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Incorporation of ^{14}C-Photosynthate into Protein during Leaf Development in Young *Populus* Plants[1]

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ABSTRACT

Gas exchange and protein metabolism were studied in expanding, mature, and near-senescent leaves of young clonal *Populus* × *euramericana* cv. Wisconsin-5 plants. Dark respiration, CO_2 evolution in the light, and CO_2 compensation concentrations were highest in unexpanded leaves but declined markedly as leaves matured and aged. Net photosynthesis was highest in nearly mature leaves. Fresh weight continued to increase after leaf expansion was complete, whereas soluble protein levels declined. Changes in the distribution of photosynthetically incorporated ^{14}C indicated that a high level of protein synthesis and rapid formation of structural components occurred only in expanding leaves. Protein turnover was slight in expanding leaves but was substantial after leaves were mature. Expanding leaves synthesized predominantly fraction I protein (ribulose diphosphate carboxylase). However, formation of this protein from photosynthate was slight once leaves matured.

An important consideration in the analysis of plant growth is the interaction of ontogeny and morphogenesis with physiological processes. Marked variations in physiological activity with leaf maturation and aging have been reported, including changes in photosynthesis and photosynthate translocation (2, 3, 12, 14, 24), photorespiration (10, 21), protein and enzyme synthesis (4, 6, 8, 15, 24, 26), and nucleic acid metabolism (17, 19, 23, 26). Our understanding of leaf developmental physiology, however, remains rudimentary.

Results of several studies of cottonwood (*Populus deltoides*) have shown that photosynthetic rate and RuDP[3] carboxylase activity seem to correlate with specific changes in electrophoretic patterns of leaf proteins and enzymes during leaf development (4, 6). To explore this relationship further, we measured CO_2 exchange and *in situ* incorporation of ^{14}C-labeled photosynthate in poplar leaves at four developmental stages. Electrophoretic profiles of ^{14}C-labeled protein from these leaves were then compared with corresponding electrophoretic staining patterns for total protein. The data indicate that developmental changes in photosynthetic rate are dependent upon synthesis of specific leaf proteins from current photosynthate.

MATERIALS AND METHODS

Plant Material. Ramets of a hybrid poplar clone (*Populus* × *euramericana* cv. Wisconsin-5) were propagated from tip cuttings rooted under mist, and were grown in an artificial soil mix consisting of peat, perlite, and vermiculite. After plants were established in pots in the greenhouse, they were transferred to growth chambers and maintained under a temperature regime of 25 C during an 18 hr day and 15 C at night. Light intensity (400–700 nm) was 500 μeinsteins m^{-2} sec^{-1} (29,800 lux). Pots were fertilized weekly with a commercial 20-20-20 soluble fertilizer supplemented with Fe-EDTA.

Plant Treatments. Leaves at a constant insertion height from the stem base (8th leaf) were treated at four stages of development as measured by LPI (13): LPI 4, an immature, rapidly expanding leaf; LPI 8, a nearly mature leaf; LPI 19, a fully mature leaf, and LPI 36, a leaf nearing senescence (Fig. 1). Attached leaves were enclosed in a water-cooled leaf chamber and connected to a closed-circuit infrared gas analysis system (3). Light intensity (400–700 nm) in the leaf chambers was 500 μeinsteins m^{-2} sec^{-1} (29,800 lux). Leaf temperature, measured by a thermocouple appressed to the lower leaf surface, was maintained within the range 24.5 to 25.5 C. After an equilibration period, measurements of net photosynthesis, dark respiration, and CO_2 evolution into CO_2-free air were taken. Then 25 μCi of $^{14}CO_2$ were introduced into the system and the leaf was allowed to photoassimilate the isotope for a minimum of 1 hr or until CO_2 compensation was reached. After treatment plants were returned to the growth chambers. Harvests were made either 3 hr or 48 hr after ^{14}C treatments were initiated. Thus, each treatment sequence consisted of eight leaves from eight different plants: four LPI's, each with two harvest times (Fig. 1). Each sequence was replicated three times.

Leaf Analysis. Leaves were excised at the base of the lamina, they were weighed, and their outlines were traced. They were then inserted in a vial, frozen in liquid N_2, and lyophilized. The dried leaf material was ground in a Wiley mill to pass a 20-mesh sieve.

Extracts of the leaf powder were prepared by a single 15-sec grinding of 100-mg samples in 5 ml of buffer (0.05 N tris-HCl, 0.1 M in sucrose) at pH 7.4 in a Duall homogenizer. The extracts were centrifuged at 105,000*g* for 1 hr. Total ^{14}C activity in the crude extract was determined by adding 0.1-ml aliquots to 15 ml of scintillation medium and counting in a liquid scintillation spectrometer.

Activity of ^{14}C in the protein fraction of the crude extract was determined by adding 1 ml of 20% (w/v) trichloroacetic acid to 1 ml of the crude extract, centrifuging at 105,000*g* for

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[3] Abbreviations: RuDP: ribulose 1,5-diphosphate; LPI: leaf plastochron index.

30 min, and counting 0.1-ml aliquots of the resulting supernatant in 15 ml of scintillation medium. Difference between activity in the trichloroacetic acid supernatant and crude extract gave activity in soluble protein. Protein determinations were run on 1 N NaOH solutions of 10% (w/v) trichloroacetic acid precipitates of the crude extracts (16). Activity of ^{14}C in the pellet resulting from the initial centrifugation was determined by dissolving 10-mg samples with 1 ml of NCS (Amersham/Searle quaternary ammonium base solubilizer) and counting as above.

Acrylamide gel electrophoresis of proteins in the crude extract was performed in a slab gel electrophoresis chamber with eight 0.1-ml aliquots of the crude extract from each of two leaves run in a single gel (6). Runs were in the direction of the anode. After electrophoresis, half of the gel from each leaf was stained with Amido black for total protein. The remaining unstained gel from each leaf was divided longitudinally into four segments and the portion of each segment between the application slot and electrophoretic front was subdivided into 5-mm sections. Each section was placed in a scintillation vial with 0.3 ml of 30% (v/v) H_2O_2. After 48 to 72 hr, the gel was depolymerized and 3 ml of NCS were added, followed by 13 ml of scintillation medium.

All labeled solutions in this study were dissolved in a scintillation medium consisting of a 5:2 mixture of toluene and methyl cellosolve plus 4 g/l of PPO and 50 mg/l of POPOP. All samples were counted to 1% error. Counting efficiency was determined with an external standard and data were corrected to dpm.

RESULTS AND DISCUSSION

Table I summarizes the CO_2-exchange data of leaves at the time of ^{14}C treatment. Photosynthetic rate was lowest in the youngest leaves, reached a maximum in the nearly fully expanded leaves (LPI 8), and declined gradually in older leaves (LPI 19 and 36). Similar patterns of photosynthesis have been shown by Dickmann (3) and Larson and Gordon (12) for *Populus deltoides* leaves. The patterns of dark respiration and CO_2 evolution into CO_2-free air in the present study were similar: a decreasing trend with leaf aging through LPI 19, then increasing slightly in the oldest leaves (LPI 36). The high evolution of CO_2 in the light by young leaves probably includes a considerable mitochondrial (dark) respiration component in addition to photorespiration. For example, young leaves of tobacco and citrus have shown low glycolate oxidase (10, 21) and slight increases in photosynthesis when external O_2 tensions are lowered (21), indicating a reduced photorespiratory capacity in immature tissues.

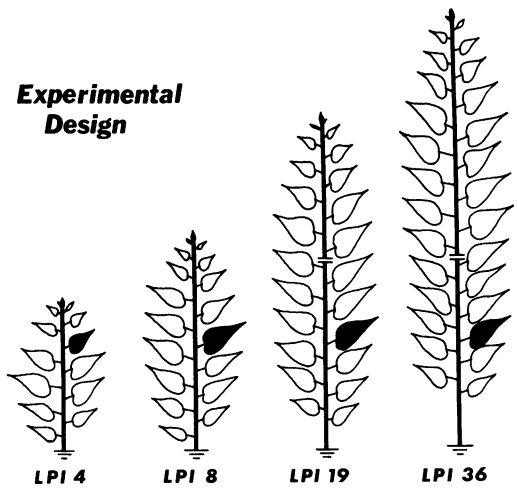

FIG. 1. Experimental design. Measurement of gas exchange patterns and exposure to $^{14}CO_2$ were performed on leaves of LPI 4, 8, 19, and 36 (darkened leaves) at a constant insertion height from the stem base on separate plants. For each LPI, one leaf was harvested three hr after inception of ^{14}C treatment and one after 48 hr. Each treatment sequence was replicated three times.

Table I. *CO_2 Exchange Characteristics for Leaves at Time of ^{14}C Treatment*

Data are averages of three replications. Average leaf temperature was 24.6 C; light intensity was (400–700 nm) 500 μeinsteins m^{-2} sec^{-1} (29,800 lux).

Leaf Age	Dark Respiration	Net Photosynthesis	CO_2 Evolution into CO_2-free Air	CO_2 Compensation Concn
LPI	*mg CO_2 hr^{-1} dm^{-2}*			*μl/l*
4	4.8	4.2	5.7	100
8	2.3	9.8	3.9	59
19	0.8	7.8	2.1	53
36	1.0	6.5	2.9	68

Table II. *Changes in Length, Fresh Weight, and Soluble Protein Content of ^{14}C-treated Leaves*

Data are averages of three replications.

Leaf Age	Harvest Time	Leaf Length		Fresh Weight	Soluble Protein
		Time of treatment	Time of harvest		
LPI	*hr*	*cm*		*mg*	*μg/mg dry wt*
4	3	7.8	7.8	563	138
4	48	8.0	10.6	1103	122
8	3	10.5	10.5	1527	114
8	48	10.4	10.6	1552	112
19	3	12.1	12.1	1710	75
19	48	12.8	12.8	1970	78
36	3	11.4	11.4	2230	99
36	48	11.6	11.6	2020	83

The CO_2 compensation concentration was highest at LPI 4, lowest at LPI 19, and increasing at LPI 36 (Table I). This morphogenic pattern is typical of young *Populus* plants, as is the minimum CO_2 compensation concentration of 53 μl/l (5). In addition, although net photosynthesis rate was maximum at LPI 8, photosynthetic efficiency, *i.e.*, the relative balance of photosynthesis and photorespiration as measured by the CO_2 compensation concentration, was maximum in older leaves of LPI 19.

In Table II certain physical and biochemical characteristics are given for leaves at the time of treatment and harvest. No detectable growth in length occurred in any leaves during the 3-hr incubation period. After 48 hr, however, a substantial extension had occurred in leaves of LPI 4, whereas leaves of LPI 8 showed only a slight increase in length. Leaves of LPI 19 and 36 were fully mature and did not grow in length. Fresh weight data reflect a different pattern. Fully mature leaves continued to increase in fresh weight, even though dimensional increase was not evident after LPI 8. Soluble protein concentrations, on the other hand, were highest in young leaves and lowest in older leaves (Table II).

Table III. *Distribution of ^{14}C Activity between Major Leaf Fractions after Extraction*

Data are averages of three replications.

Leaf Age	Harvest Time	Trichloroacetic Acid Precipitate[1]	Trichloroacetic Acid Supernatant[2]	Pellet[3]	Total
LPI	*hr*	*dpm/mg dry weight* $\times 10^{-3}$			
4	3	21	132	172	325
4	48	17	45	145	207
8	3	15	65	39	119
8	48	5	12	35	52
19	3	8	56	10	74
19	48	2	26	6	34
36	3	7	63	10	80
36	48	2	23	8	33

[1] Soluble protein.
[2] Other soluble molecules.
[3] Structural and insoluble components.

The distribution of ^{14}C (dpm/mg dry weight) among various leaf fractions after extraction is given in Table III. The trichloroacetic acid precipitate values represent activity primarily in soluble protein, whereas the trichloroacetic acid supernatant column represents activity in nonproteinaceous soluble molecules such as sugars, amino acids, and organic acids. The pellet was a heterogeneous mixture of cell fragments, cell wall components, and water-insoluble compounds.

Total leaf ^{14}C levels were highest at LPI 4 but decreased markedly with leaf age. At LPI 4 the highest proportion of ^{14}C was located in the pellet after 3 hr and, although total activity in this fraction declined after 48 hr, the percentage of total activity in the pellet at 48 hr increased (Fig. 2). A similar trend was noted at LPI 8. These trends are an indication of synthesis of structural components and other insoluble molecules during leaf expansion. But older mature leaves showed some incorporation of label into the pellet fraction (Table III and Fig. 2).

^{14}C activity in the trichloroacetic acid supernatant fraction remained high throughout the aging series (Table III), but the percentage of total activity in this fraction increased markedly in older, mature leaves (Fig. 2). However, the absolute loss of ^{14}C after 48 hr at LPI 4 and 8 was considerable. This decrease in activity in the trichloroacetic acid supernatant fraction can be attributed primarily to conversion of soluble molecules to structural components and high respiration rates in the young leaves (Table I). Loss of ^{14}C because of translocation from the leaf would be minimal at this stage (11). In contrast, a leaf of LPI 8 was almost fully expanded and was at its most effective stage for photosynthate export (11, 12). Therefore, the loss in activity from the trichloroacetic acid supernatant fraction after 48 hr at LPI 8 (Table III and Fig. 2) can be attributed primarily to translocation, although some ^{14}C-labeled components were still being converted to structural compounds. The effectiveness of translocation declines with leaf aging (12), and this is reflected by the lower loss of ^{14}C in the trichloroacetic acid supernatant fraction after 48 hr at LPI 19 and 36.

Changes in the trichloroacetic acid precipitate (soluble protein) fraction with leaf development are also given in Table III and Figure 2. Labeling in protein was highest in young expanding leaves but declined markedly with leaf age, indicating a general reduction in the level of protein synthesis from photosynthate. However, the proportion of total leaf ^{14}C activity in protein increased during leaf expansion and then remained relatively constant after leaves were mature (Fig. 2). These observations are consistent with the observed rapid protein synthesis associated with leaf expansion (15). But the present data also show that, although the size of the photosynthate pool available for *in situ* protein synthesis shrinks appreciably with leaf age, the proportion of this pool incorporated into protein varies little once leaves mature.

Table IV shows that a significant change in total protein turnover (simultaneous synthesis and degradation) occurred during leaf development. Assuming predominantly open amino acid precursor pools, loss in protein specific radioactivity with time after a pulse ^{14}C treatment is an indicator of turnover rate (7). For leaves treated at LPI 4, only 9% of the protein specific radioactivity was lost after 48 hr; in fact, when expressed as a percentage of total activity (Fig. 2), the level of ^{14}C in protein increased after 48 hr. Older leaves, however, lost nearly three-fourths of their ^{14}C in protein after 48 hr. The slight loss of ^{14}C specific radioactivity in young leaves indicated a generally lower turnover rate, or the synthesis of a major stable protein not subject to turnover. Our further data support the latter interpretation.

A densely staining band at an R_F of 0.16 to 0.18 was present in electrophoretic gels at all leaf ages. This band was faintest at LPI 4 at 3 hr but darkened considerably after 48 hr. No discernible intensification of band staining occurred in older leaves. Preliminary experiments, not reported here, indicate that this band is fraction I protein. This enzyme is a prominent constituent of photosynthetic cells, where it catalyzes both the carboxylation (9) and oxidation (1) of RuDP.

Patterns of ^{14}C distribution within electrophoretic gels are shown in Figure 3. Total ^{14}C activity recovered from the gels declined with leaf age and was less after 48 hr than after 3 hr except at LPI 4, at which more than twice the activity found at 3 hr was recovered at 48 hr. In addition, the ^{14}C labeling profile in the gels showed a distinct pattern with leaf age (Fig. 3). At LPI 4 at 3 hr, a fairly uniform profile was found throughout the gel, although a slight peak occurred in the posi-

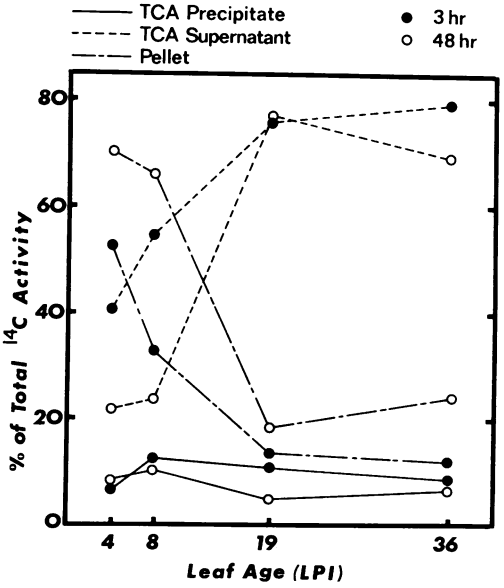

FIG. 2. Percentage of total leaf ^{14}C (dpm/mg dry weight) recovered from various leaf fractions in leaves of LPI 4, 8, 19, and 36 harvested after 3 hr and 48 hr. Points are averages of three replications. Trichloroacetic acid precipitate: soluble protein; trichloroacetic acid supernatant: nonproteinaceous soluble molecules; pellet: structural and insoluble components.

Table IV. *Changes in Specific Radioactivity of Soluble Protein (Trichloroacetic Acid Precipitate) with Leaf Age and Harvest Time*

Data are averages of three replications.

Leaf Age	Harvest Time	^{14}C Specific Radioactivity	Loss after 48 hr
LPI	*hr*	*dpm/μg protein*	%
4	3	150	
4	48	137	9
8	3	131	
8	48	48	63
19	3	106	
19	48	19	82
36	3	69	
36	48	25	64

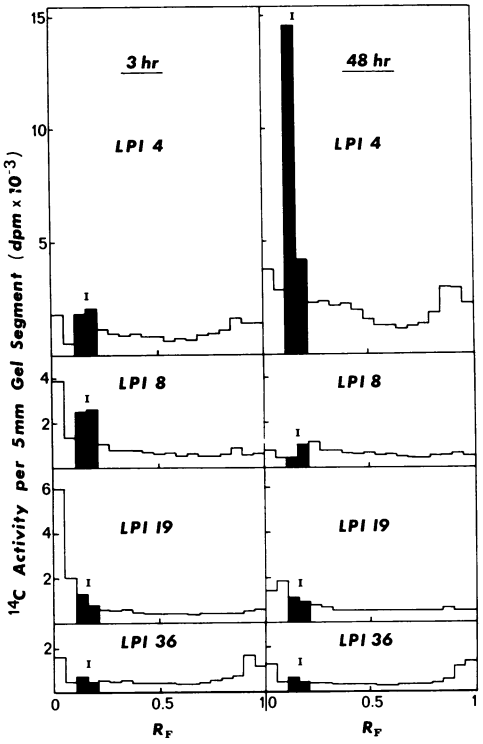

FIG. 3. ^{14}C profiles for unstained electrophoretic gels for leaves of LPI 4, 8, 19, and 36 harvested after 3 hr and 48 hr. Fraction I band is between R_F 0.1 and 0.2 (darkened bars). Figures are averages of three replications.

tion of the fraction I band. After 48 hr, however, this fraction I peak had enlarged considerably, indicating a rapid synthesis of this protein during the two-day period following treatment. A slight peak in ^{14}C activity in the fraction I position also occurred at LPI 8 at 3 hr and a lesser one occurred at LPI 8 at 48 hr. These peaks were absent at LPI's 19 and 36.

Woolhouse (26) concluded that, in *Perilla*, decrease of leaf protein with age was attributable to "switching off" of fraction I protein synthesis. The present study supports this view for *Populus* and shows furthermore that synthesis of this critical protein complex occurs predominantly in expanding leaves. Furthermore, Peterson *et al.* (20) showed that little turnover of RuDP carboxylase (fraction I protein) occurs under normal environmental conditions, as opposed to other soluble proteins which are rapidly synthesized and degraded. The data of Table IV and Figure 3 of our study support their conclusion. Therefore, any adverse environmental condition (*e.g.*, water stress or mineral deficiency) that disrupts synthesis of the fraction I complex during the critical phase of leaf expansion is likely to permanently reduce the levels of RuDP carboxylase. There is evidence that photosynthetic rate is closely correlated with RuDP carboxylase activity (4, 22, 24) and that the carboxylating step of the Calvin cycle may be rate-limiting in photosynthesis at saturating light and ambient CO_2 levels (18, 25). Thus, an expanded leaf may continue to synthesize other proteins, Chl, and various cellular components after stress is relieved, but the resulting deficiency of RuDP carboxylase may limit its potential photosynthetic capability throughout its entire life. Implicit in this conclusion is the importance of maintaining plants under conditions in which environmental stress is minimized during leaf expansion, particularly in situations where maximum plant productivity or dry weight yield is sought.

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