Time-course of Tomato Whole-plant Respiration and Fruit and Stem Growth During Prolonged Darkness in Relation to Carbohydrate Reserves

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To evaluate the relevance of a simple carbon balance model (Seginer et al., 1994, Scientia Horticulturae 60: 55–80) in source-limiting conditions, the dynamics of growth, respiration and carbohydrate reserves of tomato plants were observed in prolonged darkness. Four days prior to the experiments, plants were exposed to high or low light levels and CO2 concentrations. The concentration of carbohydrates in vegetative organs was 30–50 % lower in plants that were exposed to low carbon assimilation conditions compared with those exposed to high carbon assimilation conditions. During prolonged darkness, plants with low carbohydrate reserves exhibited a lower whole-plant respiration rate, which decreased rapidly to almost zero after 24 h, and carbohydrate pools were almost exhausted in leaves, roots and flowers. In plants with high carbohydrate reserves, the whole-plant respiration rate was maintained for a longer period and carbohydrates remained available for at least 48 h in leaves and flowers. In contrast, fruits maintained fairly stable and identical concentrations of carbohydrates and the reduction in their rate of expansion was moderate irrespective of the pre-treatment carbon assimilation conditions. The time-course of asparagine and glutamine concentrations showed the occurrence of carbon stress in leaves and flowers. Estimation of source and sink activities indicated that even after low carbon assimilation, vegetative organs contained enough carbohydrates to support fruit growth provided their own growth stopped. The time of exhaustion of these carbohydrates corresponded grossly to the maintenance stage simulated by the model proposed by Seginer et al. (1994), thus validating the use of such a model for optimizing plant growth.

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Key words: Tomato, Lycopersicon esculentum Mill., prolonged darkness, respiration, carbohydrate pools, fruit growth, carbon stress, source–sink balance.

INTRODUCTION

The growth of plants, i.e. the production of new structures, results from the acquisition of resources (mainly carbon), their temporary storage and/or translocation to the growing tissues. The activities of source or sink organs are regulated in different ways so that crop growth and yield are either source-limited or sink-limited (Ho et al., 1989). The status of carbohydrate pools may be considered to be an indicator of the source–sink balance. In conditions where sources are limiting, the size of carbohydrate pools is reduced and, when there is a shortage of carbon substrate, rates of biosyntheses decrease. In conditions where sinks are limiting, carbohydrates are stored and can inhibit the production of new assimilates (Azcón-Bieto, 1983).

Seginer et al. (1994) formalized these concepts in a simple carbon balance model with two state variables (the masses of carbon in a single carbohydrate pool and in plant structures) and four carbon fluxes (gross photosynthesis, maintenance respiration, growth respiration and production of structural biomass). The carbohydrate pool acts as a buffer with a limited capacity. When it is reduced in size, growth continues as long as gross photosynthesis is higher than maintenance respiration. If this condition is not met, structural compounds are mobilized to maintain a minimum value of respiration which, in the model, is equivalent to a negative growth rate. When the carbohydrate pool reaches its highest value, the photosynthetic inflow is limited to the outflow of carbohydrates consumed by respiration and growth. In this framework, optimizing climate control in glasshouses to maximize plant growth is possible. To this end, the carbohydrate pool should remain within its bounds; if it is not then growth is limited either by source or sink activity (Tchamitchian and Ioslovich, 2000).

In a glasshouse, climate is modified in such a way that the source–sink balance is altered (Gary and Baille, 1999). The glass limits the availability of light and the replenishment of CO2 assimilated by the crops (except when CO2 enrichment is possible) and, therefore, source activity. The increased temperature that results from the reduction in radiative and convective heat loss and, possibly, from heating of the glasshouse increases the potential growth rate and therefore the sink activity. De Koning (1994) estimated that the actual growth rate of glasshouse tomato plants is equal, on average, to about half the sink strength of their vegetative and reproductive organs. Under these conditions, glasshouse-grown crops should be more prone to a shortage,
rather than excess, of carbohydrates. Therefore, the present study focuses on the dynamics of respiration rate, carbohydrate pools and organ growth, in the absence of photosynthetic activity, in plants with contrasting amounts of initial carbohydrate reserves.

In prolonged darkness, plant tissues go through a sequence of stages (Baysdorfer et al., 1988; Brouquisse et al., 1991). First, the carbohydrate concentration and rates of respiration and growth decrease. Non-carbohydrate compounds are then used as substrates for the declining respiratory activity. Finally, irreversible processes such as the degradation of membranes occur, leading to cell death. During the first stage, if the decrease in carbohydrate content and respiration rate were concomitant, then the respiration rate would actually be limited more by the rates of biosynthesis and consequent demand for ATP (Brouquisse et al., 1991) than by carbohydrate availability. A simultaneous decrease in growth and respiration rates has been observed, e.g. in pea (Hole and Scott, 1984) and tomato (Grange and Andrews, 1995) fruits, and in alfalfa (Hendershot and Volenec, 1989) and tall fescue (Moser et al., 1982) leaves. In wheat and spinach leaves, dark respiration is correlated to the previous net assimilation of CO₂ and to carbohydrate concentration (Azcón-Bieto and Osmond, 1983; Azcón-Bieto et al., 1983). In wheat, the duration of dark treatment before the leaf extension rate (LER) starts to decrease depends on the light intensity of the previous day (Christ, 1978a). Sambo (1983) showed that in two grass species there is a critical assimilate level below which leaf growth is limited.

During the second stage of carbon starvation, substrates other than carbohydrates can be metabolized. This consumption of structural compounds can be deduced from the time-course of the carbon balance of whole vegetative tomato plants: at temperatures of 20 and 25 °C and after 24 h darkness, carbon losses by respiration were larger than the decrease in carbon content in the carbohydrate pools (Gary, 1989). At the cell scale, proteolysis was observed in maize plantlets (Brouquisse et al., 1998), and beta-oxidation of fatty acids in root tips (Dieuâdaie et al., 1993). Cells develop a survival strategy involving controlled autophagy. In sycamore cells growing in a sucrose-free medium, the number of mitochondria per cell decreased (Journet et al., 1986). It was hypothesized that the products of the breakdown of some mitochondria supplied respiratory substrates for the remaining mitochondria. Ultimately, in the third stage, this process becomes irreversible and leads to cell death.

Prolonged darkness has been used by crop modellers to estimate the maintenance component of respiration (Amthor, 1989). This ‘dark decay method’ is based on the hypothesis that, after a few hours of darkness, growth (i.e. the production of new structures) stops and the remaining respiratory activity supports only the energy cost of functions such as protein turnover and maintenance of ionic gradients. This hypothesis has been verified only for the transition between the first and second stages of carbon starvation (Gary, 1989). Later, metabolism contributes to the survival and no more to the maintenance of cell structures. Therefore, this transient stage is the time when the plant carbohydrate pool reaches its minimum value in the model of Seginer et al. (1994). To date, this model has been calibrated by using experimental data from young vegetative tomato plants only. Hence, an experiment was designed to assess the behaviour of the carbon balance of fruiting tomato plants. Relationships between the dynamics of respiration rate, carbohydrate pools and growth rate of organs in prolonged darkness were studied to identify the conditions under which carbon stress occurred at the whole-plant level and in vegetative and reproductive organs. Indicators of carbon stress, such as accumulation of the amino acid asparagine (Brouquisse et al., 1992; Chevalier et al., 1996), were examined.

MATERIALS AND METHODS

Cultivation of plants

Tomato seeds (Lycopersicon esculentum Mill. ‘Raïssa’) were sown in small rockwool cylinders on 4 Mar. 1997 (expt 1) and 21 Apr. 1997 (expt 2). Once three leaves had appeared, seedlings were transplanted to a multispans glasshouse located in southern France (INRA, Avignon). Plants were grown in a recirculating hydroponic system under the same conditions as described by Gary et al. (1998). All axillary buds were removed, and six fruits were retained per truss. When the fifth truss flowered, plants were transplanted to two similar growth chambers (8-75 m² for 10–12 d periods starting on 22 May (expt 1) and 3 July (expt 2). In both experiments, plants in one growth chamber were used for gas exchange measurements, and those in the other for growth measurements and tissue analyses. In each growth chamber, 12 plants were set in a recirculating hydroponic system consisting of 12 × 10-l pots linked to a 500-l reservoir in which the nutrient solution was circulated continuously. The nutrient solution contained the following major elements: 4.0 mol m⁻³ KNO₃, 0.9 mol m⁻³ K₂SO₄, 1.5 mol m⁻³ KH₂PO₄, 3.75 mol m⁻³ Ca(NO₃)₂ and 1.0 mol m⁻³ MgSO₄. pH was checked daily and varied between 5.5 and 6.5. Air temperature, CO₂ concentration and humidity were controlled and recorded; metal halide lamps were used to provide artificial lighting.

Climatic treatments

The experiments were designed to load plant carbohydrate reserves to a high (expt 1) or a low (expt 2) level before subjecting the plants to a long period of darkness. For this purpose, radiation values were set so that their daily integral was equal to mean values measured in Avignon, France (43°55’N, 4°52’E) in July and January, respectively, and CO₂ concentration was increased in expt 1.

Experiment 1. For 4 d, artificial lighting was provided between 0500 and 2100 h [1000 µmol m⁻² s⁻¹ PAR (photosynthetically active radiation) for the first 3 d and 500 µmol m⁻² s⁻¹ PAR for the fourth day]; air temperature was set to 20 °C in the light and 16 °C in the dark. The CO₂ concentration was controlled at approx. 960 ppm and humidity was maintained between 70 and 80 %. At the end
of the fourth day, lights were turned off for 4 d, and the temperature was set to 20 °C; the CO₂ concentration varied between 350 and 600 ppm owing to plant respiration and ventilation.

Experiment 2. As for expt 1, except that PAR was set to 200 μmol m⁻² s⁻¹ and CO₂ concentration to 350 ppm during the first 4 d.

Measurement of CO₂ exchange

One of the growth chambers was used as a closed system in which to measure CO₂ exchange of a set of 12 plants. During daylight, CO₂ was injected into both growth chambers to compensate for photosynthesis and maintain the set-point value of CO₂ concentration. In the period of darkness, the CO₂ concentration increased as a result of plant respiration; when [CO₂] reached 600 ppm the growth chambers were ventilated using air from outside the building to reduce the [CO₂] to approx. 350 ppm. As the growth chambers were not perfectly airtight, a leakage coefficient (K, m³ s⁻¹) was estimated for both chambers prior to each experiment. For this purpose, CO₂ was injected into an empty and closed growth chamber up to 1000 ppm, and the dynamics of the decrease in CO₂ concentration, Cᵢ(t) (in ppm), was analysed. The CO₂ balance of the growth chamber was:

\[ \frac{dC_i(t)}{dt} = V \left( C_o - C_i(t) \right) \]

where \( V (\text{m}^3) \) is the volume of the growth chamber and \( C_o (\text{ppm}) \) is the CO₂ concentration outside the growth chamber. Then:

\[ C_i(t) = C_o + (C_i(0) - C_o)e^{-\frac{t}{\Delta t}} \]

The leakage coefficient, K, was calculated by non-linear regression. During the experiment, at any time except when a growth chamber was ventilated, its CO₂ balance was:

\[ \frac{dC_i(t)}{dt} = N + I + K(C_o - C_i(t)) \]

where \( N (\text{m}^3 \text{s}^{-1}) \) is the CO₂ exchange rate of the plant and \( I (\text{m}^3 \text{s}^{-1}) \) is the CO₂ injection rate. N can be calculated as:

\[ N = -I - K(C_o - C_i(t - \Delta t)) \]

where \( \Delta t (300 \text{ s in both experiments}) \) is the time step of CO₂ measurements (Gary, 1988a).

Measurement of fruit and stem growth

Variations in fruit diameter were recorded simultaneously on six fruits during their period of maximum growth rate, i.e. 2 to 4 weeks after flower anthesis (fruit diameter around 50 mm). Variations in stem diameter were recorded simultaneously on six plants, on internodes exhibiting their maximum growth rate, i.e. internodes located beneath the flowering truss. Measurements were made using linear-displacement transducers (CD 4112-1; Schlumberger Enertec, Vélizy-Villacoublay, France) with a precision of ±3 μm.

Carbohydrate and amino acid analyses

Every 12 h during the period of prolonged darkness, samples of young leaves, flowers, roots and fruits from two plants were harvested for analysis. Leaflets were randomly sampled among the terminal and top-pair leaflets on one leaf of the fourth or fifth sympod, i.e. close to the last flowering cluster. The five or six available flowers per plant were pooled. Root tissue (approx. 10 g f. wt) was rinsed in distilled water. Since the timing of flowering had been noted, the age of each fruit was known. On each plant, fruits that were 4–5 and 14–16 DAA (days after anthesis) old were harvested for analyses. All samples were quickly frozen in liquid nitrogen and stored at −80 °C until analysis. Dry weight was determined after freeze-drying. Soluble carbohydrates were extracted and starch hydrolysed according to Brouquisse et al. (1991), and enzymatic analyses were carried out using the method of Kunst et al. (1984). Free amino acids were analysed by HPLC according to Cohen and De Antonis (1994).

RESULTS

Status of plants submitted to 4 d of high and low carbon assimilation

During the 4 d pre-treatment, the quite different conditions of light and CO₂ concentration led to contrasting rates of CO₂ exchange, but rates of increase in fruit diameter were more similar (Table 1). Compared with that in plants subjected to high carbon assimilation conditions, net photosynthesis was reduced by 7.9-fold and night respiration by 6.5-fold in plants pre-treated in low carbon assimilation conditions. Measurements of net photosynthesis in the day and respiration at night were consistent with values predicted by the model of Seginer et al. (1994). When using the set of parameters adopted by these authors and the mean plant dry weight and leaf area index observed in expts 1 and 2, calculated net photosynthesis was 10.3 and 2.4 μmol per plant s⁻¹ in high (1000 μmol m⁻² s⁻¹ PAR, 960 ppm CO₂) and low (200 μmol m⁻² s⁻¹ PAR, 300 ppm CO₂) carbon assimilation conditions, respectively. Calculated night-time respiration was 2.4 and 0.5 μmol per plant s⁻¹ under conditions of high and low carbon assimilation, respectively.

As expected, the status of the carbohydrate pools in the vegetative and reproductive organs differed between both pre-treatments (Table 2). In the vegetative organs (roots and...
growing leaves), concentrations of soluble carbohydrates and starch were 30–50% lower following exposure to low rather than high carbon assimilation conditions. In contrast, in flower buds and growing fruits, there were no significant differences in soluble carbohydrate concentration whereas the starch pool dropped by 50–84% in plants pre-treated in high compared with low carbon assimilation conditions.

Despite these quite different net assimilation rates and carbohydrate reserves, the expansion rate of fruits from both treatments did not differ dramatically (Table 1). On average, it tended to be higher during the day and lower at night (but not significantly so) under conditions of low compared with high carbon assimilation.

**Time-course of whole-plant respiration rate during prolonged darkness**

During the first 24 h of darkness, the average respiration rate was similar to that of the four previous nights: 1.58 and 0.21 μmol per plant s⁻¹ following high and low carbon assimilation pre-treatments, respectively (Fig. 1). The average respiration rate remained high in plants subjected to the high carbon assimilation pre-treatment, whereas it dropped by 60% during the first 6 h then levelled off in plants pre-treated in low carbon assimilation conditions.

After approx. 16 h of darkness, the respiration rate decreased exhibiting a periodic pattern of a sharp decrease followed by a plateau and/or a slight increase. After high carbon assimilation, this pattern was observed three times, starting after approx. 16, 40 and 67 h of darkness. In this experiment (expt 1), the last plateau levelled off after 79 h of darkness at 0.19 μmol per plant s⁻¹, i.e. at about 12% of the initial value and at about the same value as that observed initially in plants that had experienced conditions of low carbon assimilation (expt 2). In the latter plants, the respiration rate dropped after 16 h darkness, reaching very low values (close to the threshold of sensitivity of the CO₂ exchange measurement system) after 24 h.

**Time-course of carbohydrate and amino acid concentrations in leaves, roots and growing fruits during prolonged darkness**

In tomato leaves, starch was the major carbohydrate pool. After high carbon assimilation, its concentration decreased steadily until it was depleted after 48–60 h of darkness (Fig. 2). Meanwhile, the concentration of soluble carbohydrates decreased slowly, remaining above 60 μmol g⁻¹ d. wt after 84 h of darkness. Following low carbon assimilation, the leaf starch pool was depleted within 12 h of darkness and the soluble carbohydrate concentration dropped and remained below 50 μmol g⁻¹ d. wt after 24 h of darkness.

Tomato roots stored little starch (less than 20 μmol g⁻¹ d.wt). Their concentration of soluble carbohydrates decreased steadily irrespective of the light and CO₂ pre-treatment, reaching values of less than 50 μmol g⁻¹ d. wt after 36 h of darkness.

After high carbon assimilation, the starch concentration of flowers decreased by 80% in 24 h while the concentration of soluble carbohydrates remained above 110 μmol g⁻¹ d. wt throughout the 84 h of prolonged darkness. After low carbon assimilation, the starch pool quickly reached an all-time low level, whereas the concentration of soluble carbohydrates decreased steadily to below 50 μmol g⁻¹ d. wt after 48 h of darkness.

**Table 1. CO₂ exchanges and organ growth rates during the pre-treatment period**

<table>
<thead>
<tr>
<th></th>
<th>High C assimilation</th>
<th>Low C assimilation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Daytime</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Net photosynthesis (μmol per plant s⁻¹)</td>
<td>11.3 ± 1.3</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>Fruit growth (μm h⁻¹)</td>
<td>29.6 ± 8.7</td>
<td>40.1 ± 9.4 n.s.</td>
</tr>
<tr>
<td>Stem growth (μm h⁻¹)</td>
<td>2.6 ± 1.9</td>
<td>–</td>
</tr>
<tr>
<td><strong>Night-time</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiration (μmol per plant s⁻¹)</td>
<td>1.2 ± 0.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Fruit growth (μm h⁻¹)</td>
<td>36.2 ± 8.7</td>
<td>27.8 ± 7.0 n.s.</td>
</tr>
<tr>
<td>Stem growth (μm h⁻¹)</td>
<td>11.4 ± 3.8</td>
<td>–</td>
</tr>
</tbody>
</table>

All variables were averaged over the three day and night periods prior to the period of prolonged darkness. In the high radiation treatment, photosynthesis rate was averaged over the three daytime periods at 1000 μmol m⁻² s⁻¹ PAR. Measurements of stem diameter were not available in the low radiation pre-treatment.

**Table 2. Concentrations (nmoles glucose equivalents mg⁻¹ d. wt) of soluble carbohydrates and starch at the beginning of the period of prolonged darkness, following 4 d of high and low carbon assimilation pre-treatments**

<table>
<thead>
<tr>
<th></th>
<th>High C assimilation</th>
<th>Low C assimilation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roots</td>
<td>150 (19)</td>
<td>20 (1)</td>
</tr>
<tr>
<td>Young leaves</td>
<td>195 (28)</td>
<td>1117 (433)</td>
</tr>
<tr>
<td>Flower buds</td>
<td>195 (18)</td>
<td>253 (86)</td>
</tr>
<tr>
<td>Fruits 5 DAA</td>
<td>909 (132)</td>
<td>459 (122)</td>
</tr>
<tr>
<td>Fruits 15 DAA</td>
<td>2282 (158)</td>
<td>841 (168)</td>
</tr>
</tbody>
</table>

Mean (s.d.) of eight analyses: two plants × two extractions × two analyses.

** P < 0.01; * P < 0.05; n.s., non significant.**
In contrast to all other organs, growing fruits exhibited no changes in carbohydrate concentration throughout the period of prolonged darkness, irrespective of treatment. They stored more soluble carbohydrates than starch, with concentrations five to ten times higher than those in flower buds or vegetative organs. During the cell division stage, the concentration of soluble carbohydrates was slightly higher (mean 1230 vs. 1090 μmol g⁻¹ d. wt) and the concentration of starch slightly lower (mean 175 vs. 318 μmol g⁻¹ d. wt) after low than after high carbon assimilation. During the cell expansion stage, the concentration of soluble carbohydrates was even higher, decreasing slowly from 2200 to 1700 μmol g⁻¹ d. wt. The starch concentration remained stable, at around 840 and 440 μmol g⁻¹ d. wt after high and low carbon assimilation, respectively.

In leaves, the concentrations of asparagine and glutamine started increasing after 36 h of darkness, irrespective of previous carbon accumulation (Fig. 3). Both amino acids were present at low concentrations in roots throughout the period of prolonged darkness. In flowers, as in leaves, the asparagine concentration increased significantly after 36 h of darkness, and the rate of increase following high carbon assimilation was twice that following low carbon assimilation. In contrast, the glutamine concentration increased only very gradually throughout the period of darkness. The amino acid concentration was higher in fruits than in vegetative organs; concentrations of both asparagine and glutamine increased slowly and did not exhibit any dramatic change.

Time-course of the growth rate of fruits and stems during prolonged darkness

Despite large differences in carbohydrate storage and respiratory activity between plants in the two treatments, the growth rate of fruits was only slightly higher (not always significantly) after high than after low carbon assimilation (Fig. 4A). The change in fruit growth was comparable with the change in whole-plant respiration rate: it was high and fluctuated widely during the first 16 h (averaging 43.1 and 36.6 μm h⁻¹ after high and low carbon assimilation, respectively), then decreased by 41% during the next 16 h and by another 21% during the following 50 h. At the end of the experiment, i.e. after 82 h of darkness, the fruit growth rate was still about one-third of their growth rate during the day–night cycles, regardless of the light and CO₂ concentration conditions.

The changes in stem growth rate were more erratic (Fig. 4B). Unexpectedly, it tended to be lower during the first 40 h (yet not always significantly so), then higher in plants with high carbohydrate reserves than in those with low carbohydrate reserves. It decreased slightly and almost stopped after 70 (high carbon assimilation plants) or 50 h (low carbon assimilation plants). However, these observations cannot be considered conclusive since the range of hourly stem growth rates was close to the sensitivity of the displacement transducers.

DISCUSSION

The effects of prolonged darkness clearly differed with the initial size of the carbohydrate pools. Following high carbon assimilation, the high carbohydrate content could support growth and respiration for 20–24 h at the high levels observed during the previous day/night periods; both processes then decreased. Following low radiation conditions, carbohydrate availability was lower, and the respiration rate was maintained for 20–24 h at the low levels experienced during the previous day/night periods before a rapid decline to almost undetectable levels.

Such a response of plant respiration to assimilate availability has often been described in the literature (see review by Amthor, 1989). This response can be explained on two time scales. In the short term, a reduction in the supply of substrate limits overall metabolism. In the long term (i.e. a few hours to a few days), the respiratory capacity gradually acclimates to the new flow of assimilate supply. Williams et al. (1992) observed changes in the pattern of protein synthesis in barley roots only 24 h after shoot pruning; activities of invertase and sucrose synthase had declined. In similar conditions, McDonnell and Farrar (1992) reported a reduction in cytochrome oxidase activity.

This coarse control of respiration involves regulation of gene expression by carbohydrate availability. In tomato fruits (Baldet et al., 2002) and roots (Devaux et al., 2003), expression of some genes involved in the cell cycle and in carbohydrate and nitrogen metabolism is quickly reduced during the first 2 d of darkness. Such an acclimation occurred in our experiments after the 4 d pre-treatments. At the beginning of the period of prolonged darkness, the total carbohydrate content was reduced by 16% in fruits 15 DAA (about 30% of the plant dry mass) and by 41–49% in roots and leaves (about 40% of the plant dry mass) after low compared with high carbon assimilation conditions. At the same time, the plant respiration rate was 66% lower after low than after high carbon assimilation. This decrease in respiration rate was a more proportional to the decrease in carbohydrate content. Yet, at the organ or whole-plant scale, respiration rate is related by a linear or saturation-type curve to the carbohydrate content (e.g. Penning de Vries et al., 1989).
et al., 1979; Gary, 1988b). Accordingly, Challa (1976) characterized acclimation in vegetative cucumber plants by a response curve of respiration rate to carbohydrate content that was lower for plants grown under winter light than under spring light conditions.

The decrease in starch and soluble carbohydrate concentrations observed in vegetative organs and in flowers contrasts with their relative stability in fruits, irrespective of their physiological stage. In roots, little starch was stored and the pool of soluble carbohydrates decreased strongly during the first 24 h. However, Pressman et al. (1997) noted that starch was stored in comparable concentrations to soluble carbohydrates in roots of a different tomato cultivar grown in Israel in winter. Indeed, Hewitt and Marrush (1986) showed that there is strong genetic variability in the starch–soluble carbohydrate balance in the vegetative tissues of tomato plants. In leaves, starch is the most variable carbohydrate pool (Bertin et al., 1999). Starch was
rapidly exhausted following low carbon assimilation pre-treatment but remained at high levels for 48 h after high carbon assimilation pre-treatment. The availability of carbohydrates was not estimated in stems and petioles. These tissues mainly accumulate soluble carbohydrates (Hewitt and Marrush, 1986). They are active sinks (Khan and Sagar, 1966) and the amounts of carbohydrates they store can be similar to those stored in leaves (lower in Pressman et al., 1997; higher in Devaux, 2000).

A fairly stable concentration of soluble carbohydrates was maintained in flowers throughout the period of darkness after high carbon assimilation and for approx. 48 h after low carbon assimilation. In the last experiment and after 48 h of darkness, flowers certainly experienced a shortage of assimilates that could lead to fruit set failure (Bertin, 1995). In contrast, during the cell division and cell expansion stages, fruits could maintain their carbohydrate concentration throughout the period of darkness, irrespective of the pre-treatment. Overall, leaves and stems were the major sources of carbohydrates to support the growth of fruits and maintain their carbohydrate content. Leaf samples were harvested on the fourth and fifth sympods. Given that (1) concentrations of soluble carbohydrates vary little among leaves; (2) concentrations of starch decrease to zero on the second sympod (Bertin et al., 1999); and (3) the carbohydrate content should be similar in leaves and stems, a total content of carbohydrates available in the vegetative tissues at the beginning of the period of darkness was

![Graphs showing time-course of asparagine (triangles) and glutamine (diamonds) concentrations during prolonged darkness and after 4 d of high (open symbols) and low (closed symbols) carbon assimilation. Measurements were carried out on growing vegetative (young leaves, roots) and reproductive organs (flower buds, fruits at the stage of cell division (5 DAA) and cell expansion (15 DAA)). Mean ± s.d. of two plants.](image)
mine in fruits during cell division. These results confirm that treatments. Asparagine accumulated in flowers and glutamine accumulated in leaves but there were no clear differences related to the two pre-darkness. Asparagine and glutamine accumulated in leaves and pods. However, in the present experiments which took place in growth chambers, plants experienced no changes in temperature and water status during the period of prolonged darkness. In comparable controlled conditions, Devaux (2000) observed no changes in the dry matter concentration of tomato fruits over a 9-d period of darkness. It may be hypothesized that the fruit expansion rate measured in the present experiments reflect the import rates of assimilates.

Specific amino acids are considered to be indicators of carbon stress. During the second stage of carbon starvation when proteolysis occurs, nitrogen is stored in the form of asparagine and glutamine, which limits the accumulation of ammonium in cells (Brouquisse et al., 1992). However, neither amino acid accumulated in roots despite the apparent exhaustion of carbohydrates under prolonged darkness. Asparagine and glutamine accumulated in leaves but there were no clear differences related to the two pre-treatments. Asparagine accumulated in flowers and glutamine in fruits during cell division. These results confirm that

The most striking result is the similarity of the time-course of fruit expansion during prolonged darkness after both carbon assimilation pre-treatments. Although carbohydrate reserves were reduced by 65 % after low carbon assimilation, the fruit expansion rate was, on average, only 11 % lower throughout the period of darkness. In both experiments, the fruit expansion rate started to decrease sharply after 16 h of darkness, simultaneously with the whole-plant respiration rate, as if growth substrates where missing regardless of their availability at the beginning of the period of darkness.

Two points can be made to explain this behaviour. First, even after low carbon assimilation, sufficient carbohydrate was available to support fruit growth during 72 h of darkness. The relative growth rate of fruit was estimated from fruit expansion measurements by assuming that fruits were spheres of 50 mm diameter at the beginning of the dark period and had the same expansion rate in all three dimensions. Tomato fruit dry matter is mainly constituted by carbohydrates (Davies and Hobson, 1981). Its increase at the whole-plant scale during 72 h of darkness was estimated by hypothesizing that all fruits had the same exponential growth curve (note that the fruit growth curve is actually closer to a Gompertz function; Bertin, 1995) and, therefore, the same relative growth rate. The amount of assimilates dedicated to fruit growth was roughly estimated to be 8-7 and 4-3 g equivalent glucose per plant, after high and low carbon assimilation, respectively; these values were still lower than the amounts estimated to be available at the beginning of the dark period.

A second point of discussion is that the expansion rate of fleshy fruits, such as those of tomato, depends more on temperature and on plant water status than on the availability of assimilates (Pearce et al., 1993a, b). Grange and Andrews (1995) reported that the respiration rate of young tomato fruits is closely linked to the import rate of assimilates and weakly related to the fruit expansion rate. However, in the present experiments they took place in growth chambers, plants experienced no changes in temperature and water status during the period of prolonged darkness. In comparable controlled conditions, Devaux (2000) observed no changes in the dry matter concentration of tomato fruits over a 9-d period of darkness. It may be hypothesized that the fruit expansion rates measured in the present experiments reflect the import rates of assimilates.

**Fig. 4.** Time-course of fruit (A) and stem (B) growth during prolonged darkness and after 4 d of high (open symbols) and low (closed symbols) carbon assimilation. Diameter expansion was measured every 5 min and averaged on an hourly basis on six fruits and stems from six different plants (mean ± 90 % confidence interval).
In a context of increasing carbon starvation at the whole-plant scale, the growth rate of fruits was maintained, as indicated by the variations in fruit diameter, whereas the growth of vegetative organs was impaired, as indicated by the whole-plant respiration measurements and the variations in stem diameter.

The sequence of events observed during prolonged darkness can also be analysed in reference to the modelling of the plant carbon balance. As stated in the Introduction, the so-called maintenance status of plant metabolism should only be a transient stage between the first and second stages of carbon starvation. Values of the maintenance respiration rate at the whole-plant scale were estimated from maintenance coefficients for leaf, stem, fruit and root tissues, and acclimation of respiration to environmental conditions was calculated through a correlation with relative growth rate (Heuvelink, 1995). The average relative growth rate was estimated from the net assimilation rate measured during the 4 d pre-treatment. The calculated maintenance respiration rate was 1·125 and 0·300 μmol per plant s⁻¹ after high and low carbon assimilation, respectively. When these values were compared with the time-course of total respiration rate measured in expts 1 and 2 (Fig. 1), the maintenance stage (corresponding to the cessation of whole-plant growth and to the exhaustion of the carbohydrate pool) would have occurred after about 24 h of darkness in the first case and after approx. 5 h in the second, according to the model of Seginer et al. (1994).

The present analysis shows that modelling the carbon balance at the whole-plant scale is an over-simplification. The shortage of carbohydrates leads to changes in the carbon balance that differ in growing fruits and in vegetative organs. However, it can be argued that the stage of maintenance that was identified by existing tomato crop models (Seginer et al., 1994; Heuvelink, 1995) is not far from the time of exhaustion of the carbohydrate reserves in the vegetative organs. In fact, optimization of plant growth should be based on the ability of the vegetative apparatus not only to feed the organs of accumulation, but also to maintain the integrity of its structures. In this respect, the present results validate the existence of a lower threshold of carbohydrate reserves and its use in optimization procedures (Tchamitchian and Ioslovich, 2000).

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LITERATURE CITED


