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RESEARCH PAPER

Source–sink relationships during early crop development influence earliness of sugar accumulation in sugarcane

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Abstract

In subtropical environments where sugarcane (*Saccharum* spp.) crops are frequently limited by the duration of the growth cycle, earliness in maturity is a key genotypic trait. Using the concept of source–sink relationships, we hypothesised that earliness is controlled by the dynamics of tillering (DT), which define sink strength early in the growth cycle. Five modern commercial sugarcane genotypes with similar sucrose yields and varying degrees of earliness in ripening were grown in the field over three years and their DT, dynamics of sucrose accumulation (DS), and source–sink relationships over time were characterised. Canonical correlations and principal components analysis revealed that DT explained 68% of the total variance in DS. Early ripening genotypes exhibited the shortest thermal time to the end of tiller mortality (θTil_{mort}), the lowest tiller survival and millable tiller number, and greatest sugar content at θTil_{mort} ($S_{conc,}Til_{mort}$). The rate and duration of the sucrose accumulation phase did not explain the genotypic variation either in final sugar content or in earliness when considered in isolation without taking into account the effect of $S_{conc,}Til_{mort}$. In the set of genotypes examined, the variation in final sucrose yield was most explained by the variation in stalk number. We conclude that the dynamics of tiller appearance and senescence modified the early source–sink relationships and thus determined the differential sucrose contents around θTil_{mort} and the earliness of maximal sugar accumulation. θTil_{mort}, which was closely associated with the 14-leaf phenological stage, emerged as a candidate trait to screen for genotypic variation in early ripening, crop cycle duration, and yield.

Keywords: Genotypic traits, sucrose accumulation dynamics, ripening, *Saccharum*, sugar yield, tillering.

Introduction

Commercial hybrid sugarcane (*Saccharum* spp.) is considered one of the most efficient crops for biomass production (Waclawovsky *et al.*, 2010) and its economic importance is currently increasing because of its convenience as a bioenergy crop (Sabatier *et al.*, 2015). Development of new adapted genotypes with increased sugar yield has been achieved by means of increases in biomass at harvest (Muchow *et al.*, 1994; Jackson, 2005; Acreche *et al.*, 2015; Acreche, 2017) or increases in the extractable sugar content (Edme *et al.*, 2005; Ming *et al.*, 2006). In subtropical areas where growing seasons for sugarcane crops are generally short (up to 9–11 months) due to low temperatures or freezing stress (Eggleston *et al.*, 2004), earliness of crop ripening is also an important selection criterion for new cultivars (Mariotti, 2001; Singh and Singh, 2004). Earliness in ripening can be defined as a genotypic attribute associated with the time to maximum sucrose content during the growing

season (Elibox, 2012b; Gilbert *et al.*, 2006). Earliness is not only a valuable trait in short-season areas but it is also important for bringing forward the beginning of the annual industrial milling process. Since at the beginning of the milling season the sugarcane sucrose content is usually low, early varieties can contribute to the profitability of the crop since farmers are paid on sucrose extracted rather than on cane biomass (Mamet *et al.*, 1996; Cox *et al.*, 1998). Although it is known that genotypic variability exists for both temporal patterns of sucrose accumulation and the trait of earliness in sugarcane (Elibox, 2012a; Cardozo *et al.*, 2014), the underlying physiological and genetic mechanisms still remain unclear.

A better comprehension of the genetic and physiological basis of earliness would be very beneficial for breeding programs aimed at increasing both sugar content and earliness. Regulation of sucrose metabolism and accumulation in sugarcane has been examined through many different approaches, including agronomical, physiological, biochemical, and genomic (Waclawovsky *et al.*, 2010; Moyle and Birch, 2013; ElSayed *et al.*, 2017). Previous research on sucrose accumulation that has studied contrasting high- versus low-sugar genotypes has considered the potential role of traits associated with patterns of photosynthate allocation between growth and storage functions (Irvine, 1975; Moore, 2005; Lingle *et al.*, 2009; Wang *et al.*, 2013; Marchiori *et al.*, 2014), foliar or stalk elongation rates (Lingle and Irvine, 1994; Mamet and Galwey, 1999; Inman-Bamber *et al.*, 2008), and the form, size, or number of culms at harvest (Sinclair *et al.*, 2005; Lingle and Tew, 2008; Lingle *et al.*, 2009). In addition, factors that reduce crop growth, such as chemical regulators that diminish the elongation rate of internodes (Li, 2004; Caputo *et al.*, 2007; Fong Chong *et al.*, 2010; van Heerden *et al.*, 2015), temperature, water stress, and timing of irrigation (Inman-Bamber, 2004; Singels *et al.*, 2005a, 2005b; Inman-Bamber *et al.*, 2010), all hasten the maturity of internodes (i.e. internodes reaching their final size) and increase the commercial sugar content at later crop stages. The results of these studies support the idea that carbohydrates are mainly diverted to storage rather than to generation of new tissues, crop respiration, or elongation of internodes. Variation in the maximum sugar content has also been associated with changing proportions of young and mature internodes as the crop develops and in response to environmental effects (Lingle and Smith, 1991; Lingle and Irvine, 1994; Inman-Bamber *et al.*, 2002; Lingle and Tew, 2008). Collectively, our current knowledge points towards to an apparent trade-off between structural growth and sucrose storage (Moore, 1995; Mamet and Galwey, 1999) and an important role of source–sink relationships as regulators of sucrose accumulation (Inman-Bamber *et al.*, 2009, 2010; McCormick *et al.*, 2009; Ribeiro *et al.*, 2017; Verma *et al.*, 2017).

Although different approaches have been applied to better understand the source–sink relationships in sugarcane, their effects on sucrose accumulation are still unclear (Moore and Botha, 2013). Several conceptual models have been developed; for example, the allosteric model currently used in studies dealing with enzyme regulation (McCormick *et al.*, 2009) proposes that sucrose accumulation can be limited by the negative feedback control of sinks on photosynthesis (sink limitation). However, experiments have shown that sucrose accumulation can rise to as high as 67% of the total dry matter of full, ripened culms even in low-sucrose varieties (Muchow *et al.*, 1996b; Inman-Bamber *et al.*, 2009; Waclawovsky *et al.*, 2010; Inman-Bamber, 2013), thus reinforcing the idea that partitioning rather than photosynthesis or plant growth is the limiting factor.

Genotypic variation in final sugar content in field-grown crops is well known. Some authors have proposed that it can be explained by a source–sink model that integrates plant photosynthesis (source), elongation rate per plant, and culm number (Inman-Bamber *et al.*, 2009). However, strong quantitative relationships between source–sink dynamics and yield or earliness in ripening have yet to be developed (Singels, 2013). A particular feature of most studies that have examined the sugar accumulation process is that they have analysed processes occurring during the so-called ripening phase (Whittaker and Botha, 1997; Inman-Bamber *et al.*, 2010). The time period when the ripening process begins is not necessarily associated with a fixed phenological stage (Bonnett, 2013). However, there is some evidence that indicates that increases in sucrose accumulation rates occur concomitantly with decreases in the internode elongation rate (Lingle and Irvine, 1994). This approach does not consider the potential role of sinks on the modulation of source–sink relationships early during the establishment of the crop (i.e. when the initial number or size of culms is rapidly changing). During an important time frame in the development of the crop prior to stem elongation, multiple processes modulate sink establishment, including tiller emergence and senescence (van Dillewijn, 1952; Kang *et al.*, 1990; Bell and Garside, 2005; Vasantha *et al.*, 2012, 2014; Bonnett, 2013).

In our current study, we hypothesised that genotypic differences in tillering dynamics (i.e. rates of tillers appearance and mortality) that define the final number of sinks (i.e. millable tillers) will exert a strong effect on the early source–sink relationship, and thus modulate the pattern of sucrose accumulation and the earliness trait of the crop. Our main objectives were to characterise the temporal progress of tillering, sucrose accumulation, and source–sink relationships, and to establish associations between early source–sink relationships and candidate genotypic traits involved in earliness, sucrose accumulation, and yield. We conducted field experiments using modern commercial sugarcane varieties with similar cane and sucrose yields but with different earliness in ripening. Over three seasons, crops were grown under the most common row spacing in order to allow the natural expression of genotypic patterns of tillering (emergence, tillering, and senescence of stalks).

Materials and methods

Experimental location

One field experiment (Exp 1) was established at Famaillá, Tucumán, Argentina (27° 01′ 78′′ S, 65° 22′ 59′′W, 368 m above sea level) to study the growth and yield determination of five sugarcane genotypes over three consecutive years. The site is located in one of the most important agro-ecological areas for sugarcane production in Argentina and the soil is an Aquic Argiudoll (Zuccardi and Fadda, 1985). The climate is subtropical with monsoon rainfall distributed from October to April that delivers a total of 1300 mm year^{-1}. Annual evapotranspiration is 1330 mm and mean incident solar radiation varies from 8.3 MJ m^{-2} d^{-1} to 18.8 MJ m^{-2}

d^{-1} for June and December, respectively. Mean minimum temperature ranges from –4 °C to18 °C while maximum temperature ranges from 18 °C to 32 °C.

Genotypes and crop husbandry

Five sugarcane hybrid genotypes (*Saccharum* spp.) were selected from a database of commercially released varieties (INASE, 2015) taking into account their similarity in yield and their contrasting rates of ripening (earliness). All the genotypes were considered as being adapted to the agro-ecological area for this study, although they were initially obtained from crosses elsewhere (Table 1).

At planting (22 August, 2008), 60-cm long stem cuttings with a mean of four buds were planted by hand at a soil depth of 20 cm with a target bud density of 14.3 m^{-2}. Plots consisted of five rows of 10 m long and 1.6 m apart. Crops were grown under rainfed field conditions; water limitation was not expected since the experimental site has access to a water table that fluctuates from 30–80 cm in depth, and because rainfall was close to the annual crop evapotranspiration. The crops were fertilised each year at the beginning of tillering with 45 kg N ha^{-1} in the first year and 90 kg N ha^{-1} in the following years. No additions of P and K were made because the soil type is typically rich in these elements and meets the nutritional crop requirements of the crop. In the first year, the crop that originated from the stem cuttings (hereafter referred to as plant crops, PC) was harvested on 9 September 2009. In subsequent years, crops from regrowth (i.e. regenerated from the subterranean buds; hereafter referred to as ratoon crops, RC) were harvested on 2 September 2010 (RC1) and 25 August 2011 (RC2). We refer to PC, RC1, and RC2 collectively as the 'crop class'.

Crop phenological development

Crop development and timing to critical phenological events as affected by genotype and temperature variation among growing seasons was expressed on the basis of thermal time (day-degrees, °Cd; Ritchie and Ne Smith, 1991).

To examine whether genotypes differed in their base temperature for development (T_{base}, the lowest temperature below which no development occurs) we conducted an additional experiment under controlled conditions (Exp. 2). Stem cuttings (setts) with a single bud were grown in trays with moist soil substrate in a chamber with controlled temperature and air humidity. Four temperature treatments (12, 21, 26, and 36 °C) were applied using a split-plot design with six repetitions. The main plot was temperature and the sub-plot was the genotype. Setts were examined daily and the time taken to reach a 1-cm long sprout (shoot emergence) in at least 50% of cuttings was recorded as an estimate of the initiation of development. For each genotype, a linear regression was fitted to the relationship between development rate (inverse of time to 1-cm sprout) and temperature; and T_{base} was then determined by extrapolation to the temperature below which development was zero (Ritchie and Ne Smith, 1991).

In addition, crop phenology in Exp 1 was monitored at 2-week intervals for five primary stalks randomly selected and tagged at the sprouting phase. At each sampling date, the thermal time and the number of fully expanded leaves (i.e. with visible ligules) were recorded.

For simplicity, thermal time was calculated as the summation of mean daily temperature minus T_{base} as long as the mean temperature was greater than T_{base} (Ritchie and Ne Smith, 1991, Bonhomme, 2000). We chose to use a single T_{base} (i.e. for development initiation; Exp2) rather than varying it for different phenological phases (e.g. tillering) to avoid potential confounding effects of changes in phyllochron, crop architecture, or growth patterns on the duration of phenological phases. The approximation of using a single value of T_{base} to represent development throughout the crop life cycle is common in sugarcane crop modelling, both as a fixed (e.g. APSIM-Sugarcane model) or a genotype-dependent (DSSAT/CANEGRO) parameter (Keating *et al.*, 1999; O'Leary, 2000; Dias *et al.*, 2019).

Temporal dynamics of tillering

Tiller emergence and senescence were recorded as the evolution of tiller density over time until harvest. The number of living tillers was monitored non-destructively in the three central rows of each experimental plot at 15-d intervals until the fourth month after crop emergence, and then at 30-d intervals until harvest. A tri-linear model describing stalk density as a function of thermal time (θ, °Cd) was fitted for each experimental plot:

$$Til_N = a + Til_{rate} \times \theta, \quad \text{for } \theta \leq \theta Til_{N,max} \tag{1}$$

$$Til_N = a + Til_{rate} \times \theta Til_{N,max} + M_{rate} \times (\theta - \theta Til_{N,max}), \quad \text{for } \theta Til_{N,max} < \theta \leq \theta Til_{mort}$$

$$Til_N = a + Til_{rate} \times \theta Til_{N,max} + M_{rate} \times (\theta Til_{mort} - \theta Til_{N,max}), \quad \text{for } \theta > \theta Til_{mort}$$

where Til_N is the number of live tillers per surface area (m^2), a is the function intercept, Til_{rate} and M_{rate} are the rates of tiller appearance and mortality, respectively. $\theta Til_{N,max}$ represents the thermal time from crop emergence to the moment when the maximum tiller number is reached, and $\theta Til_{N,mort}$ represents the thermal time from crop emergence to the moment when tiller mortality ends, i.e. when the final (surviving) number of tillers is reached (i.e. $Til_{N,final}$). A full list of variables is given in Table 2.

$\theta Til_{N,max}$ and θTil_{mort} were calculated for the times at which the maximum and final tiller numbers were recorded, respectively. Percent tiller mortality (%M) was estimated as $\%M = (Til_{N,max} - Til_{N,final}) / Til_{N,max} \times 100$. From Eq 1, the duration (in thermal time) of the tiller mortality phase (θM_{dur}) was estimated as $\theta Til_{mort} - \theta Til_{N,max}$.

The temporal dynamics of tillering (DT) for each genotype and crop class (i.e. plant or ratoon crops) were represented and analysed statistically by the following parameters: tillering rate (Til_{rate}), duration of the tillering

Table 1. *Origin (breeding programs and sites of selection), earliness of maturity (ripening behaviour) and typical harvest time for the five hybrid sugarcane (*Saccharum *spp.) genotypes used in this study*

Genotype	Breeding program and site selection	Earliness of maturity	Harvest month
INTA NA 89–686	Developed by INTA, Tucumán, Argentina, from seeds obtained by Chacra Experimental Santa Rosa Salta, Argentina (Sopena *et al.*, 2015).	Intermediate-late	Early July
L 91–281	Developed by INTA, Tucumán, Argentina, from advanced clones obtained by LSU, Lousiana, USA (Sopena *et al.*, 2015).	Late	Early August
LCP 85–384	Developed by USDA-ARS Houma, Louisiana, USA, advanced clones obtained by the Agricultural Research Service, Canal Point, Florida, USA (Milligan *et al.*, 1994).	Intermediate	Late June
RA 87-3	Developed jointly by INTA and EEAOC (UIMCA agreement), Tucumán, Argentina (Costilla *et al.*, 2013).	Extra early	Late May
TucCP 77-42	Developed by EEAOC, Tucumán, Argentina, from seeds obtained by the Agricultural Research Service, Canal Point, Florida, USA (Costilla *et al.*, 2013).	Early	Early June

Table 2. *List of variables*

Variable	Definition
CY	Cane yield
M_{rate}	tiller mortality rate
$\%M$	percentage tiller mortality = $(Til_{N,max}–Til_{N,final})/Til_{N,max}$x100
θM_{dur}	duration in thermal time of the tiller mortality phase
S_{conc}	sucrose concentration
$S_{conc,max}$	maximum sucrose concentration
$S_{conc,\theta Tilmort}$	sucrose concentration at θTil_{mort}
ΔS_{conc}	sucrose accumulation after $Til_{N,final}$ is reached
S_{dur}	duration in days of the sucrose accumulation phase
S_{rate}	sucrose accumulation rate
θS_{max}	thermal time from emergence to maximum sucrose
SY	sugar yield
T_{base}	base temperature for development
Til_N	number of living tillers
$Til_{N,final}$	final tiller number
$Til_{N,max}$	maximum tiller number
Til_{rate}	tillering rate
$\theta Til_{N,max}$	thermal time from emergence to maximum tiller number
θTil_{mort}	thermal time from emergence to the end of tiller mortality phase

phase ($\theta Til_{N,max}$), mortality rate (M_{rate}), the duration of the tiller mortality phase (θM_{dur}), and the duration of the complete tillering phase (i.e. from crop emergence to final tiller number, θTil_{mort}).

In this work, we introduce θTil_{mort} as a key phenological stage in crop development. It represents a reference developmental stage that allows a more robust comparison among genotypes and crop classes since it removes confounding effects associated with genotypic differences in phenology.

Source–sink relationships

The source–sink relationships during the period from emergence until θTil_{mort} were quantified as the quotient between the crop growth rate and the number of tillers. Crop growth rate was calculated as the gain of aerial dry biomass per day (g m^{-2} d^{-1}) and was estimated from models of dry biomass accumulation over time fitted to the data obtained from destructive harvests (Supplementary Figure S1 at *JXB* online). For biomass determination, five destructive harvests were carried out for the PC plants (between 45–271 d from emergence), six for the RC1 plants (87–304 d), and seven for RC2 plants (81–310 d). Each harvest consisted of all plants in an area of 1.6 m^2 taken from the central rows of each plot, ensuring that the sampled area was bordered by other plants. The samples were weighed immediately after harvesting and then split in two sub-samples. The first sub-sample was again immediately weighed and then subsequently dried at 60 °C until constant weight in order to determine the relationship between fresh and dry biomass, which was then used to determine the dry biomass of the total sample. The second sub-sample was used to determine sucrose content (see below).

Source–sink relationships were calculated on a daily basis throughout the growing cycle each year. We selected two critical time-points ($\theta Til_{N,max}$ and θTil_{mort}; eqn 1) to represent the early source–sink relationships and to perform treatment comparisons and multivariate analysis.

Temporal dynamics of sucrose accumulation

Sucrose determination was carried out at 15-d intervals during the period from early April (PC and RC1) or early March (RC2) until final crop harvest. When sampling dates coincided with biomass determinations, the second sub-sample referred to above was used. Samples of fresh stalks (taken from an area of 1 m^{-2} in each experimental plot) were milled, and the sucrose content was determined on a fresh-weight basis as the recoverable sugar after stalk extrusion (Meade and Chen, 1977). Sugar content was measured by crushing clean and complete culms (including the apical internodes) using an experimental mill of 150 kg cm^{-2} pressure, which had the capacity to extract 50% of the juice in the first sample crushing. After filtering and mixing the juice, two 250-ml samplings were analysed for total soluble solids (ºBrix; using a Smart-1 refractometer; ATAGO Co. LTD; Japan) and the % juice sucrose concentration was determined using a digital polarimeter (Polatronic NCE – Germany) after it had been clarified with lead subacetate. Sucrose concentration (S_{conc}) was expressed as a percentage of fresh weight of tillers. Cane yield (CY) was determined as the mean weight of millable tillers from the two last destructive samplings (performed in July and August each year) and expressed in tn ha^{-1}. Sugar yield (SY) was calculated as the product of cane yield and maximum sugar content.

Bilinear models were fitted to the relationship between sucrose concentration and time after θTil_{mort}:

$$S_{conc} = S_{conc,\theta Tilmort} + (S_{rate} \times t), \text{ for } t \leq S_{dur} \quad (2)$$

$$S_{conc} = S_{conc,\theta Tilmort} + (S_{rate} \times S_{dur}), \text{ for } t > S_{dur}$$

Where t is time (d) from the end of tiller mortality, and $S_{conc,\theta Tilmort}$ and S_{rate} represent the sucrose concentration at the end of tiller mortality and the sucrose accumulation rate), respectively. S_{dur} represents duration (d) of the true ripening phase, i.e. the time interval between $S_{conc,\theta Tilmort}$ and the point at which the maximum sugar content is reached, $S_{conc,max}$.

Sucrose accumulation after the end of tiller mortality (ΔS_{conc}) was calculated as $S_{conc,max}–S_{conc,\theta Tilmort}$.

The temporal dynamics of sucrose accumulation (DS) after the end of the tillers mortality was represented and analysed statistically by the following parameters: $S_{conc,\theta Tilmort}$, S_{rate}, S_{dur}.

Earliness (θS_{max}) was quantified as the thermal time that elapsed from crop emergence until the maximum sucrose content was reached. Hence, the highest θS_{max} values correspond to the latest-ripening genotypes.

Experimental design and statistical analysis

Genotypes were arranged in a randomized complete block design with three replications. Owing to the perennial nature of sugarcane, the same experimental plots were monitored over the three years of the study. We therefore refer to the 'crop class' (CC) to distinguish between the different years, i.e. the first-year growth from planted stem cuttings (PC), and the subsequent two years of re-growth of the ratoon crop, RC1 and RC2.

The dynamics of tillering (DT), sucrose accumulation (DS), and biomass over time were modelled using the Table Curve software (Jandel Scientific, 1991).

To test whether the dynamics of tillering and sucrose accumulation differed among genotypes (G) and crop classes (CC), and to examine potential G×CC interactions, we performed two types of analysis. First, ANOVA and means comparison of the parameters that describe DT and DS (eqns 1, 2) and the yield components ($Til_{N,max}$, $Til_{N,final}$, $\%M$, θM_{dur}, $S_{conc,max}$, ΔS_{conc}, CY, SY, θS_{max}, and source–sink relationships) were investigated through mixed linear models (Di Rienzo *et al.*, 2012). G, CC, and G×CC were set as fixed effects while blocks were considered as random effects. Comparison of means was performed with Fisher's test (α=0.05). Second, to test whether DT and DS as whole processes were different among genotypes and crop classes, a multivariate analysis of variance (MANOVA) was conducted (Camacho Rosales, 1990; Balzarini *et al.*, 2008; Warne, 2014). In this analysis, the parameters describing DT (eqn 1: a, Til_{rate}, $\theta Til_{N,max}$, M_{rate}, and θTil_{mort}) or DS (eqn 2: $S_{conc,\theta Til,mort}$, S_{rate}, and S_{dur}) were used as dependent variables while CC, G, and G×CC were considered as sources of variation. Mean vectors comparisons were performed using the Hotelling–Bonferroni test (Balzarini *et al.*, 2008).

To quantitatively determine the connection between DT and DS, a canonical correlation analysis was conducted using the parameters of the fitted models for culm number (independent variables) and sucrose accumulation (dependent variables) (Hotelling, 1936; Ye and Wright, 2010; Dai *et al.*, 2015).

Finally, a multivariate analysis of principal components was fitted to explore correlations among all variables, genotypes, and crop classes. Principal components analysis is a useful tool that has significant advantages over univariate or simple relationships because it allows analysis

and interpretation of the complete data set as a whole (Di Rienzo *et al.*, 2012). In the bi-plot, two variables have a high correlation when the angle cosine between their vectors is acute (positive correlation) or obtuse (negative correlation). Statistical analysis was performed using the Infostat software (http://www.infostat.com.ar/ (accessed 22 July 2019)).

Results

Meteorological variables during the crop growing cycles

Meteorological variables during the three growing seasons are shown in Fig. 1 and were typical of the historical weather in the region. Annual rainfall was 1200, 1174, and 1424 mm for the first, second, and third year, respectively. Frost stress (temperature<2 °C) occurred in all seasons. While mild frosts occurred in late August 2009 (PC), mild and severe frosts occurred in late June and mid-July in 2010 and 2011 (RC1 and RC2).

Crop phenological development

In this study, a first step was to characterise the genotypes in terms of their base temperature, T_{base}, for development initiation in order to use thermal time as a descriptor across seasons. Initiation of development includes metabolic processes that are strongly dependent on temperature and genotype when water is not limiting (Bonhomme, 2000). For all genotypes, developmental rates were positively and linearly related to temperature within the range 12–36 °C used in Exp. 2 to determine T_{base}. T_{base} did not differ significantly between four of the five genotypes, and they had a mean value of 10.3 °C (Table 3). Only the late genotype L 91–281 exhibited a significantly lower value (8.3 °C). These values of T_{base} were in close agreement with previous studies using genotypes adapted to subtropical areas of northern Argentina (Romero *et al.*, 2001), Florida (Sinclair *et al.*, 2004), Queensland (Liu *et al.*, 1998), and Brazil (Hanauer *et al.*, 2014). Other studies, however, have reported significantly higher values ranging from 16–18 °C, for example for South African and Brazilian cultivars (Smit, 2011; Dias *et al.*, 2019). Importantly, a T_{base} of ~10 °C is within the range currently used in simulation models (8–12 °C; Keating *et al.*, 1999; O'Leary, 2000). Potential deviations of T_{base} due to our experimental approximation would have therefore had a relatively small effect on thermal time calculations, and mainly only in those cases when daily mean temperature was lower than the temperature for zero development (Bonhomme, 2000).

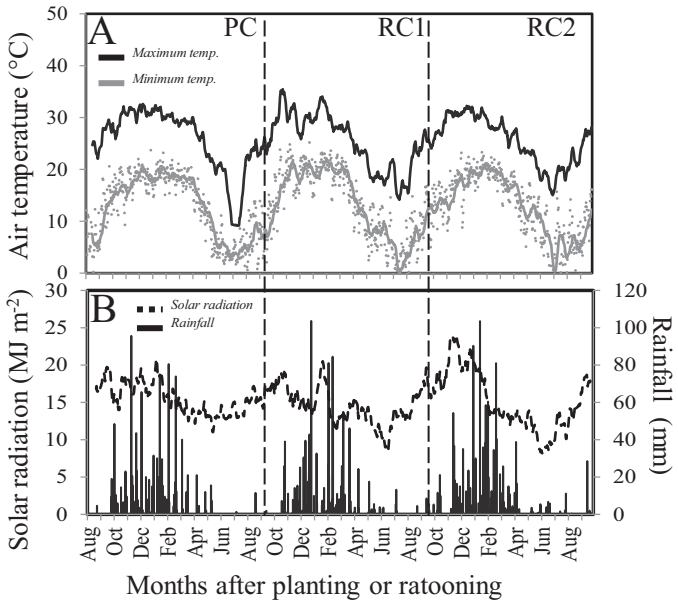

Fig. 1. Seasonal trend of (A) minimum and maximum temperature, and (B) rainfall and daily total solar radiation during three years in which the experiments were conducted at Famaillá, Argentina. PC, plant crop; RC1, first ratoon crop; RC2, second ratoon crop. The dashed vertical lines indicate the emergence of the ratoon crops.

Dynamics of tillering

For most variables associated with tillering dynamics, ANOVA showed significant G×CC interactions, which were basically explained by differences among plant and ratoon crops (Supplementary Table S1A). Hence, analyses were split according to crop class (plant and ratoons; Table 4). For all cases, tri-linear models fitted to plant or ratoon crops accurately (R^2>0.94, P<0.001) described the overall pattern of tiller appearance and senescence from crop emergence to the moment when the final tiller number was determined (θTil_{mort}, Fig. 2, Table 4). Multivariate analysis of variance to test for differences in DT among genotypes within crop classes (i.e. the complete set of parameters describing the evolution of tiller number over time in plant or ratoon crops; eqn 1, test H-B in Table 4) showed significant effects of genotype. Among ratoons, a year effect was found due to small changes in DT in the INTANA 89–686 genotype (Fig. 2). The ranking of genotypes in terms of their DT remained fairly stable across years, particularly within ratoons.

The components of DT (i.e. the individual parameters of the tri-linear models) showed that plant crops (PC) exhibited lower tillering and mortality rates, lower tiller and final stalk number, and lower cane yield than ratoon crops (RC, Table 4). Within ratoons, significant G×CC interactions were found for several variables, but they generally explained small percentages of variance in the data (~10%) and were mainly due to changes in the magnitude of responses. In contrast, genotype explained more than 90% of the data variance for most parameters.

The tillering phase (Fig. 2, Table 4) was significantly longer in PC than in RC ($\theta Til_{N,max}$=1365.2 °Cd and 1004.7 °Cd,

Table 3. *Base temperature (T_{base}) for development initiation determined for the five sugarcane genotypes used in this study*

Genotype	Base temperature (°C)
INTA NA 89-686	10.46±1.61 ab
L 91-281	8.25±1.41 b
LCP 85-384	9.61±0.62 ab
RA 87-3	10.83±0.89 a
TucCP 77-42	10.23±1.83 ab

Different letters indicate significant differences among means (Fisher's test, α=0.05).

Table 4. *Variables describing dynamics of tillering (DT) and yield components in of the five sugarcane genotypes used in this study*

Source of variation		DT (H-B test)[1]	T_{rate}	M_{rate}	$\theta Til_{N,max}$	θTil_{mort}	θM_{dur}	$Til_{N,max}$	$Til_{N,final}$	%*M*	*CY* (t ha^{-1})	Leaf number at θTil_{mort}
Crop class (CC)	**Genotype (G)**		(Tillers m^{-2} °Cd^{-1} 10^{-3})		(°Cd)			(m^{-2})				
Plant crop	INTA NA 89-686	B	7.83 e	–3.33	1453.66 a	2303.00 cd	849.33 b	11.02 d	8.47c	22.89 d	105.61 b	22.41 a
	L 91-281	A	8.99 c	–3.56	1475.23 a	2531.43 a	1056.2 ab	13.70 b	9.76 b	28.66 bc	121.30 a	19.74 b
	LCP 85-384	AB	11.65 a	–3.35	1347.03 b	2420.26 ab	1073.23 a	14.66 a	11.12 a	24.11 cd	117.20 ab	20.58 b
	RA 87-3	C	9.89 b	–4.52	1203.50 c	2191.03 d	987.53 ab	12.10 c	8.80 c	37.61 a	107.03 b	22.3 a
	TucCP 77-42	BC	8.70 d	–4.01	1346.36 b	2413.83 bc	1067.47 a	12.56 c	8.35 c	33.49 ab	123.56 a	22.17 a
Mean squares and significance of sources of variation												
	G	***	0.01***	0.00078	35073.97 ***	50256.44***	26775.85**	6.06***	4.03***	105.57***	202.15*	4.37*
	Error		0.024	0.001	3049.59	3887.94	12842.17	0.17	0.18	8.19	51.66	0.53
First ratoon crop (RC1)	INTA NA 89-686	B	20.64b	–9.93 a	1036.13 c	2118.19 d	1165.39 d	21.39 c	11.83 b	45.34 c	136.52 ab	13.79 a
	L 91-281	A	27.05a	–9.91 a	946.78 d	2430.27 a	1543.49 a	26.05 ab	11.24 b	56.36 a	122.12 c	12.88 b
	LCP 85-384	A	28.26a	–11.05 ab	944.12 d	2306.77 b	1362.58 b	28.72 a	14.60 a	49.89 b	140.60 a	14.16 a
	RA 87-3	C	13.10d	–16.82 f	1100.93 b	1842.96 h	758.03 h	21.63 b	9.57 d	56.03 a	116.60 c	13.75 a
	TucCP 77-42	B	18.59c	–14.53 e	1110.88 ab	2060.05 e	949.18 f	24.33 c	10.58 c	56.41 a	126.12 bc	14.41 a
Second ratoon crop (RC2)	INTA NA 89-686			–10.34 a	1011.14 c	2083.84 de	1082.70 e					
	L 91-281			–10.42 a	905.18 e	2414.47 a	1560.96 a					
	LCP 85-384			–11.84 bc	941.73 d	2207.12 c	1265.38 c					
	RA 87-3			–12.71 cd	1004.13 c	1904.63 gh	913.83 fg					
	TucCP 77-42			–13.38 de	1149.09 a	1997.54 f	848.45 g					
Mean squares and significance of sources of variation												
	G	***	0.025 ***	0.030***	58666.47***	267152.71***	540003.15***	57.02***	21.48***	150.21***	597.98***	2.754*
	CC	**	0.00065	0.004*	5119.78*	6809.23*	120.20	0.005	0.257	12.86	135.25	0.046
	G×CC	NS	0.0028	0.010***	3763.94**	5449.62**	17078.35***	1.486	0.322	7.47	158.46	0.385
	Error		0.001	0.00057	656.70	995.53	1961.12	0.948	0.242	12.32	76.02	0.504

[1] Hotelling–Bonferroni test for comparisons of tri-linear models fitted to DT (see Fig. 2). Different uppercase letters indicate significant differences among mean vectors (α=0.05).
Different lowercase letters indicate significant differences among means (Fisher's test, α=0.05). Significant overall effects of G, CC, and G×CC interactions were determined using MANOVA and the H-B test: *P<0.05; **P<0.01; ***P<0.001. NS indicates no significant difference according to the H-B test.
See Table 2 for list of variables.

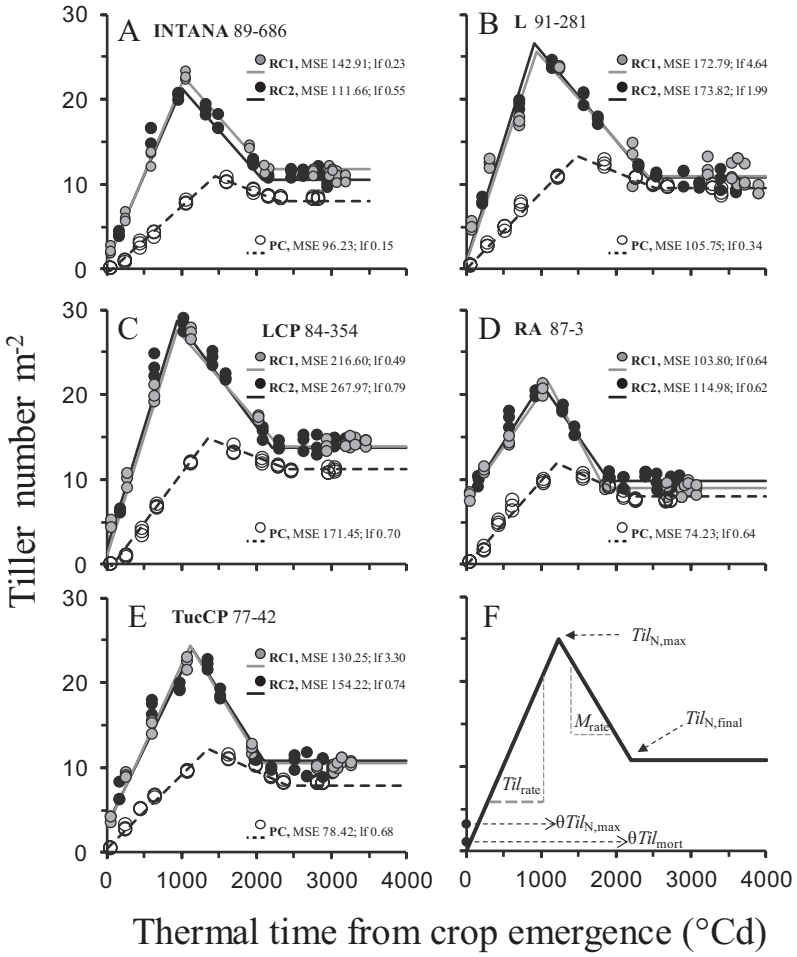

Fig. 2. Temporal dynamics of tillering in the five sugarcane genotypes (A–E) over the three years of the experiments. PC, plant crop; RC1, first ratoon crop; RC2, second ratoon crop. Symbols indicate observed values and lines show the fitted tri-linear models. (F) Schematic representation of the model and the biological significance of parameters. The base temperature varied among genotypes (Table 3). All fitted models were significant (R^2>0.94; P≤0.001). MSE, mean square error of the model; lf, lack of fit. The model parameters and comparisons among genotypes are shown in Table 4.

respectively). In contrast, duration of the tiller senescence phase was longer in RC (θM_{dur}=1145 °Cd) than in PC (θM_{dur}=1010 °Cd).

Final tiller number was mainly associated with the tillering rate and explained 61% of the variance in cane yield across all the data (Table 4). Across crop classes, the genotype RA 87-3 had the lowest $Til_{N,final}$ and LCP 85–384 had the highest.

Genotypic differences in DT were associated with θM_{dur}, $Til_{N,max}$, Til_{rate}, and θTil_{mort}. θTil_{mort} was a strong variable in defining differences among the genotypes. In ratoon crops, θTil_{mort} coincided with the 14-leaf crop phenological stage (Table 4) in four of the five genotypes, the only exception being L 91-281 where θTil_{mort} occurred at the 13-leaf stage.

Dynamics of sucrose accumulation

For most variables related to sucrose accumulation during the period from θTil_{mort} to θS_{max}, significant effects of G, CC, and G×CC interactions were found (Supplementary Table S1B) and hence analyses were split according to crop class (Table 5). In all cases, bilinear models adequately described the dynamics of sucrose accumulation (DS; Fig. 3, Table 5). Multivariate analysis of variance of DS (H-B in Table 5) showed significant effects of G and CC but no significant G×CC interactions, and hence the ranking of genotypes in terms of their patterns of sucrose accumulation was considered stable across years.

Within each crop class, most of the variance for individual variables associated with DS was explained by the genotype effect. Even when they were significant, G×CC interactions explained less than 10% of the total variance of the data (Table 5).

Genotype RA 87-3 showed the highest sucrose content at θTil_{mort} ($S_{conc,\theta Til,mort}$) but was one of the slowest genotypes in terms of sucrose accumulation rates. $S_{conc,max}$ varied between 9–12 g g^{-1} and was affected by both CC and G. There were no G×CC interactions for $S_{conc,max}$ across the three years of the study (Supplementary Table S1B), and it explained <10% of the data variance in ratoons. $S_{conc,max}$ was higher in ratoons than in plant crops. Genotypes RA 87-3 and TucCP 77-42 had the highest and lowest $S_{conc,max}$, respectively.

A significant negative relationship between initial sugar concentration and sucrose accumulation rate was found for both crop classes [S_{rate} = 0.124(±0.004) –0.01(±0.001) × $S_{conc,\theta Til,mort}$; R^2=0.74]. Sucrose gain (i.e. ΔS_{conc}, the difference between $S_{conc,max}$ and $S_{conc,\theta Til,mort}$) was accurately described by S_{dur} (R^2=0.85) or S_{rate} (R^2=0.71). A multiple regression with both variables (ΔS_{conc} = –6.87(±0.79) + 92.47(±5.93) × S_{rate} + 0.071(±0.01) × S_{dur}; R^2=0.88) accurately described the sucrose gain until maximum content was achieved.

Table 5. *Early source–sink relationships and dynamics of sucrose accumulation (DS) in stems of the five sugarcane genotypes used in this study*

Source of variation		DS (H-B test)[1]	$S_{conc,\theta Til,mort}$	$S_{conc,max}$	S_{rate} (g g^{-1} FW stalks 100 d^{-1})	S_{dur} (d)	ΔS_{conc} (= $S_{conc,max}$–$S_{conc,\theta Til,mort}$)	SY (t ha^{-1})	θS_{max} (°Cd)	Early source–sink relationship (g tiller^{-1} d^{-1})	
Crop class (CC)	**Genotype (G)**		(g g^{-1} FW tillers × 100)							at $\theta Til_{N,max}$	at θTil_{mort}
Plant crop	INTA NA 89-686	C	3.12 e	10.11 b	0.0971 a	73.56 b	7.01 a	10.68 c	2832.6 c	0.886 bc	2.888 a
	L 91-281	A	4.73 c	10.55 b	0.0674 b	93.78 a	5.59 b	12.77 a	3275.1 a	0.727 c	2.694 a
	LCP 85-384	C	3.92 d	10.55 b	0.0901 a	72.51 b	6.65 a	12.35 ab	2932.1 b	0.757 c	2.083 b
	RA 87-3	B	7.43 a	11.23 a	0.0447 c	91.90 a	3.80 c	12.03 ab	2644.4 d	1.129 a	2.409 ab
	TucCP 77-42	C	5.66 b	9.21 c	0.0712 b	57.47 c	3.54 c	11.39 bc	2768.3 c	0.910 b	2.596 a
Mean squares and significance of sources of variation											
	G	***	8.352***	1.677***	0.00134***	914.721*	7.615***	2.047*	171307.7***	0.0762***	0.2803***
	Error		0.041	0.131	0.00002	12.181	0.136	0.529	2140.5	0.0044	0.0515
First ratoon crop (RC1)	INTA NA 89-686	A	3.79 e	11.07 cd	0.0762 bcd	96.32 a	7.27 c	14.98 b	2932.6 c	0.630 b	1.978 a
	L 91-281	AB	3.93 de	11.44 bc	0.0825 ab	84.54 b	7.51 bc	14.25 b	3400.9 a	0.504 d	1.708 bc
	LCP 85-384	A	3.33 f	11.97 a	0.0885 a	101.09 a	8.65 a	16.74 a	3165.4 b	0.506 d	1.415 c
	RA 87-3	C	5.89 a	12.06 a	0.0665 e	95.13 b	6.17 d	13.87 c	2730.3 e	0.715 a	2.256 a
	TucCP 77-42	C	4.46 bc	10.05 e	0.0677 e	84.30 bc	5.59 e	12.72 cd	2830.1 d	0.587 bc	1.749 b
Second ratoon crop (RC2)	INTA NA 89-686		4.42 c	10.86 d	0.0815 abc		6.44 d		2954.9 c	0.563 c	
	L 91-281		3.99 d	11.93 a	0.0883 a		7.94 b		3372.8 a	0.504 d	
	LCP 85-384		4.33 c	11.83 a	0.0741 cde		7.5 bc		3080.4 b	0.521 d	
	RA 87-3		5.52 a	11.73 ab	0.0739 cde		6.22 d		2665.1 f	0.719 a	
	TucCP 77-42		4.61 b	10.13 e	0.0732 de		5.52 e		2787.5 d	0.542 cd	
Mean squares and significance of sources of variation											
	G	***	3.479***	3.667***	0.00027***	273.211**	6.544***	13.26***	427157.2***	0.0442***	0.6121***
	CC	***	0.657***	0.003	0.00003	230.297*	0.752**	1.99	116731.7**	0.0026*	0.0347
	G×CC	NS	0.428***	0.157***	0.00013**	16.291	0.640***	1.55	2321.87*	0.0019**	0.0939
	Error		0.043	0.052	0.00002	13.632	0.078	1.05	169.1	0.0005	0.0776

[1] Hotelling–Bonferroni test for comparisons of bi-linear models fitted to DS (see Fig. 3). Different uppercase letters indicate significant differences among mean vectors (α=0.05). Different lowercase letters in indicate significant differences among means (Fisher's test, α=0.05). Significant overall effects of G, CC, and G×CC interactions were determined using MANOVA and the H-B test:*P<0.05; **P<0.01; ***P<0.001. NS indicates no significant difference according to the H-B test.
See Table 2 for list of variables.

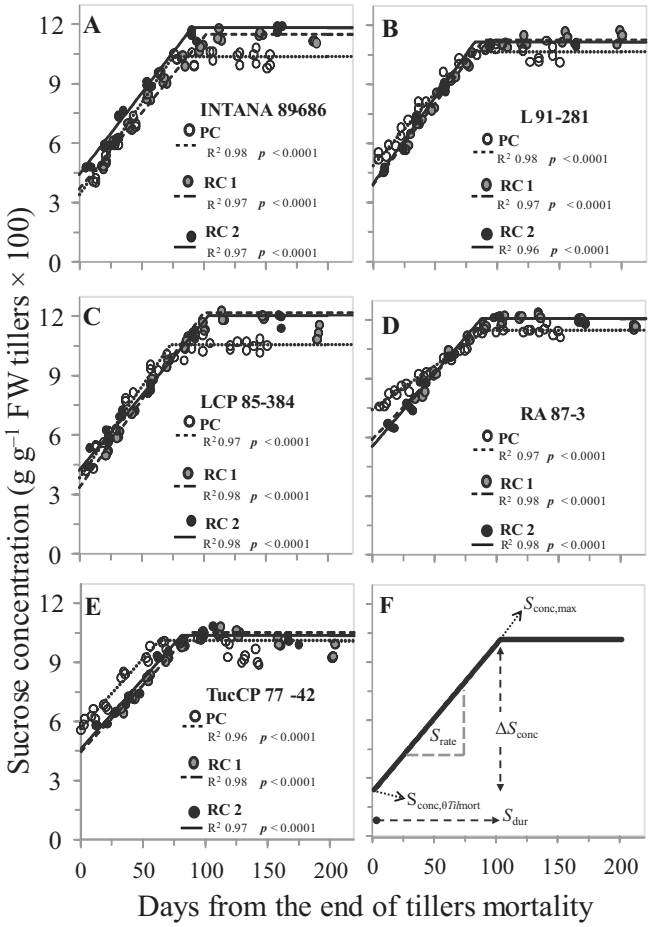

Fig. 3. Dynamics of sucrose accumulation in stems (on a FW basis) of the five sugarcane genotypes (A–E) over the three years of the experiments. PC, plant crop; RC1, first ratoon crop; RC2, second ratoon crop. Symbols indicate observed values and lines show the fitted bilinear models. (F) Schematic representation of the model and biological significance of parameters. The parameters and comparisons among genotypes are shown in Table 5.

Thermal time until the maximum sucrose content (θS_{max}) was used to quantify earliness, i.e. higher values of θS_{max} were associated with the later-maturing genotypes. θS_{max} varied between 2644 °Cd and 3458 °Cd and significant effects of G and CC were found. The G×CC interaction was significant but the genotypic effect explained more than 93 % of the total variance in earliness (Table 5). Genotypes RA 87-3 and TucCP 77-42 were found to be the earliest while L 91-281 and LCP 85-384 were the latest.

Source–sink relationships

Daily source–sink relationships showed two well-defined phases (Fig. 4). During the early stage until θTil_{mort}, source–sink relationships varied considerably due to changes in the number of tillers (see Fig. 2). The value of the relationship (expressed as g tiller^{-1} d^{-1}) was lowest at $\theta Til_{N,max}$ and became highest and constant at θTil_{mort} (Fig. 4). Among the crop classes, the source–sink relationships were highest in the plant crops. Genotypic effects always explained most of the variance in the data (Table 5). In ratoon crops, the rank of genotypes in terms of their early source–sink relationship remained fairly stable across years, with L91-281 and LCP 85-384 exhibiting the lowest values and RA 87-3 showing the highest values at both $\theta Til_{N,max}$ and θTil_{mort}.

Association between traits of tillering dynamics, early source–sink relationships, and sucrose accumulation

Canonical correlation analysis between DT and DS showed that the two dynamics were strongly associated. Two canonical correlations were significant (P<0.001) and explained 68% and 37% of the total variance in DS. Associations between DT and DS were more significant when canonical correlation analyses were run for each crop class (R^2 =0.96 and R^2 =0.88 for PC and RC, respectively).

A multivariate analysis of principal components was performed to explore the association among all variables that explained the dynamics of stalk establishment, sucrose accumulation, source–sink relationships, and yield (Fig. 5). We chose this type of analytical technique because the resulting bi-plot allows for the simultaneous integration of factors and the identification of associations between variables and genotypes (Balzarini *et al.*, 2008). When considering all the data, the first two principal components explained 76.6 % of the total variation (Fig. 5A). The first principal component split the data according to crop classes, while the second split genotypes according to their earliness and sucrose concentration. Plant crops (circles located to the left in Fig. 5A) exhibited the lowest tillering rates and final tiller number and had higher early source–sink relationship values in comparison to ratoon crops. Ratoons exhibited the highest tillering rates, final stalk number, and sucrose yield. Among the genotypes, RA 87-3 was the earliest maturing (i.e. it had the lowest θS_{max} vector) and showed the highest $S_{conc,}\theta_{Til,mort}$.

Bi-plots constructed separately for plant and ratoon crops increased the analytical power, and explained 71.7 % and 80.6 % of the data variation, respectively. In both crop classes, the first principal component split genotypes by their earliness in maturity (Fig. 5B, C). The earliest-maturing genotypes RA 87-3 and TucCP 77-42 exhibited the highest source-sink relationship values, the highest $S_{conc,\theta Til,mort}$, and the lowest tillering rate (indicated by the obtuse angles among these variables). Genotypes L 91-281 and LCP 85-384 were the latest-maturing (highest θS_{max}) and this trait was positively associated with θTil_{mort} and tillering rate and negatively associated with early source–sink relationships and $S_{conc,\theta Til,mort}$ (Fig. 5). Cane yield was explained by the final number of millable tillers ($Til_{N,final}$) and the duration of the ripening phase (S_{dur}). $S_{conc,\theta Til,mort}$ was not a variable that defined $S_{conc,max}$, and neither did it explain sugar yield. Interestingly, $S_{conc,\theta Til,mort}$ was negatively correlated with θTil_{mort}, earliness, and tillering rates (obtuse angles among these variables), but it was positively correlated with early source–sink relationships. In terms of individual relationships among variables, $S_{conc,\theta Til,mort}$ was tightly associated with the early source–sink relationship both at $\theta Til_{N,max}$ (Table 5; $S_{conc,\theta Til,mort}$ = 0.13(±0.62) + 7.35(±1.06) × Source–sink; R^2=0.63, P<0.001) and at θTil_{mort} ($S_{conc,\theta Til,mort}$ = 2.38(±0.56) + 1.09(±0.30) × Source–sink; R^2=0.32, P=0.0012).

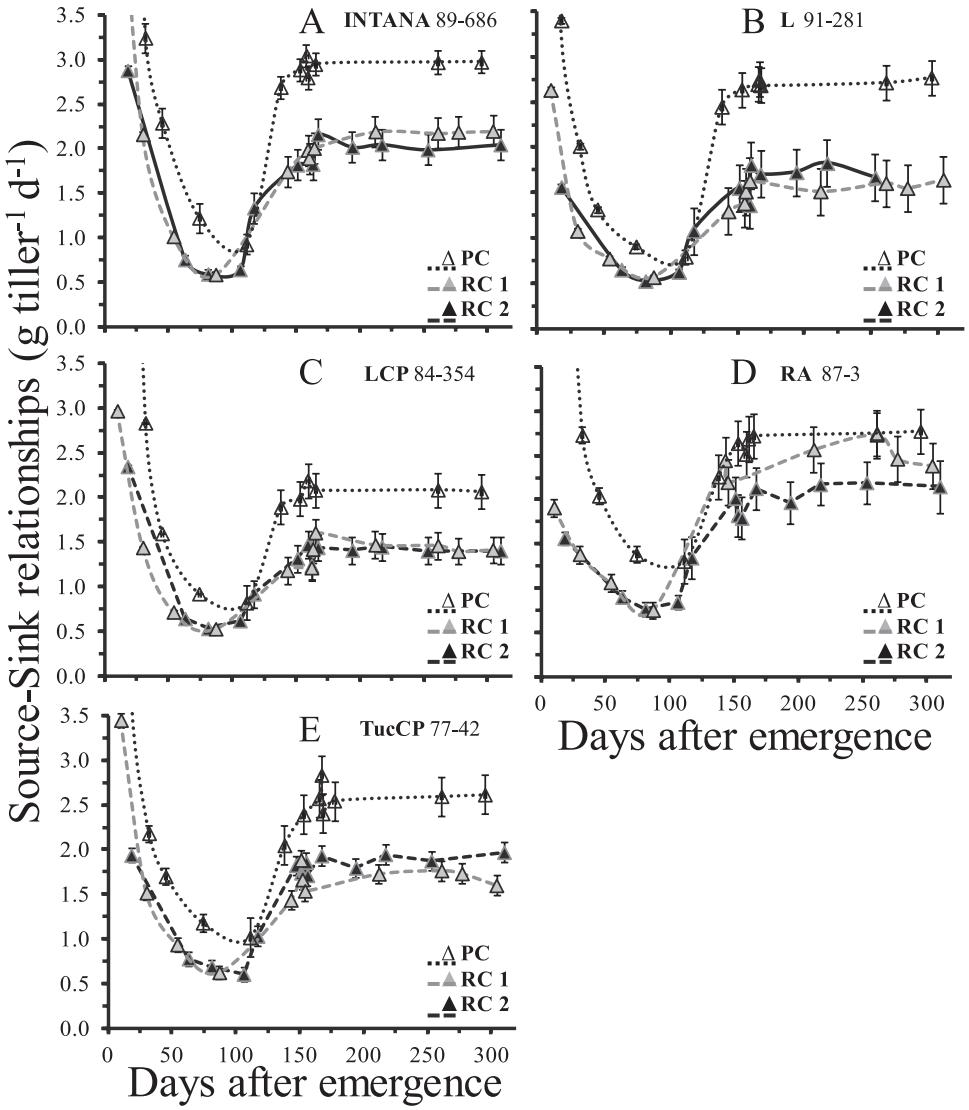

Fig. 4. Evolution of daily source–sink relationships of the five sugarcane genotypes over the three years of the experiments. PC, plant crop; RC1, first ratoon crop; RC2, second ratoon crop. Data are means (±SE) of three replicates.

Environmental variation among years (e.g. temperature) resulted in small changes in three of the four variables describing sucrose accumulation (Table 5). However, these variations did not modify the ranking of genotypes in terms of their earliness in maturity (Table 5, Fig. 5). It is likely that the use of thermal time instead of calendar days and the estimation of $S_{conc,\theta Til,mort}$ at θTil_{mort} would have integrated the potential effects of environmental factors (mainly temperature). Our new source-sink variable most robustly integrated potential environmental effects on crop growth and yield components.

Discussion

In subtropical environments where sugarcane crops are frequently limited by the duration of the growing season, breeding efforts have to be oriented towards improvements in annual biomass production (Mariotti *et al.*, 2006) and fast photoassimilate partitioning towards storage organs, i.e. sucrose accumulation in stems (Irvine, 1975; Lingle *et al.*, 2009; Wang *et al.*, 2013; Marchiori *et al.*, 2014). In addition, earliness in maturity is considered a desirable trait because of its contribution to early harvesting and milling (Mamet *et al.*, 1996; Elibox, 2012b; Cardozo and Sentelhas, 2013; Cardozo *et al.*, 2014). In this study, we aimed at establishing links between early and late processes that define yield components (final tiller number, sucrose content) in five modern high-yielding sugarcane genotypes. Field experiments were conducted over three consecutive years (i.e. plant and ratoon crops) in a subtropical environment where rainfall generally meets the crop water demand. Models for tillering dynamics (initiation and senescence), sucrose accumulation and, daily source–sink relationships were constructed to evaluate the genotypic differences. As a first step, we examined the dynamics of tillering (DT) and the dynamics of sucrose accumulation (DS) separately by means of bi- or tri-linear models (Figs 2, 3, Tables 4, 5). We chose this type of model instead of polynomial (Inman-Bamber, 1994; Gilbert *et al.*, 2006) or logistic ones (Muchow *et al.*, 1996a) in order to quantitatively compare responses in terms of genotype and crop classes (i.e. plant versus ratoon),

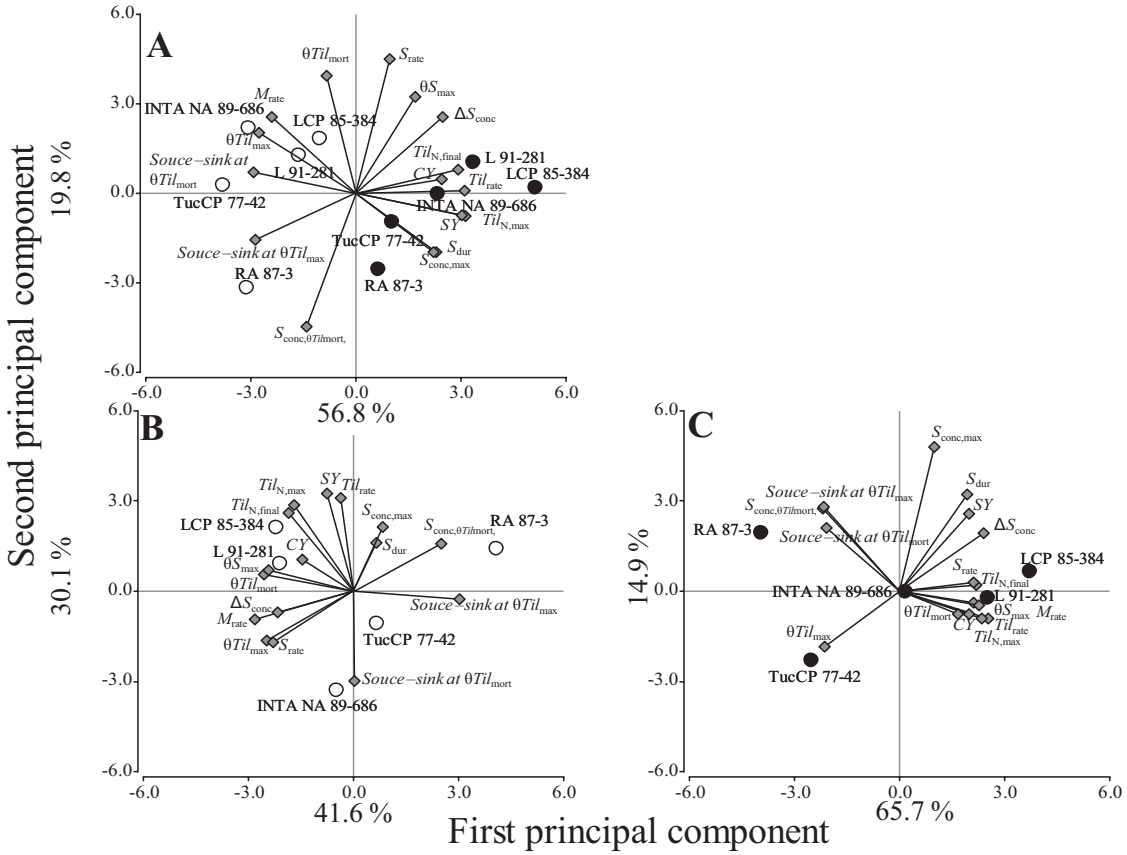

Fig. 5. Biplots of principal component analysis showing relationships between genotypes (circles) and variables (diamonds) that describe tillering dynamics and sucrose accumulation in the five sugarcane genotypes used in this study over three years of experiments. (A) Biplot showing the two crop classes, namely plant crops (open circles) and ratoon crops (filled circles). Individual biplots are shown for plant crops (B) and ratoon crops (C). The parameters and comparisons among genotypes are shown in Tables 4 and 5.

and to seek for meaningful parameters associated with traits and processes that could be used in correlation analysis or for the screening of new genotypes.

For both DT and DS, strong effects of crop class were found, and hence analyses were split according to plant and ratoon crops. In ratoons, the genotypic effect was always the most important factor in explaining variance in the parameters describing DT and DS since no inter-annual effects were found. When analysed as a whole (see the H-B test in Tables 4, 5), DT and DS discriminated genotypes by their consistent performance over this 3-year study. For instance, the genotypes LCP 85-384 and L 91-281 outperformed the other in terms of their tillering ability and stalk numbers but were consistently the latest genotypes in terms of their ripening (i.e. they showed the highest θS_{max}).

Among the DT variables, θTil_{mort} emerged as a significant trait that was strongly associated with tillering rate and final tiller number (Fig. 5). In ratoon crops, θTil_{mort} coincided with the 14-leaf stage in four of the five genotypes (Table 4). Although more genotypes need to be screened to establish this as a firm relationship, the lack of significant G×CC interactions supports our findings. Because the 14-leaf stage is rather easy to predict using the thermal time approach, we propose that θTil_{mort} be considered as a key phenological stage when performing genotypic screening. This proposal is made in the context of several studies in sugarcane that have emphasised the relevance of comparing similar sampling dates across seasons to avoid potential effects of crop phenology (Jackson *et al.*, 1995a, 1995b) and to avoid non-repeatable G×CC interactions (Kang *et al.*, 1987; Ramburan *et al.*, 2011, 2013; Ramburan, 2014).

Our observation of a reasonably constant θTil_{mort} across years and low variance due to G×CC interactions also supports the hypothesis that θTil_{mort} can be consider as a secondary genotypic trait (Table 4). Although effects of crop class and years were confounded in our study, key variables showed low variance in the G×CC interactions (e.g. Til_{rate}, $Til_{N,final}$, source–sink relationships; Tables 4, 5).

The final tiller number that was set at θTil_{mort} was a key component in defining cane yield in our set of modern, high-yielding genotypes. The strong genotypic effect for tiller number was well explained by differences in the duration and rate of the tillering phase (Fig. 5C). Interestingly, an apparent trade-off between length of the tillering phase beyond 1000 °Cd after crop emergence and yield was evident (Figs 2, 5, Table 4). Genotype L91-281 exhibited the largest θTil_{mort} but this trait did not translate to a higher $Til_{N,final}$ or *CY*. We hypothesise that an exceptionally large θTil_{mort} could be associated with asynchronous development of culms. Research by Bell and Garside (2005) demonstrated that synchrony in the development of tillers correlates with higher yields due to a greater proportion of primary stems at harvest. Inverse relationships between tillering rates and the time to maximum

tillers number (Til_{rate} and $\theta Til_{N,max}$, Fig. 5) in our study supported this idea. Hence, high tillering rates coupled with an intermediate θTil_{mort} appeared to be promising traits to select for high stalk numbers and yield. With our current approach, we were not able to determine which processes determined the rates and durations of the tillering and mortality phases. It is likely that canopy features that control attenuation of solar radiation and light quality at the basal level of the crop might be involved (Singels and Smit, 2002, 2009; Marchiori *et al.*, 2010). Given that gains in yield over time have been more frequently associated with increases in biomass rather than in sugar content (Jackson, 2005; Acreche *et al.*, 2015; Acreche, 2017), a better comprehension of the processes that define stalk number should contribute to future progress in improving yields. Interestingly, stalk number has been suggested to be a highly heritable trait (Kang *et al.*, 1990; Aitken *et al.*, 2008). Among the DS variables, $S_{conc,\theta Til,mort}$ appeared to be a second key genotypic trait that explained earliness, although not maximum sugar content ($S_{conc,max}$) or final sugar yield (Figs 3, 5). The value of $S_{conc,\theta Til,mort}$ set at ~5 months after emergence was 45% (early genotypes) or 30% (late genotypes) of the final maximum sucrose measured at harvest. $S_{conc,\theta Til,mort}$ was tightly associated with early source–sink relationships, suggesting that the dynamics of stalk generation controlled sugar accumulation at the beginning of the true ripening phase. Considering both components of the source–sink relationship, sink activity (i.e. tillering rate and stalk number) was a stronger variable in explaining $S_{conc,\theta Til,mort}$, and hence earliness, than crop growth rate.

The approach that we took for the calculation of early source–sink relationships is, to our knowledge, new and highlights the potential of $S_{conc,\theta Til,mort}$ and θTil_{mort} as traits to aid in the prediction of sucrose yields early in the growing cycle. We also demonstrated that source–sink relationships vary during crop development in two well-defined phases (Fig. 4). Before θTil_{mort}, the continued changes in sink number modified the daily source–sink relationship, and this process controlled $S_{conc,\theta Til,mort}$ (Figs 4, 5). After θTil_{mort}, the source–sink relationship became constant, although it differed among the genotypes.

Previous research in sugarcane has generally analysed processes that control sucrose accumulation during the maturation phase itself (Muchow *et al.*, 1996a; Singels *et al.*, 2005a; Lingle and Tew, 2008; Inman-Bamber *et al.*, 2009) or investigated development, growth, and sucrose accumulation in either separate (Bell and Garside, 2005; Allison *et al.*, 2007) or integrated ways (O'Leary, 2000). Similarly, a vast body of literature has previously considered the idea that genotypic and environmental effects on sucrose accumulation in sugarcane can be explained by the source–sink balance between current photosynthesis and culm growth (Singels and Bezuidenhout, 2002; Inman-Bamber *et al.*, 2009, 2010; McCormick *et al.*, 2009). For example, Inman-Bamber *et al.* (2009) proposed that final sucrose content depends on how sinks (e.g. in the form of tiller number and plant elongation rate) exert an additional demand for structural assimilates. However, mechanisms for sucrose accumulation have been difficult to quantify because there is not a well-defined phenological stage when accumulation starts and stops (Bonnett, 2013). Our study offers an analytical approach that is able to demonstrate how several aspects of sink determination are involved in defining early source–sink relationships, and thus influence earliness and sucrose yield. Further research focusing on the genetic basis and variability of some traits (e.g. θTil_{mort}, early source–sink relationship, and $S_{conc,\theta Til,mort}$) may be useful for improving selection efficiency and in bringing about genetic gains for early genotypes without potential yield penalties.

It is worth emphasising that the genotypes we used in this study all attained typically high maximum sucrose contents (10.05–12.06 % in ratoon crops, Table 5) and good sugar yields (12.7–16.2 t ha^{-1}, Table 5) through different strategies. The earliest genotypes RA 87-3 and TucCP 77-42 attained a high $S_{conc,\theta Til,mort}$ early in the season, while others exhibited large S_{rate} (L91-281) or S_{dur} (INTA NA 89–686). Interestingly, maximum sucrose content was not associated either with the individual variable S_{dur} or with S_{rate}, because $S_{conc,\theta Til,mort}$ was a third critical variable that defined sucrose accumulation (Fig. 3). An unexpected result that merits further research was the negative relationship between $S_{conc,\theta Til,mort}$ and the rate of sucrose accumulation during the ripening phase (Fig. 5). While $S_{conc,\theta Til,mort}$ seemed to reflect the ability of the crop in establishing early and rapidly changing source–sink relationships, both S_{dur} and S_{rate} would mainly reflect late source–sink relationships, i.e. during the ripening phase. During this phase, the carbon demand for structural growth has been fulfilled and hence surplus assimilates in the source would be well represented through S_{dur} and S_{rate} (Singels and Bezuidenhout, 2002).

In summary, we have quantitatively demonstrated the links between multiple traits that describe tiller production and senescence, and early sucrose accumulation and yield in five modern subtropical sugarcane genotypes. The use of a multivariate analysis rather than examining individual relationships allowed the multiple links between early and late processes to be determined, together with how they differed among the genotypes. The earliness trait was clearly explained by both $S_{conc,\theta Til,mort}$ and θTil_{mort}, variables which in turn were positively or negatively associated with early source–sink relationships. In particular, θTil_{mort} emerged as a genotypic trait involved in the determination of early source–sink relationships. No apparent trade-offs for some key traits for yield determination were found. For instance, $S_{conc,max}$ or S_{dur} were not associated (either negatively or positively) with $S_{conc,\theta Til,mort}$, earliness, or tiller number. Hence, this suggests that it is possible to select/breed for improved genotypes by means of the simultaneous selection of those traits (i.e. $S_{conc,\theta Til,mort}$ and S_{dur}).

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Analysis of variance for the main variables that describe the dynamics of tillering and sucrose accumulation for the five genotypes.

Fig. S1. Crop biomass accumulation in the five genotypes in each of the three years of experiments.

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