Allocation of carbon to growth, storage and respiration in the vegetative barley plant

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Abstract. It is proposed that the growing plant can be divided into three compartments with reference to carbon: soluble, storage and structural. Experiments carried out at 10, 15, 20 and 30 °C in the light followed changes in size of these compartments in barley plants 10–24 days old. The redistribution of 14C photo-assimilated by 10 day old plants was monitored simultaneously. The soluble and storage compartments are a higher percentage of plant weight at lower temperatures, and are turned over rapidly at all temperatures; they form the source of respired 14C. About 30% of the 14C fixed enters structural material; in the first 24 h after labelling, for each unit of 14C entering the structural compartment, between 0-9 (at 15 °C) and 3.2 (at 30 °C) units of 14C are lost by respiration. At 15 °C in the dark, respiratory loss of 14C is initially from soluble and storage compartments; thereafter respiration of 14C occurs at the expense of structural material.

Introduction

Workers modelling growth of the whole plant or crop have found it convenient to consider the plant body as consisting basically of compounds of carbon and divisible into two major components—structural material and storage material, the latter including soluble sugars (Warren-Wilson, 1972; Thornley, 1976, 1977; de Wit, Brouwer & Penning de Vries, 1971; Fick, Williams & Loomis, 1973). Whilst plants have been separated experimentally into fractions corresponding to these two components (Priestley, 1973; Smith, 1973) there has been no explicit test of the hypothesis that the growth of plants can be described adequately in this way. Nor have the kinetic parameters of such a compartmental model been derived experimentally. These omissions define the purpose of this and a following paper (Prosser & Farrar, unpublished).

Specifically, I propose a model in which the barley plant is considered to be divisible into soluble, storage and structural compartments; some possible exchanges between these compartments are prohibited (Fig. 1). The soluble and storage pools are separated, both because I believe this an important division in the plant and also to emphasize our ignorance of how the level of storage material is controlled. For purposes of interpretation, I will also assume that the compartments are homogeneous and well mixed. Such a model has previously been applied to carbon allocation in lichens (Farrar, 1978).

In the experiments here, plants were grown under controlled conditions and harvested periodically, and the sizes of the three compartments, chosen to partition carbon, determined. The redistribution of photo-assimilated 14C between the compartments was followed simultaneously. Such experiments have been conducted in continuous light at a range of temperatures, with an additional experiment in continuous darkness. The use of constant conditions, although artificial, is adopted to facilitate analysis of compartmental data.

Materials and methods

Plant growth conditions

Barley (Hordeum distichum (L.) Lam. cv. Maris Mink) seed was soaked in aerated distilled water at 20 °C for 18 h and spread on moist filter paper in a growth cabinet (Sherer-Gillet) under continuous light of 320 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) over the waveband 400–700 nm from daylight fluorescent tubes, 80% relative humidity and temperatures of 10, 15, 20 or 30 °C (±1 °C) as appropriate. Six-day old seedlings were transferred to water culture, with three seedlings per blackened pot in 300 cm\(^2\) full strength Long Ashton solution plus 10 mg dm\(^{-3}\) of sodium metasilicate. This solution was changed at 4-day intervals.

At 10 days the plants were exposed to \(^{14}\text{CO}_2\). They were transferred to a fumigation cabinet designed to give a laminar air-flow over the plants, and in which fans mixed the air rapidly and so kept the specific activity of the \(^{14}\text{CO}_2\) supplied fairly uniform, and 3.7 MBq \(^{14}\text{CO}_2\) was released into the cabinet by reacting lactic acid with Na\(^{14}\text{CO}_3\) (from the Radiochemical Centre, Amersham) The plants were exposed for 30 min in an airstream of 0.4 m s\(^{-1}\) at 25 W m\(^{-2}\) and 20°C, and then returned to the growth cabinet for 0–14 days.

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During this period they were harvested sequentially, three pots being taken per harvest.

Analysis

After harvesting and removing the seed coat, leaf area was measured by an electronic planimeter (Hayashi-Denko AAM-5). Plants were dried in a ventilated oven for 1 h at 100 °C and then to constant weight at 80°C, and the dry weight determined to 0.1 mg. They were ground in 95% ethanol in a motor-driven Potter’s homogenizer, centrifuged at 3000 g for 15 min and the ethanol extract decanted and made up to 25 cm³. The residue was air-dried in a centrifuge tube and then resuspended in 10 cm³ 0.2 mol dm⁻³ sodium acetate/acetic acid buffer, pH 4.5. To this were added 95 units of amyloligosidase (9500 units g⁻¹ from Rhizopus; Sigma Chemical Co) and the tube was stoppered and incubated at 45°C for 24 h. The reaction was stopped by centrifuging and decanting the amyloligosidase/water extract, and the residue was resuspended in a total volume of 5 cm³ 95% ethanol. The total of carbohydrate removed into these two fractions closely agrees with that removed by (a) hot 95% ethanol or 80% methanol followed by hot 0.5 N H₂SO₄, or (b) 5% TCA in 35% methanol (Priestley, 1962, 1973).

Aliquots of the ethanol extract and residue were dried and weighed to 0.1 mg. Aliquots of the culture solution, ethanol extract and amyloligosidase extracts were assayed for radioactivity before and after shaking with ion exchange resin (Amberlite monobed MB-1). A scintillant of toluene: Triton X-100: PPO: POPOP (667:333:5:0.1, v/v/w/w) was used for these fractions, whilst an aliquot of the residue was suspended in a gel of this scintillant mixed 3:2 with water. A Packard TriCarb or a Tracerlab liquid scintillation spectrometer was used, and count rates converted to Bq by quench curves prepared by both the channels ratio and external standard method.

Aliquots of the ethanol and amyloligosidase extracts were assayed for carbohydrate by the phenol/sulphuric acid method of Dubois et al. (1956).

The data were collated under three headings, as follows:
(a) soluble–neutral, 95% ethanol soluble material;
(b) storage–neutral, soluble in aqueous amylo-

The changes of weight of each fraction and of leaf area, with time, were described by fitting regressions to log-transformed data. In all cases, a linear or quadratic regression gave an adequate fit; errors could be assessed and growth analysis performed if desired (Hughes & Freeman, 1967; Elias & Causton, 1976).

Changes in the ¹⁴C content of each fraction were plotted as Bq, and the specific activity of each fraction calculated as Bq/mg. The full mathematical treatment of these data is described in a following paper.

Each point on a graph or table is a mean of three or four determinations on pots with three plants per pot, and is presented with the standard error of the mean in Table 1. In the Figures, the standard errors are omitted for clarity; the coefficient of variation is about 15%.

Results

Net allocation to the three fractions in continuous light

At 20°C, over the full experimental period, from 7–28 days, a steadily falling RGR* accounted for growth, and both LAR* and NAR* changed with time (Fig. 2). Over the period 10–20 days a constant RGR of 0.14 day⁻¹ gave a good description of growth, with LAR and NAR also constant. Over this period, however, carbohydrate levels in soluble and storage fractions did not increase proportionately with whole plant dry weight, but showed a definite minimum at 14 days. Thus the exponential dry weight increase during this period occurred simultaneously with non-exponential behaviour of component fractions (Figs 2 & 5).

Similar behaviour was seen at 15 and 30°C, where soluble and storage fractions again showed minima at 13–14 days old. At 10°C, this fall in carbohydrates was

* RGR: Relative growth rate; LAR: Leaf area ratio; NAR: Net assimilation rate.
Table 1. Net allocation to soluble, storage and structural fractions in barley at four temperatures. The data are for 16 day old plants, and the values are those estimated from fitting regressions (linear or quadratic as appropriate) to loge-transformed weights against time.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>RGR of whole plant (day⁻¹)</th>
<th>RGR of soluble carbohydrate fraction (day⁻¹)</th>
<th>RGR of storage carbohydrate fraction (day⁻¹)</th>
<th>RGR of structural carbohydrate fraction (day⁻¹)</th>
<th>Soluble carbohydrate (% d.w.)</th>
<th>Storage carbohydrate (% d.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.118 ± 0.014</td>
<td>0.098 ± 0.026</td>
<td>0.065 ± 0.011</td>
<td>0.071 ± 0.013</td>
<td>9.5</td>
<td>8.0</td>
</tr>
<tr>
<td>15</td>
<td>0.126 ± 0.006</td>
<td>0.036 ± 0.017</td>
<td>0.052 ± 0.011</td>
<td>0.128 ± 0.008</td>
<td>5.7</td>
<td>5.7</td>
</tr>
<tr>
<td>20</td>
<td>0.140 ± 0.013</td>
<td>0.122 ± 0.002</td>
<td>0.127 ± 0.016</td>
<td>0.139 ± 0.013</td>
<td>4.6</td>
<td>4.6</td>
</tr>
<tr>
<td>30</td>
<td>0.149 ± 0.014</td>
<td>0.294 ± 0.081</td>
<td>0.187 ± 0.051</td>
<td>0.150 ± 0.021</td>
<td>5.4</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Figure 2. An analysis of barley grown at 20 °C. (a) The changes in weight of the whole plant (●) and soluble (○) and storage (●) carbohydrate fractions with time; the whole plant curve is a quadratic regression and the others fitted by eye. Changes in relative growth rate (RGR, b) and leaf area ratio (LAR) and net assimilation rate (NAR) (c) are derived from quadratic regressions of ln dry weight and ln leaf area on time; points (●) refer to leaf area ratio.

not detectable (Figs 3, 4 & 6). At all four temperatures studied, overall exponential growth occurred whilst net allocation to the three fractions changed differentially with time. Temperature itself had a small but definite effect on this net allocation, as summarized in Table 1. The levels of both soluble and storage carbohydrate, expressed as percentage dry weight, were a function of temperature; the lower the temperature the higher the levels (Table 1).

Although percentage carbohydrate fell with time and this and RGR changed with temperature, there was no close relationship between RGR and carbohydrate level for either soluble or storage carbohydrate (Table 1).

Respiratory loss of ¹⁴C in continuous light

The figure for total remaining radioactivity had a large error which was the sum of errors of each component included in it. Nevertheless, the pattern of loss of radiocarbon was broadly similar at all temperatures: a rapid initial loss for about 24 h followed by a much reduced rate of loss (Figs 3–6). The total loss—about 40% of the ¹⁴C fixed—was not greatly affected by temperature, but at the higher temperatures most of the loss occurred in the initial, rapid, phase. This pattern of loss was confirmed in an experiment where respired ¹⁴C was trapped in KOH and aliquots were counted at intervals; the loss is described by a semi-log plot with two exponential components (Fig. 7).

The source of the respirated CO₂ can be seen from Figs 3–6. For the initial phase of loss, there was a close parallel between ¹⁴C loss and the decreasing radioactivity of soluble material; over longer periods, both storage and soluble materials contributed but there was no net loss of ¹⁴C from structural material.
At all temperatures the $^{14}$C detected in the rooting medium was less than 3% of the initial total activity in the plant; it has been omitted from further consideration.

**Allocation of $^{14}$C to structure and storage in continuous light**

After the labelling period, the structural fraction had only about one-fifth of its final activity. In all cases about 30% of the applied $^{14}$C eventually entered the structural fraction; nearly all of it entered in the 48 h after $^{14}$CO$_2$ feeding, whilst the fall in soluble material was most rapid. Apparently, the rise in labelling in structural material more than 24 h after labelling was associated with loss of material from the other two fractions.

Storage material showed a high labelling at the first harvest. Labelling increased for 6–12 h after $^{14}$C feeding, followed by a fall in labelling. This indicated turn-

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**Figure 3.** Changes in fraction weight (a) and $^{14}$C content (b & c) of barley plants grown in the light at 10°C. The curves for fraction weights are fitted regressions and those for $^{14}$C content are drawn by eye. Symbols: ●, total plant; ○, structural material; □, soluble material; ■, storage material.

**Figure 4.** Changes in fraction weight (a) and $^{14}$C content (b & c) of barley plants grown in the light at 15°C. Details as for Fig. 3.
over of storage material, and was much more marked at 30 than at 10°C (Figs 3–6).

Examination of specific activities (Fig. 8) showed that movement from both soluble and storage fractions into structural material was possible throughout the period examined, at all temperatures. Within 24 h of labelling, the specific activity of soluble material was nearly equal to that of storage material and the specific activity of structural material approached this after about 72 h.

The behaviour of continuously darkened plants

In another experiment, a set of plants were darkened immediately after exposure to $^{14}$CO$_2$ and maintained in the dark at 15°C for 13 days. The soluble and storage fractions showed a rapid initial loss of weight, part of which was associated with net synthesis of structural material (Fig. 9). Much of the loss was, however, respiratory.

This was reflected in the fall in $^{14}$C content of the
Figure 7. The respiratory loss of $^{14}$CO$_2$ by barley plants grown in the light at 20°C. The percentage of $^{14}$C fixed remaining in the plant (a) can be plotted as a two-phase exponential curve (b and inset) with half-times of 10 h for the initial and 400 h for the subsequent phase.

Figure 8. Changes in specific activity of three plant fractions at (a) 10°C and (b) 30°C following pulse labelling with $^{14}$CO$_2$ at 10 days. Symbols: o, structure; o, soluble; and ■, storage, fractions.

Figure 9. Changes in fraction weights (a) and $^{14}$C content (b) of barley plants maintained in the dark at 15°C following pulse labelling with $^{14}$CO$_2$ at 11 days. Details as for Fig. 3.
Table 2. The respiratory cost of manufacturing structural and storage material. Data are calculated for the first 24 h after labelling with $^{14}$CO$_2$.

<table>
<thead>
<tr>
<th>T/°C</th>
<th>$^{14}$C respired (Bq)</th>
<th>$^{14}$C gain by structural and storage fractions (Bq)</th>
<th>$^{14}$C loss by respiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>930</td>
<td>580</td>
<td>1.6</td>
</tr>
<tr>
<td>15</td>
<td>1170</td>
<td>1330</td>
<td>0.9</td>
</tr>
<tr>
<td>20</td>
<td>1130</td>
<td>880</td>
<td>1.3</td>
</tr>
<tr>
<td>30</td>
<td>1670</td>
<td>520</td>
<td>3.2</td>
</tr>
</tbody>
</table>

plants. Soluble material showed a fall to about 10% of its initial labelling in 24 h; storage underwent a transient rise and then fell, its specific activity remaining constant. Respired $^{14}$C came entirely from these fractions for 48 h after darkening; over longer times these compartments showed very low rates of loss whilst there was an appreciable fall in the labelling of structural material. Thus from 2–13 days after darkening, structural material was the ultimate source of respired $^{14}$C. In spite of the difference between the source of $^{14}$C for respiration between these plants and those in the light, the kinetics of $^{14}$C loss were very similar (Figs 7 & 9).

Discussion

The constant RGR observed over the ages 10–20 days at all temperatures occurred in spite of changing NAR and shifts in the proportion of the plant that were soluble and stored carbohydrate. The changing relative growth rates of the latter pools appear particularly complex: at temperatures of 15°C and above the pool sizes fell to a minimum at 14 day and thereafter increased at a RGR nearly equal to that of structural material, maintaining the pools at a more or less constant level relative to dry weight. The RGR of whole plants, and of structural material, rose with temperature, but the percentage carbohydrate in both fractions was greatest at low temperatures, as frequently reported (White, 1973; Ruegg & Nosberger, 1977). These measurements allow net allocation to the three compartments to be measured, but do not permit gross allocation or turnover to be estimated.

Information on gross allocation to, and turnover of, these fractions was provided by the use of radioisotopes. Of a pulse of $^{14}$C fixed photosynthetically over a 20 min period, about 50% was lost by respiration over the ensuing 7 days, at all temperatures. The higher the temperature, the greater the proportion lost in the first 24 h. As the respiratory loss of $^{14}$C was mainly from the 95% ethanol soluble carbohydrate, and this fraction was a lower level the higher the temperature, this may be a function of label dilution. The $^{14}$C lost by the plant more than 24 h after labelling came, in the darkened plants, from structural material: this was consistent with the net loss of weight of this fraction and with much earlier literature (James, 1953).

In illuminated plants there was no net loss of $^{14}$C from the insoluble fraction, but rather the respiratory loss was sustained at the expense of both soluble and storage carbohydrate.

That the respiratory loss of $^{14}$CO$_2$ can be described as a two-phase exponential decay is consistent with previous reports (Ryle, Cobby & Powell, 1976). There is a need for caution in interpreting this curve, however. Firstly, an exponential loss of isotope from a compartmental system—the type of system assumed when interpreting exponential functions—would only be expected if each compartment was labelled to constant specific activity. The present data show this assumption to be quite unfounded and the efflux kinetics to be a rather poor indication of events within the plant. This is particularly well shown by the similar CO$_2$ efflux in light and dark concealing completely different pool behaviour. Secondly, attempts to relate the rapid phase of efflux to respiration associated with growth, and the slower phase to that associated with maintenance (Ryle et al., 1976) seems unnecessarily ambitious; it is far more likely that the phases seen represent loss of $^{14}$C from soluble carbohydrate (rapid phase) and stored carbohydrate (slower phase). They are a function of a compartmental system, not of different types of respiration.

It would appear that in the illuminated plant both soluble and storage material are continually turned over. Whilst hardly surprising for soluble carbohydrate, the plant’s carbon currency, it is perhaps more so for the storage fraction. The total activity of this fraction rose for 24 h after labelling and then fell, showing turnover, this apparently being more rapid the higher the temperature. Further, the specific activity of this fraction was very close to that of soluble material, for both illuminated and darkened plants, for most of the experiment, indicating perhaps that these two pools are in isotopic equilibrium with each other and thus exchanging materials rapidly. It is necessary to determine whether both the main storage compounds—starch, mainly in the leaves, and fructosans, mainly in the stem bases (Archbold, 1938)—behave in this way. It is worth stressing that this turnover of storage material is occurring in continuous light: perhaps ‘storage’ is a misnomer and the compounds concerned play an integral part in normal plant growth. Structural material appears to be formed at the
expense of both soluble and storage pools, but this is not certain as in no case is the rise in labelling of structural material greater than the fall in either of the other fractions.

There is no obvious evidence from these data for the turnover of structural material: in illuminated plants $^{14}$C continued to accumulate in this fraction, whilst in the dark there was no compelling evidence for anything but net loss of material after more than 48 h darkening. Here, indeed, the structural fraction was the net source of respired $^{14}$C.

Adoption of the model presented in Fig. 1 enables an estimate to be made of the cost of manufacturing storage and structural materials; the $^{14}$C leaving the soluble compartment can go to respiration or to storage or structure. Respiration is given by the loss of $^{14}$C from the plant, allocation to storage and structure by the increase in labelling of these fractions; if the soluble pool is homogeneous the ratio of $^{14}$C with these fates will reflect precisely the ratio of unlabelled material. Calculations are confined to the first 24 h to reduce the effect of storage to soluble conversions, and as there is some evidence that the soluble compartment is not homogeneous but contains a more labile pool which loses its radioactivity in about 24 h (see the semi-log plot of Fig. 8, where the soluble material shows a two-component exponential loss of $^{14}$C).

The ratio ($^{14}$C to respiration: C-14 to structure and storage) rises from 0.9 at 15°C to 3.2 at 30°C (Table 2); the net cost of making plant material is temperature dependent. This ratio is similar to that obtained from respiration measurements (Farrar, 1980). There is no reason to suppose that different metabolic pathways, of higher respiratory cost, operate at higher temperatures (Penning de Vries, 1972); thus the increased cost is likely to reflect increased turnover of cellular constituents. It points to the difficulty to be met in trying to separate 'growth' and 'maintenance' respiration in any way beyond the merely empirical (Thornley, 1976; Farrar, 1980).

It is instructive to consider the implications of these data for the control of plant growth. That relative growth rate remains constant in these barley plants, in spite of the episodic production of young leaves and the changing growth rates of constituent plant fractions, argues perhaps for control by sink rather than source; it is less easy to see how such control is exercised. There is no simple control of growth by substrate level, since no overall relation between carbohydrate, neither soluble nor stored, and growth rate was found. This contrasts with the positive relationships found for tomato by Thornley & Hurd (1974). Nor is it easy to see how the level of insoluble polymer such as starch, or of polymers of variable chain length such as the fructosans, can be achieved—and yet storage carbohydrate level seems subject to such control. Perhaps the apparently rapid turnover of, and thus equilibrium between, these fractions is relevant here.

It remains to consider whether this approach to the description of plant growth, intermediate as it is between growth analysis and a biochemical approach, has merit. This depends on whether it gives useful insights into plant growth, and on whether the assumptions made are reasonable. That the storage compartment is well mixed seems reasonable, and it seems to communicate rapidly with soluble material. The dual nature of the soluble pool has been mentioned above; each may be rapidly mixed as plant size (say 50 cm) is small compared with the rate of phloem transport (approximately 1 cm min$^{-1}$; Moorry, 1977). The assumption is clearly much less appropriate for structural material, and the adoption of the suggestion of Thornley (1977) of dividing structure further into degrading (protein) and non-degrading (wall) would seem desirable; this would also result in two more homogeneous compartments. It would clearly be of interest to divide up the plant morphologically as well as chemically. I feel therefore, that this approach is of value at least for heuristic purposes. What is needed is a test of the adequacy of the simple model proposed, and an assessment of transfer coefficients between the compartments. This is provided by a following paper, in which these data are subjected to a compartmental analysis (Prosser & Farrar, unpublished).

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