Incorporation of $^{14}$C-Photosynthate into Major Chemical Fractions of Leaves and Bark of *Ceratonia siliqua* L. at Different Seasons

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**Summary**

Parts of branches of *Ceratonia siliqua* (L.) were exposed to $^{14}$CO$_2$ for 3 h during the growth period in April, after termination of growth (end of May), in the summer drought period (August) and in the cool winter (February). Twigs were harvested immediately after the end of the feeding period as well as after 48 and 144 h. Simultaneously with the last harvest of exposed plant parts, controls from outside the exposure-chamber were also gathered. The total $^{14}$C-incorporation into leaves and bark as well as the labelling and contents of soluble sugars, starch, total lipids and other precipitable soluble compounds were determined.

Total $^{14}$C-incorporation immediately after termination of $^{14}$CO$_2$ application is a measure of the assimilation rate; it was high and nearly identical in April and May, but low (only about 5% of the April value) in summer and winter. The incorporation of $^{14}$C into soluble structural components was highest in April. Uptake of total $^{14}$C and labelling of soluble compounds during the 6-day period of the experiments show that metabolic rates were very low in summer but high during spring. The turnover of soluble compounds in February was remarkably high regarding the low CO$_2$-fixation rate. The labelling of soluble sugars in leaves decreased in all experiments; the specific activity of starch remained rather stable.

The incorporation of $^{14}$C into the lipid fraction was weak in all seasons and significant alterations took place only during the growing period.

Variation in assimilation rates of *Ceratonia* at different seasons was comparable to that found for other mediterranean species using IRGA methods. There was no indication that lipids in *Ceratonia* are true storage compounds and participate in cyclic metabolic processes preventing stress effects. The weak turnover of lipids leads to the conclusion that maintenance costs for these compounds, as calculated by Merino et al. (1984), perhaps are too high, at least for *Ceratonia*. Our results do not suggest a specific adaptation of the metabolism of storage compounds to summer drought in *Ceratonia*.

**Key words: Ceratonia siliqua L., assimilation rate, $^{14}$CO$_2$-application, drought stress, lipids, maintenance cost, sugars, starch, storage.**
Material and Methods

Material

A middle-sized tree of *Ceratonia siliqua* L., about 40–50 years old, growing on the edge of the wilderness area of the Botanical Garden Diomidis, Athens, Greece was chosen for the labelling experiments. The branches used for the investigations were partly shaded when the sun was high in the sky.

Methods

Labelling procedures

The experiments took place from $10^{26} - 14^{26}$. Labelling with $^{14}$CO$_2$ was performed according to Kull and Baitinger-Haardt (1977), with some modifications for branches of trees as described by Distelbarth (1982). The terminal part (about 60 cm) of a branch (length > 1 m) with several twigs was placed in an air-tight plastic foil chamber and equilibrated for 1 h before labelling took place. Transpiration of the enclosed leaves in the chamber caused the air humidity to rise to about 80–90% during summer and 100% during the other seasons. The temperature reached maximum values about 4–5°C higher than the air temperature outside the chamber. As observed microscopically, stomata of the leaves were open in all experiments. The twigs enclosed in the chamber were allowed to only bear fully developed leaves; therefore, in the April experiment about 25% of the leaves present had to be removed and in the May experiment about 10%. This is important because growing leaves form sinks and this would lead to problems in interpretation of data (compare Kriedemann, 1969; Roberts and Menary, 1990).

After the equilibration period, 7.4 MBq (200 μCi) $^{14}$CO$_2$ were generated in the chamber (11 a.m.). After a 3-h supply of $^{14}$CO$_2$ to the branch, the chamber was opened, ventilated and then removed.

Harvesting

Immediately after removing the chamber some twigs of the branch were harvested and placed in plastic bags. In the following chase-period further harvesting took place 48 h and again 144 h after the labelling experiment. Simultaneously with the last harvest, some twigs of the branch that had remained outside the exposure-chamber were also gathered to find out how much photosynthesize was exported from the labelling area.

The branch used in the experiment was next cut at its base. In the May experiment, very little radioactivity was found at the base of the branch. No further investigation of the labelling of the branch-bases took place in the other experiments.

Quantitative estimation of substances

The plant material was separated into leaf-blades, rachis and pétioles (not investigated further), and bark. It was placed in plastic bags, dipped into boiling water for 10 min and brought to the laboratory, about 60–90 min after harvesting the material was dried in a ventilation oven at 60°C. Prior to analysis, the plant material was freeze-dried and ground to a fine powder.

Soluble sugars were analyzed by TLC and total sugar content by the anthrone method (compare Distelbarth et al., 1984). Starch content was measured using the anthrone method of McCready et al. (1950) and total lipids were quantified according to the gravimetric method of Bligh and Dyer (1959). The lipid fraction contained membrane lipids and triacylglycerols, as well as chlorophylls, carotenoids and perhaps other isoprenoids. The isoprenoid lipids constitute a rather small portion of the total lipid fraction (compare Diamantoglou and Kull, 1982), but the amount of waxes perhaps also included is not known. Mucilage and proteins were precipitated with acidic ethanol (Naglschmid et al., 1982) and determined by gravimetry.

As a gross value of (drought-)stress (compare Diamantoglou and Kull, 1982; Rhizopoulou et al., 1990), the proline content of the leaf-blades was measured according to the method of Bates et al. (1973) in the version modified for freeze-dried material by Huq and Larher (1984). In the leaves harvested in August the content was about 25% higher than in May, in those harvested in February about 15% higher.
Table 1: Total \(^{14}\)C-activity (Bq/mg dry material) in leaves and bark after feeding \(^{14}\)CO\(_2\) to twigs of Ceratonia at different seasons.

<table>
<thead>
<tr>
<th>Date of experiment</th>
<th>0 h after termination of feeding</th>
<th>48 h after termination of feeding</th>
<th>144 h after termination of feeding</th>
<th>control from outside exposure chamber (144 h after termination of feeding)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April (24. 4.) leaves</td>
<td>15.1</td>
<td>12</td>
<td>7.3</td>
<td>5.6*</td>
</tr>
<tr>
<td>May (28. 5.) leaves</td>
<td>14.5</td>
<td>11.8</td>
<td>3.6</td>
<td>0.3</td>
</tr>
<tr>
<td>August (20. 8.) leaves</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
<td>0.04</td>
</tr>
<tr>
<td>February (10. 2.) leaves</td>
<td>0.7</td>
<td>0.5</td>
<td>0.7</td>
<td>0.03</td>
</tr>
<tr>
<td>February (10. 2.) bark</td>
<td>0.6</td>
<td>0.5</td>
<td>0.6</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Controls contained growing leaves. Standard deviation measured from 8 of the samples by 3, resp. 4, separate investigations is in the range ±(0.08–0.15 Bq/mg).

Results

Total \(^{14}\)C-incorporation at different seasons

Total \(^{14}\)C-incorporation (Table 1) immediately after termination of the \(^{14}\)CO\(_2\)-feeding, which can be taken as a measure of the assimilation rate, was highest in the April experiment during the main growth period of the tree. When the fixation rate of this experiment is considered as 100%, 4 weeks later (end of May) we found 96%, during the summer drought period in August 3% and in winter (February) 5%. The \(^{14}\)CO\(_2\)-uptake was very low in the summer experiment in spite of the cuvette-climate existing in the exposure chamber during the 3 h feeding period. Also, in the cool season the assimilation rate was remarkably low and less than what we expected. In the bark, the initial activities were higher in May than in April; for summer and winter the results were comparable to those found for leaves.

To obtain some information on the incorporation of \(^{14}\)C into structural components of the leaf-tissue, the activity of water soluble compounds, total lipids and starch was summed up. The total initial activity of these compounds was highest in the May experiment. If for reasons of comparison we consider the activity of this fraction in April as 100%, the May value reached about 150%. In April, the twigs of our sample contained growing tissues (the young leaves were removed, but growing tips were still present). Therefore, a greater part of the produced assimilates was metabolized to structural components, e.g. new cell walls. In May, growth had terminated in the twigs used for the experiment. In the summer experiment the activity of soluble compounds and starch was 19%, and in February it reached 58% of the April value. From a comparison of relative values (percentages) of the total activities, one can conclude that in summer and winter the labelling of the structural components is weaker than during the growth period in April.

The dynamics of labelling during the 6-day-period of the experiments showed that metabolic rates were very low during the summer drought and low in the cool, though rather humid winter, but high during spring. In April, in the main

growth phase, the activity in the leaves during the course of 6 days declined to about 50%, whereas in the bark, due to export from the leaves, the activity doubled in this period. A high translocation rate was also recognized from the labelling of the control leaves outside the exposure chamber. About 30% of these control leaves were growing and therefore acting as sinks for assimilates. In May, the decrease of \(^{14}\)C in the leaves was still more distinct (to about 25% in 6 days). The leaves were then predominantly producing for export. In the bark, we initially found a rise and then a decline of activity, which may be explained by the transport function. In the summer experiment, the weak initial activity in the leaves was not altered significantly. The same is true for the experiment in February, but labelling was somewhat higher than in August in all samples; hence, we may infer that the reduction of metabolism is more distinct in the summer than in winter.

Specific activities of labelled compounds

The contents of sugars, starch and lipids in the samples were in general similar to those reported by Diamantoglou and Meletiou-Christou (1977, 1978), but some minor differences should be mentioned. In leaves, the starch content in our experiment was approximately the same in February and April (2.7–2.9%). The total lipid content of leaves in February (about 5%) and in April (about 5%) was less than reported earlier. In May, the content of sugars in the bark was higher and that of starch was lower than described.

The specific activities of the compounds investigated are shown in Fig. 1. To calculate rough values of the relative specific activities of the fraction of "other soluble compounds", mucilages and proteins were precipitated; the remaining activity in the supernatant was then very low. Because mucilages (carob gum) share a relatively great part of this fraction but were labelled weakly (apart from the growth period), the specific activity of this fraction should be taken only as a rough value.

A comparison of the labelling pattern in the different seasons shows that in May and August sucrose had the highest activity of all compounds, but in February and April glucose and fructose both reached higher values. The incorporation of \(^{14}\)C in the lipid fraction was weak in all cases. The "other compounds" showed a distinct labelling in April and also in February, but a very low value in August.

Looking at the dynamics of the specific activities during the period of 6 days, we found the expected decrease in sucrose in all cases, which was least distinct in April. In summer the monosaccharides showed only a weak decline. The specific activity of starch remained remarkably stable, not only during one experiment (that may be expected for a long-term storage compound), but also throughout the different seasons. Starch in the control leaves outside the chamber after 144 h had approximately the same activities as the starch of the leaves inside. In August, there seemed to be no metabolic turnover of the starch after it had been synthesized (perhaps the rather high rate of synthesis in August is a consequence of the chamber climate). In the total lipids, significant variations of the specific activities are observed only during the growing season. The low activities in August and
February (less than 1% of the initial total activity) point to a very weak metabolic rate. There was no correlation to the total lipid content, which was high in February and low in August. The heterogeneous group of other soluble compounds in all seasons except summer showed a rapid loss of activity due to compounds with rather intense metabolism. In August, the turnover of this fraction was very low.

A similar conclusion to a weak turnover in August can be drawn from calculation of the total activities of soluble compounds expressed as percentages of the initial values after the 6 days of experiment. The decrease in April led to about one third of the initial value, in May to about 15%, in February to 20% and in August to 65%. In April, the retention of growing tissues seems to be important. The turnover in February was remarkably high regarding the low CO₂-fixation rate.

In bark (Table 2), only soluble sugars attained a high labelling rate. In April, the activity seemed to be retained, whereas in May a peculiar decline from the 2nd to the 6th day was observed. The total lipids — as found for the leaves — showed a significant turnover only in April and May. The labelling patterns in the bark and their variations can be explained by the transport processes. The transport rates were low during the periods of reduced metabolic activity. From the data obtained for leaves and bark in February one may conclude that there are rather intense metabolic processes occurring within the leaves. However, transport out of the leaves was very weak, which is in accordance with the observed low CO₂-fixation rate.

**Labelling patterns of compounds (as percentage of total activity)**

Calculation of percentages of specific activities in the allocated compounds gave further information on storage processes at different seasons. The initial labelling of starch was higher in August (40% of total ¹⁴C of soluble compounds) than in the other seasons (8–13%). For the lipid fraction, in May a rise in the percentage of ¹⁴C during the 144 h experimental period from 5% to 15% was found; however, in the other experiments the percentage of activity was not altered significantly. Soluble sugars showed a decline of activity (as percentage) during the 6 days, but it was distinct only in May, probably caused by a more effective transport of the leaves. As expected, the percentage of labelling of starch rose in all experiments, predominately during the first 2 days.

**Discussion**

*Ceratonia*, in spite of growing in the driest areas of the Mediterranean region, is not a very sclerophyllous species.
The young leaves are vulnerable to water stress, so that leaf growth has to be completed before the dry season commences (Lo Gullo et al., 1986). We found high rates of photosynthesis during the growth period and low values in winter and summer, thus confirming the results from Arbutus obtained by Beyschlag et al. (1986) and very recent results from Ceratonia (Nunes et al., 1992) using an IRGA-method. From their data these authors conclude that in winter the photosynthetic rate may be low due to low temperature and in summer due to the severe drought. We also observed the highest rate of photosynthesis in adult leaves of Ceratonia during the period of intense growth. During the feeding-experiment in August, the stomata of the leaves inside the exposure chamber were open (as proved by microscopy) and transpiration took place (seen from a rise of air humidity in the chamber); therefore, the low assimilation rate cannot be attributed to a tight stomatal closure. Non-stomatal mechanisms of the reduction of photosynthetic rates already were discussed by Larcher et al. (1981) for Olea and then, in a more general way comprising the data from literature, by Farquhar and Sharkey (1982) and Farquhar et al. (1989). A shift in the physiological response of photosynthesis to drought is not influenced by short time changes of leaf water potential (Gollan et al., 1985; Tenhunen et al., 1985), which in the August experiment could have been effective in the exposure chamber. However, there seem to be major influences of soil water content (Sharp and Davies, 1989). Nunes et al. (1992) showed that in Ceratonia a marked reduction in photosynthesis rate occurred only after depletion of all the available water in the upper soil layer. According to Tenhunen et al. (1990), such physiological adjustments lead to large savings of water and to a more efficient water use under severe stress; if so, they may be substantial in terms of carbon gain.

Variations in the labelling of sugars in Ceratonia leaves are comparable to those described for leaves of growing and non-growing Populus plants (Dickson and Nelson, 1982). During the growth period, most of the 14C initially found in sugars is metabolized or exported. Therefore, in Ceratonia after 144 h the activity of sugars in non-exposed leaves of the branch was as high as in the 14CO2 fed leaves.

A function of lipids as storage compounds is not clear and an accumulation, as described for Ceratonia by Diamantoglou and Meletiou-Christou (1977), is not in all cases a true storage (Hetherington et al., 1984; Chapin et al., 1990). Fully expanded leaves of Glycine show a slow lipid turnover (Kawara and Wong, 1985) and we obtained comparable results for Ceratonia in spring. The metabolic rates of lipids in Ceratonia in the summer and winter experiments almost may be neglected. Perhaps only a fairly small portion of the lipid fraction (e.g. the membrane lipids) has a high turnover rate and other components are rather inert. Therefore, an important portion of the fraction may not be used as readily available resources. Larcher and Thomaser-Thin (1988) showed that the lipid accumulation in sclerophylls is not influenced by environmental factors. From our experiments, a function of components of the lipid fraction as long term storage substances with only a weak metabolism cannot be fully ruled out. However, in any case they are less important as an energy storage than carbohydrates. Because of their weak turnover, the values for maintenance costs for the total lipids as calculated by Merino et al. (1984) seem to be too high for Ceratonia, especially in the cool and dry seasons. Perhaps this may also be true for other sclerophyllous species.

Severe stress conditions may cause a reinforcement of cyclic metabolism. Under conditions of drought and high light intensity in sclerophylls, a recycling of CO2 by photosynthesis could maintain carbon metabolism behind closed stomata. This mechanism provides a sink for the dissipation of photochemical energy, preventing damage of the photosynthetic system (Osmond et al., 1980). Anoxic stress may cause cyclic metabolic processes with participation of lipids (Kennedy et al., 1992). The decrease in lipid content in leaves of Ceratonia during the drought period led us to suppose that degradation of lipids could increase the CO2-production for recycling (Diamantoglou and Kull, 1982). Our labelling experiments described here do not give any indication of such a process. Our results in Ceratonia do not suggest a specific adaptation of the metabolism of storage compounds to summer drought. Biophysical mechanisms may be important for drought stress tolerance of leaves (Wyn Jones and Pritchard, 1989). According to recent results from Ceratonia leaves (Nunes et al., 1989) such mechanisms, as e.g. a change in the elastic modulus of cell walls, may be efficacious for avoiding the results of severe water stress.

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References


