The effect of transient and continuous drought on yield, photosynthesis and carbon isotope discrimination in sugar beet (\textit{Beta vulgaris} L.)

A. Monti$^1$,*, E. Brugnoli$^2$, A. Scartazza$^2$ and M. T. Amaducci$^1$

$^1$ Department of Agroenvironmental Science and Technologies (DiSTA), Viale G. Fanin 44, I-40127 Bologna, Italy
$^2$ CNR, Institute of Agroenvironmental Biology and Forestry (IBAF), Via Marconi 2, I-05010 Porano (TR), Italy

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Abstract

Stable carbon isotope discrimination ($\Delta^{13}C$), photosynthetic performance ($A$), dry matter accumulation (DW), and sucrose yield ($Y_s$) of sugar beet were evaluated in a glasshouse experiment under transient (TS) and permanent (PS) water stress. $A$ was significantly reduced under drought, to an extent depending on stress duration. The reduced $A$ was strictly associated with a low DW and $Y_s$, the later being 42% lower in PS than control plants (C). Restoring water steeply increased $A$ and the associated leaf traits (RWC, leaf water potential etc.), but the increase of $Y_s$ was negligible. Therefore, the negative effects of severe water stress in the early growth period, though reversible on gas-exchange and most leaf traits, can drastically reduce $Y_s$ of sugar beet. Furthermore, $A$ seems not to be effective in predicting sucrose accumulation, although it was very effective in detecting the occurrence of plant water stress. The $A/C_i$ model was used to assess the photosynthetic adjustments to continuous or transient drought by calculating the photosynthetic parameters $V_{cmax}$ and $J_{max}$ and then compared with $\Delta^{13}C$. Mesophyll conductance ($g_m$) was estimated by comparing $\Delta^{13}C$ measured on soluble sugars and gas-exchange data. This approach confirmed the expectation that $g_m$ was limiting $A$ and that there was a significant drop in [CO$_2$] from the substomatal cavities and the chloroplast stroma both in favourable and drought conditions. Therefore, the carbon concentration at the carboxylation site was overestimated by 25–35% by conventional gas-exchange measurements, and $V_{cmax}$ was consistently underestimated when $g_m$ was not taken into account, especially under severe drought. Root $\Delta^{13}C$ was found to be strictly related to sucrose content (brix%), $Y_s$ and root dry weight, and this was especially clear when $\Delta^{13}C$ was measured on bulk dry matter. By contrast, leaf $\Delta^{13}C$ measured in soluble sugars ($\Delta_s$) and bulk dry matter ($\Delta_{dm}$) were found to correlate weakly to brix% and yield, and this was not surprising as the integration time-scale of leaf $\Delta_s$ and $\Delta_{dm}$ were found to be shorter than that of root $\Delta^{13}C$ in bulk dry matter. The effect of water stress on diffusive and biochemical limitations with different integration times ranged from 1 d (leaf $\Delta_s$) to more than 1 month (root $\Delta_{dm}$).

Key words: $A/C_i$ curve, drought, gas exchange, mesophyll conductance, mesophyll limitation.

Introduction

It is well documented that drought is the major limiting factor for sugar beet yield (Pidgeon \textit{et al.}, 2005). By contrast, the sensitivity of sugar beet to water deficit has been poorly studied (Ober \textit{et al.}, 2003), and little is known about physiological traits which can be used to assess the effects of drought. Understanding the physiological responses to water stress and the traits associated with it is therefore strongly desirable to develop mechanistic forecasting...
systems for sugar beet growth. Furthermore, water stress reduces the yield to such an extent that it is likely to depend on stress duration and phenological stage. For instance, Milford and Lowlor (1975) showed that short-term water stress may lead to a significant yield loss, and this occurred particularly when drought affected young plants (Brown et al., 1987). The authors also pointed out that early water shortage, although transient, can significantly reduce the adventitious roots, and this was found negatively to affect the photosynthetic capacity even when favourable conditions were restored (Brown et al., 1987).

Therefore, the main objective of the present study was to quantify the dry matter and sucrose yield losses of sugar beet under transient and continuous drought. Moreover, the photosynthetic adjustments were assessed as a possible quantitative indicator of yield loss or as a reliable gauge of the occurrence of drought stress. In order to do this, the model developed by Farquhar et al. (1980) was used, the so-called ‘A/C\textsubscript{i} curve’, coupled with leaf and root carbon isotope discrimination measured on bulk dry matter and soluble sugars.

The A/C\textsubscript{i} model has been widely used and tested in many studies at different levels of complexity, ranging from cell to global levels (for a recent review see von Caemmerer and Quick, 2000), and it has been incorporated as the mechanistic basis in many physiological models on the photosynthetic response to acclimation (De Pury and Farquhar, 1997; Pitman, 2003; Manter and Kerrigan, 2004). However, a limitation of the A/C\textsubscript{i} model is that, in most applications, it considers the mesophyll conductance to CO\textsubscript{2} (g\textsubscript{m}) as infinite. That is to say that the [CO\textsubscript{2}] at the substomatal cavities (C\textsubscript{i}) and at the carboxylation site (C\textsubscript{c}) would not be significantly different. Conversely, in some cases this assumption was found not to be correct (Evans and Loreto, 2000, and references therein), and this assumption can lead to the overestimation of C\textsubscript{c} at the carboxylation site and the underestimation of V\textsubscript{cmax} calculated by the conventional A/C\textsubscript{i} model. As a consequence, the decrease in A caused by a stress might be erroneously attributed to biochemical limitations, while it may simply be due to increased diffusive limitations (Wilson et al., 2000, and references therein). Therefore, in this experiment g\textsubscript{m} was assessed and its influence upon parameters calculated by the A/C\textsubscript{i} model was quantified.

Among several methods used to determine g\textsubscript{m}, the isotopic method was found to perform better over a wide range of situations (Evans et al., 1986; Loreto et al., 1994). This is based on the well-described discrimination effects against 13\textsubscript{C} (\Delta\textsuperscript{13}C\textsubscript{i}), occurring during photosynthesis in C\textsubscript{3} plants, which is closely related to the ratio of chloroplastic to atmospheric CO\textsubscript{2} concentrations (C\textsubscript{i}/C\textsubscript{a}) (Farquhar et al., 1982). C\textsubscript{i} may be calculated by comparing the \Delta\textsuperscript{13}C observed in a plant (\Delta\textsubscript{obs}), with that predicted from conventional gas-exchange measurements based on the value of C\textsubscript{i}/C\textsubscript{a} (Evans et al., 1986; Lloyd et al., 1992). The value of \Delta\textsubscript{obs} can be accurately determined using \Delta measured in leaf-soluble carbohydrates, as shown previously in other herbaceous and tree crops (Brugnoli et al., 1998; Scartazza et al., 1998). Therefore, in this research g\textsubscript{m} was determined from the \Delta\textsuperscript{13}C of leaf-soluble sugars as given by Evans et al. (1986).

Furthermore, previous studies have shown that \Delta\textsuperscript{13}C of soluble sugars may also reflect short-term changes in the C\textsubscript{i}/C\textsubscript{a} ratio due to their fast turnover (Brugnoli and Björkman, 1992; Brugnoli et al., 1998). Conversely, the \Delta\textsuperscript{13}C of structural carbon gives a long-term integrated value of the C\textsubscript{i}/C\textsubscript{a} ratio, which is generally weakly affected by short-term environmental fluctuations. Therefore use was made of the \Delta\textsuperscript{13}C of soluble sugars and bulk dry matter to assess the photosynthetic adjustments under transient and longer term water stress, and \Delta in soluble carbohydrates or bulk dry matter of different biomass parts was found to be effective in estimating \Delta integrated over different time-scales, in sugar beet.

Materials and methods

Plant material and growth conditions

The experiment was carried out in a greenhouse at CNR-IBAF, Porano (42° 41’ N, 12° 06’ E), Italy. Plants were grown in pots under sunlight and the natural photoperiod from 31 March to 12 July. Irradiance, relative humidity, and air temperature inside the greenhouse were measured every 15 min by an automatic meteorological station placed near the pots. Mean day/night temperatures were 28.1±2.8 °C and 18.4±2.3 °C. During the day the relative humidity ranged from 22% to 50%; while the peak of photosynthetic photon flux density (PPFD) was 1160 to 1630 μmol photon m\textsuperscript{-2} s\textsuperscript{-1}.

Plants of Beta vulgaris L. (var. Monodoro) were sown in large pots (50 l) containing a mixture of garden soil and sand (1:3 v/v). The sowing was done on 31 March, using five seeds per pot. All plants had expanded the two cotyledon leaves 7 d after sowing (CEBEST 25; Misonne, 1989). CEBEST (CEentesima BEet STage) is a growing scale from 0 to 89 mainly based on the number of fully expanded leaves and this was used to classify the phenological stages of sugar beet. A nutrient solution as described by Hewitt and Smith (1975) with ammonium nitrate instead of potassium nitrate was supplied weekly to each plant.

Three different water regimes were applied according to a completely randomized design with six replications. Before the drought period all plants were kept well-watered until CEBEST 25 (stress imposition). Treatments and stress imposition were: the control (C) that was always kept close to the optimal water status, i.e. at 90% of field capacity (FC); transient drought stress (TS), where the irrigation was reduced to allow the soil water content to decrease to 30% of FC for a period of 37 d (DAS, days after stress imposition), and subsequently, irrigation was reinstated (CEBEST 50) so that the soil water content recovered to 90% of FC; permanent drought stress (PS), where the soil moisture was maintained permanently at 30% of FC starting from CEBEST 25.

At CEBEST 25 (DAS 1), three plants for each plot were sampled, while the remaining two plants were maintained until CEBEST 50 (37 d later), the stage when drought stress in TS was stopped. Leaves, petioles, and tap root of harvested plants were weighed and some relevant biometric traits (tap root length and diameter, leaves number etc.) were measured.
Leaves on which gas-exchange had previously been measured were collected at 16.00 h, frozen in liquid nitrogen, and then stored at −30 °C until carbon isotope analysis. Carbon isotope analysis was performed on leaf bulk dry matter and soluble sugars on 5, 38, and 71 DAS. Carbon isotope composition of roots bulk dry matter and soluble sugars was analysed at the final harvest (71 DAS). Over the 71 DAS the mean water additions were 33, 22, and 16 l pot−1 for C, TS, and PS, respectively.

Gas-exchange measurements
Gas-exchange measurements were taken during the experiment and A/Ci curves were generated during the treatment periods using a portable open photosynthesis system (CIRAS-1, PP-Systems, Hertfordshire, UK). The leaf area of photosynthetically active leaves was measured using the LI-3100C area meter (Li-Cor Inc.). Measurements were repeated several times per day from 09.00 h to 14.00 h on the youngest fully expanded leaves with similar exposition. During measurements the air flowing entering the chamber was 250±4 cm3 min−1. PPFD (natural light) 1500±21 μmol m−2 s−1, relative humidity <50%, and leaf temperature between 26±1.8 °C. A/Ci curves were measured with short-term measurements (~6–8 min for each data point) as the response of photosynthesis (A) to varying Ci and hence Ci, under high light. Measurements started at Ci of 350 μmol mol−1 then CO2 in the incoming air was decreased to about 50 μmol mol−1. Subsequently, the CO2 concentration was progressively increased up to about 2000 μmol mol−1 using a CO2 mass flow controller. Each data of A was measured at a given CO2 concentration waiting until measurements were stable (2% of coefficient of variation was considered tolerable). Generally, 10 data points were collected for each A/Ci curve. Vcmax and Jmax were determined according to the biochemical model by Farquhar et al. (1980). Briefly, the model represents the mechanistic relationship between leaf net assimilation rate (A, the dependent variable) and intercellular CO2 concentration (Ci, the driving variable). The model relies on the concept that A is co-limited by three processes: (i) the activity and kinetics of Rubisco (W); (ii) the regeneration of ribulose-1, 5-bisphosphate (RuBP) supported by the electron transport chain (Wp); and (iii) the availability of triose phosphates for the Calvin cycle (Wp). That is:

\[
A = \left(1 - \frac{\Gamma^*}{\Gamma^*_{\text{opt}}}\right) \min(W_c, W_i, W_p) - R_d
\]  

(1)

where Γ* is the CO2 compensation point in the absence of dark respiration; \(R_d\) is the so-called ‘day respiration’, i.e. the mitochondrial respiration during illumination. In this experiment Γ* and \(R_d\) were determined on control plants using the direct method described by Laish (1977) as the intercept of four A/Ci curves generated at different low PPFDs (from 110 to 420 μmol m−2 s−1) at Ci values below 15 Pa. During the measurements \(T_{\text{leaf}}\) was kept at 26 °C and the relative humidity near 50%. \(\Gamma^*\) and \(R_d\) was 3.46±0.09 Pa (CO2) and −0.86±0.09 μmol m−2 s−1, respectively. The model runs iteratively: \(V_{\text{cmax}}\) was initially estimated by solving the \(W_c\) curve, then \(J_{\text{max}}\) and triose phosphate utilization (TPU) were obtained by solving the \(W_i\) and \(W_p\) curves. It was assumed here that the threshold between the \(W_i\) and \(W_p\) curves was 20–25 Pa (Wullschleger, 1993), although Manter and Kerrigan (2004) showed inter- and intra-specific variations for the transition from the Rubisco-limited to the RUBP-regeneration limited parts of the A/Ci curve.

Sugar extraction and carbon isotope analysis
Carbon isotope composition was measured both on soluble sugars and on bulk-leaf material on the same leaves previously used for gas-exchange measurements on DAS 5, 38, 53, and 71. Extraction of leaf sugars was done as described by Bruognli et al. (1988). Briefly, a subsample of 80 mg of leaf was boiled at 80 °C for 30 min in 35 ml of aqueous ethanol 80% (v/v); then the extract was centrifuged for 3 min at 12 600 g at 40 °C. This procedure was repeated four times. Then, the supernatant, mostly formed by water, amino acids, organic acids, and soluble sugars, was collected and subjected to sequential purification of soluble sugars. The pellet was extracted in 80% ethanol and analysed for carbon isotope composition. Soluble sugars were purified using two resins Dowex-50 (H+) and Dowex-1 (Cl−) in sequence. The eluates after filtration were freeze-dried and analysed for carbon isotope composition.

Plant material was combusted using an elemental analyser (model NA 1500, Carlo Erba, Milan, I), coupled with a dual-inlet mass spectrometer (model SIRA II, GV-Instruments, Middlewich, UK). Carbon isotope ratios of samples were compared with that of a working standard CO2 calibrated against the international standard Vienna-Pee Dee Belemnite (VPDB). Possible fractionation during combustion was controlled by using a working standard sucrose (Sigma Chemicals, USA) with a carbon isotope composition (δ13C) of −25.09±0.06‰. Carbon isotope composition of the air CO2 inside the glasshouse was −7.5‰. Carbon isotope discrimination (\(\Delta_{\text{iso}}\)) was calculated as:

\[
\Delta_{\text{iso}} = \left[\frac{\delta_i - \delta_p}{1 + \delta_i}\right]
\]

(2)

where \(\delta_i\) and \(\delta_p\) are the isotope compositions of source air and plant material, respectively, relative to VPDB.

Estimation of mesophyll conductance and \(C_c\)
Mesophyll conductance \(g_m\) was calculated according to Evans et al. (1986) and modified by Lloyd et al. (1992) as the discrepancy between \(A\) of soluble sugars (\(\Delta A\)) and the \(\Delta\) expected on the basis of gas-exchange measurements (\(\Delta E\)) and assuming that \(C_c\) is equal to \(C_i\):

\[
g_m = \frac{(b - e - a)\frac{\Delta A}{\Delta E}}{\Delta C_i - \Delta C_s}
\]

(3)

where \(b\) is the discrimination associated to carboxylation reactions (28.2 per mil \(\%_{\text{iso}}\); Farquhar et al., 1982), \(e\) is the fractionation occurring during the dissolution of CO2 (1.1%); Mook et al., 1974), \(a\) is discrimination caused by the diffusion of CO2 in the liquid phase (0.7%\%_{\text{iso}} O’Leary, 1981), \(C_s\) is the CO2 concentration in the ambient air, \(f\) is the discrimination during photorespiration (8%\%_{\text{iso}} Griffiths et al., 1999).

The expected \(\Delta A\) was calculated as given by the model of Farquhar et al. (1982):

\[
\Delta A = a + (b - a)\frac{C_i}{C_s}
\]

(4)

where \(a\) is the fractionation occurring during diffusion in air (4.4%\%_{\text{iso}}; Craig, 1953); \(C_s\) is the CO2 concentration in the substomatal cavities obtained by gas-exchange measurements, and assuming that \(C_i = C_s\).

Mesophyll conductance was then used to determine the CO2 concentration at the site of carboxylation (\(C_i\)):

\[
C_i = C_s - \Delta A / g_m
\]

(5)

Statistical analysis
The experimental error appeared normally distributed and the variances homogeneous (Kolmogorov–Smirnov’s and Bartlett’s tests, respectively). Therefore, all the measured and derived data were subjected to the analysis of variance with repeated measurement (ANOVA). This method takes into account the variation among individuals, allowing concentration of the effect of the treatments rather than the differences between individuals. When ANOVA showed significant differences among means, Tukey’s test with a
probability of 5% was used to separate the means. Pearson’s correlation coefficient was used to test the significance of correlations.

Results

Effect of water stress on growth

The difference in TDW between C and stressed plants was proportional to the stress duration. At the end of S-1, TDW of C was 16% higher than stressed plants, while at the end of S-2 it was 34% and 52% higher than TS and PS, respectively (Fig. 1). About 75% of these differences were explained by the tap root.

As expected, the sucrone content (brix%) was higher in stressed plants, and this was very clear at the end of S-2 when PS showed brix% values of 20.7 compared to TS (18.7) and C (17.7) (Fig. 2). Conversely, leaf number was similar in PS and C (19.0 and 18.3 respectively), while it was significantly higher in C (22.3).

The difference in A between C and stressed plants was appreciable 1 week after the imposition of water stress (Fig. 2). A being, on average, 120% higher in C than in stressed plants. RWC decreased concurrently with A from 81% in C to about 65% in PS only 2 d after water stress imposition (Fig. 2). As expected, the leaf water potential (Ψl) also decreased from −0.8 ± 0.2 MPa in C to −1.7 ± 0.3 MPa in PS (Fig. 2). VPD was relatively constant in C, while it progressively increased in stressed plants up to 3.7 kPa by the end of S-2.

The negative effect of water stress appeared fully reversible. Restoring water sharply increased A, RWC, and Ψl. Thus C and TS showed similar values during S-2, while PS was significantly lower (Fig. 2).

Carbon isotope discrimination

Carbon isotope discrimination of bulk dry matter (Δdm) was generally higher than that of soluble sugars (Δs) (Table 1); this was only partly true in roots where Δdm and Δs often appeared very similar. However, a correlation between Δdm and Δs was observed, irrespective of organs and treatments (Fig. 3).

Leaf Δs enabled metabolic changes to be detected within 1–5 d of stress imposition or after restoring irrigation (Table 1). Differences in leaf Δs between TS and PS were

Fig. 1. Sucrose yield (Ys) and dry weight (DW) of tap roots, petioles and leaves at 1, 38, and 71 d after stress imposition (DAS). Different letters at the top of the histograms mean significant differences on total DW among treatments, while different letters inside the histograms indicate differences in DW of single biomass organs among treatments (P ≤0.05). C, PS, and TS mean control, transient, and permanent stress, respectively. S means stressed plants as PS and TS were not yet differentiated at the end of S-1. Values between brackets indicate the sucrose content expressed as brix%.

Fig. 2. Course of leaf photosynthesis (A), relative water content (RWC) and leaf water potential (Ψl) under transient (TS) and permanent stress (PS) compared with control plants (C). DAS are the days after stress imposition. Vertical bars indicate standard deviations of means. S-1 and S-2 indicate the two periods of transient water stress and recovering water, respectively. Different letters mean significant differences among treatments (Tukey’s test for P ≤0.05).
Table 1. Carbon isotope discrimination determined on bulk dry matter (Δdm/‰) and soluble sugars (Δs/‰)

C, control plants (never stressed); TS, transient drought stress; PS, permanent drought stress. DAS are the days after stress imposition. Δdm, carbon isotope discrimination of bulk dry matter; Δs, carbon isotope discrimination of soluble sugars. Stress imposition occurred on DAE 25, CEBEST 25. Vertically different letters indicate significant differences among values (LSD Fisher’s test, P < 0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaves</th>
<th>Leaves</th>
<th>Leaves</th>
<th>Leaves</th>
<th>Roots</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Δdm</td>
<td>Δs</td>
<td>Δdm</td>
<td>Δs</td>
<td>Δdm</td>
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<tr>
<td>C</td>
<td>21.6 a</td>
<td>19.9 a</td>
<td>20.5 a</td>
<td>19.2 a</td>
<td>20.3 a</td>
</tr>
<tr>
<td>TS</td>
<td>—</td>
<td>—</td>
<td>19.7 b</td>
<td>18.2 b</td>
<td>19.7 b</td>
</tr>
<tr>
<td>PS</td>
<td>21.1 a</td>
<td>18.4 b</td>
<td>19.6 b</td>
<td>17.1 c</td>
<td>19.3 b</td>
</tr>
</tbody>
</table>

Fig. 3. Relationship between carbon isotope discrimination determined on soluble sugars (Δs) and bulk dry matter (Δdm) of leaves (blank symbols) and roots (dark symbols). C, TS, and PS indicate control plants (well-watered plants), transient, and permanent drought stress, respectively. Broken line represents one to one ratio.

As expected, leaf Δs was much more effective than leaf Δdm in predicting C/Ca ratio as given by the model by Farquhar et al. (1982). Slopes and intercepts of the regressions between leaf Δs and C/Ca were close enough to the expected values (Table 2). Conversely, the correlations between leaf Δdm, as well as root Δs (data not shown), and C/Ca were always weak with low regression coefficients and intercepts larger than expected from the model (Table 2). Furthermore, during crop ageing the biochemical component of the discrimination process (slope of the regression between leaf Δs and C/Ca) decreased from 21.0 to 17.7 after 71 d of treatment (Table 2). Therefore, the discrimination during carboxylation became more and more underestimated if compared with the model by Farquhar et al. (1982).

Leaf Δdm and leaf Δs did not correlate with A/E, i.e. the instantaneous water use efficiency, conversely they appeared significantly correlated with A/s, i.e. the intrinsic water use efficiency (Fig. 4). This seems to indicate that stomatal closure was significant in response to drought and that it caused the decrease of the C/Ca ratio and, consequently, A. However, the decrease of C/Ca did not parallel that of transpired water, and this was explained by the increasing VPD values in drought conditions.

Root Δ showed an overall better correlation with sucrose content (brix%) and yield (Ys) than leaf Δ (Fig. 5). Root Δdm and Δs were very closely related with brix% values and reasonably related with Ys, while leaf Δdm and Δs appeared moderately related with brix% and not significantly associated with Ys (Fig. 5). Root Δdm was also very closely correlated with root dry weight (RDW), and this was also observed for leaf Δdm, although it was at a significantly lower extent (Fig. 6). By contrast, root Δs was much less correlated with RDW (r=0.56*, data not shown) than root Δdm, while leaf Δs was insignificantly related with RDW (r=0.21).

Mesophyll conductance

Mesophyll conductance (g_m) was negatively influenced by severe water stress, on average being 0.33 mol m⁻² s⁻¹ in C and TS (not statistically different) and 0.16 mol m⁻² s⁻¹ in PS. Moreover, while g_m marginally changed in C, it progressively decreased in PS from 0.27 (DAS 5) to 0.12 mol m⁻² s⁻¹ (DAS 71), indicating a possible ‘water-regime × plant-age’ interaction on g_m. Mesophyll conductance was found to be significantly lower in stressed conditions, however, when the optimal water condition was restored (S-2 phase), TS showed g_m values very similar
in this study, related with viable when water stress was released. These results show that drought can be very detrimental to the market-beet plants. According to Nevins and Loomis (1970), the negative effects of drought mostly concerned the marketable organ (i.e. the tap root), which was, respectively, 30% and 40% lower in TS and PS than C. Similarly, the sucrose yield was significantly lower in TS (−35%) and PS (−41%) than C.

Despite the fact that leaf photosynthetic capacity was strictly associated with water availability, it seems not to be effective in predicting sucrose yield. A was steeply modulated by water conditions, but when A increased following the stress interruption (S-2 phase), it mostly caused the growth of the leaf apparatus (+34% in TS than PS at the end of S-2), followed by the structural part of the tap roots and, possibly, the adventitious roots. By contrast, the increase of sucrose accumulation during S-2 phase was negligible, and this was in agreement with the priority order of assimilate allocation in sugar beet given by Snyder and Carlson (1978).

The literature on drought tolerance of sugar beet in relation to plant age is very scarce and only in one case has the effect of stress period on yield and photosynthesis been studied (Brown et al., 1987). The authors showed that major yield loss occurred when water stress affected young plants. This is in agreement with this study’s results, however, the authors also observed a lower photosynthetic capacity than the potential even when the favourable water conditions were restored, and they concluded that this was somewhat related to the reduction of the root apparatus caused by water stress. In this research the adventitious roots were not measured but it is possible that the high assimilation rates of TS during the S-2 phase were addressed to recover the root loss during the drought period (S-1). This would partially explain the negligible increase of sucrose yield despite the high photosynthetic rates in TS after water restoration.

In summary, it may be concluded that even a transient water stress, when it occurs in the early growth stages, may drastically reduce the tap root and sucrose yield in sugar beet. Leaf photosynthesis was not related with sucrose yield, but it was very effective in detecting the occurrence of water stress over a short period, and this may be crucial in preventing the loss of sucrose.

Carbon isotope discrimination measured in leaf soluble sugars (∆s) or bulk dry matter (∆dm) can be a valuable tool in revealing photosynthetic adjustments over short- or long-time periods (for a recent review see Brugnoli and Monti et al., 1982).

Table 2. Statistical analysis of parameters of the linear regression between carbon isotope discrimination (Δ) and C1 to C3 ratio (Farquhar et al., 1980)

<table>
<thead>
<tr>
<th>Regression parameter</th>
<th>DAS 5</th>
<th>DAS 38</th>
<th>DAS 53</th>
<th>DAS 71</th>
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<td></td>
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<td>∆s</td>
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<tr>
<td>R²</td>
<td>0.39</td>
<td>0.74</td>
<td>0.55</td>
<td>0.65</td>
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<tr>
<td>Slope</td>
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<td>n.s.</td>
<td>21.0</td>
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<tr>
<td>Intercept</td>
<td>16.5</td>
<td>n.s.</td>
<td>6.3</td>
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</table>

Fig. 4. Correlation between leaf carbon isotope discrimination determined on bulk dry matter (∆dm) and instantaneous (∆/E) and intrinsic (∆/gs) water use efficiency. Equally, leaf ∆e was only correlated with ∆/gs, (r = −0.72**).

to C indicating that changes in gs were somewhat reversible when water stress was released.

Mesophyll conductance was found to be significant related with A (Fig. 7). Within the range of data measured in this study, gs caused a significant decrease in CO2 partial pressure from the intercellular air spaces to the sites of carboxylation. This caused an overestimation of the CO2 concentration inside the chloroplast (Ci) from 25% in control plants to 35% in permanent stress, as calculated by conventional gas-exchange measurements. Consequently, Vcmax was also significantly overestimated by the A/Ci model, and spurious differences in Vcmax occasionally resulted between C and PS when gs was ignored (see DAS 29 and 38 in Table 3).

Discussion

These results show that drought can be very detrimental to biomass accumulation and sucrose yield of young sugar beet plants. According to Nevins and Loomis (1970), the negative effects of drought mostly concerned the marketable organ (i.e. the tap root), which was, respectively, 30% and 40% lower in TS and PS than C. Similarly, the sucrose

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</table>

Fig. 4. Correlation between leaf carbon isotope discrimination determined on bulk dry matter (∆dm) and instantaneous (∆/E) and intrinsic (∆/gs) water use efficiency. Equally, leaf ∆e was only correlated with ∆/gs, (r = −0.72**).
The present work confirmed that the diurnal turnover of leaf soluble sugars was fast enough to detect photosynthetic discrimination against $^{13}$C over a period of one to a few days (Brugnoli et al., 1988). These results are in agreement with that on other crops (Brugnoli and Bjorkman, 1992; Scartazza et al., 1998; Arndt and Wanek, 2002), showing that $\Delta_s$ measured in different parts of the plant can enable distinct drought stress durations and integration times to be detected. In the present work, leaf $\Delta_s$ was very sensitive to short-term changes in $C_i/C_a$ and was significantly affected after about 1 d, while leaf $\Delta_{dm}$ changed significantly only after 30 d of drought imposition. As a consequence, the transient stress treatment was not differentiated by leaf $\Delta_{dm}$ as it remained similar to the values found in PS or C in drought or favourable periods, respectively. Root $\Delta_{dm}$ also appeared to be a promising parameter for the integration of gas-exchange, being significantly different among the three water regimes at the end of the experiment (34 d after restoring irrigation in TS). In summary, these results show
attributed to translocation and phloem loading and unloading isotopes. Other possible fractionation effects could be metabolites was probably due to the effects of isomerase sucrose content and yield. Root (higher brix %). According to this, root though the latter can be more concentrated in small roots expected as plants with higher photosynthetic rates generation. Furthermore, the gradual decrease in the slope of the position of leaves compared with roots, the latter being more concentrated in small roots (higher brix %). According to this, root $\Delta_{dm}$ was also positively associated with root dry weight. By contrast, leaf $\Delta_{lm}$ and $\Delta_{dm}$ were found to weakly or insignificantly correlate to sucrose content and yield, and this was not surprising as the time-scale of integration of leaf $\Delta_{s}$ and $\Delta_{dm}$ was found to be shorter than that of root $\Delta_{lm}$.

Leaf dry matter always showed higher $\Delta$ than leaf soluble carbohydrates (Table 1); this means that other plant components must show higher discrimination compared with sugars and to the bulk biomass. In fact, if other discrimination processes occur during metabolism then there must be some metabolite that is enriched in $^{13}$C, while others should be depleted (Brugnoli and Farquhar, 2000). Similar effects will also be evident in any metabolic branching if isotopic fractionation occurs during respiration (Ghashghaie et al., 2003; Tcherkez et al., 2003). In this experiment, the cause for this intra-leaf isotopic distribution could not be unequivocally identified, but the present results are in good agreement with previous reports (Brugnoli et al., 1988; Gleixner et al., 1993; Scartazza et al., 1998). In particular, Gleixner et al. (1993) found similar results in sugar beet and concluded that the effect may be caused by isotopic effects during aldolase–fructose 1,6-bisphosphate reaction. Previously, Brugnoli et al. (1988) had concluded that the lower $\delta^{13}$C in secondary metabolites was probably due to the effects of isomerase and aldolase with uneven distribution of heavy and light isotopes. Other possible fractionation effects could be attributed to translocation and phloem loading and unloading, but no conclusive proof exists on this matter (Pate and Arthur, 1998; A Scartazza and E Brugnoli, unpublished results). In this experiment, root $\Delta_{s}$ was very similar compared with leaf $\Delta_{s}$ and this would indicate that there is no fractionation during translocation to the root. However, despite the fact that $\Delta_{s}$ was similar in roots and leaves, $\Delta_{lm}$ was statistically lower in roots than in leaves (about $2%_{\text{so}}$). This may be explained by specific enzymatic processes and/or by the different chemical composition of leaves compared with roots, the latter being much richer in soluble carbohydrates (mainly sucrose). Differences in respiratory fractionation between roots and shoots could also explain these differences (E Brugnoli, personal communication).

The regression coefficients of $\Delta_{s}$ versus $C_{i}/C_{a}$ ratio was always lower than that expected from the theory (Farquhar et al., 1982). The significant deviation between measured ($\Delta_{s}$) and expected $\Delta$ could be associated with different factors: (i) patchy stomatal conductance and finite cuticular conductance; (ii) fractionation processes associated with respiration and photorespiration; (iii) $g_{m}$ leading to a decrease of $\left[\text{CO}_{2}\right]$ at the site of carboxylation. Patchy stomatal conductance (for an extensive review see Mott and Buckley, 1998) is possible but seems unlikely as a considerable deviation between measured $\Delta_{s}$ and expected $\Delta$ was also found under optimal water conditions. The effects of respiration and photorespiration are still being debated. Lin and Ehleringer (1997) showed negligible fractionation during respiration on proplasts, while Duranceau et al. (1999) showed consistent enrichment in $^{13}$C of the $\text{CO}_{2}$ respired. These results were confirmed by Ghashghaie et al. (2001) and Griffiths et al. (1999) and it now appears obvious that leaves with a complex metabolism have a significant fractionation during respiration. The lower values of measured $\Delta$ compared with the expected $\Delta$ may also be due to the overestimation of $\text{CO}_{2}$ partial pressure at the site of carboxylation. In the present study, the fractionation due to photorespiration was assumed as $8%_{\text{so}}$ (Griffiths et al., 1999), but the deviation between measured and expected $\Delta_{s}$ was still high. Therefore $g_{m}$ seems to be causing the most reduction in $\text{CO}_{2}$ partial pressure at the site of carboxylation. Furthermore, the gradual decrease in the slope of the regression of $\Delta_{s}$ versus $C_{i}/C_{a}$ over time (Table 2) may explain the observed drop of $A$ over the growing season (data not shown). This corroborates previous results (Loreto et al., 1994; Scartazza et al., 1998) showing that the decrease in $A$ and $\Delta$ during the ontogeny may be due to $g_{m}$. Besides, $g_{m}$ was found to be reversed when optimal

| Treatment | DAS 29 | | DAS 38 | | DAS 50 | | DAS 64 |
|-----------|--------|--------|--------|--------|--------|--------|
|           | $V_{cmax_{Ci}}$ | $V_{cmax_{Cc}}$ | $V_{cmax_{Ci}}$ | $V_{cmax_{Cc}}$ | $V_{cmax_{Ci}}$ | $V_{cmax_{Cc}}$ |
| C         | 91.a   | 106 a  | 83 a   | 94 a   | 94 a   | 96 a   |
| TS        | –      | –      | –      | –      | 91 a   | 106 a  |
| PS        | 78.b   | 101 a  | 70 b   | 87 a   | 65 b   | 74 b   |

Table 3. Maximum carboxylation rate ($\mu$mol m$^{-2}$ s$^{-1}$) of Rubisco determined on $C_{i}$ ($V_{cmax_{Ci}}$) and $C_{c}$ ($V_{cmax_{Cc}}$) basis
water conditions were restored, according to other experiments (Scartazza et al., 1998; Delfine et al., 1999). This should be taken into account when interpreting the seasonal variation of \( A \) as biochemical limitations can sometimes be erroneously associated with a lower efficiency of the photosynthetic apparatus. Again, \( g_m \) is often not included in the calculation of \( V_{\text{max}} \) or \( J_{\text{max}} \) as given by the A/C\( i \) model and this can lead to spurious results (Ethier and Livingston, 2004, and references therein). In this research it is shown that \( g_m \) should not be ignored as \( C_i \) was up to 35\% lower than \( C_c \). As a result, \( V_{\text{max}} \) was considerably underestimated by the conventional model (Table 3).

In conclusion, it has been shown that if water stress occurs in young sugar beet plants it can drastically reduce the assimilation rate and sucrose yield. Restoring water did not allow sucrose yield loss to be recovered, therefore irrigation may be recommended when water stress is expected to occur in the early growth stages. Photosynthesis capacity was very effective in revealing the occurrence of plant stress, but it was ineffective in predicting sucrose accumulation as, when favourable water conditions were restored, a steeply increased while the sucrose accumulation rate remained low. Carbon isotope discrimination determined on soluble sugars or bulk dry matter of different parts of the biomass appeared to be an easy-to-use tool for investigating the integrated photosynthetic adjustments over different time-scales ranging from 1 d (leaf \( \Delta_c \)) to more than 1 month (root \( \Delta_{im} \)). Besides, leaf \( \Delta_c \) can be used for estimating \( g_m \) that, if it is not negligible, may lead to spurious photosynthetic parameters (e.g. \( V_{\text{max}} \), CO\( \text{2} \)-operational point and \( J_{\text{max}}/V_{\text{max}} \) ratios).

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