) and many others (Myster and Moe, 1995). A higher positive DIF is thought to increase the stem extension rate by increasing cell elongation without cell division (Erwin *et al.*, 1994). The study on the effect of DIF on sugarcane stem extension is limited. In this study, DIF was similar (10°C) in all the three temperature levels and in spite of the uniform DIF, differences in stem extension and other morphological growth parameters occurred on the sugarcane plants grown at the different temperatures, with the exception of total plant weight.

3.3 Assimilation

3.3.1 Total biomass

It was not possible to meaningfully assess total biomass per plant for spring because sampled plants for this trial did not have a uniform number of shoots per plant since some shoots were snipped off and there were variable larger shoots per stool. Therefore it would not be sensible to plot graphs of plant weight over time for such plants. In autumn the dry weights of the five shoots that were retained per plant increased with time after temperature differentiation at all temperatures (Fig. 3.3.1.1).

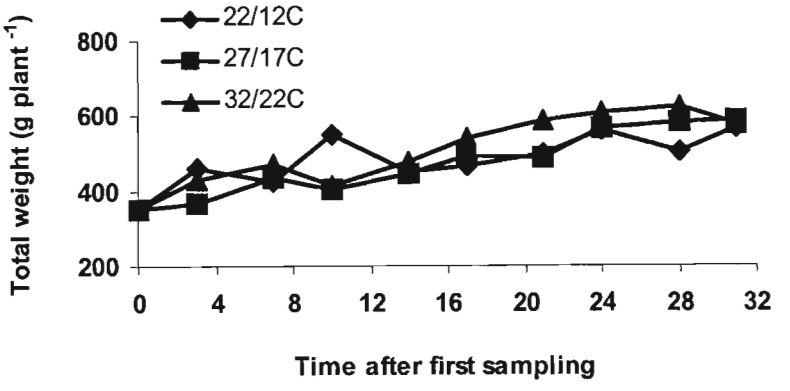


Figure 3.3.1.1: Total above ground dry weight per plant of plants grown over the autumn equinox. Mean of two plants per data point.

Although there was a higher vegetative growth rate in the two higher temperatures (W and H) than in the cold temperature (C), in terms of stalk extension, leaf emergence, leaf area development and internode appearance per shoot (data not shown), this effect was not reflected in the total dry weight of plants per shoot at the three temperature levels. There were no significant total dry weight differences, over the one month period of temperature treatment between plants grown at the three temperatures. This obviously explains the importance of specifying the parameters used as a measure of plant growth. Mongelard and Mimura (1972) noted that the increase in dry matter is the reliable means of measuring plant growth in plants that are beyond the germination phase. However, in the case of sugarcane, whereby the economic yield is in terms of the measure of sucrose,

an increase in dry matter may not be a suitable measure of plant growth. Depending on the environmental conditions, increase in sugarcane dry mass may occur due to dry matter accumulation in the cell wall materials in the expense of sucrose accumulation. Also, the rapid stalk extension observed at high temperature and moisture conditions (as observed in this study at H) may be due to cell division and elongation, also at the expense of sucrose accumulation.

Mongelard and Mimura (1972) used cumulative water transpired by sugarcane plants to estimate cumulative growth, assuming that dry weight accumulation and transpired water were closely related variables, and observed that water consumption was higher in the warm temperature than in the cool temperature. Although water consumption was not quantified in this study, there was a higher demand for frequent watering in the two higher temperatures (W and H) than in the cold temperature (C), suggesting that biomass accumulation should have been higher in the upper temperature levels than in the cold temperature. Since water loss in plant-water relations is due to soil evaporation and transpiration, it is reasonable to assume that not all the water applied to plants in the warm and hot temperatures was used up for plant growth. Part of it was lost though surface evaporation.

It is well known that respiration loss of photoassimilates is higher in plants grown at higher temperatures than in the cool temperatures (Glover, 1972b). Although respiration rates were not measured in this study, it is probable that a considerable amount of photoassimilates in the higher temperatures were lost through respiration. Hence, the seemingly higher growth rate in the higher temperatures was nullified by increased rates of respiration.

3.3.2 Leaf mass

It was only possible to assess leaf mass per plant over time for the autumn crop for the same reason as explained for biomass per plant. Since there was a lower rate of leaf appearance at C than at W and H, green leaf biomass was lower at C than at the two

higher temperature levels (Fig. 3.3.2.1). Green leaf biomass per plant over time at W and H followed nearly the same pattern. There was no observable change in green leaf biomass over time at the two higher temperature levels. This was expected since the number of green leaves that appeared at the top of the shoots induced senescence on the leaves at the bottom of the shoots, causing the rate of leaf appearance and senescence to be closely similar. The slight increase could only be due to the leaf size differences between early leaves at the bottom of the shoots and late leaves at the top of the shoots. The late appearing leaves were obviously larger in size than the early bottom leaves, as indicated by the stalk profile of leaf area (Section 3.2.3).

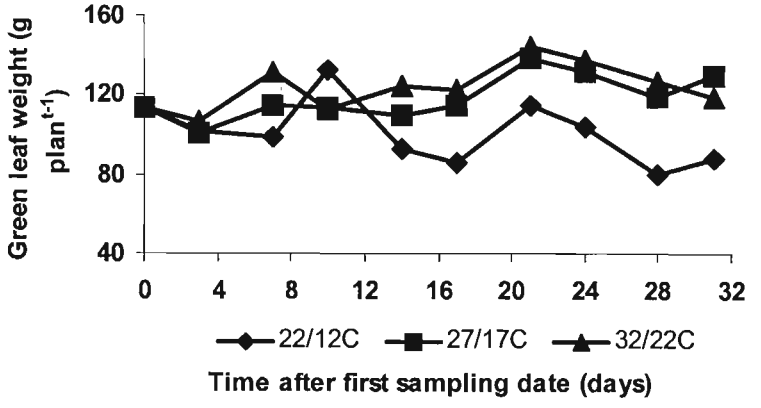


Figure 3.3.2.1: Green leaf biomass per plant over time of plants grown over the autumn equinox. Each data point represents the total leaf biomass of five shoots averaged for two plants.

3.3.3 Stalk mass

Temperature differences did not have any marked effect on the stalk biomass even though stalk extension was slower in the cool temperature (Fig. 3.3.3.1a). The fewer internodes observed in the cool temperature were short but thick, and were heavier than the internodes of plants at the warm and hot temperatures. This indicated that plants in the warm and hot temperatures used their assimilates for making more internodes with less weight, whereas plants in the cold temperature used their assimilates in concentrating mass on the fewer internodes that they had. The same effect was observed when stalk biomass was measured on a per plant basis (Fig. 3.3.3.1 b).

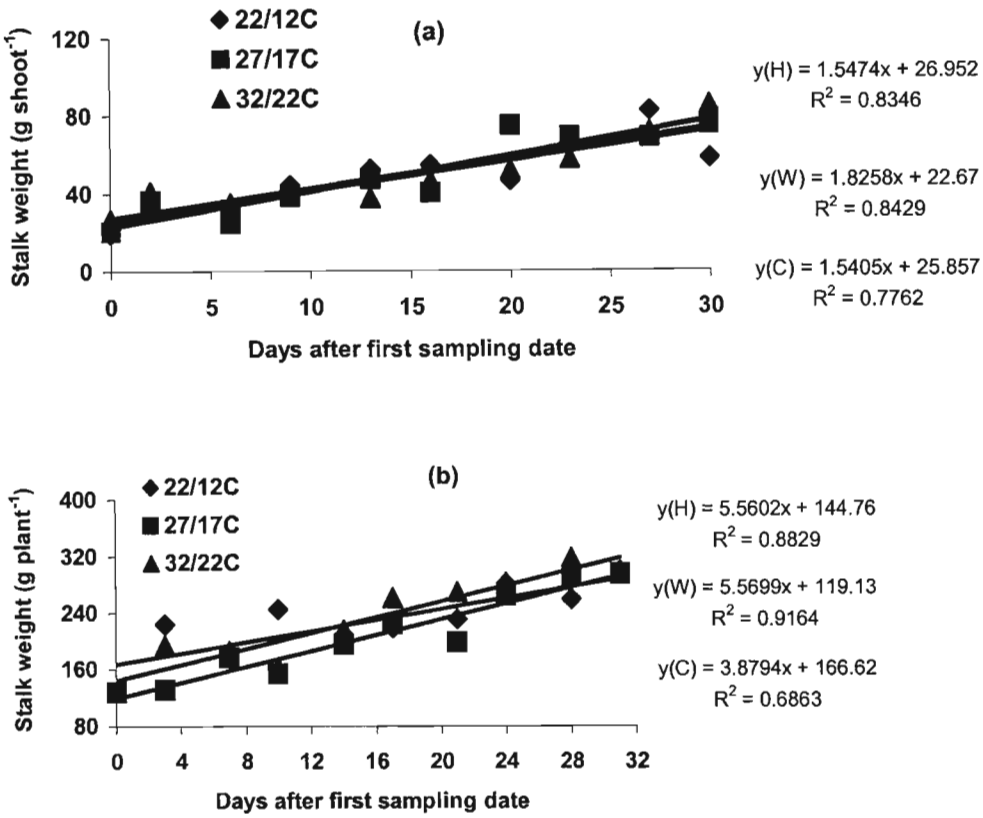


Figure 3.3.3.1: Dry weight (a) per shoot of stalk of sugarcane plants grown over the spring equinox and (b) per plant of five shoots for plants grown over the autumn equinox.

3.4 Assimilate partitioning to stalk and herbage

The above ground biomass in sugarcane mainly comprises the stalk and the leaf components. The stalk is composed of soluble and non-soluble substances, namely, fibre, sucrose and non-sucrose materials. The fibre makes the structural part of the stalk which maintains the plant's rigidity. The non-sucrose substances include the reducing sugars, primarily glucose and fructose, proteins and some organic and amino acids. The leaf component consists of the leaf sheath and the leaf blades of the mature and immature leaves. A leaf is designated mature once it develops a collar. The distribution of assimilates between the stalk component and the leaves in the above ground structure differed between the three temperatures. The cool temperature (C) favoured assimilate partitioning to the stalk; whereas the higher temperatures (W and H) favoured assimilate

partitioning to the leaf production. In autumn, which explicitly showed the effect of temperature on assimilate distribution between the stalk and the leaves, it was shown that partitioning (gg^{-1}) to the stalk declined from a peak value of 0.71 at C to 0.70 at W, and then further down to 0.67 at H, with corresponding partitioning coefficients of the leaf structure as 0.29, 0.30 and 0.33, in the same order of temperature (Fig. 3.4.1 d, e, f).

In spring the assimilate partitioning coefficient to the stalk decreased from 0.87 at C to 0.82 at W, and then increased to 0.87 at H (Fig. 3.4.1 a, b, c). The difference between the slopes of Fig. 3.4.1 indicated the partitioning of assimilates to the stalk relative to the leaves. In autumn the difference between the assimilate partitioning coefficients of stalk and leaves was highest (0.43) at the cold temperature (C) and it decreased to 0.40 at W and further decreased to 0.33 at H. In spring the difference between the partitioning coefficients decreased from 0.73 at C to 0.63 at W and then increased to 0.73 at H. The higher partitioning coefficient to leaves at H in spring cannot easily be explained, except that the differences in partitioning between the three temperature treatments were in general not that large.

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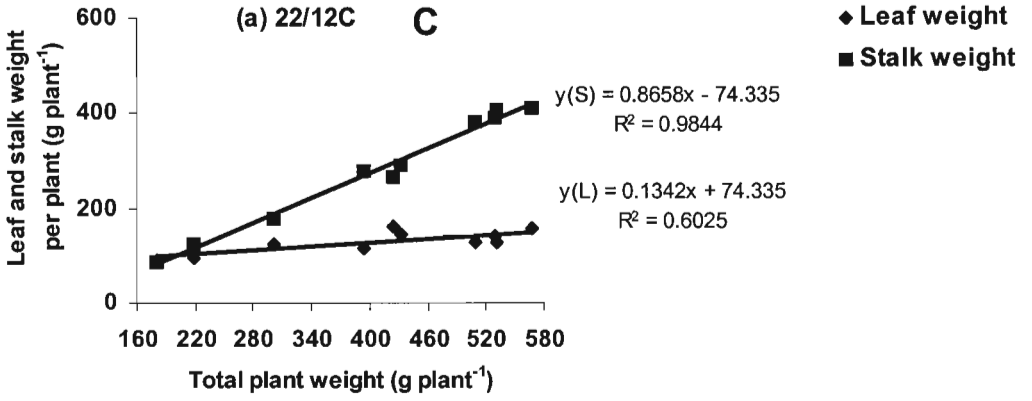
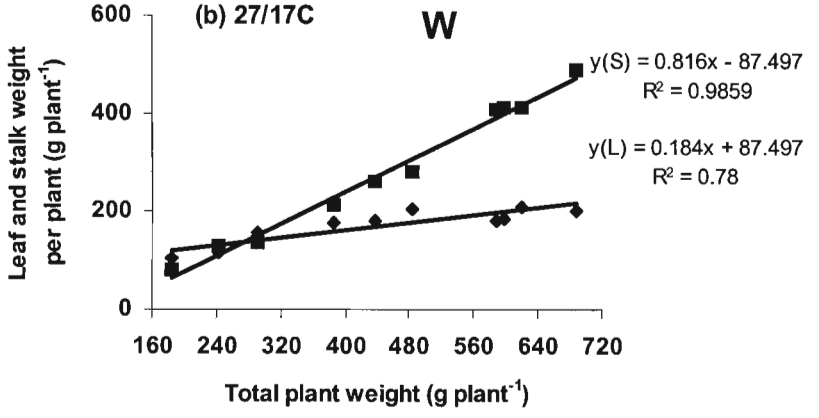
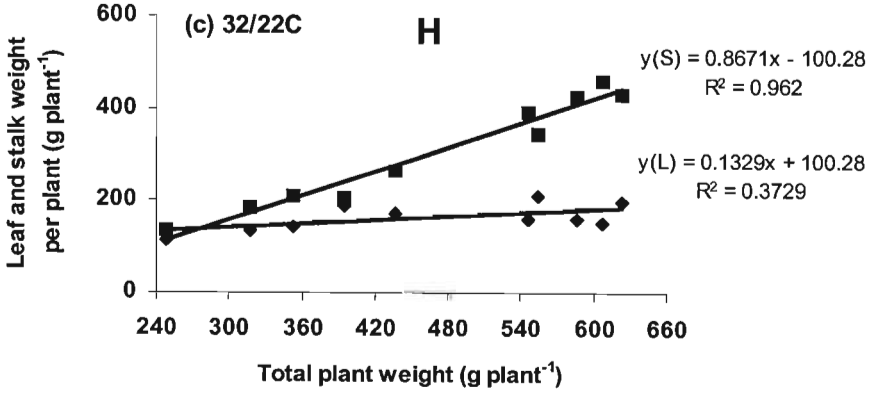

(a) 22/12C
C
◆ Leaf weight
■ Stalk weight
y(S) = 0.8658x - 74.335
R² = 0.9844
y(L) = 0.1342x + 74.335
R² = 0.6025
Leaf and stalk weight per plant (g plant⁻¹)
600
400
200
0
160 220 280 340 400 460 520 580
Total plant weight (g plant⁻¹)


(b) 27/17C
W
y(S) = 0.816x - 87.497
R² = 0.9859
y(L) = 0.184x + 87.497
R² = 0.78
Leaf and stalk weight per plant (g plant⁻¹)
600
400
200
0
160 240 320 400 480 560 640 720
Total plant weight (g plant⁻¹)


(c) 32/22C
H
y(S) = 0.8671x - 100.28
R² = 0.962
y(L) = 0.1329x + 100.28
R² = 0.3729
Leaf and stalk weight per plant (g plant⁻¹)
600
400
200
0
240 300 360 420 480 540 600 660
Total plant weight (g plant⁻¹)

AUTUMN

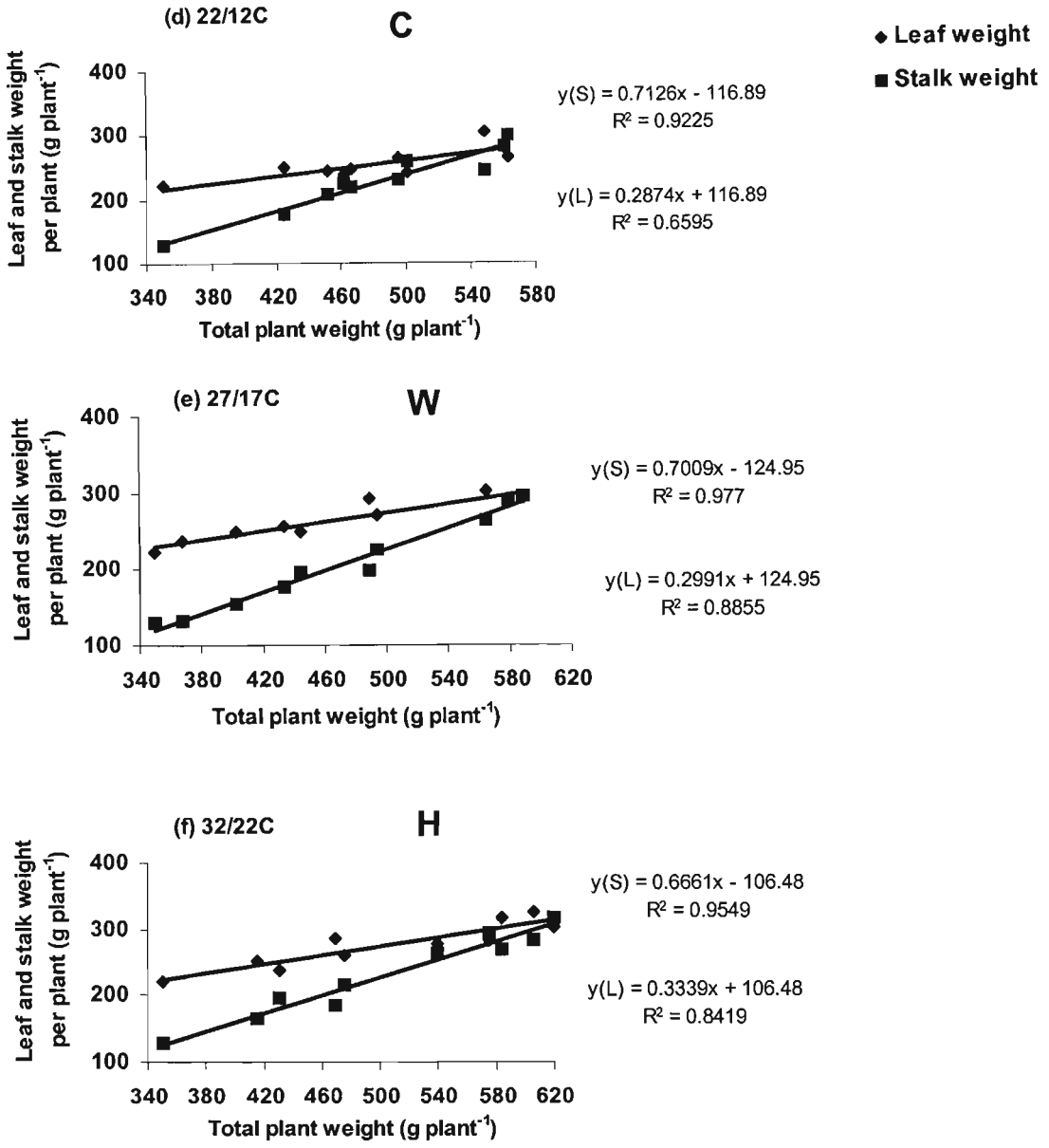


Figure 3.4.3.1: Partitioning of assimilates to stalk and leaves (dead and green leaves) of sugarcane plants grown over the spring (a), (b) and (c) and autumn (d), (e) and (f) equinoxes at the cold temperature 22/12°C (C), warm temperature 27/17°C (W) and hot temperature 32/22°C (H). Partitioning coefficients are represented by the regression line coefficients. In the regression line equations S denotes stalk and L denotes leaves.

It is well known that low temperature lowers the rate of leaf emergence and leaf development, and that when the temperature is not too low to inhibit photosynthesis, the stalk structure may continue to accumulate biomass. This explains the shift in assimilate distribution in favour of the stalk at low temperature. The coefficients obtained in this study, ranging from 0.67 to 0.71, with decreasing temperature closely match with the range of coefficients that have been reported in earlier studies, 0.7-0.8 (Cheroo-Naymuth *et al.*, 2000), 0.66 (Evenson *et al.*, 1997; Thompson, 1978), 0.59-0.71 (Thompson, 1988), 0.8 (Robertson *et al.*, 1996a). Differences could probably be accounted for by the different environmental conditions in which the plants were grown, ranging from field conditions to controlled environmental conditions. Inman-Bamber (2002) reported a stalk fraction of 0.85, reached by cultivars NCo 376 and Q117, obtained from both irrigated and droughted plants. The partitioning coefficients of the leaf component obtained in this study, also compared well with the range obtained by Inman-Bamber (2002), which was obtained from plants grown up to 16 months.

3.5 Stalk profiles

3.5.1 Size (length, diameter and volume)

The general observation common to both spring and autumn was that the haulms of plants grown in the hot temperature were longer and thinner, whereas in the cool temperature they were shorter and thicker (Figs. 3.5.1.1 and 3.5.1.2). There was not much difference between the diameter of internodes of plants in the W and hot H temperatures in both trials (Fig. 3.5.1.2 d-f and j-l). As was the case for the number of leaves per shoot, the number of internodes per shoot was higher at H and lower at C. In an almost similar study by Ebrahim *et al.* (1998a), suboptimum temperature (15°C) resulted in plant stalks with shorter and fewer internodes, whereas plants that were grown in supra-optimum temperature (45°C) had numerous internodes, as in the optimum temperature (27°C), but internodes were shorter as well.

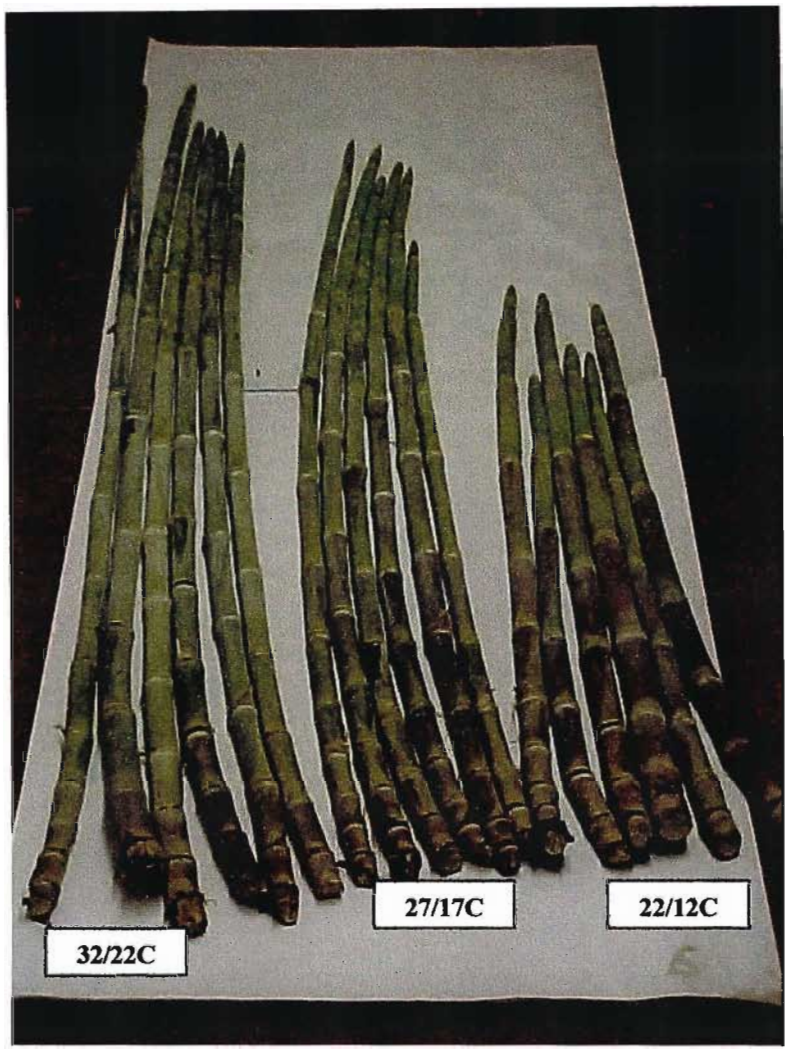


Figure 3.5.1.1: Shoots harvested after 30 days of growth at three temperatures over the spring equinox following an initial growth period under natural environmental conditions.

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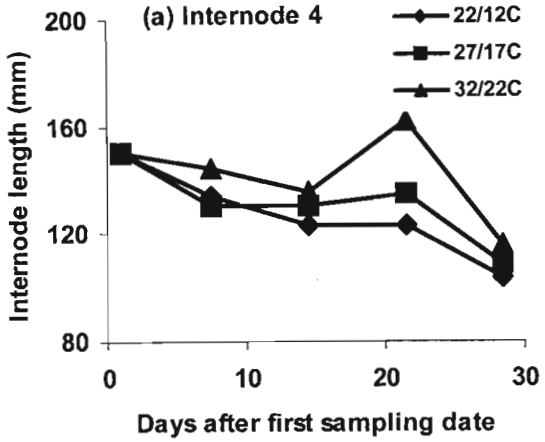
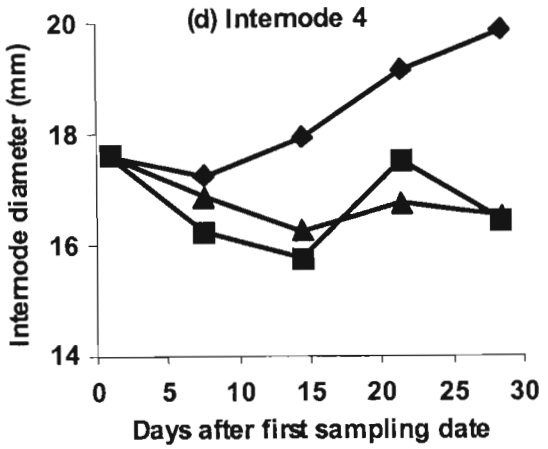
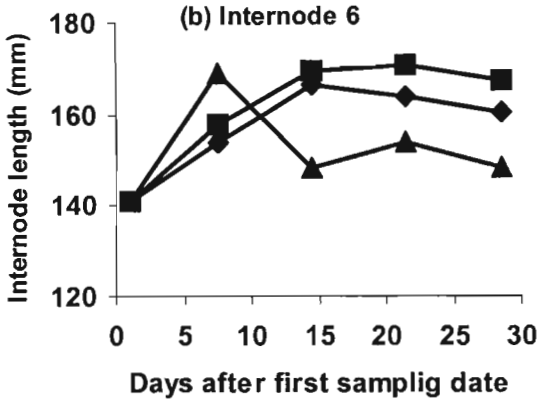
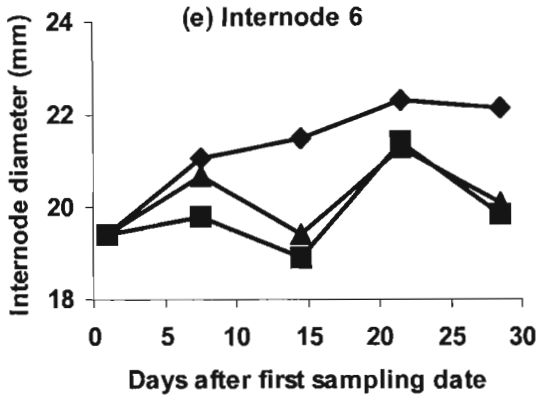
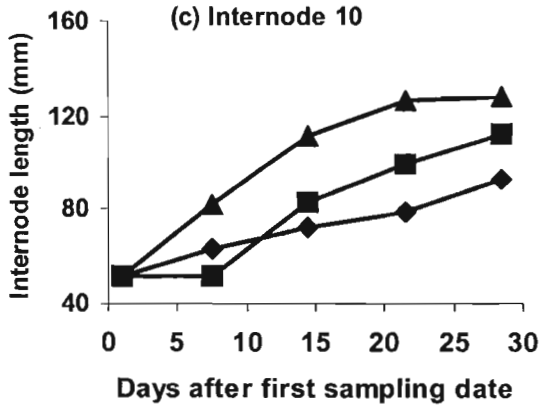
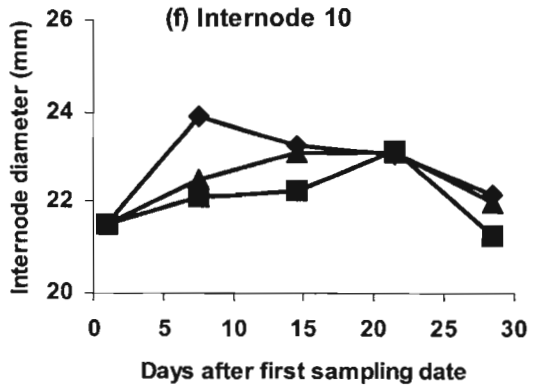

(a) Internode 4
22/12C
27/17C
32/22C
Internode length (mm)
200
160
120
80
0
10
20
30
Days after first sampling date


(d) Internode 4
Internode diameter (mm)
20
18
16
14
0
5
10
15
20
25
30
Days after first sampling date


(b) Internode 6
Internode length (mm)
180
160
140
120
0
5
10
15
20
25
30
Days after first samplig date


(e) Internode 6
Internode diameter (mm)
24
22
20
18
0
5
10
15
20
25
30
Days after first sampling date


(c) Internode 10
Internode length (mm)
160
120
80
40
0
5
10
15
20
25
30
Days after first sampling date


(f) Internode 10
Internode diameter (mm)
26
24
22
20
0
5
10
15
20
25
30
Days after first sampling date

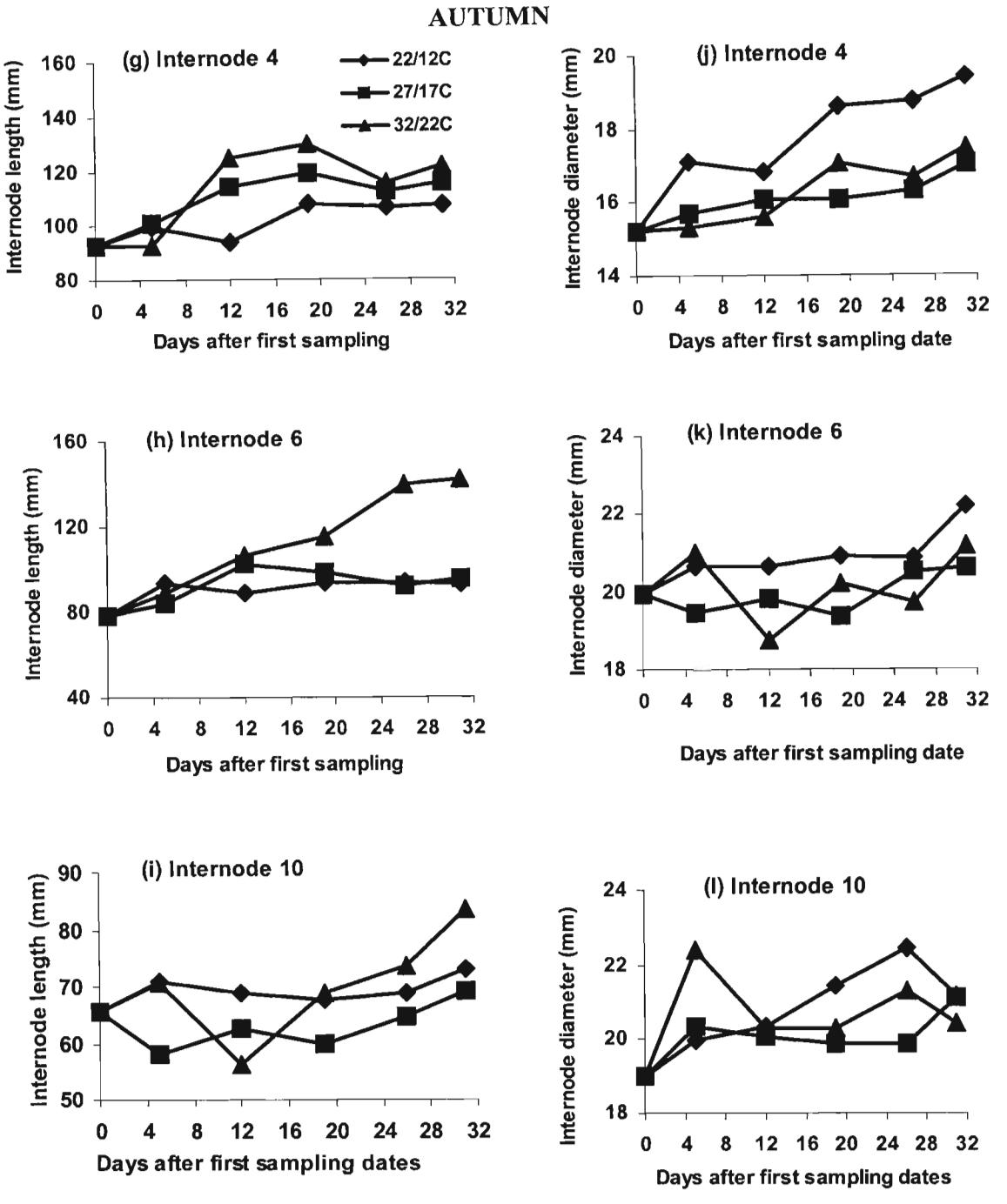


Figure 3.5.1.2: Length and diameter of internodes 4, 6 and 10 (counting from the top of shoot) of sugarcane plants grown in three temperatures over the spring (a-f) and autumn (g-l) equinoxes. Each data point represents the mean of two internodes of primary haulms and a mean of two sampling dates. On the first day of temperature differentiation, the diameters and lengths of internodes in the three temperatures were adjusted to a common value.

The data in Fig. 3.5.1.2 for autumn were presented, numbering the internodes from the bottom of the shoots (Fig. 3.5.1.3). In this method the position of the internodes from the top (i.e., the physiological age of the internodes) was kept fixed but numbered according to their position from the bottom of the shoot. This method illustrated the magnitude of change between the number of internodes from the start to the end of the experiment in the three temperatures. They also illustrated the magnitude of change between the internode length and diameter at the beginning and the end of the experiment. The advantages of this method are outlined in Section 3.5.3.

There was a mean difference of 4.33 internodes between the number of internodes per shoot at the start and at the end of the experiment at H. At W and C, the differences were 3.83 and 1.33 internodes, respectively. The difference between internode length at the start of the experiment and at the end was highest at H and lowest at C. The highest internode length was achieved by internode 6 at H on both trials (Fig. 3.5.1.2 b, h), indicating that cell elongation was more responsive at this region of the stalk (internode 6) than the region next to the apical meristem (internode 4) and the region that comprised mature tissue (internode 10), as one would expect. The changes in diameter, length and internode number per shoot from the start to end of experiment have been summarized (Table 3.5.1.1).

The dry weights of all the selected internodes (internodes 4, 6 and 10) were higher for the plants subjected to the cold treatment (C) than they were for the warm (W) and the hot (H) temperature treatments (Fig. 3.5.1.4). This observation was not surprising since each internode in the cold temperature had an extended period of biomass accumulation before a subsequent internode would be developed. This observation was common for both trials.

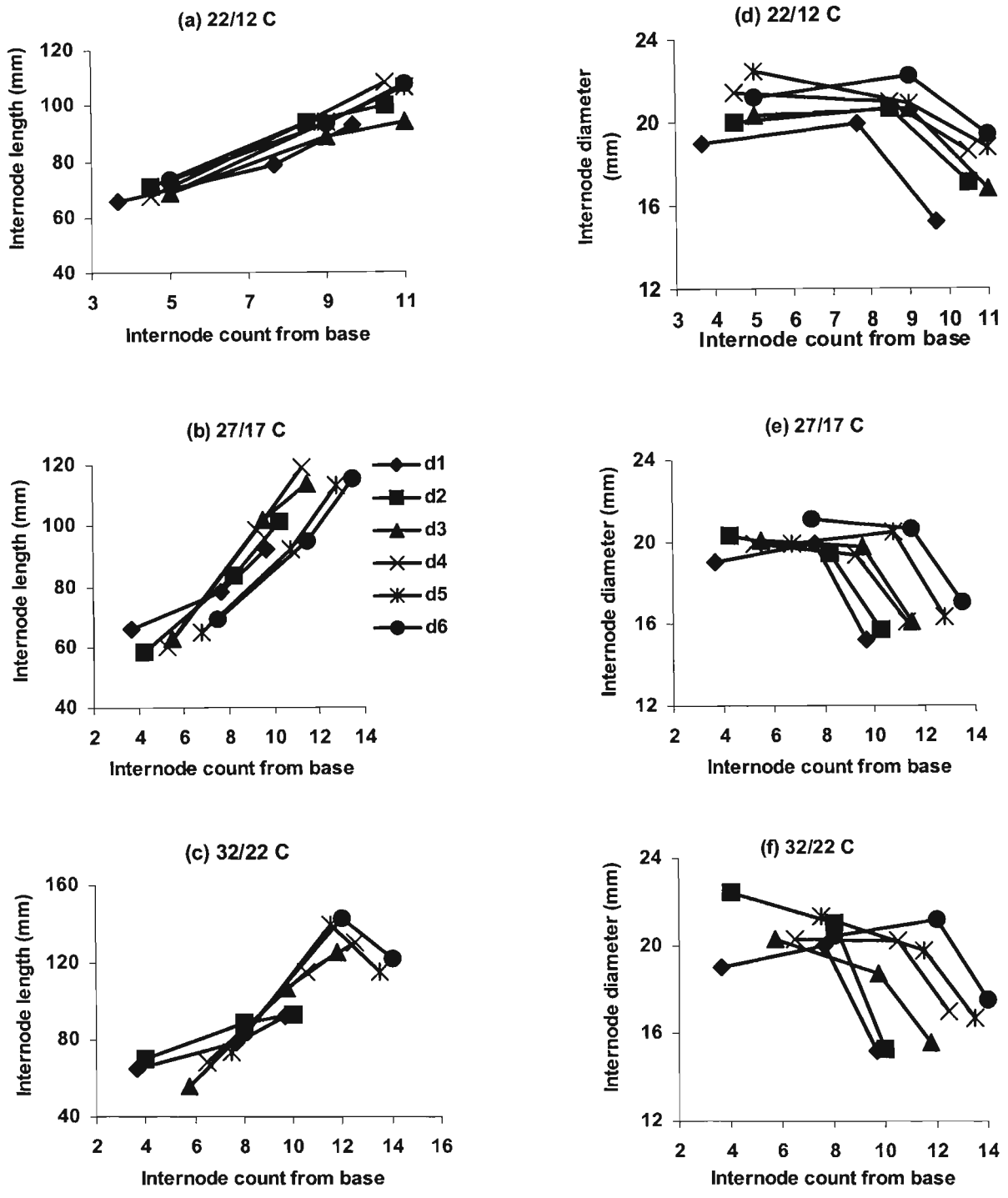


Figure 3.5.1.3: Length (a, b, c) and diameter (d, e, f) of selected internodes (counting from bottom) of sugarcane plants grown in three temperatures over the autumn equinox. d1, d2, d3, d4, d5 and d6 represent 0, 5, 12, 19, 26 and 31 days, respectively, after temperature differentiation.

Table 3.5.1.1: Changes in internode diameter, length and number of internodes per shoot from the day of temperature differentiation to the last sampling day of plants grown in three temperatures over the autumn equinox. Values are means of two internodes per sampling date and mean of two sampling dates.

Temperature	Internode No.	Δ diameter (mm)	Δ length (mm)	Δ internode No.
22/12°C	4	4.17	14.7	1.33
22/12°C	6	2.26	14.7	
22/12°C	10	2.12	7.3	
27/17°C	4	1.81	18	3.83
27/17°C	6	1.28	16.7	
27/17°C	10	2.09	3.3	
32/22°C	4	2.28	3.27	4.33
32/23°C	6	0.26	63.7	
32/24°C	10	1.39	17.8	

Δ = difference.

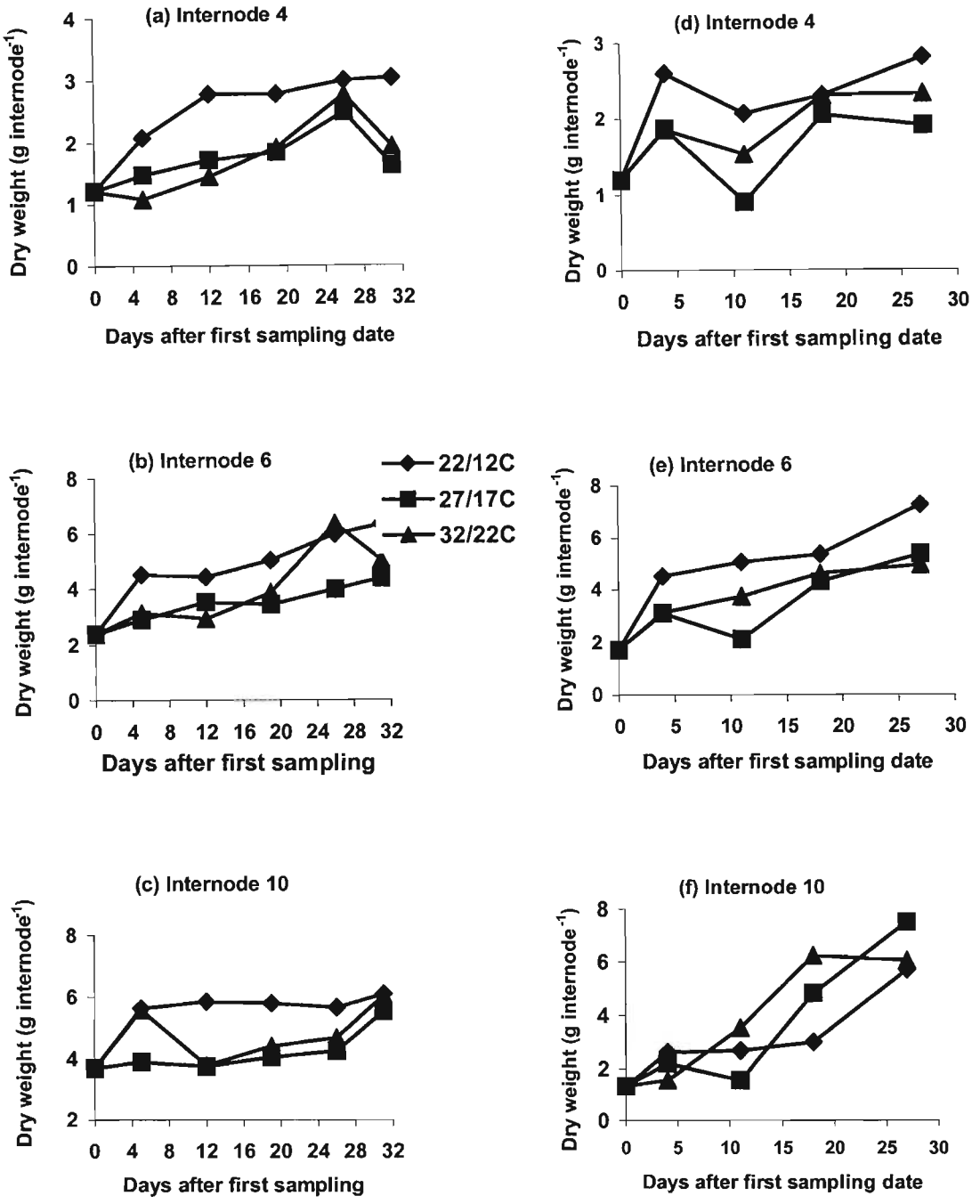


Figure 3.5.1.4: Dry weights of internodes 4, 6 and 10 (count from shoot top) of the primary haulms of sugarcane plants grown at three temperatures over the autumn (a), (b) and (c) and the spring equinox (d), (e) and (f).

3.5.2 Sugar changes and interrelationships

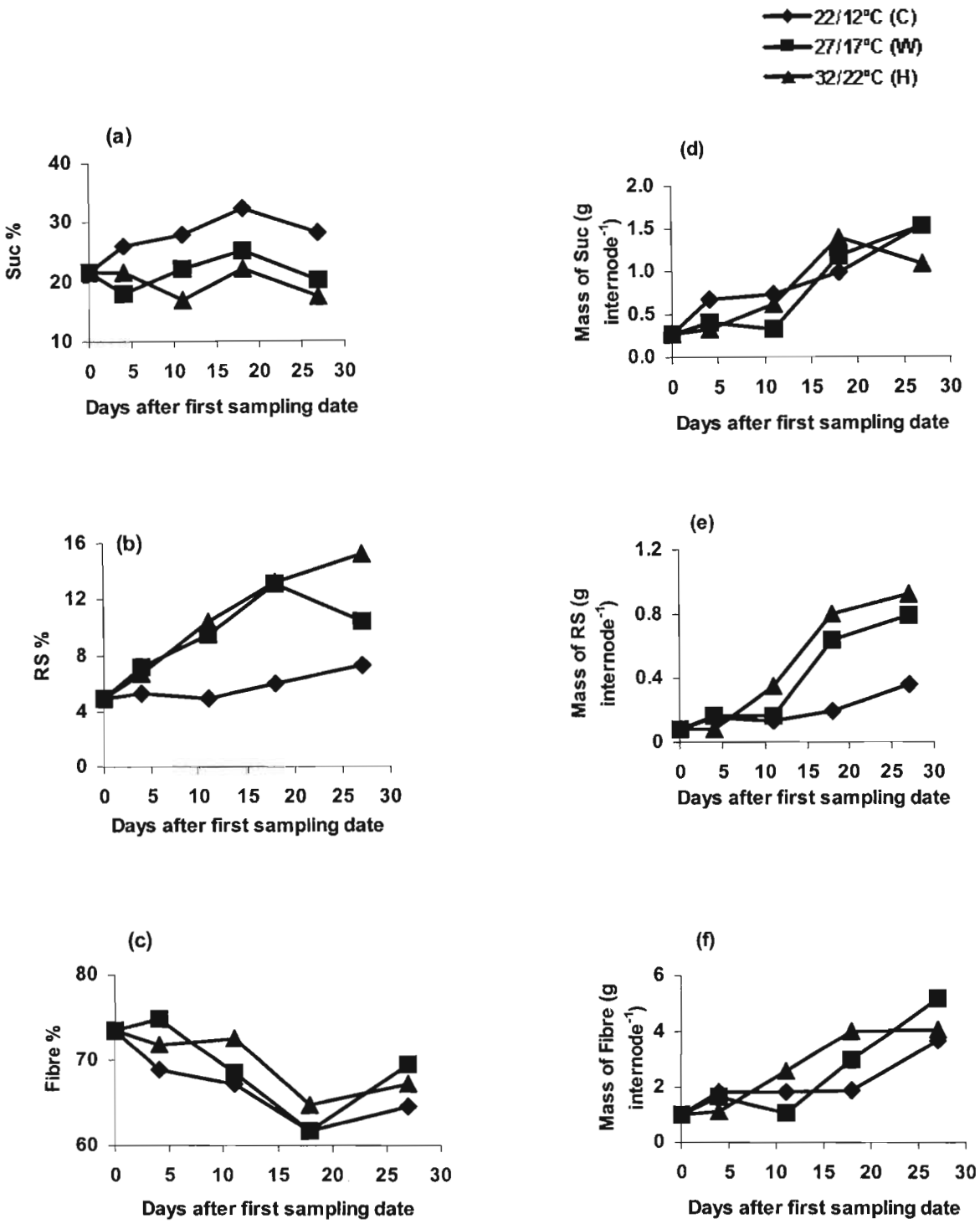
3.5.2.1 Mature tissue (Internode 10)

Statistical analysis showed that the temperature effect on the sucrose concentration (%) of the mature internodes of the primary haulms was significant (P=0.003) (Appendix 1 (a) (iii)) for the plants that were sampled over the autumn equinox. The mean sucrose concentration in the mature internodes of the autumn plants was highest (35.5%) at the cold temperature (C) and lowest (25.5%) at the hot temperature (H). The mean sucrose concentration at the intermediate temperature (W) was 29.2%. This corresponded to a significantly (P=0.002) (Appendix 1 (b) (iii)) higher level of RS concentration at H (13.3%) than at W (9.8%) and C (5.7%). The corresponding values of fibre % in the three temperatures were significantly different (P=0.015) (Appendix 1 (c) (iii)), higher at the higher temperatures (61.3% at H and 61.1% at W) than at the cold temperature (58.8%).

The differences between the concentrations of the stalk component of the mature internodes due to the temperature effect in plants that were sampled over the spring equinox were not as marked as those in autumn (Fig. 3.5.2.1.1b). Values of sucrose concentration of these internodes were 27.8, 20.9 and 19.9% at C, W and H, respectively, and they were statistically not different at 5% (P=0.055) (Appendix 2 (a) (iii)). Non-significant (P=0.065) concentrations of the RS of 5.9, 9.76 and 10.9% were observed at C, W and H, respectively (Appendix 2 (b) (iii)); and corresponding fibre % of 66.3, 69.4 and 69.2% at C, W and H, respectively, which were also non significantly different (P=0.36) (Appendix 2 (c) (iii)). The absence of significance in spring was probably due to the high level of variability between the primary shoot sizes of plants within a temperature treatment, which was observed at the start of experimentation. Variability in stalk components and stalk growth was variable in this experiment, as stools were only debudded of new tillers at the commencement of temperature treatments. This resulted in stools having variable haulm numbers in contrast to the situation in autumn where pruning was done to achieve a constant number of haulms per stool (Chapter 2). Also, haulms in the autumn equinox were more developed, having been grown over the summer as opposed to winter period in the pre-temperature-treatment phase. Hence,

counting from the top, internode 10 was less basal and larger and heavier in autumn than in spring. The immature internodes (4 and 6) were comparable physiologically between the two trials, but this was not strictly true for internode 10, even though they were both mature in both trials. The mean sucrose and RS concentration and fibre % of the mature internodes at W and H were similar in both trials. This implies that even the lower temperature of W relative to H was still high enough to alter the sucrose metabolism activity and probably alter the source-sink relationships among the stalk components to a level that was achieved at H. This means that both W andH temperatures raised the activity of SAI to a level above the threshold to a similar extent.

As expected, the mature internodes (internode 10), accumulated higher levels of sucrose relative to the reducing sugars. These internodes had stopped elongating (Moore, 1995), with the exception of internodes 10 of plants in spring of which their basal internode length showed increase with time (Fig. 3.5.1.2c). Ripening of sugarcane is accelerated by a decrease in internode extension (Lingle and Irvine, 1994). Extension in sugarcane occurs at about six internodes that are below the internode that is subtended by the top visible dewlap (TVD) leaf and on the ensheathed internodes above the TVD leaf (Moore, 1995). This means that there was no extension in the mature internodes (internode 10) that were investigated in this study, and hence no utilization of sugars for growth (Qudsieh *et al.*, 2001). Sucrose that was translocated and unloaded into these internodes was either accumulated as storage sugar in the vacuoles of the storage parenchyma cells or cleaved into the energy sugars (glucose and fructose) and remobilized to the growing parts of the plant (Glasziou and Gayler, 1972). This is dependent on the growth conditions.


22/12°C (C)
27/17°C (W)
32/22°C (H)
(a)
Suc %
Days after first sampling date
(b)
RS %
Days after first sampling date
(c)
Fibre %
Days after first sampling date
(d)
Mass of Suc (g internode-1)
Days after first sampling date
(e)
Mass of RS (g internode-1)
Days after first sampling date
(f)
Mass of Fibre (g internode-1)
Days after first sampling date

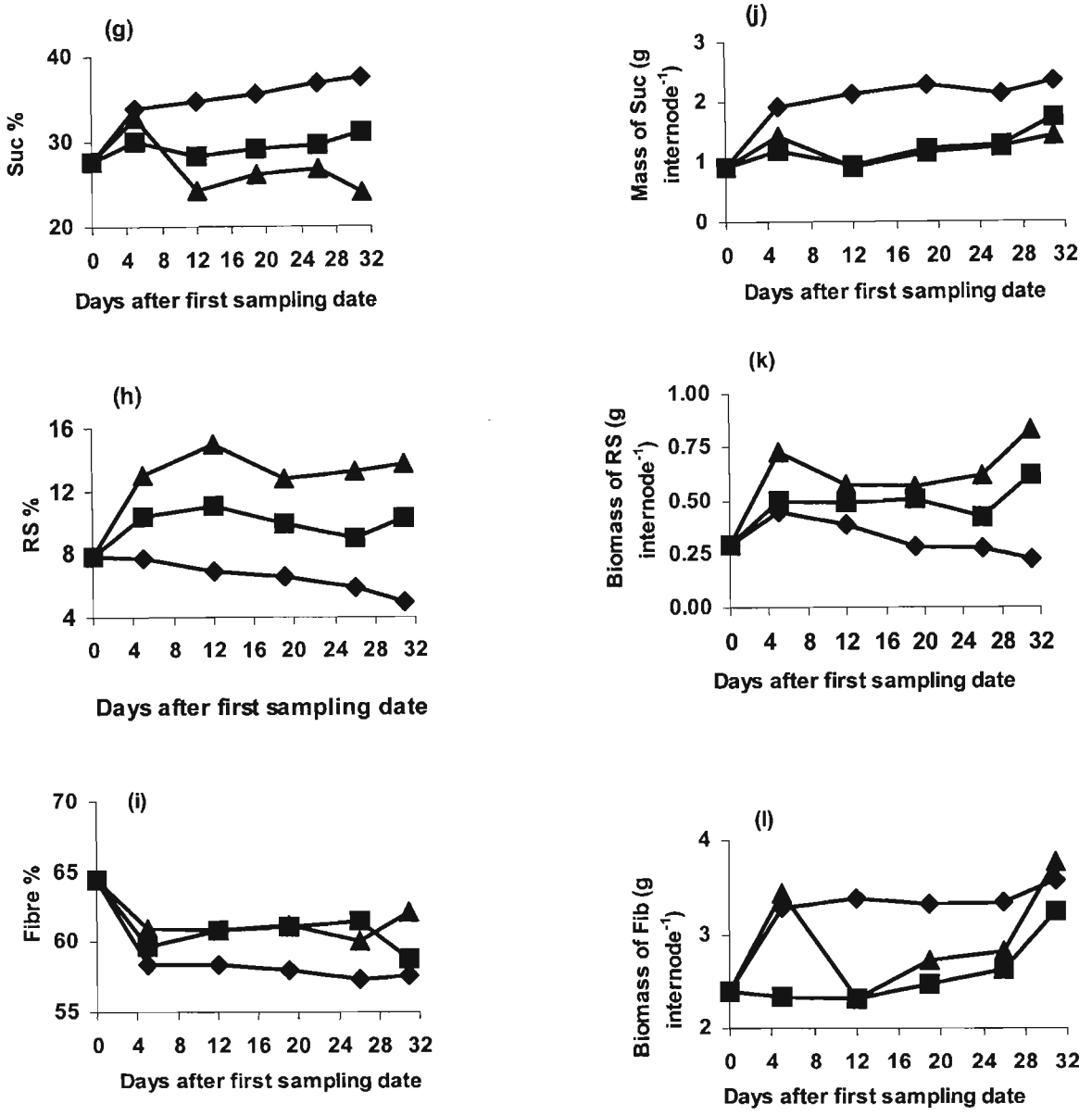


Figure 3.5.2.1.1: Sucrose (Suc), reducing sugars (RS) and Fibre (Fib) % and mass of internode 10 (count from shoot top) of the primary haulms of sugarcane grown at three temperatures over the spring (a-f) and autumn (g-l) equinoxes. Each data point represents the measurements of internode 10's of the primary haulms of four plants. The letters C, W and H in the legend denote cold (22/12°C), warm (27/17°C) and hot (32/22°C) temperatures, respectively. On the first day of temperature differentiation, the concentrations and masses of the internodes of plants in the three temperatures were adjusted to a common value.

In both trials the cold temperature treatment (C) appreciably reduced the accumulation of RS in the mature internodes, as compared to the higher temperatures (W and H). Since there was no more extension in these internodes; it implies therefore, that the energy sugars obtained from the cleavage of sucrose at the higher temperatures were not utilized for growth of the bottom internodes, but instead to meet with the energy demand by the top actively growing internodes. This also indicated that internodal sucrose can be remobilized either acropetally to the top internodes or basipetally to the root meristem. This action was noted by Hartt (1965b) and was described as most favoured by high temperature, moisture and the dark. The bottom mature internodes also have the advantage of accumulating sucrose earlier than the top immature internodes. The pH of the mature internodes, which is approximately 7 is not conducive for the activity of soluble acid invertase (SAI) that facilitates sucrose hydrolysis (Lingle and Smith, 1991). Such pH is optimum for the neutral invertase enzyme (NIE) activity which is associated with sucrose translocation into the stalk storage cells (Glasziou and Gayler, 1972). The concentrations of the RS that were observed in this study in all three temperatures were typical of levels of RS found in sugarcane at harvest (Robertson *et al.*, 1996a).

The W and H temperatures had little effect on sucrose concentration in the mature internodes in either trial (Fig. 3.5.2.1.1 a and g). However, these temperatures induced a noticeable increase in the concentration of RS in the mature internodes (3.5.2.1.1 b and h). On the plants that were sampled across the spring equinox the increase in RS concentration with time in the mature internodes due to the warm and hot temperature treatments was almost linear, whereas in the plants that were sampled across the autumn equinox, the effect was a sudden increase on temperature differential and then it remained relatively constant throughout the sampling period. This effect was more pronounced at H than W (Fig. 3.5.2.1.1h). The increase in RS concentration of the mature internodes due to the higher temperatures (W and H) did not seem to be in the expense of sucrose since the increase in RS concentration did not correspond to a concomitant decrease in sucrose concentration. This indicates that the higher temperatures up-regulated the SAI activity in the vacuolar space without affecting the SPS activity at the metabolic compartment (cytoplasm) of the storage parenchyma cells of the mature tissue. There is thus an

increase in SAI activity relative to SPS, thereby increasing the rate of vacuolar sucrose cleavage into the reducing sugars in order to supply energy to the rapidly growing zones at the shoot and root meristems and leaves. This explains the lower concentration of sucrose and high concentration of RS that was observed in the mature internodes of plants that were treated with the warm (W) and the hot (H) temperatures (Fig. 3.5.2.1.1 a and g) and *vice versa* at the cold (C) temperature in both trials (Fig. 3.5.2.1.1 b h).

The onset of the cold temperature treatment induced a sudden increase in sucrose concentration in the mature internodes. This observation was common for both trials (Fig. 3.5.2.1.1 a and g). As was the response of sucrose and RS concentration on the higher temperatures (W and H), the sudden increase in sucrose concentration due to the cold temperature did not correspond to a sudden decrease in RS concentration. This indicated that the additional sucrose in the mature internodes that resulted from the treatment of plants with the cold temperature was not in the expense of the RS. This effect was clearer in spring, whereby the concentration of the RS in the mature internodes still increased gradually over the sampling period, in spite of the treatment of plants changed to the cold temperature (Fig. 3.5.2.1.1b). The additional sucrose may have resulted from an up-regulated SPS activity in the cytoplasm of the storage parenchyma cells of the mature internodes, without affecting the SAI activity, so that the sucrose that was unloaded in the storage parenchyma cells of the mature internodes was not cleaved in the vacuolar storage space.

The sudden induction of sucrose accumulation in the mature internodes that was observed in both trials (Fig. 3.5.2.1.1 a and g) seem to be in disagreement with the reports of field trials in South Africa, which have indicated that the rise in sucrose concentration in the stalks of sugarcane is delayed by 4-6 weeks after the onset of the cold and dry winter season (Glover, 1971; Singels *et al.*, 2005a). The sudden ripening effect of cold temperature that was observed in this study was also in disagreement with the results of Lingle (2004) who showed that a brief cold temperature treatment (24 hours at 10°C) did not increase sucrose % in the sugarcane stalk. Although the enzyme activity was not measured in this current study, it is believed that the sudden rise in sucrose accumulation

in the mature internodes of plants grown in the cold temperature was due to the effect of cold temperature on the interaction of sucrose metabolism enzymes, especially SAI and SPS; otherwise, the sudden shift in assimilate allocation more to the storage sucrose due to the cold temperature accounted for this observation.

The sugar interactions in sugarcane tissue are complex and still unknown. It is well known that the relative amount of the various sugars in the stalk of sugarcane is controlled by the balance between the activity of SAI and SPS (Zhu *et al.*, 1997). However, the role of temperature on the enzymatic activity is not clear. Lingle (2004) observed that transient chilling temperature (10°C) slightly increased the concentration of sucrose in the internodes of sugarcane. However, the chilling effect did not alter the activity of the sucrose metabolism enzymes SAI, NI, SS, SPS, and CWAI *in vitro*. The slight increase in sucrose concentration was accounted for by the suppression of sucrose cleavage enzymes *in planta*. The results of Ebrahim *et al.* (1998a) showed that the primary sucrose metabolism enzyme activities (SAI, NI, SS and SPS) were higher at 27°C (regarded as optimum temperature) than at 15°C. Lingle (2004) argued that the discrepancy between the results of the two studies was due to the fact that temperature was constant over 10 months on the latter study, whereas in the former it was transient, implying that the effect of temperature on the sucrose metabolic enzyme activities was cumulative over an extended period in the study by Ebrahim *et al.* (1998a). The enzyme activity in this study was not measured and their discussion in this thesis is thus reserved.

In part, sucrose accumulation in the mature internodes of sugarcane can be explained by the source-sink relation in response to temperature. In this study the partitioning of assimilates to the reducing sugars and fibre increased with increasing temperature, and partitioning to sucrose increased with decreasing temperature (Chapter 3.6). The demand of energy for growth was higher at the higher temperatures, causing the plants at these temperatures to partition their assimilates to the energy sugars (RS) to supply for the high temperature-stimulated rapidly growing organs. The meristematic sink demand of the plants that were treated with the cold temperature was not as high since the cold temperature was suppressing to growth. This induced a shift in the direction of

assimilates towards storage sucrose in the parenchyma cells. In principle, this indicates that the response of the different stalk components due to temperature is most likely to be due to assimilate distribution among the plant components than it can be explained by enzymatic activity.

Fibre is insoluble and cannot be converted to other substances in the stalk of sugarcane. The lower internodal fibre % in the cold temperature was merely due to dilution by sucrose. It is clear that even though the fibre % was lower in the cold temperature in the mature internodes of the autumn crop (Fig. 3.5.2.1.1 l); its weight was consistently higher throughout the sampling period than in the two higher temperatures. It can also be deduced from that in both trials, the fibre % of the mature internodes seemed lower in the cold temperature when the dilution effect of sucrose was not eliminated; whereas on sucrose-free basis fibre % is higher at the cold temperature and decrease with increasing temperature (Fig. 3.5.2.1.2).

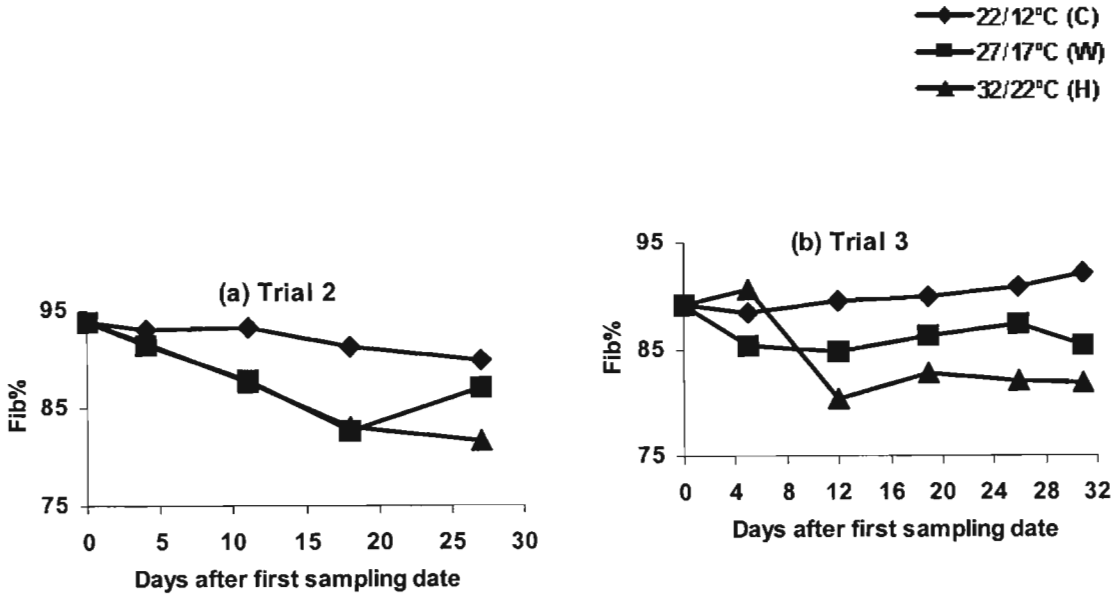


Figure 3.5.2.1.2: Fibre % of internode 10 (count from shoot top) of the primary haulms of sugarcane grown at three temperatures over the spring (a) and autumn (b) equinoxes when the dilution effect of sucrose was eliminated. The letters C, W and H in the legend denote cold (22/12°C), warm (27/17°C) and hot (32/22°C) temperatures, respectively.

The comparison of the results of the two seasons indicated that the RS in the mature internodes of cold treated plants of spring gradually increased from 4.9% on the first day of temperature treatment to 7.3% after 27 days (Fig. 3.5.2.1.1b), whereas in autumn it gradually decreased from 7.8% to 5.0% after 31 days. This seasonal difference might have been interpreted as that there are possible effects of the increasing daylength and solar radiation across the spring equinox and the effect of the decreasing daylength and solar radiation across the autumn equinox on the flux of the different sugars in the sugarcane stalk. There were also marked differences in the concentrations of the mature stalk components between the two seasons. The autumn crop had higher levels of sucrose concentration in their mature internodes than the spring crop in all the temperatures (Table 3.5.2.1.1). This is most likely due to the physiological age differences between the stock plants of the two trials, as well as the differences in the management of plants before temperature differential. On average, the primary haulms of the spring stock plants had 16.5 internodes, compared with 14 internodes for the stock plants of autumn at the start of experimentation. The bottom internodes (internode 10's) of the two studies

were not comparable as the stock plants were better grown in autumn than the stock plants of spring. The graphs of internode length over the sampling period (Fig. 3.5.1.2) indicated that internode 10 of the spring plants was continuously elongating over the sampling period, whereas in autumn the length of internode 10 was almost stable. The continuous extension of the mature internodes of the spring crop may be accountable for the low sucrose concentration that was observed in spring than in autumn.

Table 3.5.2.1.1: Mean sucrose concentrations in the mature internodes (internode 10) in the primary haulms of plants treated with three temperature levels (22/12°C, 27/17°C and 32/22°C) for one month during spring and autumn.

	22/12°C	27/17°C	32/22°C
Spring	27.8%	20.9%	19.9%
Autumn	35.5%	29.1%	25.5%

3.5.2.2 Immature tissue (Internode 4 and 6)

As would be expected, the immature internodes accumulated less sucrose than the mature internodes. Instead the immature internodes accumulated higher levels of the reducing sugars, particularly at the higher temperatures (W and H). Relatively high concentrations of reducing sugars in the sugarcane internodes is indicative of rapid internode extension, or high SAI activity (which may occur even in non-elongating internodes) under conditions that stimulate rapid stem extension. The immature internodes that were analyzed for sugars in this study (internodes 4 and 6) were within the rapidly elongating section of the sugarcane haulm, which was described by Moore (1995) for sugarcane stems. Lingle (2004) showed that there are significant differences between sucrose metabolism enzymatic activities of internodes of different maturity. The CWAI activity, which was correlated with the substrate-facilitated entrance of glucose and fructose into the apoplastic space of the storage cells (Glasziou and Gayler, 1972), was higher in the mature internodes than in the immature internodes. Robertson *et al.* (1996a) showed that the concentration of reducing sugars (which results from high activity of SAI) was higher (50-80%) in a younger crop of sugarcane than in a 12 months old crop (30-50%).

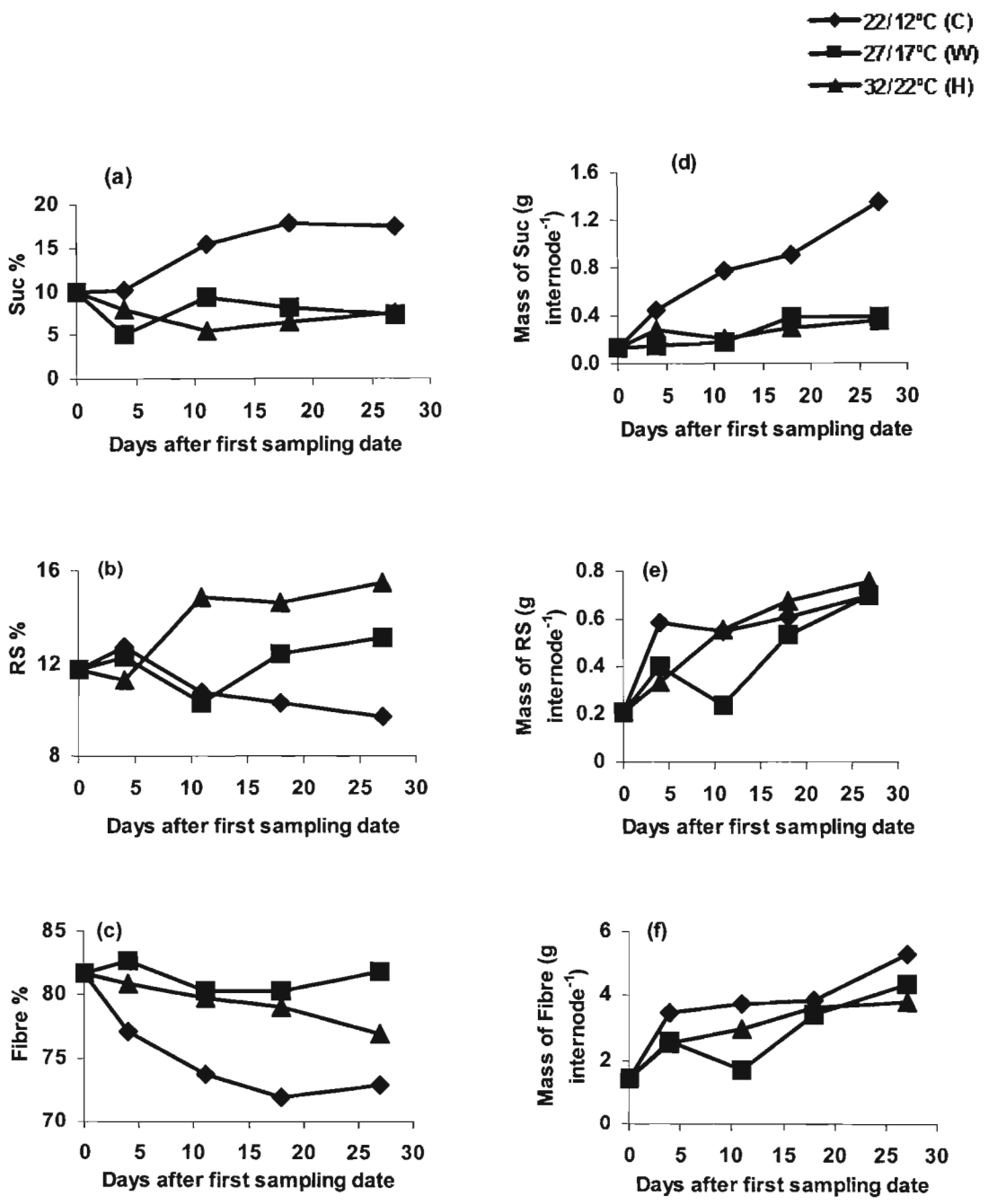
The effect of temperature on the concentration of the various sugars in the immature internodes was not as pronounced as in the mature internodes, and this was particular in autumn. Although not measured, the activity of soluble acid invertase (SAI) were high in the immature tissue of sugarcane, and the vacuolar sucrose was being rapidly broken down into the energy sugars to supply for the rapid growth demand of these internodes (Glasziou and Gayler, 1972). Hence, the effect of cool temperature on sucrose accumulation was nullified by the rapid break down of sucrose molecules by SAI. However, there were cases of significant effect of temperature on the various sugars in the immature internodes. This response was regarded important in sugarcane management in determining the optimum topping height of sugarcane during harvest, so that sucrose loss from topping too low below the shoot apex, and transporting non sucrose matter to the mill from topping too high, would be minimized. The latter is a more important consideration when transporting cane over long distances to the mill (Inman-Bamber and Wood, 1987).

In spring sucrose concentration in internode 6 was significantly affected by temperature (P=0.046) (Appendix 2 (a) (ii)). The mean sucrose % over the sampling period in the three temperatures was 14.9%, 7.3% and 7.6% at C, W and H, respectively. In spite of the high activity of SAI in this portion of the stalk, it can be deduced that the lower temperature (C) effectively reduced the breaking down of sucrose by this enzyme, relative to the two higher temperatures (W and H). The concentrations of sucrose in internode 6 of plants grown at W and H temperatures were closely similar over the sampling period (Fig. 3.5.2.2.1a). As noted earlier for the mature internodes, this indicated that the warm temperature affected the sucrose metabolism enzyme activities in the same way as the hot temperature. The mean concentration of RS in internode 6 of the spring crop was significantly higher (P=0.046) at H (14.1%) and lowest at C (10.6%) (Appendix 2 (b) (ii)). The RS concentration at the intermediate temperature (W) was 12.3%. The corresponding mean values of fibre % in the three temperatures for this trial were 74.5%, 81.3% and 79.3% at C, W and H, respectively, and were significantly different (P=0.024) (Appendix 2 (c) (ii)). The cold-temperature-induced significant increase in sucrose concentration in internode 6 (Fig. 3.5.2.2.1a) was accompanied by

only a slight decrease in RS (Fig. 3.5.2.2.1b) concentration and a noticeable decrease in fibre % (Fig. 3.5.2.2.1c).

The effect of temperature on the relative proportions of the various sugars in internode 6 of autumn plants was significant (P=0.016) for fibre % only (Appendix 1 (c) (ii)). Fibre % in the three temperatures were 71.7%, 74.7% and 74.0% at C, W and H, respectively. Even then, there was no clear trend of the fibre % over the sampling period in all the plants treated with the three temperatures (Fig. 3.5.2.2.1i). Temperature effect on the concentrations of sucrose and RS in internode 6 of the plants that were harvested over the autumn equinox was not significant (P=0.100 and P=0.811, respectively) (Appendices 1 (a) (ii) and 1 (b) (ii)), with the mean sucrose concentration values of 13.6%, 10.8% and 11.8% and RS % of 14.8%, 14.5% and 14.3% at C, W and H, respectively. The masses of the three stalk components were consistently higher in the cold temperature than in the two higher temperatures in either trial (Fig. 3.5.2.1. d-f and j-l). This was due to the fact that the chronological age of the corresponding internodes of plants grown in the cold temperature was older than the internodes in the higher temperatures, which allowed carbohydrate deposition over an extended period in the internodes of plants grown in the cold temperature.

A dilution effect of fibre by sucrose was observed in internode 6. Fibre % was lowest in the cold temperature (C) when the dilution effect of sucrose was not eliminated, in spite of the continuous increase in fibre mass of this internode over the sampling period, which was higher on plants at 22/12°C than at W and H (Fig. 3.5.2.2.1 f and l). The dilution effect of sucrose on fibre in the immature internodes was not as strong as in the mature internodes, and for that reason, it was not discussed further. The rate of RS mass accumulation in internode 6 of the spring plants was not different between the three temperatures (Fig. 3.5.2.2.1e), indicating that the differences that were observed in the relative proportions of RS were due to the dilution effect of sucrose at the cold temperature.


22/12°C (C)
27/17°C (W)
32/22°C (H)
(a)
Suc %
Days after first sampling date
(b)
RS %
Days after first sampling date
(c)
Fibre %
Days after first sampling date
(d)
Mass of Suc (g internode-1)
Days after first sampling date
(e)
Mass of RS (g internode-1)
Days after first sampling date
(f)
Mass of Fibre (g internode-1)
Days after first sampling date

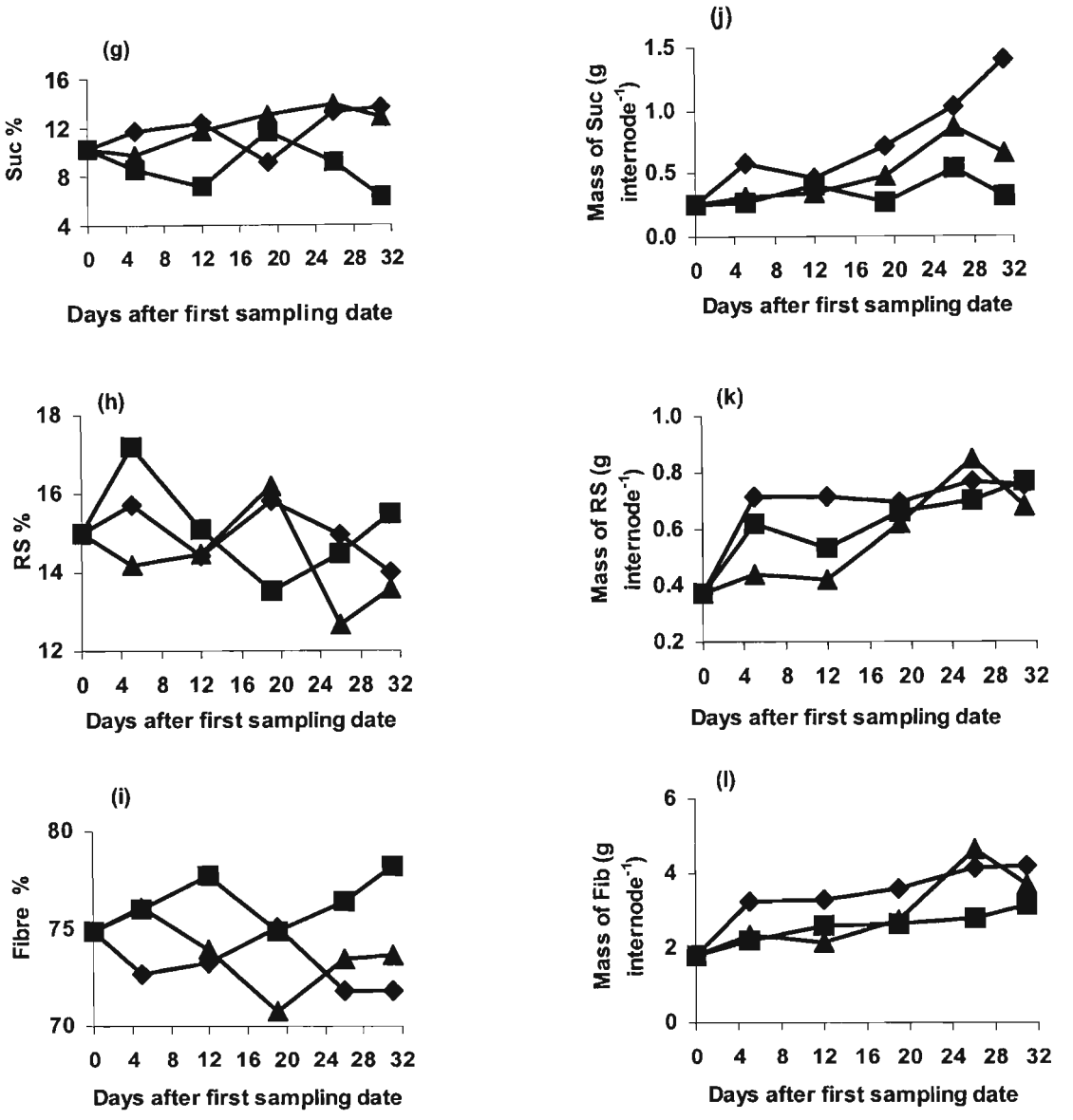
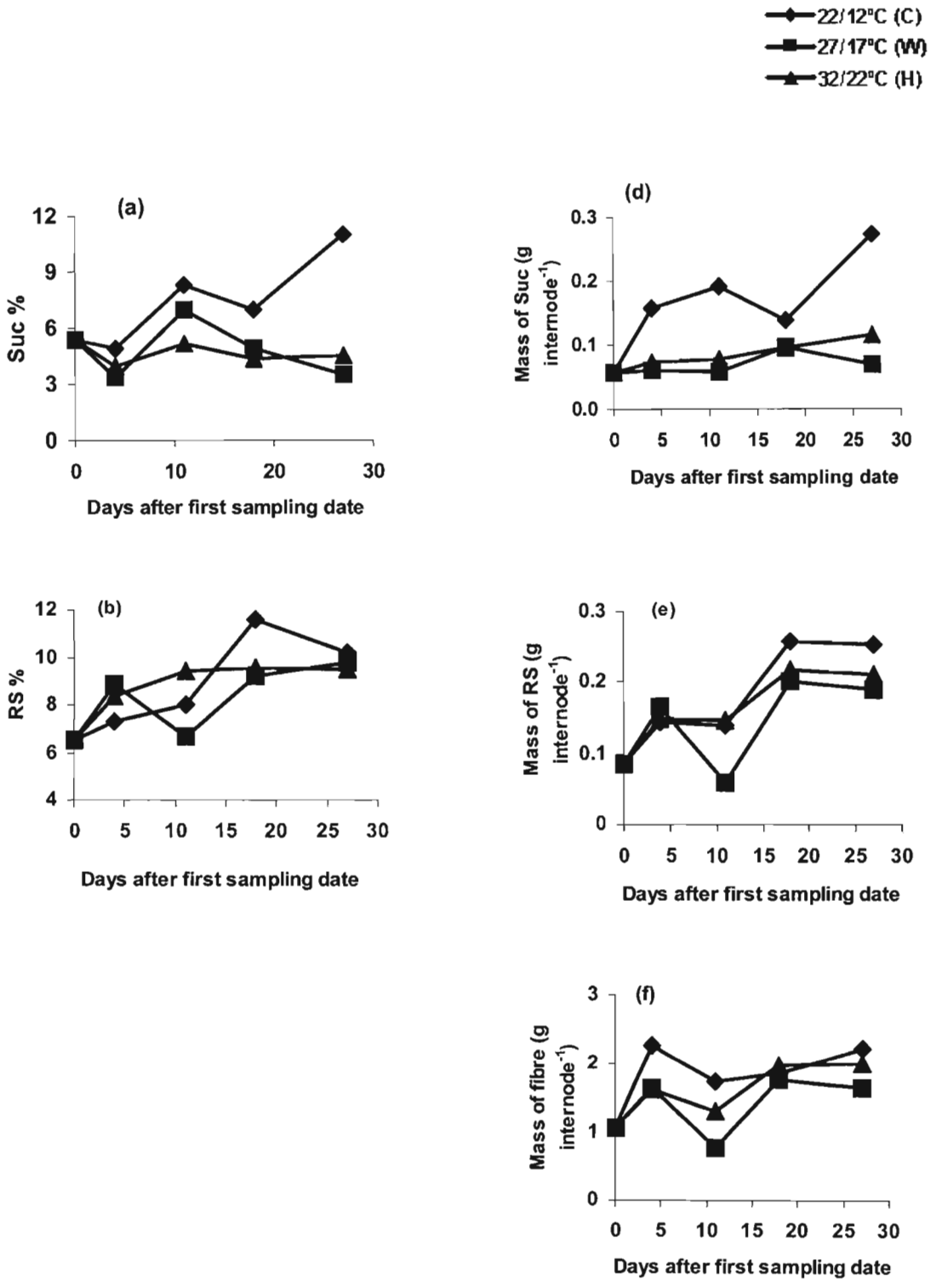
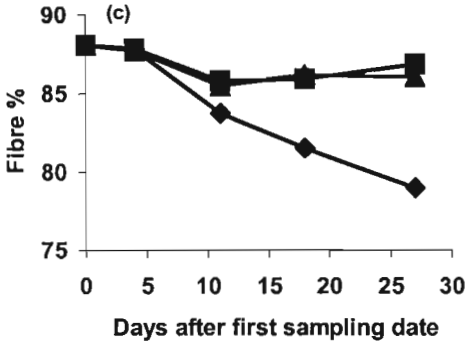


Figure 3.5.2.2.1: Sucrose (Suc), reducing sugars (RS) and Fibre (Fib) % and mass of internode 6 (count from shoot top) of the primary haulms of sugarcane grown at three temperatures over the spring (a-f) and autumn (g-l) equinoxes. Each data point represents the mean of four observations. The letters C, W and H in the legend denote cold (22/12°C), warm (27/17°C) and hot (32/22°C) temperatures, respectively. On the first day of temperature differentiation, the concentrations and masses of the internodes of plants in the three temperatures were adjusted to a common value.

In general, there was no distinction in the concentration of the stalk components between the three temperatures in the most immature internodes, although the trends in their masses indicated that the mass of all the three stalk components were consistently higher at C than at W and H (Fig. 3.5.2.2.2). The same explanation as for the mature internodes applies for this observation. Sucrose concentration was significantly higher (P=0.014) in the plants grown in the cold temperature of the autumn crop (Appendix 1 (a) (i)). The mean sucrose concentrations in the three temperatures were 8.4%, 6.8% and 6.1%, corresponding to non-significant (P=0.159) fibre % of 82.9%, 84.3% and 84.9% (Appendix 1 (c) (i)); and non-significantly different (P=0.819) RS % of 8.7%, 8.9% and 9.0% (Appendix 1 (b) (i)) at C, W and H, respectively. In spring, the effect of temperature on internode 4 sucrose concentration was not significant (P=0.056) with mean values of 7.4%, 4.4% and 4.71% at C, W and H, respectively. However biological differences were observed and showed that sucrose % in the internodes of plants that were grown at 22/12°C was progressively increasing over the sampling period and it was consistently higher than sucrose % of the plants grown at W and H (Fig. 3.5.2.2.2a). The corresponding concentrations of RS were 8.8%, 8.5% and 8.8% at C, W and H, respectively. These were not significantly different (P=0.409) (Appendix 2 (b) (i)). The Fibre % in the three temperatures were significantly different (P=0.035) (Appendix 2 (c) (i)) with the means of 83.8%, 87.0% and 86.5% at C, W and H, respectively.


22/12°C (C)
27/17°C (W)
32/22°C (H)
(a)
Suc %
Days after first sampling date
(b)
RS %
Days after first sampling date
(d)
Mass of Suc (g internode-1)
Days after first sampling date
(e)
Mass of RS (g internode-1)
Days after first sampling date
(f)
Mass of fibre (g internode-1)
Days after first sampling date


(c)
90
85
80
75
Fibre %
0
5
10
15
20
25
30
Days after first sampling date

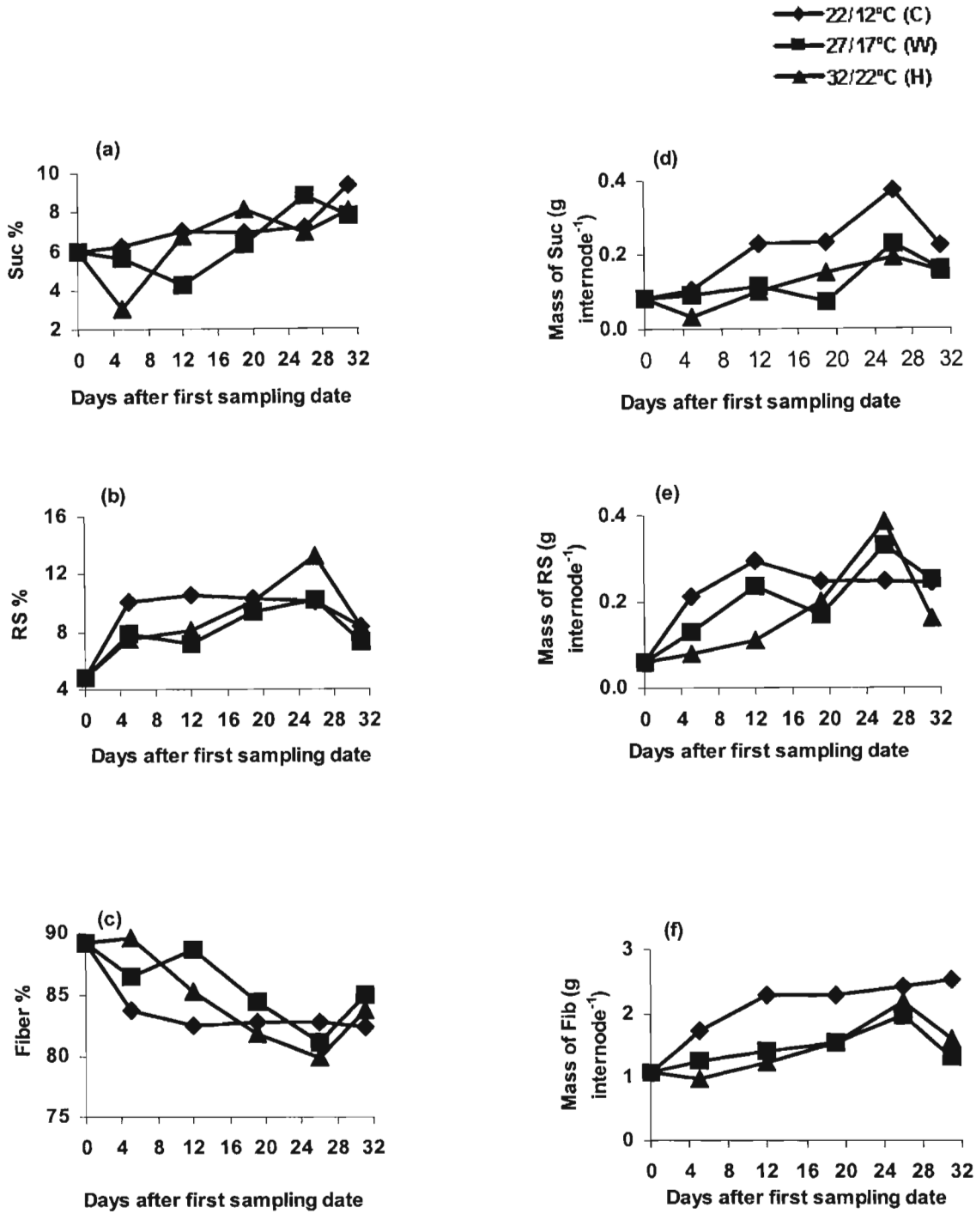


Figure 3.5.2.2.2: Sucrose (Suc), reducing (RS) and Fibre (Fib) % and mass of internode 4 (count from shoot tp)of the primary haulms of sugarcane grown at three temperatures over the spring (a-f) and autumn (g-l) equinox. Each data point represents the mean of four observations. The letters C, W and H in the legend denote cold (22/12°C), warm (27/17°C) and hot (32/22°C) temperatures, respectively.

3.5.3 Review of the sugar changes and interrelations

The significant sucrose %, RS % and fibre % differences in the three selected internodes that were reported for this study was exclusively due to temperature treatment and was obtained from well watered plants with no moisture stress. Obviously, there may be confounded environmental factors that may have influenced the results of the experiments. Such factors include the increasing and decreasing daylength during the spring and autumn, respectively, and the increasing and decreasing solar radiation during spring and autumn, respectively. As it is widely accepted that sucrose % in sugarcane stalk is increased by conditions that restrict internode extension more than photosynthesis (Glover, 1971; Hatch and Glasziou, 1964; Johnson, 1966; Inman-Bamber *et al.*, 2002; Inman-Bamber and De Jager, 1988; Legendre, 1975; Singels *et al.*, 2000; Singels *et al.*, 2005a), it was expected that sucrose % would increase in the stalks of plants grown in the cold temperature treatment. Under such conditions structural growth is reduced and the plant accumulates more sucrose in the storage cells of the sugarcane stalks, a phenomenon known as "ripening" (Lingle and Irvine, 1994). Cold temperature, reduced soil moisture and low nitrogen fertilization are well known causal for increased sucrose % in sugarcane (Lingle, 1997; Lingle and Irvine, 1994; Singels *et al.*, 2000; Singels *et al.*, 2005a). Under such conditions, the activity of SAI is down-regulated and this has an effect of increasing the difference in the activity of SPS and SAI in favour of SPS, a key factor of internodal sucrose accumulation (Zhu *et al.*, 1997). The SAI activity is high on tissue that is rapidly elongating (i.e., immature internodes, root and shoot meristematic tissues), and when SAI is present at a level above a critical threshold level, then sucrose accumulation is hindered (Zhu *et al.*, 1997). When demand for growth is high under high temperature, moisture and nutrient conditions, storage sucrose from the vacuoles of the storage parenchyma cells of mature internodes may be cleaved by SAI and result in remobilization of sucrose from mature internodes to the growing sites (Glasziou and Gayler, 1972).

However, it still remains unclear whether sucrose % in the stalk of sugarcane responds more to rainfall (soil water) than temperature. Singels *et al.* (2003) attributed the good season of high sucrose yield in 2002-2003 to reduced rainfall which was evenly

distributed over the harvesting season. In the report of Singels *et al.* (2003) it was concluded that the major factor for sucrose concentration in sugarcane was rainfall, rather than temperature. This may imply that the rise in sucrose % that is observed during the winter period is merely due to reduced soil moisture and not by temperature drop. Glover (1971) argued that the 4-6 weeks delay of sucrose % peak after the onset of the winter season was due to the buffering effect of soil moisture, which remains in the soil for a duration of time after rainfall has ceased.

Lingle (2004) showed that a 24 hour cold treatment of sugarcane at 10°C did not significantly alter the sucrose metabolism in the internodes 2, 4, 6 and 8 (counting from the top of stalk), and hence did not affect the sucrose:total sugar ratio in the internodes. Lingle (2004) argued that the significant difference in enzyme activity between plants grown at 27°C and 15°C, reported by Ebrahim *et al.* (1998a), was only because temperature treatment in the Ebrahim *et al.* study was continuous over time, otherwise transient temperature treatment was not effective on sucrose metabolism. Her idea could be supported by the results of Glover (1971) who showed that the effect of cold temperature treatment was linked to sucrose % (fresh mass basis) measured three months later after a cold spell.

The high sucrose concentration that was observed in the mature internodes at the cold temperature could partly be explained in terms of the chronological age (time in normal days) of the internodes. Since the rate of internode appearance was lower in the cold temperature, it is most likely that the internodes of plants in the cold temperature had the advantage of an extended period to accumulate sucrose in the same internode before the development of the next internode. This is to say, that the numbering of internodes using their physiological age (internode number from shoot top) ignores the fact that the positioning of the internodes on the stalk could be the same for the different temperatures, whereas they have different age in normal time.

One approach of internode numbering would be to analyse sugars on fixed internodes, whereby the internodes that are to be sampled for sugar analysis are identified and

marked at the beginning of the experiment. This method compares internodes of similar chronological age, which may have different physiological ages. The advantage of this method is that it avoids errors when assuming internodes of similar physiological age using the position of the internodes from the top of the shoot. The disadvantage of using fixed internodes for sugar analysis is that internodes of plants from different temperatures may have different physiological age (GDD) at any given time. Another disadvantage of this method is that it can limit comparison of late developing internodes that otherwise may have not been produced during the time when the internodes were identified.

The other internode numbering, which was used in this study was to number the internodes of similar physiological age in the different temperatures from the bottom of the shoot. In this method the positions of the internodes 4, 6 and 10 when counting from the top of the shoot, were determined when counted from the bottom of the shoot. This method clearly showed the profile of the sugar content on subsequent internodes (Figs. 3.5.3.1, 3.5.3.2, 3.5.3.3, 3.5.3.4 and 3.5.3.5). The variability in shoot sizes at the start of the experiment was exposed by this method. This was particularly evident in spring. The bottom internode that was sampled on the first day was position 8 from the bottom of the shoot, yet the 'subsequent' internode was position 7, indicating that at the start of the experiment, the primary shoots of plants that were sampled on day 1 were physiologically older than those of plants which were sampled on day 2 (Fig. 3.5.3.1a). In autumn the accumulation of sucrose increased in the subsequent internodes at the bottom of the shoot in the plants that were grown at C (Fig. 3.5.3.2a). There was no change in sucrose concentration in the subsequent internodes at the W temperature (Fig. 3.5.3.2b), and at H sucrose concentration slightly declined on subsequent internodes (Fig. 3.5.3.2.c). Changes in the sucrose concentration in the immature internodes (internodes 4 and 6) were not pronounced.

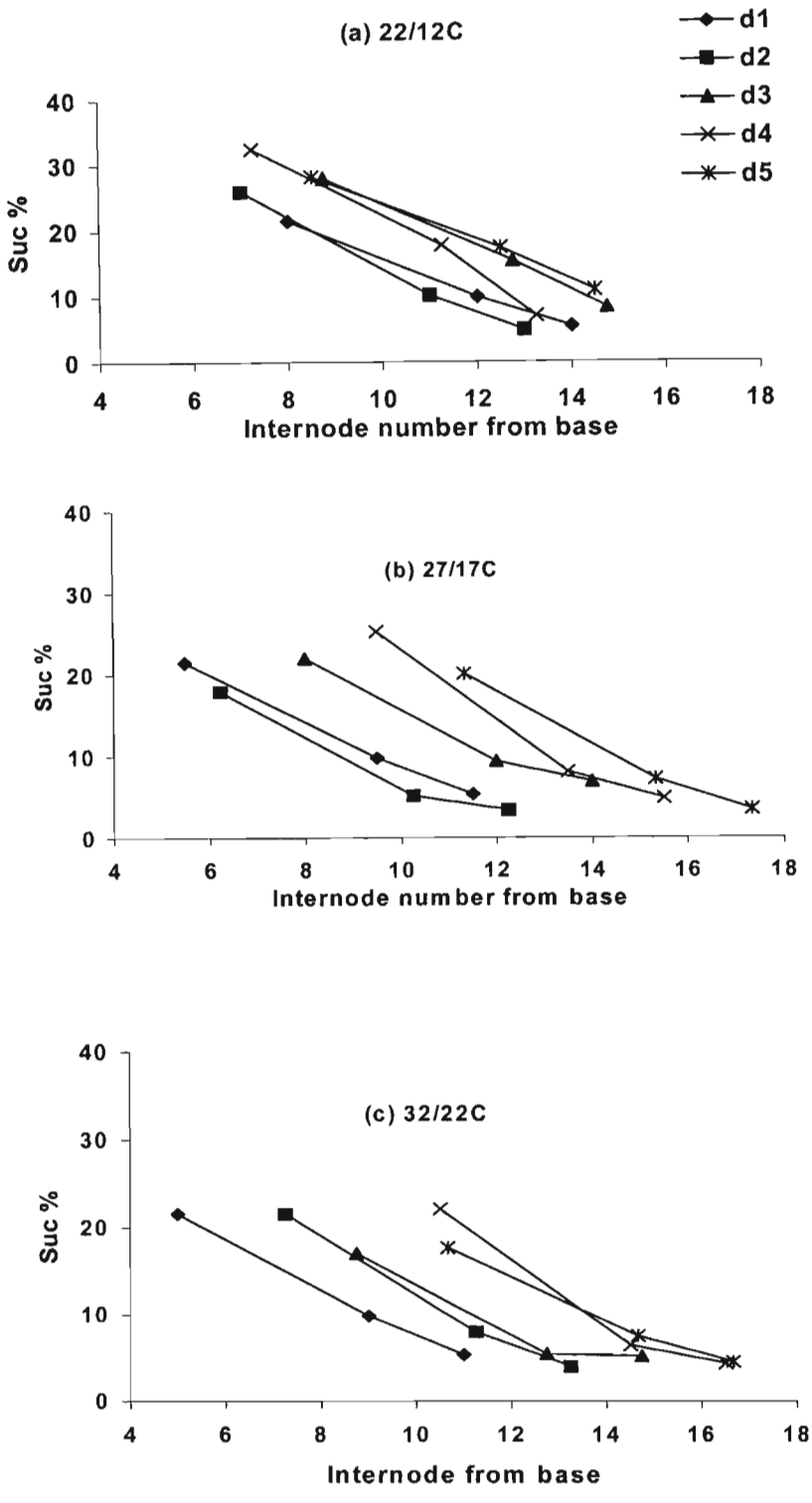


Figure 3.4.3.1: Sucrose concentration in selected internodes of the primary shoots of sugarcane plants grown at 2212°C (a), 27/17°C (b) and 32/22°C (c) over the spring equinox. d1, d2, d3, d4 and d5 represent 0, 4, 11, 18 and 27 days, respectively, after temperature differentiation.

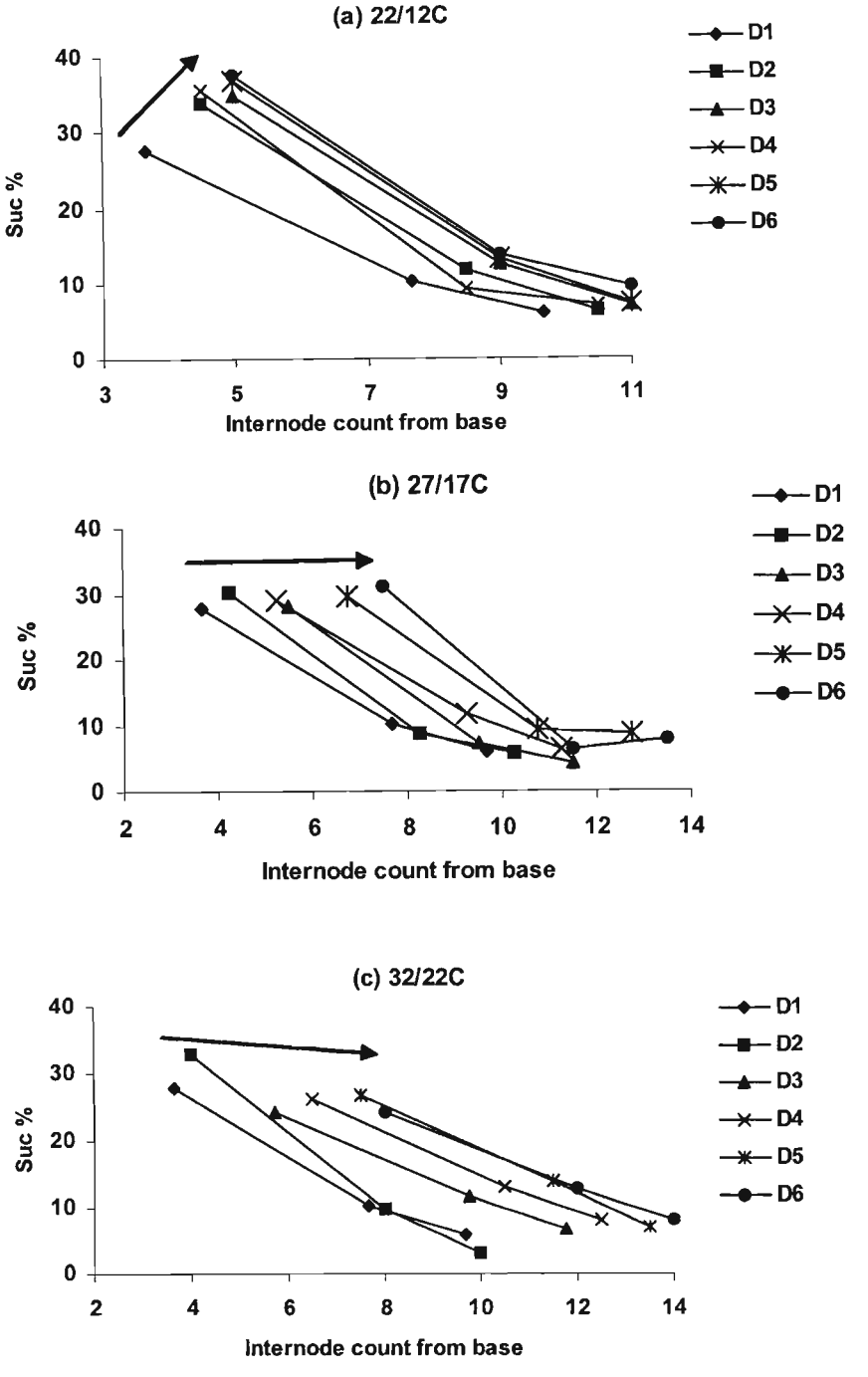


Figure 3.5.3.2: Sucrose concentration in selected internodes of the primary shoots of sugarcane plants grown at 2212°C (a), 27/17°C (b) and 32/22°C (c) over the autumn equinox. The arrows in the graphs indicate the general change in sucrose % in the selected internodes when affected by temperature. D1, D2, D3, D4, D5 and D6 represent 0, 5, 12, 19, 26 and 31 days, respectively, after temperature differentiation.

The concentration of RS did not decrease down the primary shoots in a similar way as sucrose decreased up the shoot stalk. The concentration of the RS was lowest in the mature internodes (internode 10), and it increased up the shoot at internode 6, and then decreased at the most immature tissue of internode 4. This was common in all the temperatures and for both trials (Figs. 3.5.3.3 and 3.5.3.4). Since the lower concentrations in the immature internodes (internode 4) could not be accounted for by the increase in sucrose concentration, it indicates that there may be other soluble substances that were present in significant amounts in these particular internodes, which were not accounted for. Tejera *et al.* (In Press) showed that in addition to the soluble sugars there are other soluble substances (mainly N compounds) that are present in the sugarcane sap, which support plant growth. Asis *et al.* (2003) noted that the relative proportions of the various compounds vary with different cultivars, growth stage and internode position. The higher RS % in internode 6 than internode 4 may indicate that the extension rate of internode 6 was higher than that of internode 4. Probably cell division was predominant in internode 4, whereas cell elongation was predominant in internode 6 (Berghage, 1998).

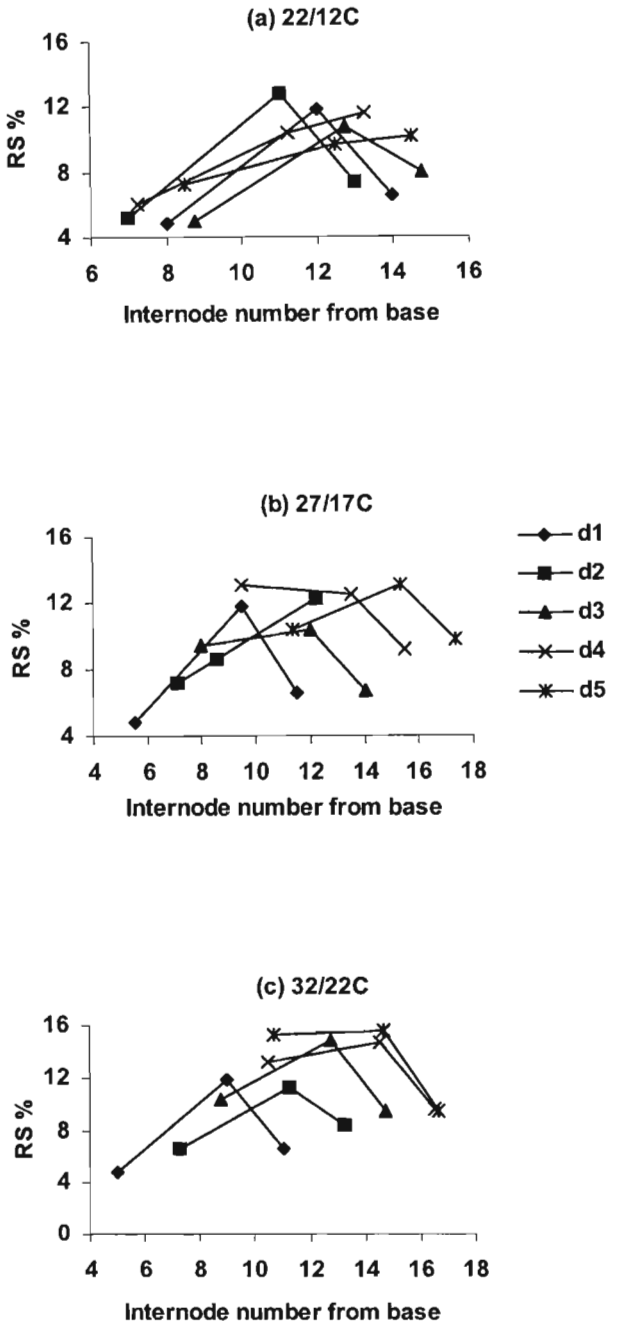


Figure 3.5.3.3: Concentration of reducing sugars in selected internodes when count is from the bottom of the primary shoot stalks of sugarcane plants grown in three temperatures over the spring equinox. The arrows show the change in RS% between the first and last day of sampling. d1, d2, d3, d4 and d5 represent 0, 4, 11, 18, and 27 days, respectively, after temperature differentiation.

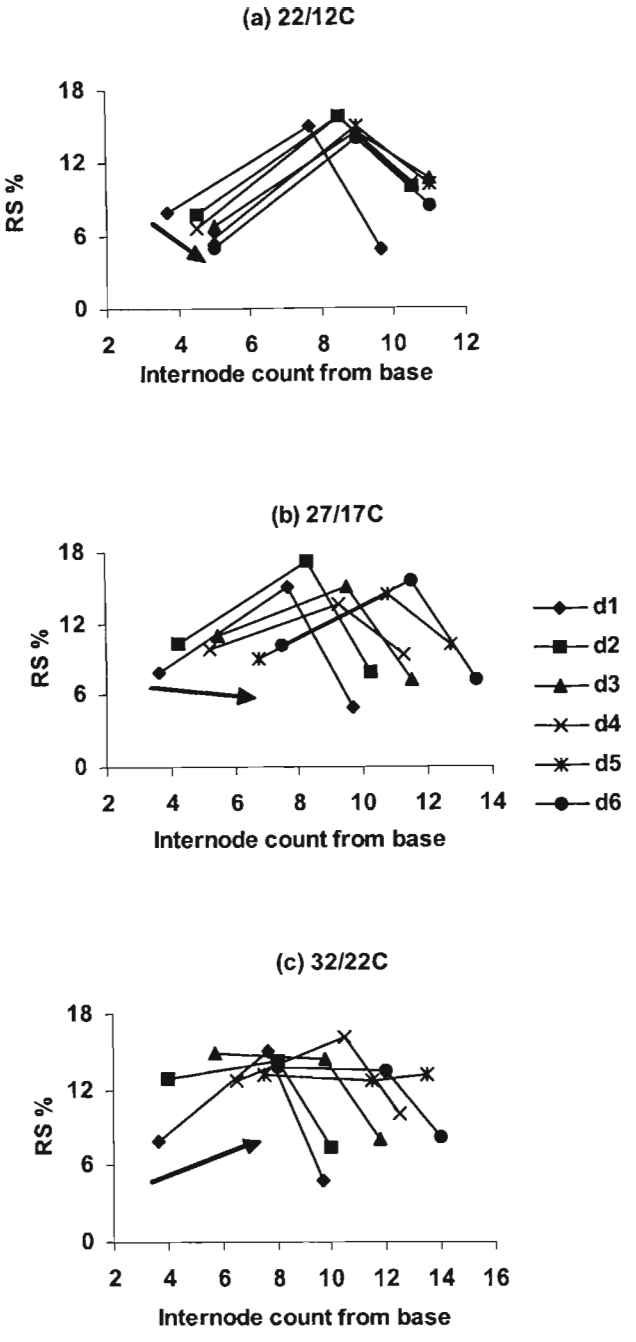


Figure 3.5.3.4: Concentration of reducing sugars in internodes 4, 6 and 10 when count is from the bottom (a, b, c) of primary shoot stalks of sugarcane plants grown in three temperatures over the autumn equinox. The arrows show the change in RS% between the first and last day of sampling. d1, d2, d3, d4, d5 and d6 represent 0, 5, 12, 19, 26 and 31 days, respectively, after temperature differentiation.

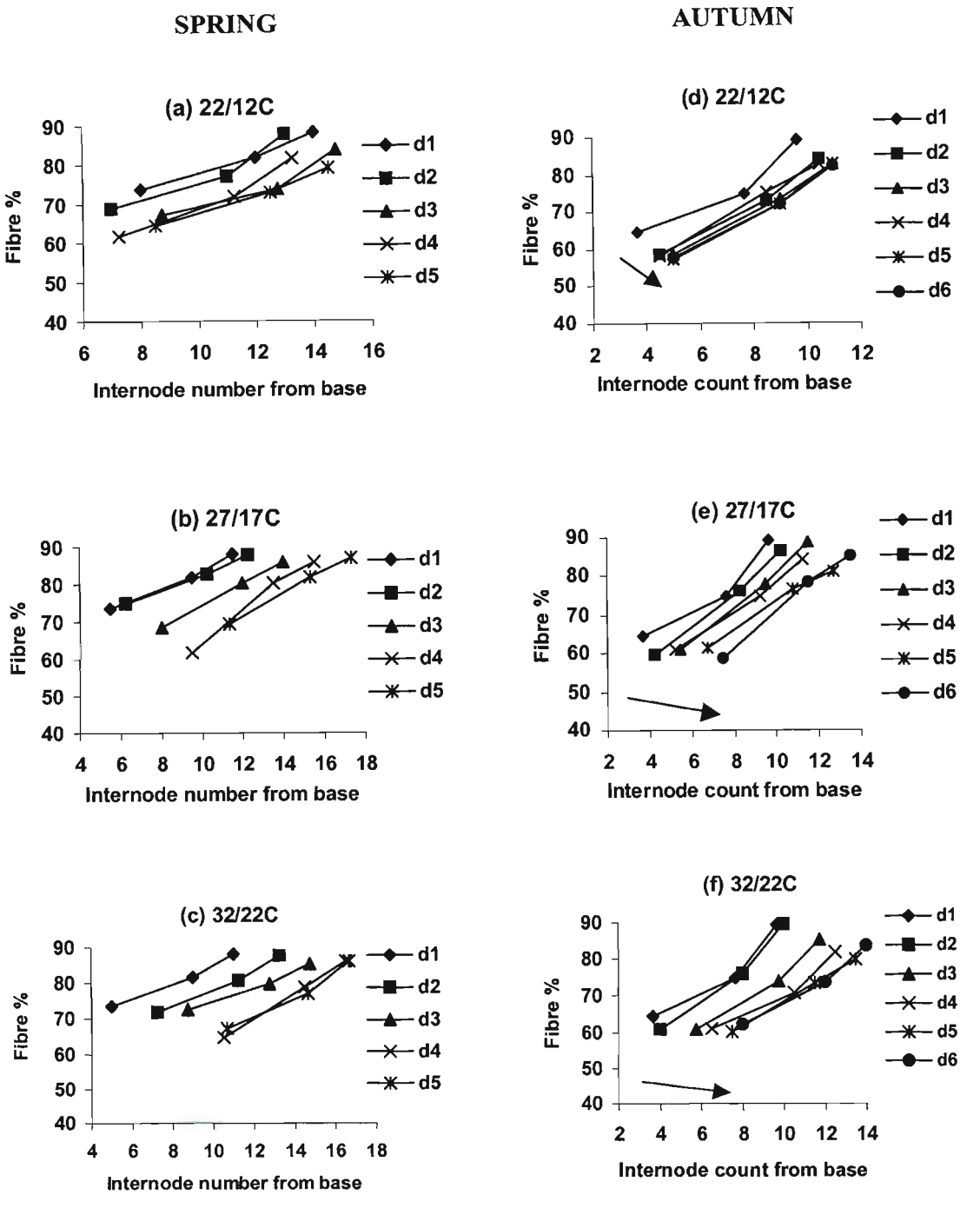


Figure 3.5.3.5: Fibre % in selected internodes (counting from the base) of primary shoots of sugarcane plants grown over the spring equinox (a, b, c) and autumn equinox (d, e, f) at three temperature regimes. The arrows show the change in RS% between the first and last day of sampling.d1, d2, d3, d4 and d5 in spring represent 0, 4, 11, 18, and 27 days, respectively, after temperature differentiation for; and in autumn d1, d1, d2, d3, d4, d5 and d6 represent 0, 5, 12, 19, 26 and 31 days, respectively, after temperature differentiation.

3.6 Partitioning of assimilates to sucrose, non-sucrose and fibre

Photoassimilates in the stalk of sugarcane are distributed among three stalk components, namely sucrose, non-sucrose and fibre. The photoassimilate partitioning between these three components of the sugarcane stalk were determined on each of the selected internodes (internode 4, 6 and 10). The partitioning coefficients were determined on the basis of dry weight of each component relative to the dry weight of the respective internodes (gg^{-1}). No attempt is made to portray any relationship between the weight of the respective internodes and the three stalk components (Figs. 3.6.1 to 3.6.3) (sucrose, non-sucrose and fibre), but data were used for the purpose of illustrating the fractional distribution of biomass between sucrose, non-sucrose and fibre, as the internodes accumulate weight. The partitioning fractions of each of the stalk components were indicated by the slopes of each line.

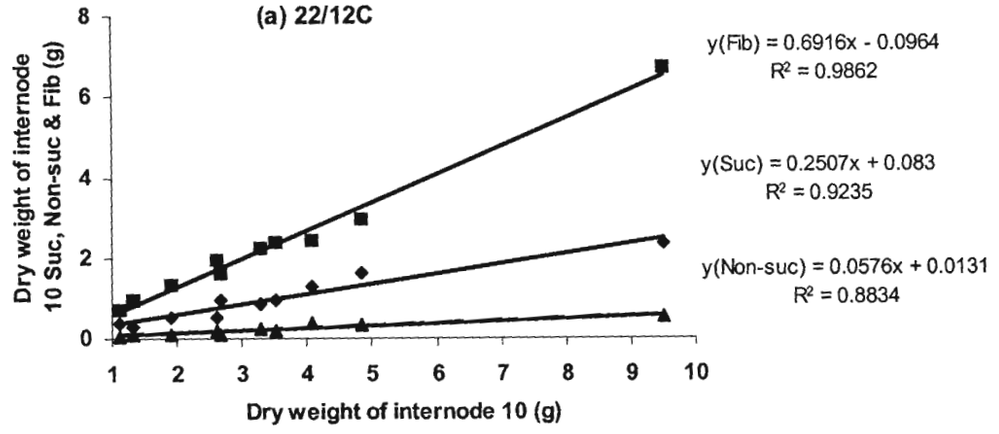
In spring the coefficient of assimilates partitioned to sucrose in the mature internodes (internode 10) decreased with increasing temperature from 0.25 at C (Fig. 3.6.1a) to 0.21 at W (Fig. 3.6.1b) and then to 0.20 at H (Fig. 3.6.1c). This corresponded with coefficients of assimilates partitioned to soluble non-sucrose of 0.06, 0.12 and 0.16 at C, W and H, respectively. The coefficient of assimilates partitioned to fibre in the mature internodes decreased from 0.69 at C to 0.67 at W and then to 0.64 at H. The results of spring were similar to autumn, with the exception of the partitioning coefficients of fibre. Whereas the coefficient of assimilates partitioned to fibre in the mature internodes of the spring crop decreased with increasing temperature, in autumn it increased from 0.51 (Fig. 3.6.1d) to 0.55 (Fig. 3.6.4e) and to 0.65 (Fig. 3.6.4f) at C, W and H, respectively. The latter observation was likely due to internode 10 in this study still being in the extension phase of its growth.

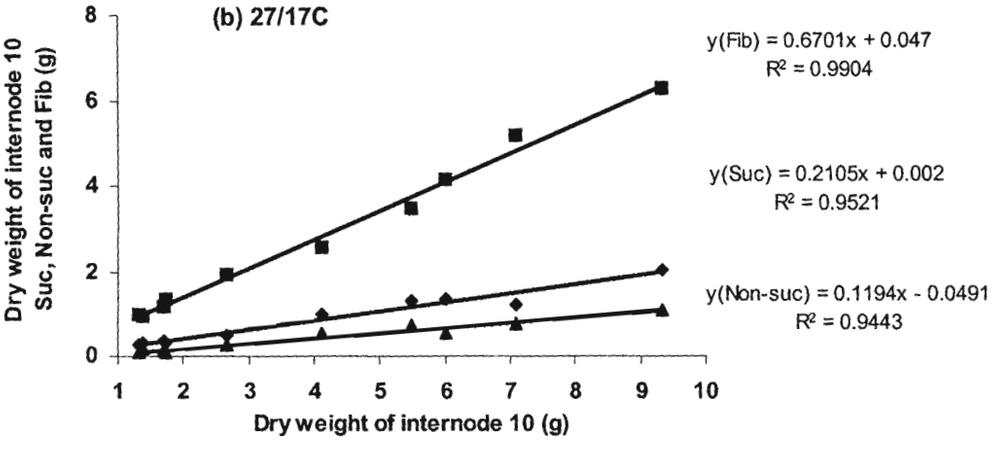
In the sugarcane plants that were harvested over the autumn equinox the partitioning coefficient to sucrose in the mature internodes decreased from 0.50 at C (Fig. 3.6.1d) to 0.32 at W (Fig. 3.6.1e) and then down to 0.21 at H (Fig. 3.6.1f). This corresponded to an

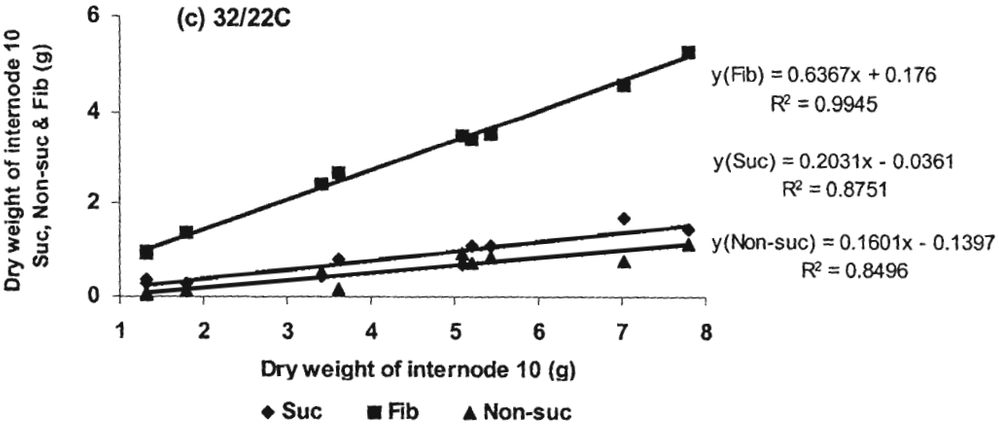
increasing trend in the coefficient of soluble non-sucrose substances from -0.009 at C to 0.13 at W and to 0.14 at H. There seemed to be an increase in the partitioning coefficient to fibre with increasing temperature, as indicated above.

The partitioning coefficient to sucrose in internode 6 in the spring crop decreased rapidly between C and W, from 0.24 at C (Fig. 3.6.2a) to 0.07 at W (Fig. 3.6.2b), then it further decreased but slightly to 0.068 at H (Fig. 3.6.2c). This corresponded to a sudden increase of the coefficient of assimilates partitioned to the non-sucrose substances between C and W, from 0.09 at C to 0.15 at W, and then increased only slightly to 0.16 at H. The partitioning fraction to fibre in internode 6 increased from 0.68 at C to 0.78 at W and then decreased to 0.77 at H.

A similar trend was observed in the sucrose partitioning coefficient in internode 6 in autumn. The partitioning coefficient decreased from 0.26 at C (Fig. 3.6.2d) to 0.17 at W (Fig. 3.6.2e) and then to 0.14 at H (Fig. 3.6.2f), with a corresponding increase in the partitioning coefficient to non-sucrose substances from 0.10 at C to 0.14 at W and then a slight decrease to 0.13 at H. The coefficient of assimilates partitioned to fibre followed the general increasing trend with temperature, as in the mature internode. It increased from 0.64 at C to 0.70 at W and then to 0.72 at H.


(a) 22/12C
Dry weight of internode 10 Suc, Non-suc & Fib (g)
Dry weight of internode 10 (g)
y(Fib) = 0.6916x - 0.0964
R² = 0.9862
y(Suc) = 0.2507x + 0.083
R² = 0.9235
y(Non-suc) = 0.0576x + 0.0131
R² = 0.8834


(b) 27/17C
Dry weight of internode 10 Suc, Non-suc and Fib (g)
Dry weight of internode 10 (g)
y(Fib) = 0.6701x + 0.047
R² = 0.9904
y(Suc) = 0.2105x + 0.002
R² = 0.9521
y(Non-suc) = 0.1194x - 0.0491
R² = 0.9443


(c) 32/22C
Dry weight of internode 10 Suc, Non-suc & Fib (g)
Dry weight of internode 10 (g)
y(Fib) = 0.6367x + 0.176
R² = 0.9945
y(Suc) = 0.2031x - 0.0361
R² = 0.8751
y(Non-suc) = 0.1601x - 0.1397
R² = 0.8496
◆ Suc ■ Fib ▲ Non-suc

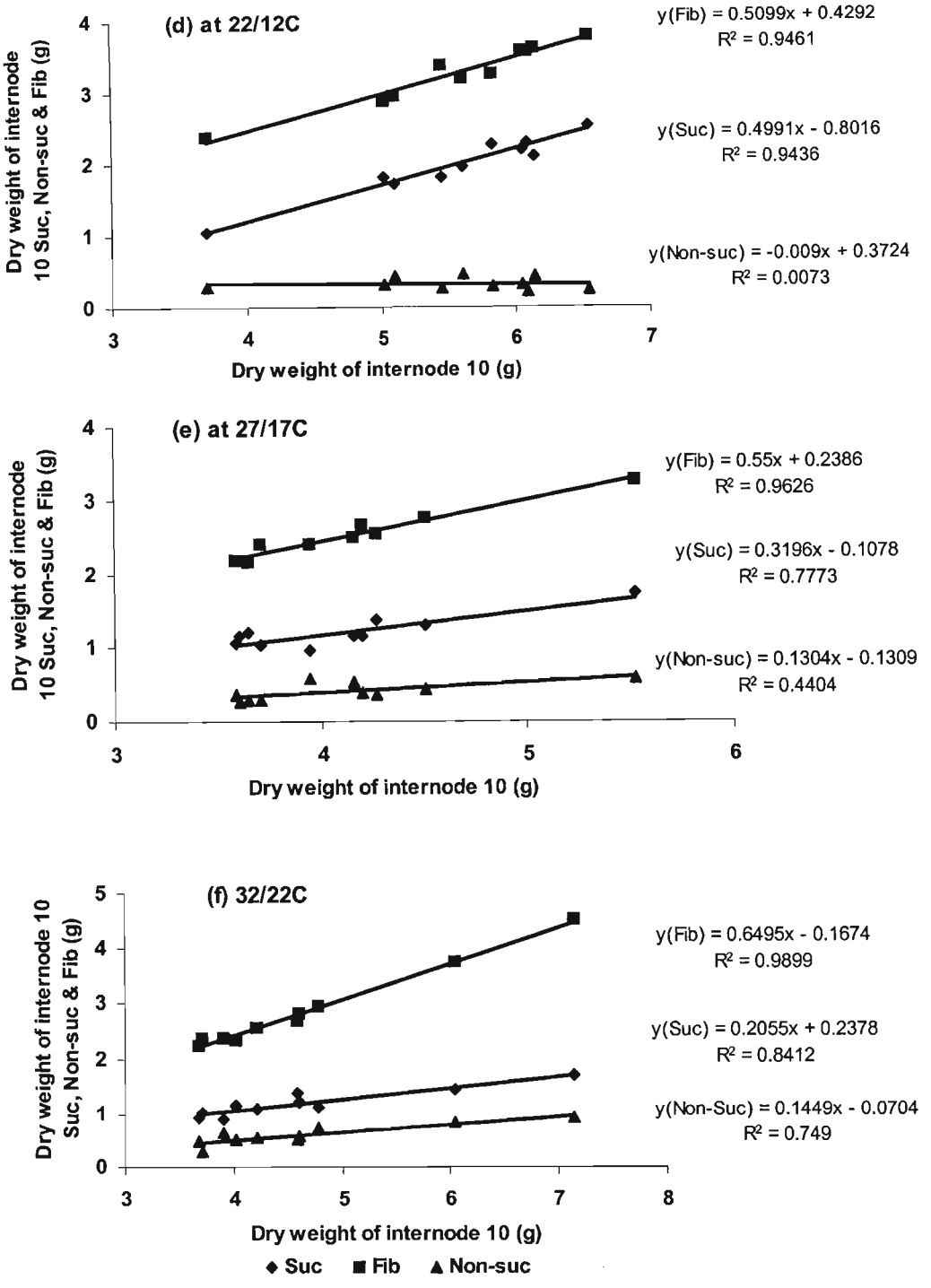
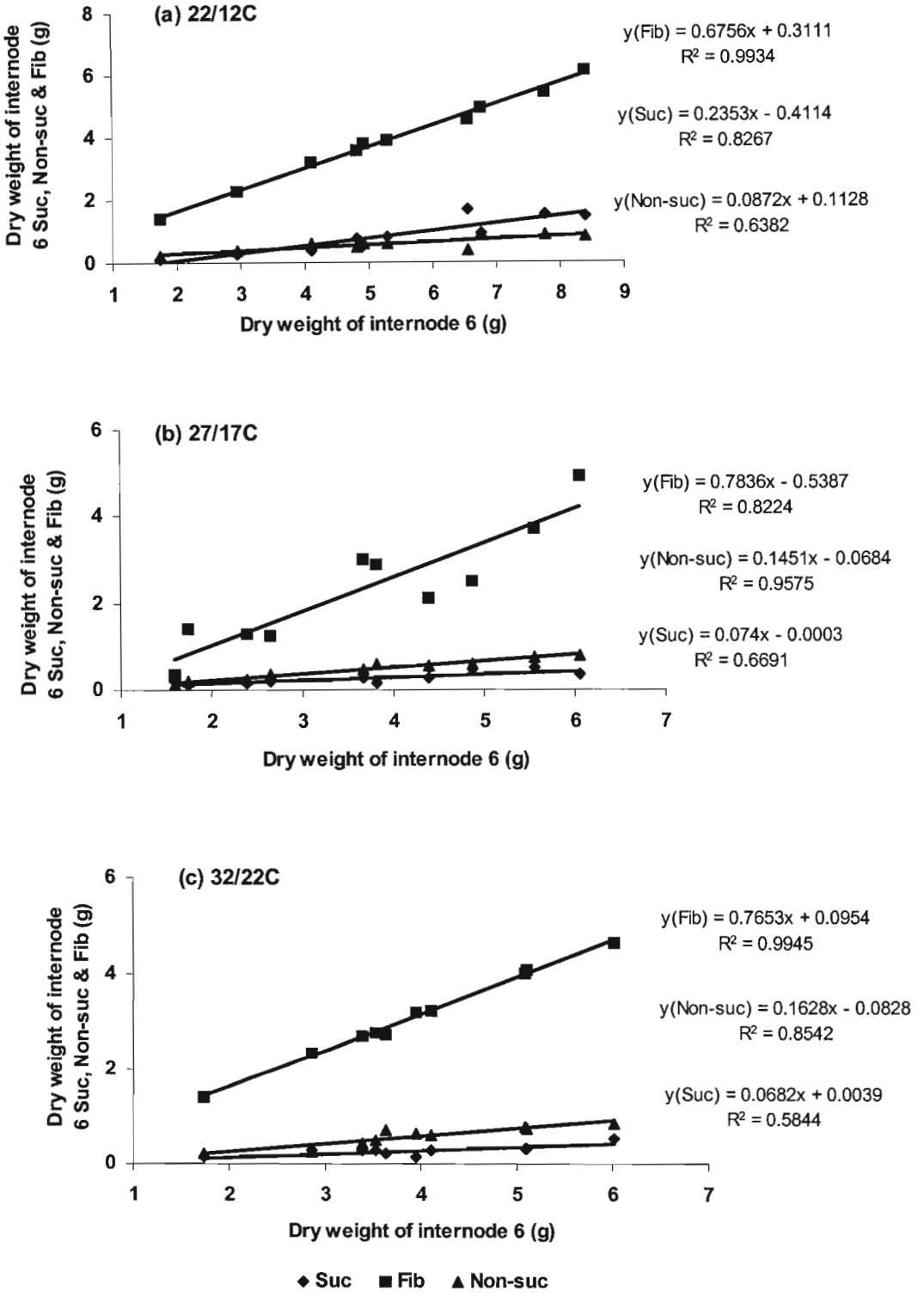


Figure 3.6.1: Assimilate partitioning to sucrose (Suc), non-sucrose (Non-suc) and fibre (Fib) in internode 10 (count from top of shoot) at 22/12°C, 27/17°C and 32/22°C in sugarcane plants grown over the spring (a, b, c) autumn (d, e, f) equinoxes. Partitioning coefficients of the respective stalk components are represented by the regression coefficients on the regression line equations.


(a) 22/12C
Dry weight of internode 6 Suc, Non-suc & Fib (g)
Dry weight of internode 6 (g)
y(Fib) = 0.6756x + 0.3111
R² = 0.9934
y(Suc) = 0.2353x - 0.4114
R² = 0.8267
y(Non-suc) = 0.0872x + 0.1128
R² = 0.6382
(b) 27/17C
Dry weight of internode 6 Suc, Non-suc & Fib (g)
Dry weight of internode 6 (g)
y(Fib) = 0.7836x - 0.5387
R² = 0.8224
y(Non-suc) = 0.1451x - 0.0684
R² = 0.9575
y(Suc) = 0.074x - 0.0003
R² = 0.6691
(c) 32/22C
Dry weight of internode 6 Suc, Non-suc & Fib (g)
Dry weight of internode 6 (g)
y(Fib) = 0.7653x + 0.0954
R² = 0.9945
y(Non-suc) = 0.1628x - 0.0828
R² = 0.8542
y(Suc) = 0.0682x + 0.0039
R² = 0.5844
◆ Suc ■ Fib ▲ Non-suc

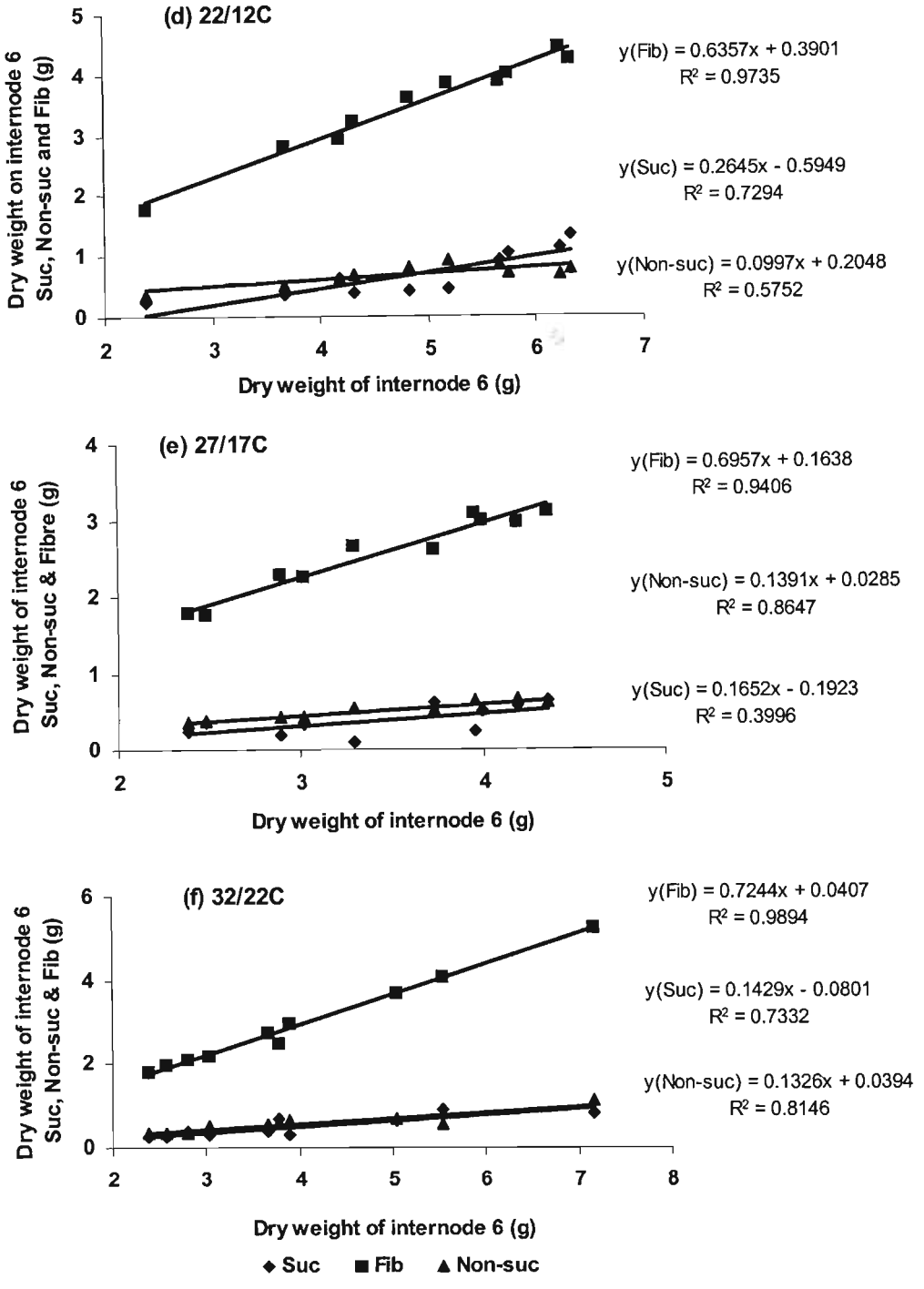
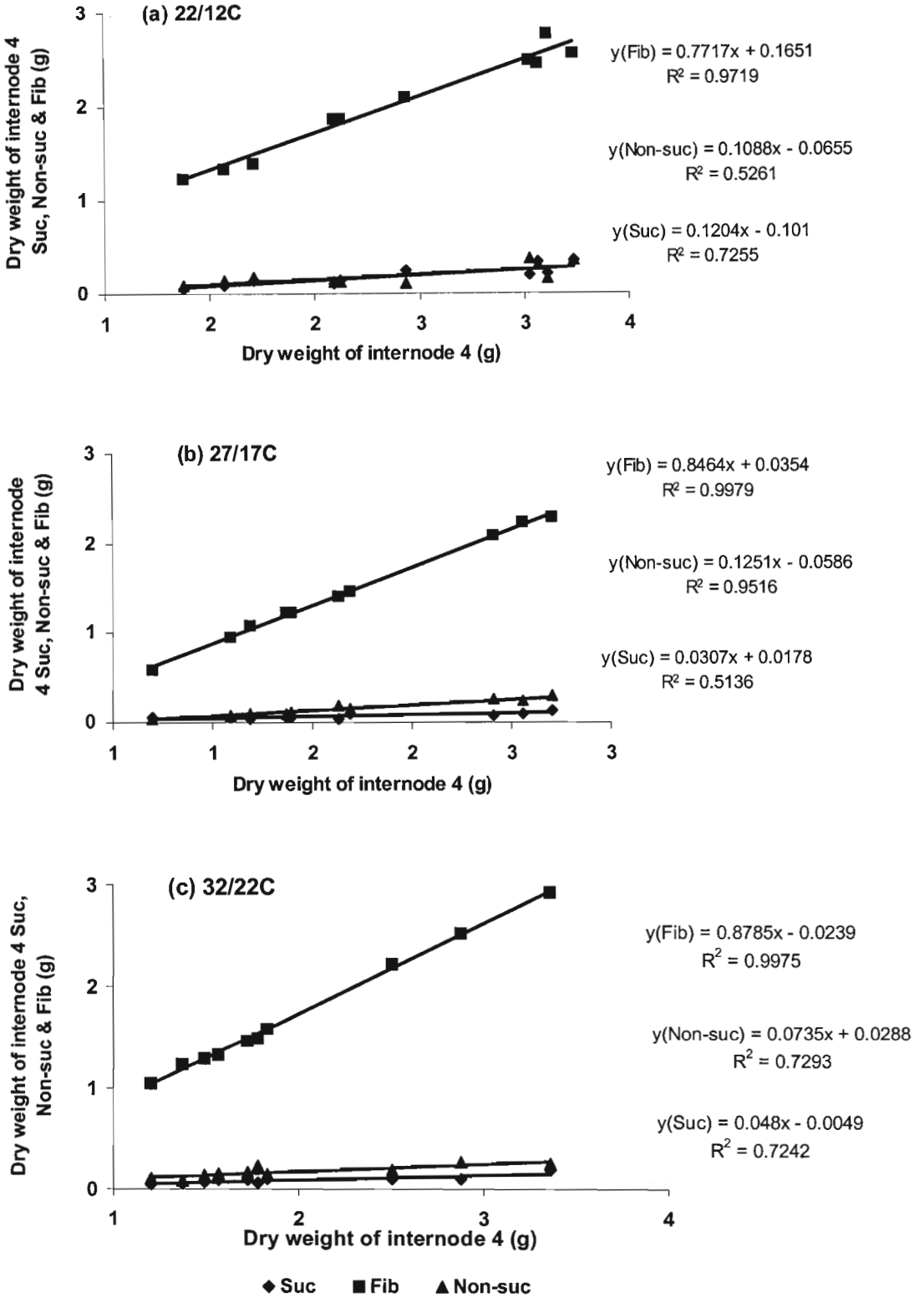


Figure 3.6.2: Assimilate partitioning to sucrose (Suc), non-sucrose (Non-suc) and fibre (Fib) in internode 6 (count from top of shoot) at 22/12°C, 27/17°C and 32/22°C in sugarcane plants grown over the spring (a, b, c) autumn (d, e, f) equinoxes. Partitioning coefficients of the respective stalk components are represented by the regression coefficients on the regression line equations.

There was no definite order of assimilate partitioning to the stalk components of the immature internode (internode 4) over the three levels of temperature in either trial. In spring the partitioning coefficients to sucrose were 0.12 at C (Fig. 3.6.3a), 0.03 at W (Fig. 3.6.3b) and 0.05 at H (Fig. 3.6.3c). The partitioning coefficient to non-sucrose solutes were 0.10, 0.13 and 0.07, respectively, in the same order of temperature. Partitioning to fibre increased with increasing temperature from 0.77 at C to 0.85 at W and then to 0.88 at H. In autumn the assimilate partitioning coefficient to sucrose was 0.09 (Fig. 3.6.3d), 0.10 (Fig. 3.6.3e) and 0.09 (Fig. 3.6.3f) at C, W and H, respectively; and to the fibre component the partitioning coefficients were 0.79, 0.72 and 0.73, in the same order of temperatures.

The results of this study show that temperature had a role on the partitioning of assimilates to the various components of the stalk. Partitioning of assimilates to the component of interest in this study (sucrose) was higher in the mature internodes that were treated with cold temperature, indicating a negative relationship between temperature and assimilate partitioning to sucrose. There seemed to be a positive response of the non-sucrose and fibre components to increasing temperature, indicating that low temperature favoured assimilate partitioning to sucrose, whereas high temperature favoured assimilate partitioning to non-sucrose and fibre. The results also show that assimilate partitioning is affected by the maturity of the internodes. Higher assimilate partitioning coefficients were obtained at the more mature internodes than the immature internodes.


(a) 22/12C
Dry weight of internode 4 Suc, Non-suc & Fib (g)
Dry weight of internode 4 (g)
y(Fib) = 0.7717x + 0.1651
R² = 0.9719
y(Non-suc) = 0.1088x - 0.0655
R² = 0.5261
y(Suc) = 0.1204x - 0.101
R² = 0.7255
(b) 27/17C
Dry weight of internode 4 Suc, Non-suc & Fib (g)
Dry weight of internode 4 (g)
y(Fib) = 0.8464x + 0.0354
R² = 0.9979
y(Non-suc) = 0.1251x - 0.0586
R² = 0.9516
y(Suc) = 0.0307x + 0.0178
R² = 0.5136
(c) 32/22C
Dry weight of internode 4 Suc, Non-suc & Fib (g)
Dry weight of internode 4 (g)
y(Fib) = 0.8785x - 0.0239
R² = 0.9975
y(Non-suc) = 0.0735x + 0.0288
R² = 0.7293
y(Suc) = 0.048x - 0.0049
R² = 0.7242
◆ Suc ■ Fib ▲ Non-suc

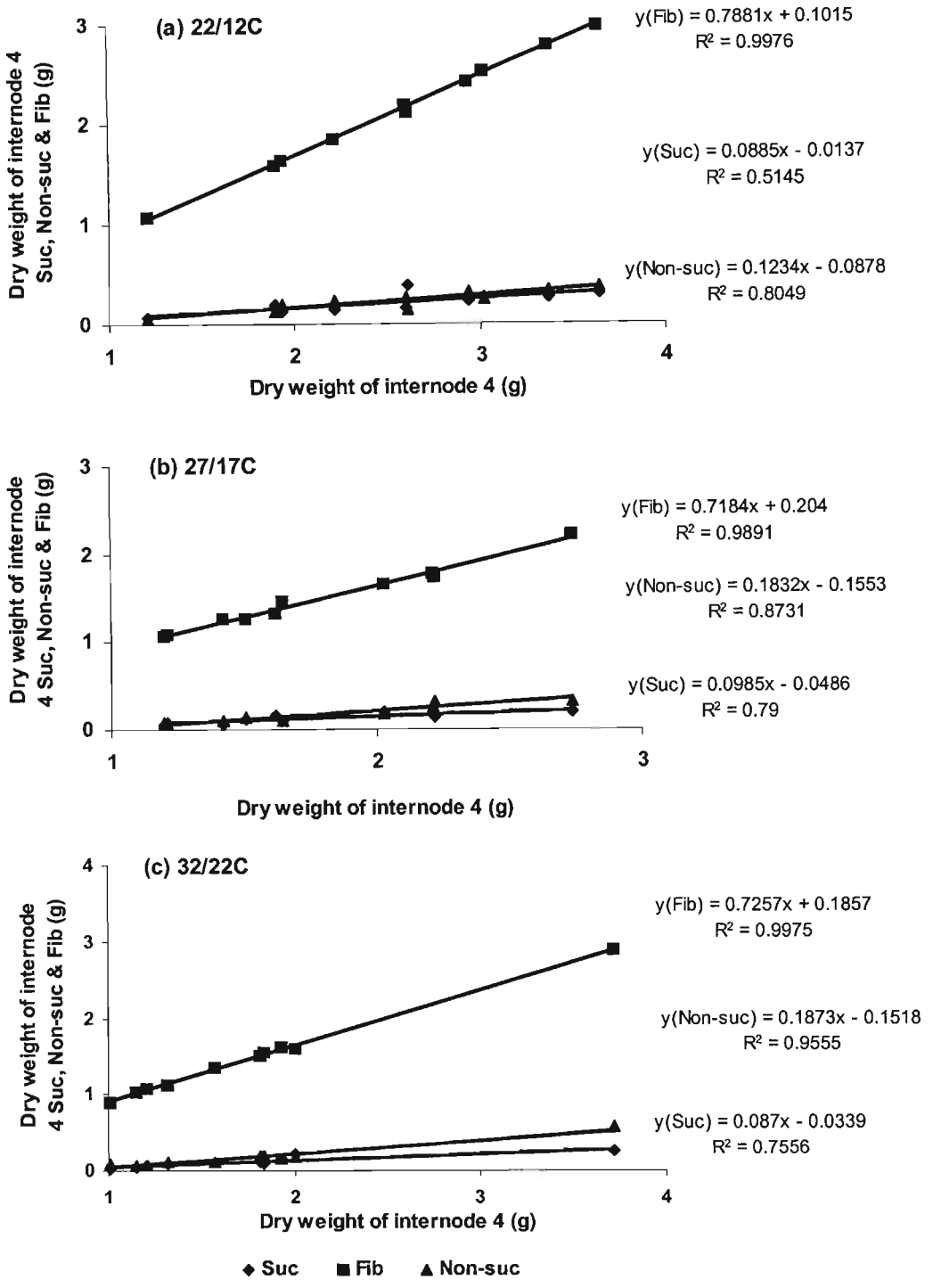


Figure 3.6.3: Assimilate partitioning to sucrose (Suc), non-sucrose (Non-suc) and fibre (Fib) in internode 4 (count from top of shoot) at 22/12°C, 27/17°C and 32/22°C in sugarcane plants grown over the spring (a, b, c) autumn (d, e, f) equinoxes. Partitioning coefficients of the respective stalk components are represented by the regression coefficients on the regression line equations.

3.7 Seasonal comparison of sucrose flux and partitioning

Seasonal comparison showed that the coefficient of assimilates partitioned to sucrose at the mature internodes (base stem) was lower in the spring crop than in the autumn crop. This does not accord with the results of Singels *et al.* (2005a). It is thought that this could have been caused by the fact that the basal internodes of the spring equinox plants were still in their extension phase during the sampling period, whereas those of the autumn equinox plants had ceased extension. Studies that attempt to address the partitioning of assimilates to sucrose and to the other stalk components include those by Cherro-Nayamuth (2000), Evenson *et al.* (1997), Inman-Bamber (2002), Singels *et al.* (2005a), Singels and Inman-Bamber (2002), and Thompson (1978). Results of these studies indicated that the distribution of assimilates between the stalk components is controlled by a range of factors, including temperature, soil moisture content, fertilizers (particularly nitrogen) and maturity, all of which regulate growth in sugarcane. The extension of the basal internodes of spring plants relative to autumn plants is more likely to be accountable for the lower sucrose concentration in internode 10 of spring plants than of autumn plants.

The results reported in this study show the temperature response in isolation. The other factors that influence the development and assimilate partitioning in sugarcane were not included. A complementary study was conducted on the same plants by the South African Sugar Research Institute, in which the seasonal incident and intercepted radiation in the three temperatures were quantified and correlated with the development characteristics of sugarcane.

4 GENERAL DISCUSSION AND CONCLUSIONS

Lisson *et al.* (2005) emphasized the importance of both field and controlled-environment physiological studies for sugarcane in the provision of a clearer understanding of the plant. This understanding relates the interaction between the environment and key physiological processes (some of which are genetically determined) which determine the economic yield of the crop. The knowledge provided by such studies is of direct use in the crop's agronomic management, such as the scheduling of planting and harvesting dates, application of chemical ripeners, harvesting management, etc. It is also used indirectly to develop and improve simulation models. Singels *et al.* (2005a) expressed a concern about the inaccuracy of the currently used sugarcane models (including Canegro), which are widely used to simulate sugarcane growth and sucrose content over the cropping season in South Africa. The study reported here and the associated complementary study by the SASRI staff were part of an ongoing project that seeks to correct some of the shortcomings of current sugarcane simulation models, particularly the effect of temperature on the partitioning of assimilate between harvestable and non-harvestable components of the crop.

The results of this study have shown that the morphological development of sugarcane is primarily dependent on temperature when other factors are non-limiting. The slower rate of leaf appearance and leaf expansion that was observed at C, relative to W and H, explains the lower rate of canopy development observed in the field situation during the cool winter season than during the warm summer season. Hence, sugarcane cultivars exhibiting rapid canopy development and rapid tillering resulting in higher haulm density, as reported by Singels and Smit (2002), are an important consideration for a sugarcane crop that is established or ratooned during the cool winter season.

The ripening effect of cold temperature on sucrose accumulation, which has been reported in previous studies (Singels *et al.*, 2005a; Inman-Bamber *et al.*, 2002; Truen, 1972; Rostron, 1971), was supported by the results of this study. However, this effect would only be beneficial to the sugarcane grower on plants having adequate stalk growth.

This is to say that one requires the sugarcane crop to exhibit an initial period of rapid growth and development to establish both the leaf area potential (source) and the stalk (sink) structures that are of adequate size and strength. Having achieved that condition, a crop subjected to cold would achieve the relevant ripening. In the field situation, the rapid growth and subsequent ripening of a sugarcane crop is regulated by the seasonal fluctuation of the environment. Both the growing and the harvesting seasons of sugarcane are a function of temperature, radiation, rainfall and photoperiod, all of which are influential on sugarcane growth and sucrose accumulation. Data could theoretically be generated from the assessment of all the above variables and their interaction effects on the biochemical systems involving the interaction of hormonal biosynthesis and activity, sucrose metabolism enzyme activity and sucrose accumulation in sugarcane. This information would be highly valuable but practically impossible to attain. For instance, such a study would shed some light, at a bio-molecular level, on how low temperature increases the concentration of sucrose without affecting the concentration of the reducing sugars (RS), and how high temperatures increase the concentration of the RS without having a marked effect on the concentration of sucrose. A study of this form might elucidate the unclear understanding surrounding the 4-6 weeks lag of the sucrose response to the environmental changes reported by Glover (1971). It would also be interesting to investigate the role of the stress hormones such as abscisic acid on sugarcane ripening. As much as such a study would give comprehensive results, it would be impossible to carry out in one study. In a complex study of this sort, future complex computer models could incorporate all the influential factors to reflect the possible morphological and physiological development of sugarcane.

The higher concentration of reducing sugars (RS) obtained even in the mature internodes of sugarcane at the higher temperatures (W and H), which is typical of the field situation during the hot and wet season, reduces the economic value of harvested sugarcane for South African growers, since it reduces the recoverable value (RV) of sugarcane. However, it is likely that this RS content would have additional economic significance to sugarcane growers once the South African sugar industry begins to grow sugarcane for the production of ethanol. This event would require an adjustment of the payment system

of sugarcane consignments in a way that it would not be based on the RV of sucrose alone but would additionally need to account for total fermentable sugars. Studies on the cultivation of sugarcane for both raw sugar and ethanol production and the feasibility of using ethanol as fuel for automobiles in South Africa are still underway. These studies were initiated in the 1970's following a depressed sugar market (Ortmann, 1985), and the fuel price increase of ca. 400% within two decades (Buchanan, 1979). The development of combustion engines that are able to use ethanol as fuel were intensified by the realization that petroleum oil deposits are finite and mineral oil reserves will run out at some stage in the future (Buchanan, 1979). However, there are different opinions about the use of ethanol and its blends as fuel. Ortmann and Nieuwoudt (1987) indicated that the replacement value of ethanol was lower than its then cost of production. Thompson (1979) reported that ethanol production from sugarcane may be feasible, but cited references to the problems of stillage disposal. Oliveira *et al.* (2005) reported that the use of ethanol as a substitute for fuel would not be sustainable in that its production had direct and indirect environmental hazards that exceeded its value as a replacement fuel. On the other hand there are positive reports about using ethanol as a replacement for petrol (Ramos and Wilhelm, 2005). One-half of the sugarcane crop cultivated in Brazil today is used for ethanol production, and as a result Brazil is said to be more flexible in responding to changes in the international sugar and fuel market markets (Bolling and Suarez, 2001). Of course, any consumption of the use of sugar for conversion to fuel will in time affect the world price of sucrose, making it difficult to predict the most economic proportion of the crop to be used for ethanol or sucrose production.

The value of this study is that:

1. It has shown that cold temperature directly lowers the rate of leaf and hence canopy development of sugarcane and therefore planting/ratooning dates should, where possible, be scheduled in a way that the early growth does not coincide with the cool season.
2. Controlling the number of haulms per stool through pruning considerably reduced the measured variability of measured growth components between tillers of the same hierarchy and between stools subjected to the same temperature treatment in

comparison with stools left to grow out normally. The latter stools (data not reported from the initial study) exhibited substantial variation in number of haulms produced per stool, despite being grown out from uniformly selected transplants.

3. The study has shown the importance of determining the partitioning response between harvestable (fibre, sucrose & RS) and non-harvestable (leaf and sheath) components in relation to temperature. This knowledge will enhance better management of sugarcane by efficient scheduling of planting/ratooning and harvesting dates in a way that the growth phase of sugarcane is targeted at the warm season and the ripening and harvesting phase is directed towards the cool season.
4. The economic value of sugarcane is higher during the cool season when sucrose concentration in the stalk is high. The use of chemical ripeners and drying off of crop may be necessary for crops that are harvested during the hot summer months.
5. The sugarcane crops that are harvested in warm conditions contain a higher concentration of non-sucrose substances. If substantive alternative products to sucrose were to be produced from sugarcane in South Africa using additional sugars, such as glucose and fructose, then the consignment payment system would need to incorporate the value of the total fermentable sugars. Provided with this payment situation, many entrenched sugarcane management practices would have to be adjusted in a way that targeted summer harvests for the production of fermentable sugars and the winter harvests for sucrose.

This study was not designed to directly compare the sugar concentrations between the two (autumn and spring equinox) seasons as there was a difference in the pre-temperature treatment growth of the stock plants for the two trials. As a result, the lower concentration of sucrose and the lower partitioning coefficient of sucrose in the mature internodes of plants that were grown during the spring equinox cannot be regarded as a true reflection of the real field situation. In fact, many studies have shown that sucrose concentration in sugarcane is higher in spring than in autumn (Glover, 1971; Inman-Bamber *et al.*, 2002; Singels *et al.*, 2005a), which is the opposite of the results reported for this study. As

indicated earlier in this thesis study, the higher amounts of sucrose in the basal internodes of the plants grown in the spring equinox was due to their still being in an extension phase of growth as compared with the already matured basal internodes of the autumn equinox crop. The reported 4-6 weeks lag of stalk sucrose content in the field in response to the cold effect of the winter season was not supported by the results of this study, where the concentration of sucrose responded rapidly to the onset of the cold temperature treatment (C) and then increased steadily afterwards. Therefore, the observed field effect is unlikely to be directly related to temperature but rather to alternative stress responses and their interactions. The study could also not establish the fundamental basis of the compared sugar interactions as the relevant enzyme activities were not measured.

The downfall of this study in its initial stages was excessive plant to plant variability, in part due to the number of replications being restricted, but particularly associated with the high variability of tillering between stools. Variability in measured growth components was markedly improved by shoot pruning to a constant number of haulms per stool. In the future, the precision of results from a study such as this could be further improved by increasing the number of replications per sampling date and repeating the experiment over many more seasons than just two. However, this would necessitate larger and/or many more controlled-environment glasshouses and technical staff, which is not likely to be economically feasible. Pre-trial management of plants would also have to be as uniform as possible for different seasons through producing the initial plant material under controlled environment conditions. The study also needs to be extended to other commercial cultivars of sugarcane. The latter development is important to ascertain whether cultivar-specific models or generic models would be satisfactory for prediction of growth and sucrose accumulation in the sugarcane stalk.

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Appendix 1 (a) (i) Analysis of variance of internode 4 sucrose concentration for sugarcane grown at three temperatures over the autumn equinox.

Analysis of variance

Variate: Sucrose %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	7.114	7.114	17.07	
Reps.Subject stratum					
Temperature	2	56.950	28.475	68.33	0.014
Residual	2	0.833	0.417	0.06	
Time	9	150.662	16.740	2.29	0.188
Time.Temperature	18	156.915	8.717	1.19	0.405
Residual	27	197.505	7.315		
Total	59	569.978			

Table of means

Variate: Sucrose %

Time	Cold	Hot	Warm
1	8.52	3.59	5.66
2	5.99	3.25	7.25
3	6.45	2.73	4.05
4	7.58	6.96	4.39
5	6.25	6.52	8.18
6	8.11	4.52	9.38
7	10.57	11.64	6.19
8	7.65	6.72	7.46
9	14.57	7.05	6.52
10	8.65	8.00	9.31
Mean	8.44	6.10	6.84

	Time	Temperature	Time.Temperature
s.e.d.	1.562	0.204	2.574
l.s.d.	3.891	0.878	6.392

CV%=38%

Appendix 1 (a) (ii) Analysis of variance of internode 6 sucrose concentration for sugarcane grown at three temperatures over the autumn equinox.

Analysis of variance

Variate: Scrose %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	29.81	29.81	6.93	
Reps.Subject stratum					
Temperature	2	77.00	38.50	8.95	0.100
Residual	2	8.60	4.30	0.29	
Time	9	361.53	40.17	2.69	0.174
Time.Temperature	18	590.23	32.79	2.19	0.222
Residual	27	403.91	14.96		
Total	59	1471.07			

Table of means

Variate: Scrose %

Time	Cold	Hot	Warm
1	11.28	8.09	11.18
2	8.41	10.00	13.94
3	14.90	9.47	3.08
4	9.79	13.20	11.18
5	8.40	10.11	12.08
6	17.99	7.44	6.17
7	9.15	18.62	6.39
8	16.39	16.18	16.39
9	17.88	11.60	13.30
10	21.28	12.77	14.48
Mean	13.55	11.75	10.82

	Time	Temperature	Time.Temperature
s.e.d.	2.233	0.656	3.727
l.s.d.	6.018	2.821	9.837

CV%=32.1%

Appendix 1 (a) (iii) Analysis of variance of internode 10 sucrose concentration for sugarcane grown at three temperatures over the autumn equinox.

Analysis of variance

Variate: Sucrose %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	20.51	20.51	12.61	
Reps.Subject stratum					
Temperature	2	1028.37	514.18	316.19	0.003
Residual	2	3.25	1.63	0.13	
Reps.Subject.Time stratum					
d.f. correction factor 0.1621					
Time	9	182.81	20.31	1.67	0.278
Time.Temperature	18	146.15	8.12	0.67	0.608
Residual	27	328.17	12.15		
Total	59	1709.25			

Table of means

Variate: Sucrose %

Time	Cold	Hot	Warm
1	31.93	24.49	26.61
2	34.24	23.77	32.64
3	33.53	28.56	27.50
4	36.01	22.89	24.48
5	34.95	25.55	31.93
6	38.67	25.72	28.92
7	36.19	26.43	29.27
8	33.17	23.24	27.50
9	38.85	30.16	31.76
10	37.61	24.12	31.05
Mean	35.52	25.49	29.17

	Time	Temperature	Time.Temperature
s.e.d.	2.013	0.403	3.332
l.s.d.	5.404	1.735	8.856

CV%=11.6%

Appendix 1 (b) (i) Analysis of variance of internode 4 RS concentration for sugarcane grown at three temperatures over the autumn equinox.

Analysis of variance

Variate: RS %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	0.075	0.075	0.04	
Reps.Subject stratum					
Temperature	2	0.882	0.441	0.22	0.819
Residual	2	3.988	1.994	0.30	
Time	9	208.879	23.209	3.54	0.129
Time.Temperature	18	215.500	11.972	1.82	0.277
Residual	27	177.139	6.561		
Total	59	606.463			

Table of means

Variate: RS %

Time	Cold	Hot	Warm
1	6.62	4.16	3.76
2	9.75	6.49	8.62
3	10.28	8.41	7.08
4	10.81	8.62	7.22
5	9.81	7.55	11.48
6	10.35	11.07	8.81
7	6.29	9.09	5.62
8	10.02	11.07	11.88
9	5.09	15.40	14.67
10	8.15	8.27	9.48
Mean	8.72	9.01	8.86

	Time	Temperature	Time.Temperature
s.e.d.	1.479	0.447	2.471
l.s.d.	4.039	1.921	6.595

CV%=28.9%

Appendix 1 (b) (ii) Analysis of variance of internode 6 RS concentration for sugarcane grown at three temperatures over the autumn equinox.

Analysis of variance

Variate: RS %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	4.119	4.119	0.85	
Reps.Subject stratum					
Temperature	2	2.259	1.129	0.23	0.811
Residual	2	9.713	4.856	0.83	
Time	9	59.075	6.564	1.12	0.384
Time.Temperature	18	167.563	9.309	1.58	0.304
Residual	27	158.597	5.874		
Total	59	401.325			

Table of means

Variate: RS %

Time	Cold	Hot	Warm
1	18.57	14.31	12.08
2	16.55	14.85	14.85
3	14.85	13.57	16.13
4	14.00	11.87	14.11
5	17.62	17.09	12.91
6	12.29	16.45	16.02
7	15.70	15.91	14.96
8	15.16	10.06	13.89
9	10.91	15.28	15.71
10	11.98	13.57	14.00
Mean	14.76	14.30	14.46

	Time	Temperature	Time.Temperature
s.e.d.	1.399	0.697	2.403
l.s.d.	3.561	2.998	5.870

Appendix 1 (b) (iii) Analysis of variance of internode 10 RS concentration for sugarcane grown at three temperatures over the autumn equinox.

Analysis of variance

Variate: RS %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	8.081	8.081	12.49	
Reps.Subject stratum					
Temperature	2	569.451	284.725	440.04	0.002
Residual	2	1.294	0.647	0.17	
Time	9	88.421	9.825	2.57	0.168
Time.Temperature	18	110.164	6.120	1.60	0.300
Residual	27	103.293	3.826		
Total	59	880.704			

Table of means

Variate: RS %

Time	Temperature	Cold	Hot	Warm
1		5.23	11.08	7.19
2		6.83	13.03	8.07
3		8.60	12.86	12.50
4		5.05	16.59	14.64
5		8.07	13.22	7.36
6		3.64	13.22	9.84
7		6.30	12.33	9.84
8		4.70	15.17	9.31
9		5.05	11.26	8.60
10		3.63	13.75	10.20
Mean		5.71	13.25	9.76

	Time	Temperature	Time.Temperature
s.e.d.	1.129	0.254	1.873
l.s.d.	2.869	1.094	4.711

CV%=20.4%

Appendix 1 (c) (i) Analysis of variance of internode 4 fibre concentration for sugarcane grown at three temperatures over the autumn equinox.

Analysis of variance

Variate: Fibre %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	5.729	5.729	1.38	
Reps.Subject stratum					
Temperature	2	43.994	21.997	5.30	0.159
Residual	2	8.298	4.149	0.65	
Time	9	463.770	51.530	8.02	0.028
Time.Temperature	18	293.566	16.309	2.54	0.167
Residual	27	173.575	6.429		
Total	59	988.932			

Table of means

Variate: Fibre %

Time	Temperature	Cold	Hot	Warm
1		84.86	92.25	90.58
2		84.27	90.26	84.13
3		83.27	88.85	88.86
4		81.60	84.41	88.39
5		83.93	85.92	80.34
6		81.54	84.40	81.80
7		83.13	79.27	88.19
8		82.33	82.20	80.67
9		80.34	77.55	78.81
10		83.20	83.73	81.20
Mean		82.85	84.89	84.30

	Time	Temperature	Time.Temperature
s.e.d.	1.464	0.644	2.490
l.s.d.	3.737	2.772	6.143

CV%=3%

Appendix 1 (c) (ii) Analysis of variance of internode 6 fibre concentration for sugarcane grown at three temperatures over the autumn equinox.

Analysis of variance

Variate: Fibre %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	11.77	11.77	14.64	
Reps.Subject stratum					
Temperature	2	99.38	49.69	61.83	0.016
Residual	2	1.61	0.80	0.07	
Time	9	192.21	21.36	1.99	0.236
Time.Temperature	18	454.79	25.27	2.35	0.203
Residual	27	289.78	10.73		
Total	59	1049.53			

Table of means

Variate: Fibre %

Time	Temperature	Cold	Hot	Warm
1		70.14	77.59	76.74
2		75.04	75.15	71.21
3		70.25	76.96	80.79
4		76.21	74.93	74.72
5		73.98	72.81	75.01
6		69.72	76.11	77.81
7		75.15	65.46	78.66
8		68.44	73.76	69.72
9		71.21	73.12	70.99
10		66.74	73.66	71.52
Mean		71.68	73.95	74.71

	Time	Temperature	Time.Temperature
s.e.d.	1.891	0.283	3.121
l.s.d.	5.074	1.220	8.325

CV%=4.5%

Appendix 1 (c) (iii) Analysis of variance of internode 10 fibre concentration for sugarcane grown at three temperatures over the autumn equinox.

Analysis of variance

Variate: Fibre %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	54.340	54.340	95.97	
Reps.Subject stratum					
Temperature	2	76.705	38.352	67.73	0.015
Residual	2	1.132	0.566	0.06	
Time	9	177.023	19.669	2.19	0.217
Time.Temperature	18	51.518	2.862	0.32	0.804
Residual	27	242.541	8.983		
Total	59	603.260			

Table of means

Time	Temperature	Cold	Hot	Warm
1		62.83	64.43	66.20
2		58.93	63.20	59.28
3		57.87	58.58	59.99
4		58.93	60.52	60.88
5		56.98	61.23	60.70
6		57.69	61.06	61.23
7		57.51	61.24	60.88
8		62.13	61.59	63.19
9		56.09	58.57	59.64
10		58.75	62.13	58.75
Mean		58.77	61.25	61.07

	Time	Temperature	Time.Temperature
s.e.d.	1.730	0.238	2.853
l.s.d.	4.687	1.024	7.691

CV%=5%

Appendix 2 (a) (i) Analysis of variance of internode 4 sucrose concentration for sugarcane grown at three temperatures over the spring equinox.

Analysis of variance

Variate: Sucrose %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	11.154	11.154	3.42	
Reps.Subject stratum					
Temperature	2	109.999	55.000	16.85	0.056
Residual	2	6.528	3.264	0.50	
Time	9	64.113	7.124	1.08	0.394
Time.Temperature	18	110.696	6.150	0.94	0.500
Residual	27	177.278	6.566		
Total	59	479.769			

7.42 4.71 4.42

Table of means
Variate: Sucrose %

Time	Temperature	Cold	Hot	Warm
1		5.91	6.70	3.49
2		4.67	3.83	3.38
3		5.13	4.05	3.33
4		8.00	4.45	4.73
5		8.56	5.86	9.12
6		6.37	5.46	4.84
7		7.55	3.21	4.96
8		10.85	4.84	3.61
9		6.14	5.41	4.39
10		11.10	3.27	2.36
Mean		7.42	4.71	4.42

	Time	Temperature	Time.Temperature
s.e.d.	1.479	0.571	2.497
l.s.d.	3.688	2.458	6.073

CV%=46.4%

Appendix 2 (a) (ii) Analysis of variance of internode 6 sucrose concentration for sugarcane grown at three temperatures over the spring equinox.

Analysis of variance

Variate: Sucrose %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	87.68	87.68	4.86	
Reps.Subject stratum					
Temperature	2	745.79	372.89	20.67	0.046
Residual	2	36.07	18.04	0.71	
Time	9	133.39	14.82	0.58	0.571
Time.Temperature	18	369.18	20.51	0.81	0.556
Residual	27	685.81	25.40		
Total	59	2057.92			

Table of means

Variate: Sucrose %

Time	Temperature	Cold	Hot	Warm
1		10.09	13.51	5.95
2		9.75	8.29	3.78
3		10.63	7.65	6.48
4		14.50	3.24	6.76
5		16.39	7.57	11.98
6		19.73	6.76	8.56
7		15.77	6.12	7.57
8		13.51	9.07	9.01
9		17.30	8.02	6.76
10		21.35	5.49	6.00
Mean		14.90	7.57	7.29
s.e.d.		2.910	1.343	4.966
l.s.d.		7.345	5.778	12.106

CV%=50.8

Appendix 2 (a) (iii) Analysis of variance of internode 10 sucrose concentration for sugarcane grown at three temperatures over the spring equinox.

Analysis of variance

Variate: Sucrose %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	0.48	0.48	0.02	
Reps.Subject stratum					
Temperature	2	746.42	373.21	17.19	0.055
Residual	2	43.43	21.71	1.82	
Time	9	336.79	37.42	3.13	0.141
Time.Temperature	18	732.35	40.69	3.40	0.119
Residual	27	322.93	11.96		
Total	59	2182.40			

Table of means

Variate: Sucrose %

Time	Temperature	Cold	Hot	Warm
	1	21.32	25.07	18.01
	2	26.73	15.62	17.87
	3	25.07	27.33	18.01
	4	20.42	21.92	20.87
	5	35.44	12.01	23.12
	6	33.18	19.82	24.77
	7	31.53	24.32	25.67
	8	31.53	19.97	21.92
	9	24.47	17.71	16.67
	10	28.38	15.01	21.77
Mean		27.81	19.88	20.87
s.e.d.		1.997	1.474	3.597
l.s.d.		5.273	6.340	8.778

CV%=15.1%

Appendix 2 (b) (i) Analysis of variance of internode 4 RS concentration for sugarcane grown at three temperatures over the spring equinox.

Analysis of variance

Variate: RS %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	0.831	0.831	1.56	
Reps.Subject stratum					
Temperature	2	1.538	0.769	1.44	0.409
Residual	2	1.065	0.532	0.07	
Time	9	156.591	17.399	2.22	0.215
Time.Temperature	18	86.431	4.802	0.61	0.633
Residual	27	211.908	7.848		
Total	59	458.364			

Table of means

Variate: RS %

Time	Temperature	Cold	Hot	Warm
	1	8.24	5.29	6.09
	2	8.68	7.66	9.57
	3	5.97	9.01	8.11
	4	10.70	9.29	7.04
	5	5.24	9.46	6.25
	6	12.50	9.74	10.81
	7	10.59	9.35	7.61
	8	9.24	7.72	10.03
	9	5.76	7.78	7.94
	10	11.10	12.90	11.26
Mean		8.80	8.82	8.47
s.e.d.		1.617	0.231	2.668
l.s.d.		4.393	0.993	7.206

CV%=32.2%

Appendix 2 (b) (ii) Analysis of variance of internode 6 RS concentration for sugarcane grown at three temperatures over the spring equinox.

Analysis of variance

Variate: RS %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	11.511	11.511	3.87	
Reps.Subject stratum					
Temperature	2	123.099	61.550	20.70	0.046
Residual	2	5.946	2.973	0.44	
Time	9	82.775	9.197	1.35	0.328
Time.Temperature	18	223.327	12.407	1.82	0.245
Residual	27	183.904	6.811		
Total	59	630.561			

Table of means

Variate: RS %

Time	Temperature	Cold	Hot	Warm
	1	9.19	12.88	13.24
	2	13.67	12.43	15.32
	3	11.80	10.09	9.19
	4	11.44	15.68	13.69
	5	10.09	14.05	6.94
	6	10.81	14.32	11.35
	7	9.82	14.86	13.51
	8	13.42	13.69	13.60
	9	9.82	13.51	12.70
	10	5.68	19.28	13.00
Mean		10.57	14.08	12.25
s.e.d.		1.507	0.545	2.535
l.s.d.		3.699	2.346	6.097

CV%=21.2

Appendix 2 (b) (iii) Analysis of variance of internode 10 RS concentration for sugarcane grown at three temperatures over the spring equinox.

Analysis of variance

Variate: RS %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	1.737	1.737	0.18	
Reps.Subject stratum					
Temperature	2	276.890	138.445	14.44	0.065
Residual	2	19.171	9.585	1.03	
Time	9	356.009	39.557	4.24	0.090
Time.Temperature	18	322.049	17.892	1.92	0.248
Residual	27	251.774	9.325		
Total	59	1227.631			

Table of means
Variate: RS %

Time	Temperature	Cold	Hot	Warm
	1	4.50	2.85	7.21
	2	4.80	7.21	9.76
	3	5.71	6.01	4.50
	4	6.01	4.50	10.36
	5	3.90	16.22	8.56
	6	6.61	15.62	13.82
	7	5.41	10.81	12.31
	8	9.01	13.81	8.71
	9	6.01	14.57	10.81
	10	6.76	17.27	11.56
Mean		5.87	10.89	9.76
s.e.d.		1.763	0.979	3.058
l.s.d.		4.579	4.212	7.539

CV%=34.6

Appendix 2 (c) (i) Analysis of variance of internode 4 fibre concentration for sugarcane grown at three temperatures over the spring equinox.

Analysis of variance

Variate: Fibre %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	4.192	4.192	2.02	
Reps.Subject stratum					
Temperature	2	116.195	58.098	27.93	0.035
Residual	2	4.160	2.080	0.25	
Time	9	207.594	23.066	2.80	0.163
Time.Temperature	18	149.718	8.318	1.01	0.467
Residual	27	222.113	8.226		
Total	59	703.973			

Table of means

Variate: Fibre %

Time	Temperature	Cold	Hot	Warm
	1	85.85	88.00	90.43
	2	86.65	88.51	87.05
	3	88.90	86.94	88.56
	4	81.31	86.26	86.60
	5	86.20	84.69	84.91
	6	81.13	84.79	84.34
	7	81.87	87.44	87.44
	8	80.12	87.44	86.37
	9	88.17	86.82	87.67
	10	77.81	83.84	86.37
Mean		83.80	86.47	86.98
s.e.d.		1.656	0.456	2.759
l.s.d.		4.410	1.962	7.218

CV%=3.3%

Appendix 2 (c) (ii) Analysis of variance of internode 6 fibre concentration for sugarcane grown at three temperatures over the spring equinox.

Analysis of variance

Variate: Fibre %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	53.07	53.07	9.11	
Reps.Subject stratum					
Temperature	2	481.16	240.58	41.29	0.024
Residual	2	11.65	5.83	0.28	
Time	9	200.95	22.33	1.06	0.399
Time.Temperature	18	215.80	11.99	0.57	0.682
Residual	27	566.66	20.99		
Total	59	1529.29			

Table of means

Variate: Fibre %

Time	Temperature	Cold	Hot	Warm
	1	80.72	83.60	80.81
	2	76.58	79.28	80.90
	3	77.57	82.25	84.32
	4	74.06	81.08	79.55
	5	73.52	78.38	81.08
	6	69.46	78.92	80.09
	7	74.42	79.01	80.36
	8	73.07	77.21	77.39
	9	72.88	78.47	80.54
	10	72.98	75.23	87.50
	Mean	74.52	79.34	81.25
s.e.d.		2.645	0.763	4.413
l.s.d.		6.648	3.284	10.921

CV%=5.8%

Appendix 2 (c) (iii) Analysis of variance of internode 10 fibre concentration for sugarcane grown at three temperatures over the spring equinox.

Analysis of variance

Variate: Fibre %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	1	0.33	0.33	0.01	
Reps.Subject stratum					
Temperature	2	119.69	59.84	1.77	0.360
Residual	2	67.43	33.72	2.72	
Time	9	914.66	101.63	8.19	0.021
Time.Temperature	18	418.48	23.25	1.87	0.239
Residual	27	334.90	12.40		
Total	59	1855.49			

Table of means

Variate: Fibre %

Time	Temperature	Cold	Hot	Warm
	1	74.17	72.08	74.78
	2	68.47	77.18	72.37
	3	69.22	66.67	77.48
	4	73.58	73.57	68.77
	5	60.66	71.77	68.32
	6	60.21	64.56	61.41
	7	63.06	64.86	62.01
	8	59.31	66.22	69.37
	9	69.52	67.72	72.52
	10	64.88	67.72	66.67
	Mean	66.31	69.23	69.37
s.e.d.		2.033	1.836	3.812
l.s.d.		5.034	7.900	8.853

CV%=5.2%