

Functional and Structural Changes in Senescing *Populus deltoides* (Bartr.) Chloroplasts¹

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ABSTRACT

Chloroplasts isolated from *Populus deltoides* leaves were used to study age-dependent changes in the rate of cyclic photophosphorylation. Single leaves were used to measure CO₂ fixation by leaf discs, chlorophyll concentration, and ATP synthesis. The ability of chloroplasts to synthesize ATP diminished steadily from the time of full leaf expansion, regardless whether the results are expressed on a leaf area or chlorophyll basis. This decline in the rates of ATP synthesis was paralleled by the decline in the rate of CO₂ fixation. The results suggest that the efficiency of the membrane-bound ATP synthesizing system declines with age.

MATERIALS AND METHODS

Individual plants were used to evaluate age-dependent changes in the rates of photosynthetic processes. CO₂ fixation, ATP synthesis, and chlorophyll content were measured using an individual leaf for all determinations. This avoided variation arising from mixing leaves of the same age from different plants while preparing the chloroplasts. Variation was further reduced by use of clonal material. Cottonwood plants were grown as described previously (9). Newly emerging leaves were marked with small labels. Leaves were harvested after a period of 5 to 6 hr in the light. For each experiment, three leaves of different age were taken from one plant and processed individually. The leaves were freed of midribs and leaf margins and cut into halves. One-half of a leaf was used for chlorophyll determination and CO₂ fixation, and the other half for chloroplast isolation for cyclic ATP synthesis. Chlorophyll was measured as described by Arnon (1).

Photosynthetic and respiratory rates vary with leaf age (8, 13, 14, 22, 23, 26). In leaves of dicotyledon plants after an initial rise (3), the photosynthetic capacity gradually declines after leaf expansion (6, 16, 19, 20). This decline in CO₂ assimilation with age is parallel to a decrease in RNA, protein, and DNA contents of leaves (27).

Molish (14) observed during yellowing of leaves the disappearance of chlorophyll and protein, the disorganization of the chloroplast structure, and the appearance of fat droplets. He found that leaves in a very advanced stage of senescence were unable to fix CO₂ and suggested a correlation between structure and function in the chloroplast. Fine structure changes of senescing leaves have been studied in several plants (11, 21). Senescence of birch tree leaves was associated with a change in shape and volume of chloroplasts in addition to an increase in the number of lipid globules (4).

Nearly all the studies of the relationship between photosynthesis and leaf age have been conducted with intact leaves or leaf extracts. However, Smillie and Krotkov (24) have isolated chloroplasts from pea plants and evaluated the changes in photophosphorylation with age.

The present investigation on foliar senescence of *Populus deltoides* Bart. attempts to relate CO₂ fixation and chlorophyll content of leaf discs to cyclic photophosphorylation by isolated chloroplasts and changes in chloroplast structure as seen under the electron microscope.

CO₂ Assimilation. CO₂ assimilation of leaves was measured with discs 1.2 cm in diameter and floated on water with the adaxial surface downward in a flask equipped with a side arm (12). Two hundred μ moles NaHC¹⁴O₃ (9×10^6 – 1×10^7 cpm) were placed in the side arm of the flask. Five ml of air were withdrawn from the sealed flask with a syringe to create a partial vacuum. Flasks were placed in a Plexiglas-bottomed constant temperature water bath and preilluminated for 5 min before the release of ¹⁴CO₂ gas from the side arm by injection of 0.2 ml 9 N H₂SO₄. The partial pressure of CO₂ in the flask was approximately 7%. Illumination from above was provided by incandescent light at an intensity of about 50,000 lux (4.5 w cm⁻²) at 20 C. Under these conditions CO₂ assimilation progressed linearly for up to 30 min. Photosynthesis was stopped by the injection of 95% ethanol. The leaf discs were extracted in boiling 80% (v/v) ethanol; the radioactivity in the extracts and in the ethanol extracted leaf discs was determined with a scintillation spectrometer (9). Radioactivity in the ethanol-insoluble fraction was between 10 and 15% of total activity.

ATP Synthesis. One-half leaf was placed in 30 ml of homogenizing medium consisting of 0.35 M sorbitol, 50 mM Tricine, pH 7.6, 2 mM EDTA, 20 mM Na-isoascorbate, and 1.5% polyethylene glycol 4000 (w/v) and vacuum infiltrated to preserve enzymatic activity. The deveined leaf pieces were then disrupted for 30 sec in a Waring Blendor at full speed. ATP synthesis by the isolated chloroplast fragments was measured in triplicate as described earlier (9). Conditions used for ATP synthesis were found to be close to optimum for young, mature, and old leaves. Photophosphorylation was defined as the ATP formed in the light minus the ATP formed in the dark.

¹ This study is drawn from the Ph.D. thesis research of R. Hernández-Gil.

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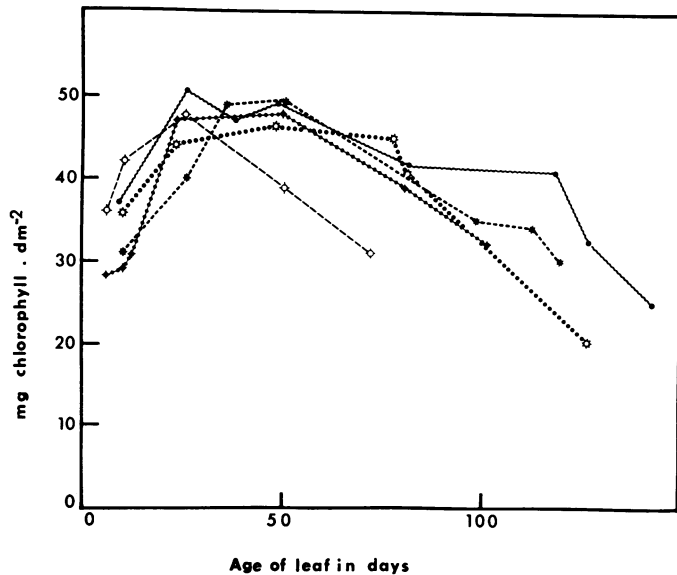


FIG. 1. Effect of leaf age on total chlorophyll content of cottonwood leaf discs. Each curve represents an individual plant.

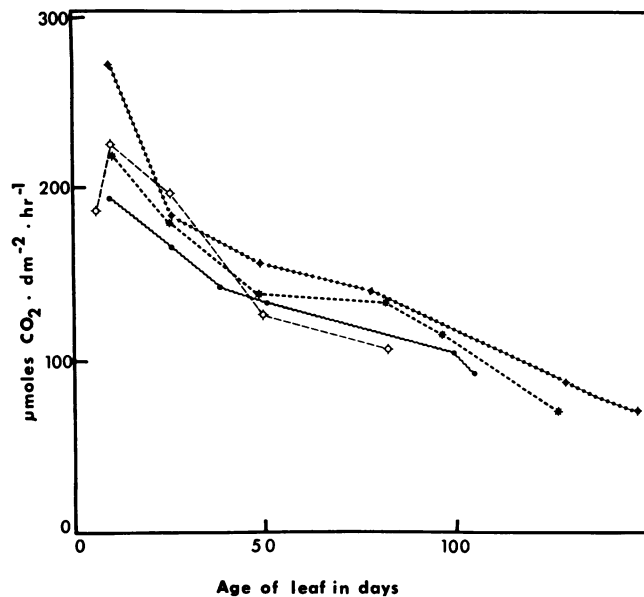


FIG. 2. Effect of leaf age on the rates of CO_2 assimilation of leaf discs from cottonwood. Each point represents the average of two determinations. Each curve represents an individual plant.

Electron Microscopy. Leaves from three stages of development were selected for senescence studies: young leaves (1 day after unfolding); mature leaves (15 days old); and senescing leaves (60 days old).

Leaf samples for electron microscopy were cut from between the veins with a razor blade in strips 1 to 2 mm wide and 10 mm long. The tissue slices were infiltrated for 60 min under vacuum in 4% glutaraldehyde buffered by 0.15 M phosphate at pH 7.2. The glutaraldehyde-fixed tissue was washed thoroughly two to three times with distilled water. Secondary fixation was carried out in 2% OsO_4 buffered by 0.1 M phosphate at pH 7.0 for 17 hr. The samples were then washed two to three times with distilled water and dehydrated through 15, 30, 50, 70, and 95% ethanol to absolute ethanol. The tissue slices were immersed in two changes of propylene oxide. Embedding medium was Araldite 50L.

Sections were cut with a diamond knife on a Porter-Blum MT-28 ultramicrotome, floated on water, and collected on formvar-coated copper grids. Sections from glutaraldehyde- OsO_4 -fixed material were poststained on the grid with lead citrate for 15 min (18). All observations were made with an RCA EMU-3 electron microscope which operated with an accelerating voltage of 100 DV.

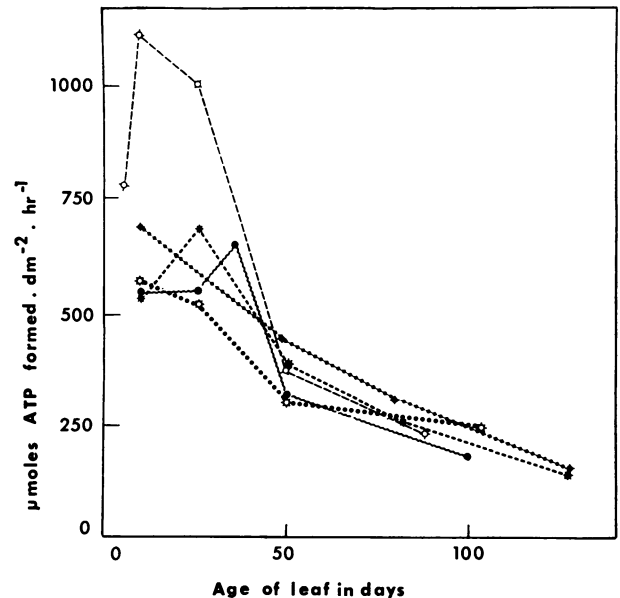


FIG. 3. Effect of leaf age on the rate of cyclic ATP synthesis per surface area by chloroplasts isolated from cottonwood. Reaction time was 5 min, and the light intensity was 50,000 lux. Temperature was 20 C. Reaction mixture contained in a final volume of 1.7 ml, P, (labeled with P^{32}), 5 μmoles ; ADP, 12 μmoles ; PMS, 0.1 μmole ; MgCl_2 , 6 μmoles ; Na-isoascorbate, 25 μmoles ; Tricine-NaOH buffer, pH 8.2, 210 μmoles , and chloroplast suspensions containing between 9 to 10 μg chlorophyll/ml. Each point is the average of three replications. Each curve represents an individual plant.

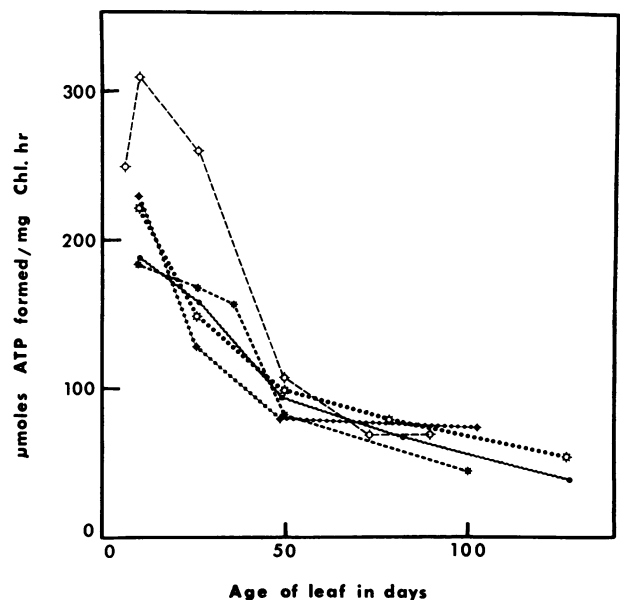


FIG. 4. Effect of leaf age on the rates of cyclic ATP synthesis on a chlorophyll basis by isolated chloroplasts from cottonwood. Reaction conditions as in Fig. 3. Each point is the average of three replications. Each curve represents an individual plant.

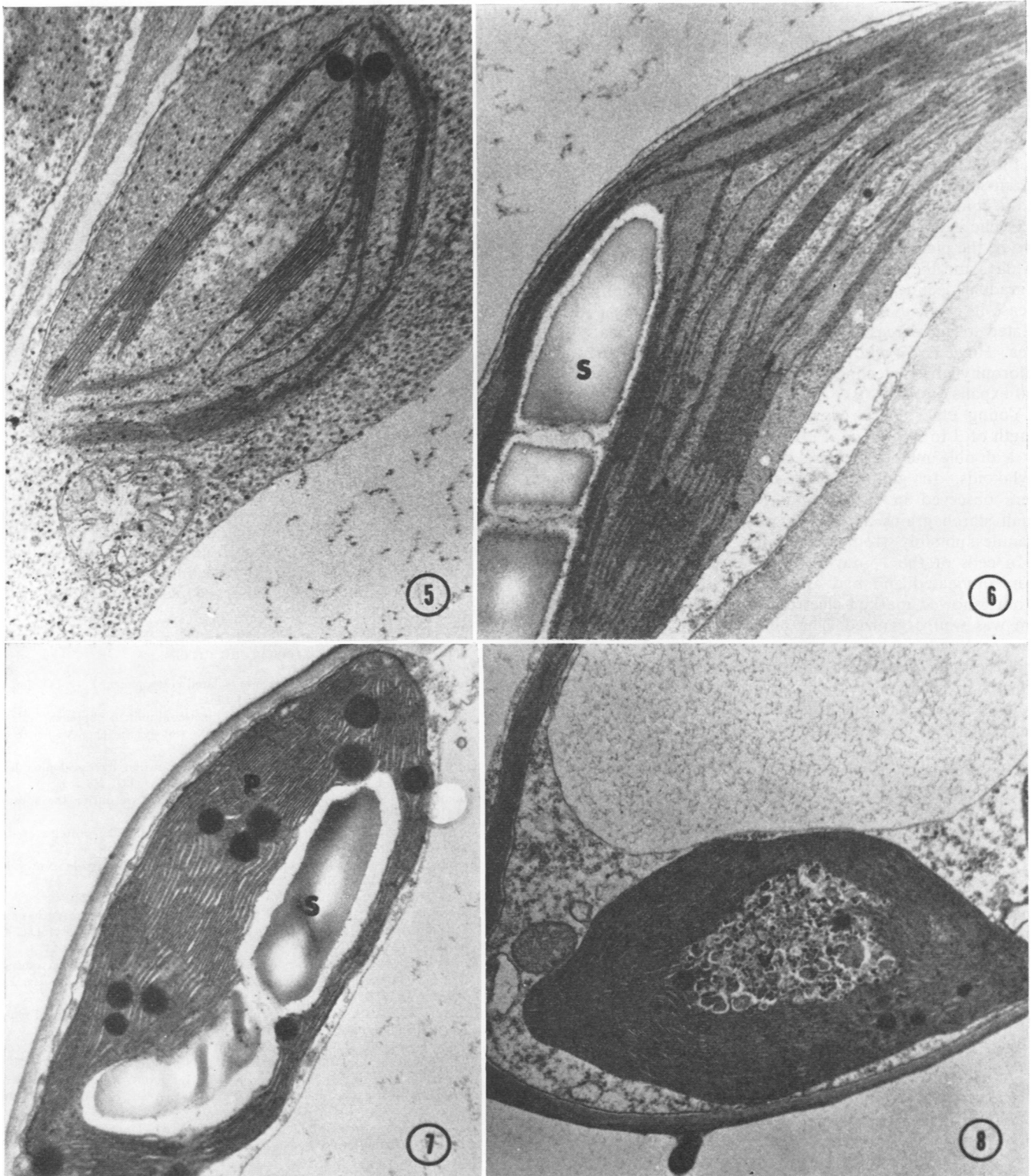


FIG. 5. Chloroplast from 1-day-old leaf. Note the well developed thylakoid system organized into grana; stromal ribosomes are also observed. $\times 31,240$.

FIG. 6. Chloroplast from a 15-day-old leaf. The grana-thylakoids are well developed. Starch grains (S) are present between the lamellar system $\times 31,240$.

FIG. 7. Chloroplast from a 60-day-old leaf. The chloroplast are densely packed with membranes. Grana thylakoid are poorly defined. Characteristically present are big osmiophilic plastoglobuli (P) and starch grains (S). $\times 35,500$.

FIG. 8. Chloroplast from a senescing leaf showing the stroma highly vesiculated (v). Chloroplast show intact outer membranes and are closely packed with membranes. $\times 23,750$.

RESULTS

Populus deltoides leaves reached full expansion from 10 to 12 days after leaf unfolding. The chlorophyll content per surface area increased during the period of leaf expansion, reached a peak between 30 to 40 days after leaf unfolding, and then declined slowly until leaf abscission (Fig. 1).

The rate of CO₂ assimilation declined steadily from the time of full leaf expansion (Fig. 2). This decline in CO₂ fixation rate began some 20 days before there was any significant decrease in the chlorophyll content of the leaf.

Cyclic ATP synthesis on an area basis (Fig. 3) increased in two of the plants from leaf expansion for approximately 15 to 25 days and then declined sharply. Three of the plants showed a gradual decline for 100 days from leaf expansion. The increase in cyclic ATP synthesis in two samples appeared to be related to an increase in chlorophyll content per leaf surface area. However, cyclic ATP synthesis when expressed on a chlorophyll basis declined continuously from the time of full leaf expansion (Fig. 4).

Young chloroplasts, usually lens-shaped, had an average length of 3 to 3.5 μ (Fig. 5). Each chloroplast was surrounded by a double membrane and contained well organized grana thylakoids. In glutaraldehyde-OsO₄ fixation, lipid globules were observed in the stroma. Most chloroplasts contained small starch grains as well as numerous electron dense-free granules, possibly stromal ribosomes.

In cells of the 15-day-old leaf (Fig. 6), chloroplasts were more elongated and had an average size of 5 to 6 μ . The stroma showed marked electron density and the lamellar system was well organized. The number of thylakoid per grana was increased; such increase can be an indication of a high photosynthetic activity at this stage. Some chloroplasts showed a lamellar system not organized in grana and lying parallel to the long axis of the organelle. Chloroplasts from leaves harvested after 12 hr in light showed big, lens-shaped starch grains.

The cytoplasm of senescing leaves (Fig. 7) showed reduction to a very thin layer around the periphery of the cell, contained fewer and less organized organelles, and was highly vesiculated. The chloroplasts exhibited strong electron density and were more closely packed with membranes than in earlier stages of development, thus making the resolution of the lamellar system very difficult. Still some grana structure was evident, but with apparently thinner membranes. Ribosomal particles were not evident in the stroma. The average size and number of the osmophilic globules increased. Starch grains were large and numerous. In some senescing chloroplasts, anomalous electron opaque areas were found in the stroma (Fig. 8). These areas could have been produced by the action of hydrolytic enzymes (15).

DISCUSSION

Leaf expansion in young *Populus deltoides* plants grown under defined environmental conditions was complete 10 to 12 days after leaf unfolding. The chlorophyll content per surface area, however, continued to increase for another 30 to 40 days. This rise in chlorophyll content was not reflected in higher photosynthetic rates. The rates of cyclic ATP synthesis and leaf disc CO₂ fixation when compared on a surface area basis declined steadily for over 100 days. Thus the performance of the photosynthetic system in cottonwood leaves, after an initial rise as shown by Dickman (3), declined steadily after full leaf expansion. The slight increase in cyclic ATP synthesis on a surface area basis observed in chloroplasts isolated from leaves of two of the plants could be the reflection of a rapid

increase in leaf chlorophyll, since no such increase is evident when the results are expressed on a chlorophyll basis (Fig. 4).

The decline in the rate of cyclic photophosphorylation with leaf or plant age (24) may well reflect the observed (Figs. 5–8) changes in the composition and structure of the thylakoid membranes (7). With advancing age, chloroplasts developed an increasingly vesiculated stroma and contained a larger number of osmophilic plastoglobuli. The number of thylakoid membranes increased, but their thickness appeared to decrease. Since older chloroplasts contained more layers of membrane coincident with reported decline in leaf chlorophyll and protein content (27), the thinning of membrane in older chloroplasts could be the consequence of the reduction in chlorophyll and protein content of the membranes. Such membranes would also be functionally defective (12). This suggests that the decline in photosynthetic performance of chloroplasts is not only the result of changes in the soluble enzyme fraction, but reflects also a decline in the synthetic performance of the membrane-bound ATP synthesizing system.

It has been reported that cyclic photophosphorylation is an important component of photosynthetic ATP production. "Cyclic" ATP synthesis may contribute ATP to CO₂ assimilation (2), glucose uptake, acetate uptake, and potassium uptake (25). Moreover, it can meet the energy requirements of the plastid for nucleic acid synthesis, protein synthesis, lipid synthesis, and other ATP-dependent biosynthetic reactions (17). The rapid decline in the cyclic photophosphorylation capacity with leaf age and the associated decline in synthetic reactions (5) could contribute to the observed parallel decrease in CO₂ assimilation.

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