Dynamics of Photoassimilated Carbon in Douglas Fir Seedlings

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ABSTRACT

The relations between CO₂ uptake, translocation, and carbon accumulation in several vegetative components of Douglas fir seedlings (Pseudotsuga menziesii [Mirb.] Franco) have been quantified using ¹⁴CO₂. Seedlings were exposed to a constant specific radioactivity of ¹⁴CO₂ and a repeating daily pattern of temperature and light for 4 consecutive days. Results of ¹⁴C analysis, which indicated a transitory pattern of photoassimilated carbon movement, were extrapolated to a “steady rate” using a compartment analysis. Accumulation rates of photoassimilated carbon, relative to tissue carbon, were new needles, 0.94%/day, old needles, 1.14%/day, new shoots 0.38%/day, stem, 0.16%/day, and roots, 0.50%/day. Therefore, the source of carbon, the needles, is also the strongest sink.

The relations between structure and function in higher plants are ultimately linked to the carbon source, or photosynthesis. Rates of net photosynthesis have been reported extensively, especially in relation to environmental variables such as light, temperature, and water potential (2, 9), but quantitative data in support of the links between photosynthesis and plant growth functions are not as ubiquitous. In fact, a quantitative picture of carbon movement following its capture does not exist for a given plant (10). To understand the relations between photosynthesis and plant structural development, the links between photosynthesis and translocation must be coupled to those of utilization and respiration at various tissue sites in the plant. These links are difficult to quantify because the components of the plant system are so interdependent that unless experiments are conducted on undisturbed plants the results are hard to interpret (3).

Movement of photoassimilated carbon in intact plants has been studied extensively with ¹³CO₂: Schier (12) and Shiroya et al. (13) are two examples. This material is easy to apply and to assay with destructive sampling techniques. Results from single plants are readily attainable, but more difficult to attain are estimates of rates of carbon movement that require a time-intensive approach obtained from discrete destructive sampling. Usually, the high variation among plants and poor control over specific radioactivity of ¹³CO₂ during uptake results in variation too high to quantify transfer rates (7). Further, plants assayed for radioactivity several weeks or days after exposure to ¹³CO₂ have the radioactivity diluted by subsequent photosynthesis and respiration while plants sampled too soon after short term exposures show only the transient behavior of the tracer (6). This results in a “logarithmic distribution” of labeled material from the site of absorption, which is not representative of long term carbon movement following CO₂ uptake.

These technique and interpretation problems have made it difficult to quantify the rate that photoassimilated carbon accumulates in various plant parts. Without this comprehensive information on whole plants, understanding growth in terms of photosynthesis, respiration, translocation, and utilization is not possible.

In this paper, the dynamics of photoassimilated carbon are presented for intact seedlings of Douglas fir. To obtain rates of carbon assimilation and translocation, seedlings were exposed to constant specific radioactivity of ¹³CO₂ for 4 days. The results, which are shown to be of a transitory nature, are applied to a system of difference equations which are solved numerically to produce a “steady rate” of carbon accumulation in several of the vegetative components.

MATERIALS AND METHODS

Douglas fir seedlings (Pseudotsuga menziesii [Mirb.] Franco). 1 year old, were obtained from a nursery in December and planted in a soil block 60 × 60 × 20 cm. The sides and bottom of the soil block were encased in stainless steel screen to allow nutrient and H₂O movement from a surrounding natural soil profile. The block was partitioned into four compartments, seven seedlings per compartment, and the seedlings were grown outside (14). During the following November, the seedlings were placed in a 1780-liter controlled environment chamber and exposed to a constant specific radioactivity of ¹³CO₂ for 4 consecutive days.

The controlled environment chamber and the instrumentation for monitoring and controlling ¹³CO₂ and ¹³CO₂ in the chamber have been detailed previously (15). Briefly, light intensity in the chamber is supplied with a single 6000-w xenon arc. Intensities of 0.8 cal cm⁻² min⁻¹ are possible at plant level, and the intensity can be reduced to lower levels with filters that operate automatically. Air temperature and humidity are controlled independently. Concentrations of both the ¹³CO₂ and ¹³CO₂ in the chamber are monitored and controlled with systems located external to the controlled environment chamber. Air is pumped slowly from the chamber through an infrared gas analyzer for monitoring ¹³CO₂, and in another gas flow system, air is circulated through an ionization chamber for ¹³CO₂ measurements. As photosynthesizing plants remove ¹³CO₂ or ¹³CO₂ from the chamber atmosphere, both gases are automatically replaced from pressurized bottles containing high concentrations of either ¹³CO₂ or ¹³CO₂ in N₂. A custom-built controller, which receives the output of the infrared gas analyzer and the ionization chamber, activates solenoids inside the chamber, and small pulses of each gas are added as required. In the study reported here, ¹³CO₂ was controlled to 0.017 μCi/l ± 5% and ¹³CO₂ was between 320 and 335 μl/l. The environmental conditions in the chamber were programmed to be consistent with those on the growing site for con-
RESULTS AND DISCUSSION

Photoassimilated carbon increased significantly in all tissue components during the exposure period. The data for new needles are shown in Figure 1 and the variation is representative of other tissues. A linear regression was fitted to the data, which showed that approximately 5% of the carbon in the new needles was assimilated during the 4-day exposure to $^{14}$CO$_2$. The rate of accumulation was 1.29%/day based on total carbon. The accumulation rate, or the regression slope coefficient, was significantly different from zero at the 1% level, using a standard $t$ test, while the intercept was not significant. The $r^2$ was 0.84. A quadratic regression was fit to the data but the error term was not reduced.

The regressions for all tissues are shown in Figure 2. The coefficients and $r^2$ values for these regressions are tabulated in Table I. Accumulation rates, on a per day basis, were significantly different from zero at the 1% level for all tissue types. New needles accumulated carbon at the highest rate, 1.29%/day, followed by old needles at 0.88%/day. New stems accumulated carbon at 0.55%/day followed by the roots at 0.38%/day and the stems at 0.24%/day. The $r^2$ value was highest for the old needles at 0.90 and lowest for the stems at 0.59. Values of $C_f/C_t$ were found to be independent of the amount of tissue in each component. Intercept values, although not significant for new needles and old needles, were both positive, while intercepts for new shoots and stems were both negative, although neither was significant. The latter, however, indicates that a lag is occurring before carbon is translocated into the stems. This lag is magnified for roots, where the intercept in Figure 2 has a negative value greater than for new shoots or stems and is statistically significant.

\[ C_f/C_t = K \cdot \mu \text{Ci}^{14}\text{C}/\text{mg Tissue} \cdot \text{mgCO}_2 \text{ chamber}/\mu \text{Ci}^{14}\text{C} \text{ chamber} \] (1)

The constant, $K$, is a proportionality constant to convert from a $\text{CO}_2$ and dry weight basis to a carbon basis. The results of the analysis formed a time series which showed the accumulation of photoassimilated carbon in each tissue component during the exposure period.

![Figure 1](image1.png)

FIG. 1. Accumulation of photoassimilated carbon in new needles of 2-year-old Douglas fir seedlings. $C_f/C_t$ is the ratio of photoassimilated carbon ($C_f$) to tissue carbon ($C_t$).

ditions in November. The photoperiod was 9.5 hr long and total shortwave radiation was 340 cal/cm$^2$-day. Temperature was programmed to approximate a sine function with the maximum of 13°C at 4 AM and a minimum of 6.5°C at 4 AM. Humidity was held constant at a 6.5°C dew point.

After 24 hr in the controlled environment chamber, and just before the beginning of each light cycle, one-quarter of the plants were removed from the soil block and sectioned into components of new needles, old needles, new shoots, stem, and roots. The new needles of these 2-year-old seedlings were those that had developed in the second growing season. New shoots were stem material that elongated from the terminal and lateral buds which had set after the first growing season. Old needles were those developed in the first growing season. The stem was material elongated in the first growing season as well as growth from cambial differentiation in the second growing season.

After sectioning, the material was freeze-dried, weighed, and ground with a Wiley mill. The assay for $^{14}$C was performed with a dry combustion in-vial technique after Gupta (8), with modification by Webb (14). These samples were counted with a liquid scintillation spectrometer. Results were converted to a ratio of carbon fixed ($C_f$) during the exposure period to total carbon in a particular tissue ($C_t$) using the following relation and assuming 50% of the dry weight to be carbon.

![Figure 2](image2.png)

FIG. 2. Accumulation of photoassimilated carbon ($C_f$), relative to tissue carbon ($C_t$), in Douglas fir seedlings. Statistical parameters from these regressions are listed in Table I.
Table I. Carbon Accumulation in Douglas Fir Seedlings

Summary of linear regressions from Figure 2.

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Regression Coefficients</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intercept</td>
<td>Slope Y/Co/d</td>
</tr>
<tr>
<td>New needles</td>
<td>0.0036</td>
<td>0.0129</td>
</tr>
<tr>
<td>Old needles</td>
<td>0.0019</td>
<td>0.0088</td>
</tr>
<tr>
<td>New shoots</td>
<td>-0.00045</td>
<td>0.0055</td>
</tr>
<tr>
<td>Stem</td>
<td>-0.00065</td>
<td>0.0024</td>
</tr>
<tr>
<td>Roots</td>
<td>-0.0029</td>
<td>0.0038</td>
</tr>
</tbody>
</table>

The results of the regressions represent net accumulation rates of photoassimilated carbon. They are somewhat conservative estimates of actual accumulation since some carbon assimilated during the exposure of ¹⁴C0₂ will be turned over or respired back to the environment. The turnover rate seems slow since the intercepts of the regressions, with the exception of the roots, are not significant. A rapid turnover of photoassimilated carbon would result in a decreasing rate of net accumulation, since daily CO₂ exchange was held constant by repeating the temperature and light regime in the exposure chamber. Therefore, fitting the data with a linear expression would result in a positive intercept that would be proportional to the turnover rate. Ross (11) exposed branchlets of Douglas fir to ¹⁴C0₂ and recovered only 0.5% of the activity in respiration during a 6-hr period after labeling.

To determine if regressions in Figure 2 were significantly different from each other, the principal of "extra sum of squares" was followed (4). The following general model was used.

\[ Y = b_0X_1 + b_0X_2 + b_0X_3 + b_0X_4 + b_0(1 - X_1 - X_2 - X_3) + b_1(1 - X_1 - X_2 - X_3)X_1 + b_3X_2 + b_3X_3 + b_3X_4 + b_3(1 - X_1 - X_2 - X_3)X_4 \]

\[ X_1 \text{ through } X_4 \text{ are dummy variables with values of 0 or 1, and, in combination, are reference data for new needles, old needles, new shoots, stem, and roots. } X_1 \text{ is time in days and } Y \text{ is } C_7/C_1. \]

To test for differences, the parameters for any two regressions adjacent to one another in Figure 2 were set equal, thereby reducing model 2 to one with two less parameters. Then, the sum squares error terms for model 2 and the reduced model were used to calculate an F statistic. Several tests were made in this fashion and the results showed that the regression for new needles differed from old needles which, in turn, were different from new shoots. The regression on new shoots was different from stem and roots. Stem and roots were significantly different, but only at the 10% level while the others were different at the 1% level.

The demonstrated differences in accumulation rates shown in Figure 2 can be ascribed to two factors. Different tissues have separate carbon requirements for maintenance and tissue construction (10), and there are also differences in accumulation rates due to the lags inherent in translocation times. Studies have shown that new needles characteristically accumulate more ¹⁴C0₂ than do needles from previous growing seasons (11). The carbon requirements for these two seem to be the most directly comparable since there is no translocation lag. This conclusion assumes that translocation from them is identical.

The differences between the regressions for roots and stem show, to a degree, the dynamics of translocation and accumulation. Roots are receiving photoassimilated carbon later than the stems, but are accumulating carbon faster than stems. This represents a movement of carbon through a tissue with a low carbon requirement, the stem, into a tissue with a higher requirement, the roots.

These data make clear that the ¹⁴C tracer is showing a transient condition of accumulation and transfer among plant components and is not representing a steady rate condition. Results from regressions representing this 4-day period will not adequately represent true carbon dynamics, but are only useful for comparing transient states of each tissue.

To obtain estimates of the steady rate accumulation of photoassimilated carbon in various tissue components, a series of difference equations representing a compartment model was established. The compartments and the flow of carbon into and among them is represented in Figure 3. Both the representation and the DYNAMO simulation language for solving the difference equations numerically are after Forrester (5). Atkins (1) discusses the use of tracers in compartment analysis. In his model photoassimilated carbon moves from one organ to an adjacent one, with each organ retaining a portion of what it has received and passing on another portion. The system is a donor-controlled one in which the amount of carbon transferred from one compartment to the next is based on the amount in the first and a transfer coefficient, which represents the relation between accumulation and translocation. The result is a system of difference equations, one equation for each compartment, that are coupled together through the transfer coefficients. As an example, the equation for the stem tissue, or compartment, is

\[ \Delta ST/\Delta t = aNS + bON - cST \]  \[ (3) \]

NS, ON, ST, are variables representing carbon in the new shoots, old needles, and stem. The transfer coefficients a, b, and c are computed from the data in Figure 2.

To determine the transfer coefficients, a quadratic expression without a constant term was fitted to the data for each of the tissue types. This forced the regression through the origin, consistent with the fact that no ¹⁴C0₂ was absorbed into the plant at time zero. The average tissue weight, determined from all seedlings, was multiplied by the respective quadratic functions to give total amount of photoassimilated carbon accumulating in each tissue as a function of time. The first derivative of these regressions was then evaluated at time zero to give an estimate of the accumulation rate of photoassimilated carbon in each tissue.

Carbon flows, in mg/day, between compartments were calculated by subtracting accumulations in adjacent compartments.
In the simplest case, the daily flow of carbon from stem to roots was obtained by subtracting the daily accumulation in the stem from that of the roots. Transfer coefficients were obtained by dividing flows by accumulations.

The daily input of 16.6 mg of carbon, obtained by adding the accumulation rates of all tissues, was fractioned between new and old needles based on the relative mass of each and the fact that old needles are 70% as efficient in uptake as the new needles (16). Transfer coefficients for the feedback from roots to needles were determined by obtaining the best fit of the model to the data.

The scheme in Figure 3 probably is the simplest representation of carbon translocation and accumulation in an intact plant. The movement of carbon is represented as moving from the needles toward the roots, with utilization occurring in various sinks. The feedback from roots to needles represents the upward movement of various compounds in the xylem sap. A major constituent of the xylem sap is nitrogenous compounds formed by nitrate reduction in the roots (17). Compartments are specified based on their homogeneity of carbon accumulation. New needles, as an example, will metabolize quite differently on a weight basis than old needles where cell wall structure is completed.

The transfer coefficients in Figure 3 illustrate quantitatively the relation between tissue structure and the translocation and accumulation of photoassimilated carbon. The new needles, which are the most metabolically active, transfer only 44.4% of the carbon that enters directly from photosynthesis or indirectly from the roots. At the other extreme, the stem, which is composed largely of conducting tissue, transferred over five times as much carbon as the new needles.

Table II. Steady Rate Increase of Photoassimilated Carbon in Douglas Fir Seedlings

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Average Total Carbon</th>
<th>Increase of Photoassimilated Carbon</th>
<th>Increase of Photoassimilated/Total Carbon</th>
</tr>
</thead>
<tbody>
<tr>
<td>New needles</td>
<td>573</td>
<td>5.39</td>
<td>0.0094</td>
</tr>
<tr>
<td>Old needles</td>
<td>157</td>
<td>1.79</td>
<td>0.0114</td>
</tr>
<tr>
<td>New shoots</td>
<td>192</td>
<td>0.73</td>
<td>0.0038</td>
</tr>
<tr>
<td>Stem</td>
<td>445</td>
<td>0.71</td>
<td>0.0016</td>
</tr>
<tr>
<td>Roots</td>
<td>1592</td>
<td>7.98</td>
<td>0.0050</td>
</tr>
</tbody>
</table>

Fig. 4. Accumulation of photoassimilated carbon in Douglas fir seedlings. Lines in A, B, and C are results from compartment model shown in Figure 3. Data points in B and C are means of four observations.
carbon as it retained. Old needles, which are usually less efficient in CO₂ uptake than new needles, also transferred more carbon than new needles. New shoots are transferring 3.29 times as much carbon as they retain. Terminal buds were included in new shoots which may account for the high retention of carbon in this tissue relative to the stem component. Roots retained 50% of the carbon they received, and are transferring 50% back to the needles. Probably much of this retained carbon is in a storage component since most confers store photosynthate in the roots during the fall (13).

The dynamics of carbon accumulation for each compartment are shown in Figure 4. Figure 4A is the result of the compartment model in Figure 5. Figure 4, B and C, compare the data and model output. Data are averages of four observations and the variation is proportional to that in Figure 2, and that shown by r² values in Table I.

The ¹³C results represent the uptake and movement during the 4-day labeling period. The model formulates the assumptions of translocation and accumulation, and, with the transfer coefficients obtained from the data, is used as a basis to extrapolate the ¹³C data to a rate of constant increase of photoassimilated carbon in each tissue type. This steady rate of increase, which is sometimes termed a dynamic steady state, represents the real accumulation of carbon resulting from the uptake and distribution pattern as opposed to the apparent rates obtained directly from the tracer results. In this instance, the extrapolation required to obtain steady rates of increase was 2 days beyond the 4-day labeling period.

The transient condition of carbon movement following uptake is most easily seen for the new needles and roots. In new needles the accumulation rate is high initially and declines to a steady rate of increase after 6 days, i.e. the rate of increase approaches linearity. The photoassimilated carbon increase in roots has a low rate initially and increases to a higher steady rate after about 6 days. The other tissues have transient conditions that occur in the first 6 days, but they are not readily discernible in Figure 4. Dynamics in old needles is similar to new needles. The photoassimilated carbon in stems and new shoots is a very flat S shape. Increases are initially low, reach a maximum in about 3 days, and decline to a steady rate in about 6 days. The transient behavior of the system is complete within 6 days. After this point, photoassimilated carbon is increasing at essentially constant rates in all five tissues (Table II).

Roots received the most carbon per day, 7.98 mg, followed by new needles at 5.39 mg/day. Old needles accumulated carbon at 1.79 mg/day followed by new shoots and stems at 0.73 mg/day and 0.71 mg/day, respectively. Total accumulation of carbon per day was 16.6 mg, or the same as that obtained by adding accumulation rates for all tissues. These rates are the average accumulation rates for each tissue and reflect the total average carbon in each tissue as well as the demand of each for photoassimilated carbon on a per unit of tissue basis. In Table II, column 3, the photoassimilated carbon per day per unit of tissue is listed. Both new needles and old needles accumulated carbon at high rates, 0.90%/day and 1.14%/day. Roots were lower, 0.50%/day followed by new shoots, 0.38%/day and stems, 0.16%/day.

The average increase, weighted by the respective tissue weights, is 0.56%/day. These rates are estimates of daily carbon require-ments for each tissue. Translocation lags have been removed since the system has reached a steady rate of increase.

Accumulation rates for both old and new needles is consistent with pholology. Ross (11), using a pulse-labeling technique, found that the difference in sink strengths between current year's foliage and previous years' decreased during the growing season. Extractions of old needles and new needles for cellulose and lignin showed the concentrations of each to be about 10% in both tissue types (unpublished data). Therefore, the non-cell wall fractions in the old and new needles were identical during the exposure to ¹⁴CO₂. Accumulation rates for old needles is slightly higher than new needles, and may indicate a storage of carbohydrates. New shoots have a much higher accumulation rate than stems, 0.38%/day as opposed to 0.16%/day. This may indicate a higher growth rate in new shoots relative to the stem, where a larger percentage of daily carbon accumulation is probably used for maintenance functions. Generally the lower rates for roots, stems, and new shoots, compared to needles, reflects the much higher metabolic activity, on a per unit weight basis, in the needles. Thus, the needles, commonly considered the source of carbon in the classic source-to-sink concept of translocation, are clearly the major sink for photoassimilated carbon as well as its source.

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LITERATURE CITED