Plant Physiol. (1990) 93, 1184–1190
0032-0889/90/93/1184/07/$01.00/0

Received for publication August 25, 1989
and in revised form November 27, 1989

Photosynthesis and Chlorophyll Fluorescence Characteristics in Relationship to Changes in Pigment and Element Composition of Leaves of *Platanus occidentalis* L. during Autumnal Leaf Senescence[1]

William W. Adams III*[2], Klaus Winter, Ulrich Schreiber, and Peter Schramel

Institut für Botanik und Pharmazeutische Biologie , Universität Würzburg, Mittlerer Dallenbergweg 64, 8700 Würzburg, Federal Republic of Germany (W.W.A., K.W., U.S.); *and GSF München, Ingolstädter Landstr. 1, 8042 Neuherberg, Federal Republic of Germany* (P.S.)

ABSTRACT

The loss of chlorophyll and total leaf nitrogen during autumnal senescence of leaves from the deciduous tree *Platanus occidentalis* L. was accompanied by a marked decline in the photosynthetic capacity of O_2 evolution on a leaf area basis. When expressed on a chlorophyll basis, however, the capacity for light- and CO_2-saturated O_2 evolution did not decline, but rather increased as leaf chlorophyll content decreased. The photon yield of O_2 evolution in white light (400–700 nanometers) declined markedly with decreases in leaf chlorophyll content below 150 milligrams of chlorophyll per square meter on both an incident and an absorbed basis, due largely to the absorption of light by nonphotosynthetic pigments which were not degraded as rapidly as the chlorophylls. Photon yields measured in, and corrected for the absorptance of, red light (630–700 nanometers) exhibited little change with the loss of chlorophyll. Furthermore, PSII photochemical efficiency, as determined from chlorophyll fluorescence, remained high, and the chlorophyll *a*/*b* ratio exhibited no decline except in leaves with extremely low chlorophyll contents. These data indicate that the efficiency for photochemical energy conversion of the remaining functional components was maintained at a high level during the natural course of autumnal senescence, and are consistent with previous studies which have characterized leaf senescence as being a controlled process. The loss of chlorophyll during senescence was also accompanied by a decline in fluorescence emanating from PSI, whereas there was little change in PSII fluorescence (measured at 77 Kelvin), presumably due to decreased reabsorption of PSII fluorescence by chlorophyll. Nitrogen was the only element examined to exhibit a decline with senescence on a dry weight basis. However, on a leaf area basis, all elements (C, Ca, K, Mg, N, P, S) declined in senescent leaves, although the contents of sulfur and calcium, which are not easily retranslocated, decreased to the smallest extent.

The loss of Chl and decline in photosynthesis are two of the phenomena associated with the process of senescence in leaves of higher plants (6, 19, 24, 29, 31, 34). Photosynthesis at saturating photon flux densities is generally independent of Chl content throughout the greater portion of a green leaf's life span and is, instead, dependent on factors such as the capacity for the enzymatic fixation of CO_2 into starch and sucrose and the capacity for photosynthetic electron transport (4, 30). At limiting PFDs, photosynthesis based on incident light (the apparent photon yield) has been found to be dependent on Chl content (4, 5, 13). When corrected for changes in absorptance, however, the photon yield is unaffected by changes in Chl content, at least in the range found in healthy green leaves (4, 5). In pale green to yellow leaves such corrections may be more complicated due to an increase in the proportion of light which is absorbed by components of the leaf other than Chl.

Furthermore, during senescence, it is possible that the active breakdown of chloroplasts and catabolism of Chl and other components of the photosynthetic apparatus could result in a lowering of the capacity and efficiency of photosynthetic energy conversion for those components remaining in the leaf. We have examined the changes in a number of photosynthetic characteristics from leaves of *Platanus occidentalis* during the natural course of autumnal senescence to evaluate the ability of the leaf to maintain high levels of photosynthetic efficiency in those components (particularly Chl) which remain as degradation progresses.

MATERIALS AND METHODS

Leaves from a large tree of *Platanus occidentalis* L., growing in the Würzburg Botanical Garden, were examined during October of 1987 and September and October of 1988. During 1987 many leaves were collected prior to dawn, and the majority of October was overcast. In both years care was taken to use leaves which were not receiving direct illumination from the sun at the time of collection in order to avoid the possibility of any high-light induced reductions in photochemical efficiency (1, 2, 8). The leaves of *P. occidentalis* do not senesce synchronously, and thus leaves with different Chl contents (*i.e.* in various stages of senescence) could be examined at a given time during the period of this study. Furthermore, only leaves with a uniform color were chosen, and no leaves were examined after minimum nocturnal temperatures reached 0°C.

[1] This work was supported by the Deutsche Forschungsgemeinschaft and by the Bayerisches Staatsministerium für Landesentwicklung und Umweltfragen. W. W. A. gratefully acknowledges the support of Fellowships from the North Atlantic Treaty Organization and the Alexander von Humboldt-Stiftung.

[2] Present address: Department of Environmental, Population, and Organismic Biology, University of Colorado, Boulder, CO 80309.

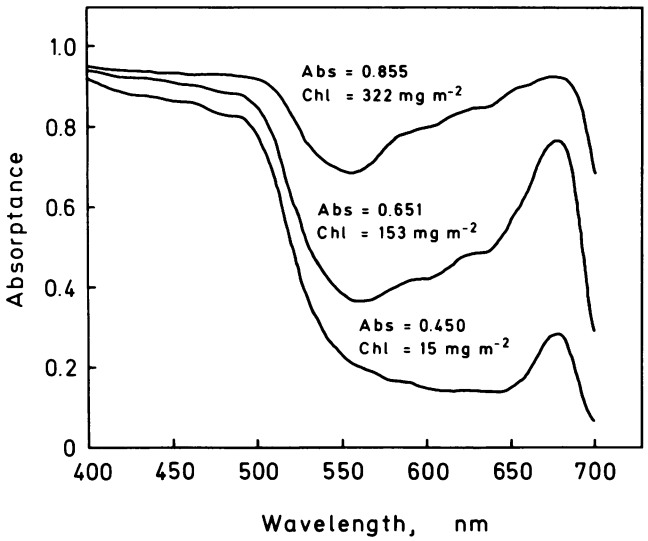


Figure 1. Spectral absorptance curves obtained from the upper surface of three different leaves of *P. occidentalis* at various stages of senescence in 1987, with the integrated absorptance (400–700 nm) and Chl content for each leaf indicated. The upper curve was obtained from a healthy green leaf on October 5, the middle curve from a yellow-green leaf on October 20, and the lower curve from a very yellow leaf on October 16.

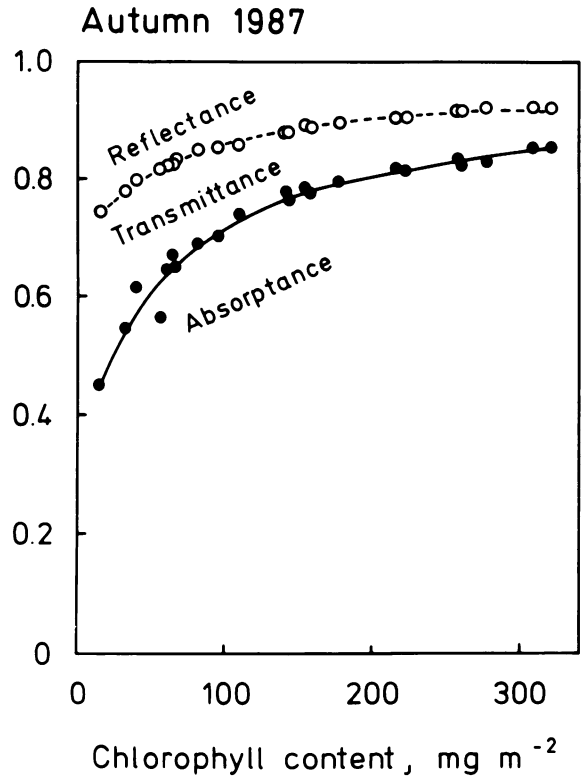


Figure 2. Changes in absorptance, transmittance, and reflectance in relationship to changes in Chl content for leaves of *P. occidentalis* at various stages of senescence during 1987. The region between the open circles and the solid circles represents the fraction of light which was abosorbed by each of the leaves, the region been the solid circles and the open circles represents the fraction of light which was transmitted by each of the leaves, and the region between the open circles and 1.0 represents the fraction of light which was reflected by each of the leaves.

Upon collection, a leaf was placed in a Petri dish containing moist filter paper and immediately taken into the laboratory. During October 1987, one disc (10 cm^2) was removed for measurements of O_2 exchange, reflectance, transmittance, and Chl content, and up to six adjacent and smaller discs were used to examine fluorescence characteristics from Chl associated with both PSII and PSI. In 1988 discs were again removed for measurements of O_2 exchange, reflectance, transmittance, as well as Chl and carotenoid contents, while one to three additional discs from each leaf were used for elemental analyses.

Measurements of photon yield and photosynthetic capacity (rate of O_2 evolution between 1000 and 1700 μmol photons $m^{-2}s^{-1}$, defined as A_{max}[3]) of leaves illuminated from the upper surface were made at 25°C with 5% CO_2 in a leaf-disc O_2 electrode (LD-2 and LS-2 light source; Hansatech, King's Lynn, Norfolk, UK) as described by Björkman and Demmig (5). Photon yields of O_2 evolution in red light were obtained using a red cut-off filter (RG630; Schott Glaswerke, Mainz, FRG). The light source was calibrated with a quantum sensor (Ll-190SB; Li-Cor, Lincoln, NE). Reflectance, transmittance, and absorptance through the upper surface of the leaves, over the range 400 to 700 nm (1987 and 1988) and 630 to 700 nm (1988), were determined with an integrating sphere (Ll-1800–12; Li-Cor) and spectroradiometer (LI-1800; Li-Cor). Pigment contents were determined by grinding each leaf disc (previously frozen in liquid N_2) in a chilled buffer solution of 50 mM Hepes at pH 7.5 (KOH) in dim light followed by extraction in 80% acetone at 4°C in darkness, and estimated by spectrophotometric analysis of the extracts according to Arnon (3; Chl) and Röbbelen (23; carotenoids). The Chl contents of the leaves estimated from the equations of Röbbelen (23) (data not shown) were slightly lower than those following Arnon (3).

Chl fluorescence was determined from small discs removed from the leaf following the determination of the photon yield and photosynthetic capacity (*i.e.* after a minimum of 2.5 h in room light). Discs were removed from the area surrounding that which was used in the measurements of O_2 exchange. Two or three discs were used to measure fluorescence at room temperature, and fluorescence from both PSII and PSI was measured from two to three additional discs frozen to 77K. Chl *a* fluorescence at room temperature (primarily PSII) was measured using a PAM Chl Fluorometer (Walz, Effeltrich, FRG; 28). Leaf discs were darkened for 5 min, after which F_O and F_M were ascertained (7). Fluorescence at 77K was measured using the instrument described in Demmig-Adams *et al.* (8) from discs darkened for 5 min and then frozen in liquid nitrogen for 5 min.

After digestion of the organic material using a pressure ashing technique with nitric acid (26), Ca, K, Mg, P, and S analyses were preformed by inductively coupled plasma emis-

[3] Abbreviations: A_{max}, photosynthetic capacity (CO_2-saturated rate of O_2 evolution between 1000–1700 μmol photons m^{-2} s^{-1}); F_O, instantaneous fluorescence emission; F_M, maximum fluorescence emission; F_V, variable fluorescence emission; Q, primary electron acceptor of PSII.

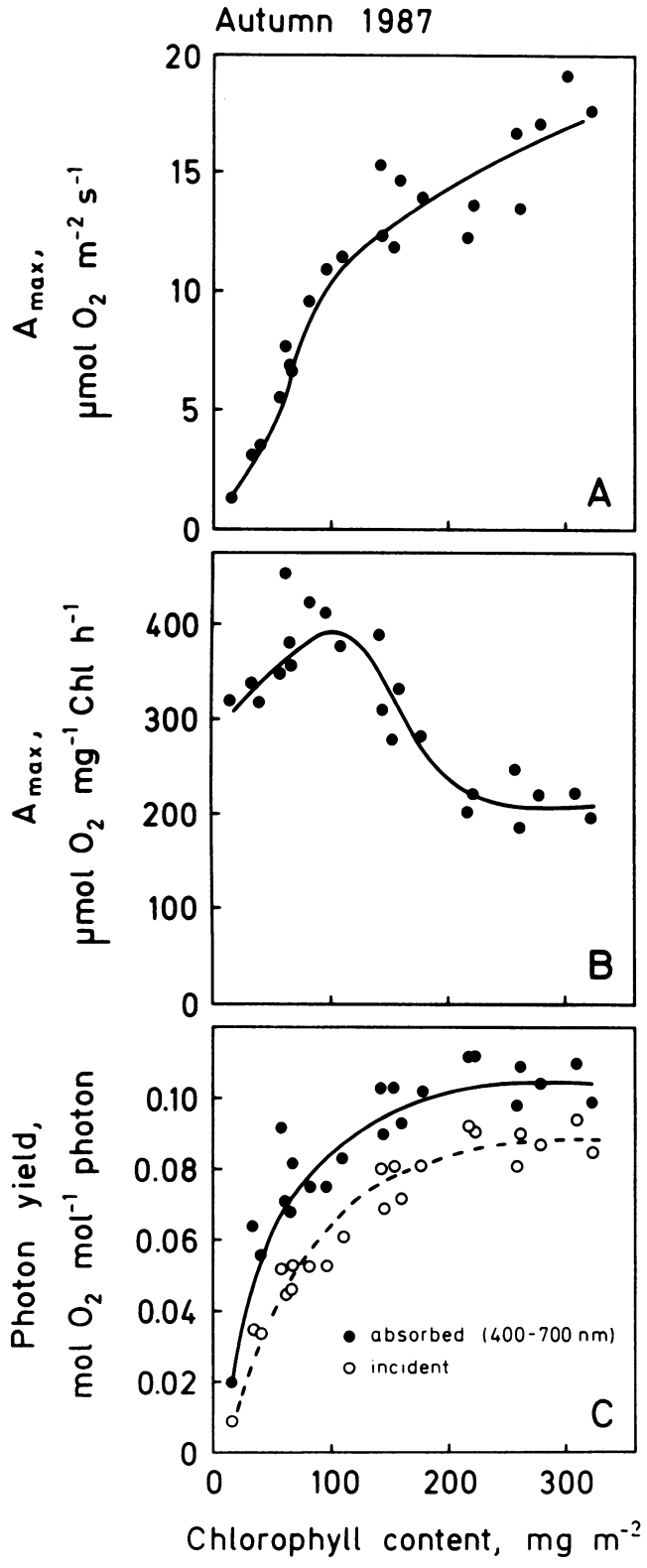


Figure 3. Changes in the light- and CO_2-saturated rate of O_2 evolution on (A) a leaf area basis and (B) a Chl basis, and in the photon yield of O_2 evolution in white light on (C) both an incident and an absorbed (400–700 nm) basis in relationship to changes in Chl content for leaves of *P. occidentalis* at various stages of senescence during 1987.

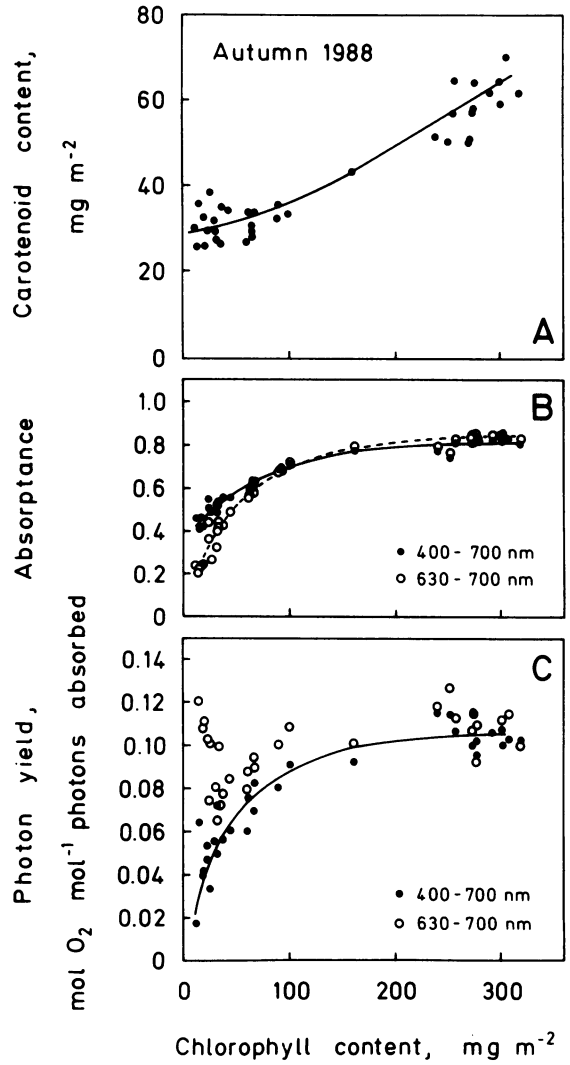
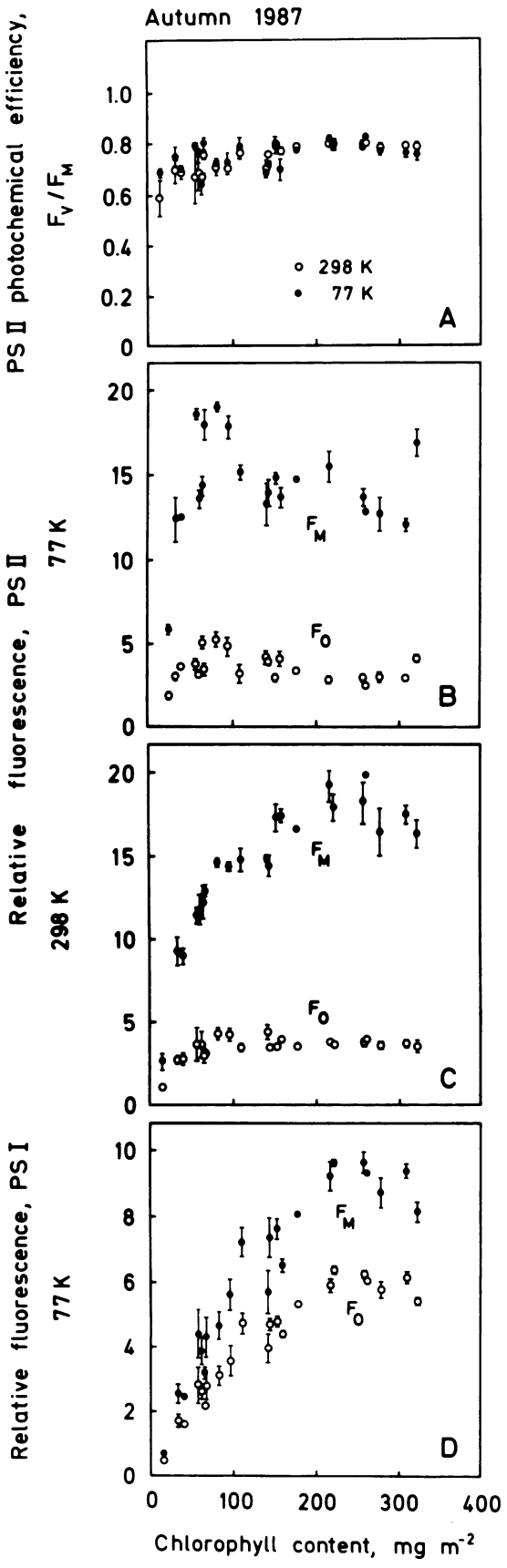


Figure 4. Changes in (A) the carotenoid content, (B) the absorptance of white (400–700 nm) and red (630–700 nm) light, and (C) the photon yield of O_2 evolution (absorbed light basis) measured in white and red light in relationship to changes in Chl content for leaves of *P. occidentalis* at various stages of senescence during 1988.

sion spectrometry (25) with a sequential spectrometer (model JY 38 Plus, Jobin Yvon, France). Total C and N were determined using an elemental analyzer (model CHN 1106, Carlo Erba, Italy; 27).

RESULTS

Typical spectral absorptance curves (400–700 nm) for the upper surface of three leaves of *Platanus occidentalis* in various stages of senescence, from deep green to very yellow, are shown in Figure 1. The decrease in integrated absorptance across this range was only 24% with a 50% decrease in Chl content, and no greater than 50% in the leaf which lost 95% of its Chl. It is clear that there were massive losses of Chl from these leaves during senescence with little reduction in absorptance from 400 to 500 nm, which is the range in which the carotenoids absorb light strongly. The decrease in integrated absorptance (400–700 nm), as Chl content decreased,



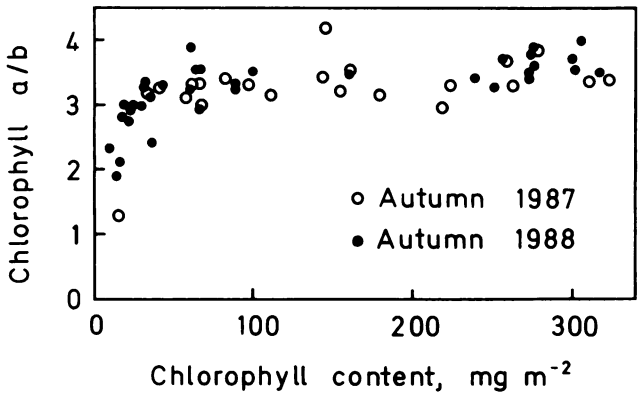


Figure 6. The relationship between the Chl *a*/*b* ratio and total Chl content in leaves of *P. occidentalis* at various stages of senescence.

was due to an increase in both transmittance and reflectance (Fig. 2).

Changes in the light- and CO_2-saturated rate of O_2 evolution and in the photon yield measured with white light in relationship to changes in leaf Chl content are depicted in Figure 3. There was a marked decrease in the capacity for photosynthesis on a leaf area basis which accompanied the loss of Chl (Fig. 3A). However, when expressed on a Chl basis, the capacity for photosynthetic O_2 evolution increased as leaf Chl content declined during senescence (Fig. 3B). Similar results were obtained in 1988 (data not shown), except that the decline in photosynthetic capacity (area basis) appeared to be more linear over the range of 250 to 50 mg Chl m^{-2}, and the increase in photosynthetic capacity on a Chl basis with decreasing leaf Chl content was more pronounced (data not shown).

The photon yield of O_2 evolution remained fairly constant between 300 and 150 mg Chl m^{-2}, but declined with further decreases in Chl content (Fig. 3C). The photon yields based on absorbed light (400–700 nm) exhibited a similar pattern of change with leaf Chl content to that based on incident light, although the values on an absorbed basis were higher. Since these photon yields were based on absorptances that included a high degree of absorption by the carotenoids, which became proportionally greater with decreases in Chl content (Figs. 1 and 4), photon yields were reexamined during the autumn of 1988 using red light.

A loss of 95% of the Chl content during senescence of these *P. occidentalis* leaves was accompanied by only a 50% decline in carotenoid content (Fig. 4A). The decline in the integrated absorptance of white light (400–700 nm) with decreasing Chl content was similar to that observed in the leaves examined during the autumn of 1987 (*cf.* Figs. 1 and 4B). The absorptance of red light (630–700 nm) was slightly higher than that of white light in leaves with high Chl contents, but declined to a much greater degree than the absorptance of white light

Figure 5. Changes in (A) the photochemical efficiency of PSII, (B) the absolute levels of F_O and F_M from PSII measured at 77K, (C) the absolute levels of F_O and F_M from (primarily) PSII measured at room temperature, and (D) the absolute levels of F_O and F_M from PSI measured at 77K in relationship to changes in Chl content in leaves of *P. occidentalis* at various stages of senscence during 1987. Standard deviations are indicated where $n = 3$, otherwise $n = 2$.

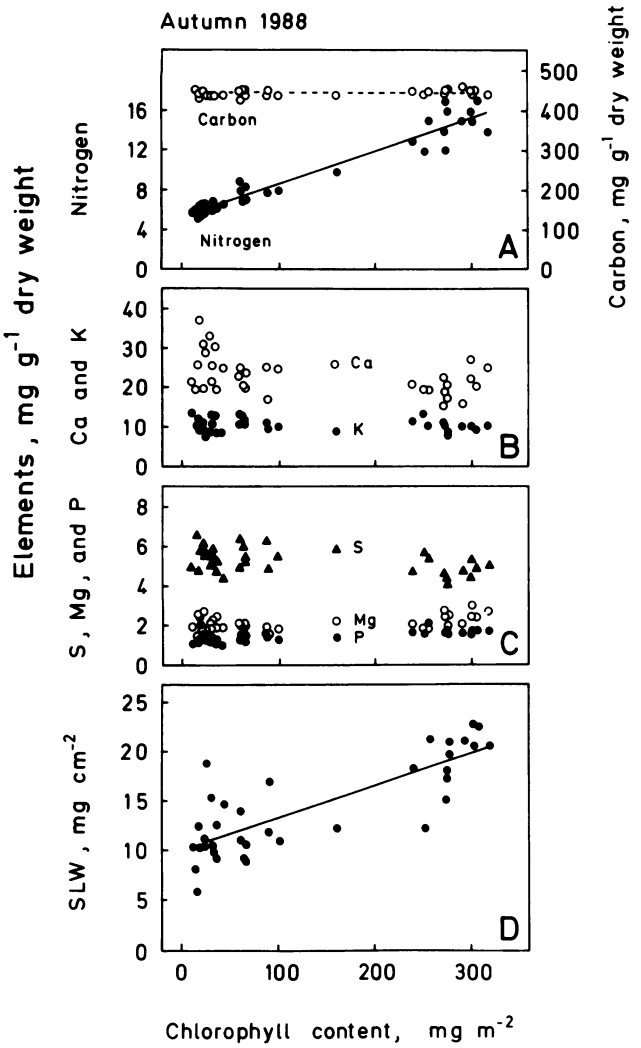


Figure 7. Changes in (A) the carbon and nitrogen ($r^2 = 0.94$) contents, (B) the calcium and potassium contents, (C) the sulfur, magnesium, and phosphorus contents, and (D) the specific leaf weight ($r^2 = 0.65$) in relationship to changes in Chl content for leaves of *P. occidentalis* at various stages of senescence during 1988.

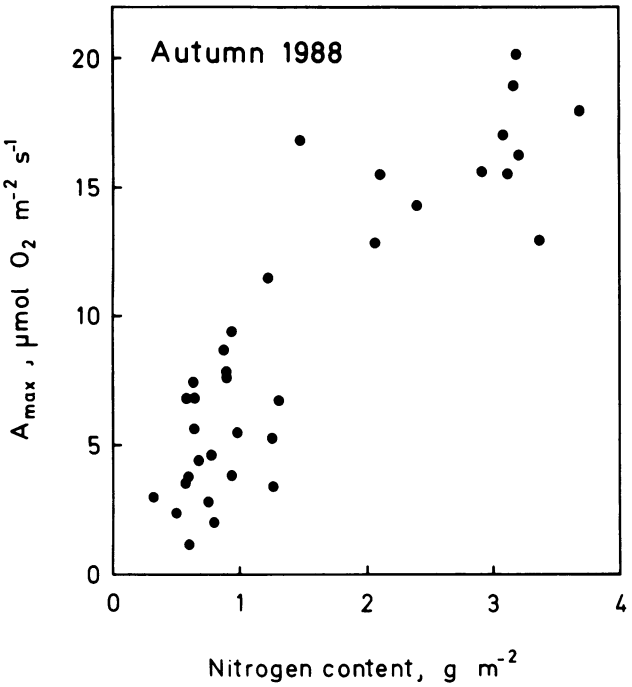


Figure 8. The relationship between the light- and CO_2-saturated rate of O_2 evolution and total nitrogen content in leaves of *P. occidentalis* at various stages of senescence during 1988.

Table I. *Contents of the Major Elements in Green (240–320 mg Chl m^{-2}) and Senescent (<100 mg Chl m^{-2}) Leaves of* Platanus occidentalis *during the Autumn of 1988*

Values are means ± SD. Probabilities are the results of *t*-tests.

Element	Content of Elements		Decrease
	Green leaves (n = 13)	Senescent leaves (n = 21)	
	gm^{-2}		%
Carbon	87.0 ± 14.2	51.4 ± 13.3***	−40.9
Nitrogen	2.90 ± 0.69	0.79 ± 0.26***	−72.8
Phosphorus	0.33 ± 0.07	0.15 ± 0.04***	−53.2
Magnesium	0.45 ± 0.11	0.24 ± 0.06***	−46.9
Potassium	1.99 ± 0.22	1.23 ± 0.23***	−38.2
Sulfur	0.92 ± 0.16	0.63 ± 0.14***	−31.5
Calcium	3.99 ± 0.98	2.94 ± 1.06**	−26.3

** $P < 0.01$. *** $P < 0.001$.

in leaves with very low Chl contents (Fig. 4B; see also Fig. 1). Whereas the photon yield based on absorbed light exhibited similar changes to those observed in the previous year (Figs. 4C and 3C), the photon yield measured in, and corrected for the absorptance of, red light exhibited little change with the decline in Chl content (Fig. 4C).

Characteristics of Chl fluorescence from both PSII and PSI were examined in leaves of *P. occidentalis* at various stages of senescence during 1987 (Fig. 5). There was little change in the photochemical efficiency of PSII (F_V/F_M; see ref. 18), measured at either 77K or room temperature, with the progressive loss of Chl (Fig. 5A). There were no significant changes in the absolute levels of F_O or F_M from PSII measured at 77K (Fig. 5B). On the other hand, both F_O and F_M from PSI measured at 77K exhibited marked decreases as leaf Chl content declined (Fig. 5D). F_M, and to a smaller extent F_O, from fluorescence measured at room temperature also declined during senescence (Fig. 5C).

During both 1987 and 1988 the Chl *a/b* ratio of the senescing leaves of *P. occidentalis* remained remarkably constant as Chl was degraded, and declined only at extremely low Chl contents (Fig. 6).

There was a clear decline in leaf nitrogen content, when expressed on either a dry weight basis (Fig. 7A) or on a leaf area basis (Table I), which accompanied the loss of Chl during leaf senescence. All other examined elements (C, Ca, K, Mg, P, S) exhibited no significant changes per unit dry weight (Fig. 7, A, B, and C), although since the specific leaf weight declined markedly (Fig. 7D), there was a loss of all nutrients per unit leaf area during senescence (Table I). There was a concomitant decline in photosynthetic capacity and in leaf nitrogen content on a leaf area basis (Fig. 8).

DISCUSSION

The natural senescence of *Platanus occidentalis* leaves was characterized by a decrease in Chl content and photosynthetic

capacity on a leaf area basis. However, the decline in capacity did not appear to involve either a decrease in photosynthetic capacity on a Chl basis or a decrease in the efficiency of photosynthetic energy conversion. In fact, the light- and CO_2-saturated rate of photosynthetic O_2 evolution per Chl increased as the Chl content of the leaves decreased. The photon yield of O_2 evolution for (red) light which was specifically absorbed by the Chl also remained fairly constant, and the photochemical efficiency of PSII (F_V/F_M) remained high despite large decreases in Chl content. Thus, there was a continued efficient use of the light captured by the remaining Chl during senescence.

The capacity for photosynthetic O_2 evolution on a Chl basis increased during senescence, which indicates that, as senescence proceeded, the biochemical capacity of the leaves to fix CO_2 declined more slowly than the capacity to harvest light energy. Thus, during senescence, a relatively greater proportion of the photons which were captured by the remaining Chl could be used for CO_2 fixation at light saturation. Therefore, a sufficient capacity for the enzymatic reduction of CO_2 was maintained as Chl was degraded. These results are, however, in contrast to those of Jenkins and Woolhouse (14) who found that the capacity for photosynthetic electron transport (Chl basis) at light saturation declined markedly in chloroplasts isolated from bean leaves at various stages of senescence. This discrepancy may reflect species-specific differences, differences in growth conditions (natural *versus* growth cabinet), or it may be due to the fact that isolated chloroplasts were used in the previous study.

The fact that the photon yield of O_2 evolution based upon the light which was actually absorbed by the Chl remained fairly high during senescence, supports the suggestion that senescence of leaves is a controlled process of degradation which leaves the remaining components functional (19). The maintenance of a high photon yield is also consistent with studies which have suggested that, at low PFDs, the efficiency of electron transport in chloroplasts isolated from leaves at various stages of senescence remained constant (9, 17).

The photochemical efficiency of PSII (F_V/F_M) remained virtually constant during the senescence of *P. occidentalis* leaves, as had been observed previously in bean (16). The absolute levels of F_O and F_M from PSII at 77K also remained fairly constant with Chl loss. There was, however, a distinct decline in F_M from PSII measured at room temperature, and both F_M and F_O from PSI (measured at 77K) declined with decreasing Chl content. Decreases in F_M from PSII at room temperature which are not accompanied by decreases in F_M from PSII at 77K have previously (8) been attributed to a decrease in the activity of the water-splitting complex. However, these differences in F_M were accompanied by pronounced differences in F_V/F_M (8), whereas F_V/F_M values were similar at 77K and at room temperature in the present study, and the photon yield also remained high. In addition, Jenkins and Woolhouse (15) have reported that the water-splitting complex was not affected during senescence of bean leaves. An alternative explanation for a decline in PSII fluorescence measured at room temperature (predominately at wavelengths >720 nm) *versus* that measured at 77K (at 699 nm, 3 nm half-bandwidth) may be derived from considerations of fluorescence reabsorption within the leaf.

The absorptance spectrum of Chl overlaps that of Chl fluorescence emission such that there is considerable reabsorption of fluorescence emitted below 700 nm (further enhanced by the scattering properties of leaf tissue) and virtually no reabsorption of fluorescence emitted above 720 nm (32, 33). Thus, a decrease in fluorescence emission (at 699 nm) from PSII due to the loss of Chl may have been matched by a concomitant decrease in the proportion of fluorescence which was reabsorbed, such that no net changes in PSII fluorescence emission from the leaves frozen to 77K were detected during senescence. The decline in room temperature fluorescence (>720 nm), which is almost exclusively associated with PSII (as the high values of F_V/F_M also indicate), more closely reflects the actual loss of PSII units accompanying the degradation of Chl, since fluorescence at these wavelengths is relatively free of reabsorption phenomena. The decrease in fluorescence emission from PSI at 77K (>720 nm) with the loss of Chl is also consistent with the fact that it experiences little reabsorption within the leaf. Therefore the presented data are consistent with the conclusion that there is a parallel loss of PSI and PSII throughout senescence, which is further supported by the observed constancy of the Chl *a*/*b* ratio.

Of all of the examined elements, only nitrogen content exhibited a decline on a dry weight basis during leaf senescence. This loss of nitrogen was closely correlated with the loss of Chl. Consequently, a decline in nitrogen content was accompanied by a decline in photosynthetic capacity on a leaf area basis, resulting in a relationship between nitrogen content and photosynthetic capacity similar to those which have been observed in other species under non-senescing conditions (10–12). Only total leaf nitrogen was measured and, therefore, it is unknown whether the proportion of nitrogen represented by soluble proteins (*e.g.* ribulose bisphosphate carboxylase) *versus* that in thylakoid membrane proteins was altered during senescence.

The majority of the decrease in the leaf content of the elements examined during the period of this study was probably due to degradation and retranslocation into the tree for storage, although a small amount of leaching was also possible. Sulfur, and particularly calcium, are not easily translocated in the phloem (20, 21), and they exhibited the smallest decline on a leaf area basis, and a slight trend for an increase on a dry weight basis.

The results from this study indicate that, in *P. occidentalis*, leaf senescence under natural conditions progresses such that the loss of Chl and other chloroplastic components does not result in an impairment to the functioning of those components which are still retained within the leaf. Light which is captured by the Chl in a yellowing leaf can still be used efficiently in photosynthesis. This may be important to the continued mobilization of degradation products out of a senescing leaf for deciduous species such as *P. occidentalis* which experience periods of dormancy. In evergreen species, the leaves of which may experience a very prolonged senescent phase (2), the ability to continue contributing to the carbon gain of the plant could be even more important. It should be cautioned, however, that the observations in this study may not apply to all species or environmental situations. Whereas the Chl *a*/*b* ratio remained very stable in the leaves of *P. occidentalis*, in most species the loss of Chl during senescence is accompanied by a decline in this ratio (34). Furthermore,

senescence of leaves under different environmental conditions, such as in high irradiance habitats (22), *i.e.* involving potentially photooxidative processes, would be expected to lead to a decrease in the efficiency of photochemical energy conversion.

ACKNOWLEDGMENTS

We thank Maria Lesch for technical assistance and Dr. U. Heber for suggesting that we examine the photon yields in red light. We are also grateful to Dr. Barbara Demmig-Adams for reviewing the manuscript.

LITERATURE CITED

1. **Adams WW III, Díaz M, Winter K** (1989) Diurnal changes in photochemical efficiency, the reduction state of Q, radiationless energy dissipation, and non-photochemical fluorescence quenching in cacti exposed to natural sunlight in northern Venezuela. Oecologia **80:** 553–561
2. **Adams WW III, Terashima I, Brugnoli E, Demmig B** (1988) Comparisons of photosynthesis and photoinhibition in the CAM vine *Hoya australis* and several C_3 vines growing on the coast of eastern Australia. Plant Cell Environ **11:** 173–181
3. **Arnon DI** (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris.* Plant Physiol **24:** 1–15
4. **Björkman O** (1981) Responses to different quantum flux densities. *In* OL Lange, PS Nobel, CB Osmond, H Ziegler, eds, Encyclopedia of Plant Physiology (New Series), Vol 12A: Physiological Plant Ecology I. Springer-Verlag, Berlin, pp 57–107
5. **Björkman O, Demmig B** (1987) Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta **170:** 489–504
6. **Camp PJ, Huber SC, Burke JJ, Moreland DE** (1982) Biochemical changes that occur during senescence of wheat leaves. I. Basis for the reduction of photosynthesis. Plant Physiol **70:** 1641–1646
7. **Demmig B, Winter K, Krüger A, Czygan F-C** (1987) Photoinhibition and zeaxanthin formation in intact leaves. A possible role of the xanthophyll cycle in the dissipation of excess light energy. Plant Physiol **84:** 218–224
8. **Demmig-Adams B, Adams WW III, Winter K, Meyer A, Schreiber U, Pereira JS, Krüger A, Czygan F-C, Lange OL** (1989) Photochemical efficiency of photosystem II, photon yield of O_2 evolution, photosynthetic capacity, and carotenoid composition during the "midday depression" of net CO_2 uptake in *Arbutus unedo* growing in Portugal. Planta **177:** 377–387
9. **Drury S, Park RB** (1968) The effect of leaf senescence on quantum efficiencies of photosynthetic light reactions. Plant Physiol **43:** S-29
10. **Evans JR** (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia **78:** 9–19
11. **Field CB** (1987) On the role of photosynthetic responses in constraining the habitat distribution of rainforest plants. Aust J Plant Physiol **15:** 343–358
12. **Field CB, Mooney HA** (1986) The photosynthesis-nitrogen relationship in wild plants. *In* TJ Givnish, ed, On the Economy of Plant Form and Function, Cambridge University Press, Cambridge, pp 25–55
13. **Gabrielsen EK** (1948) Effects of different chlorophyll concentrations on photosynthesis in foliage leaves. Physiol Plant **1:** 5–37
14. **Jenkins GI, Woolhouse HW** (1981) Photosynthetic electron transport during senescence of the primary leaves of *Phaseolus vulgaris* L. I. Non-cyclic electron transport. J Exp Bot **32:** 467–478
15. **Jenkins GI, Woolhouse HW** (1981) Photosynthetic electron transport during senescence of the primary leaves of *Phaseolus vulgaris* L. II. The activity of photosystems one and two, and a note on the site of reduction of ferricyanide. J Exp Bot **32:** 989–997
16. **Jenkins GI, Baker NR, Bradbury M, Woolhouse HW** (1981) Photosynthetic electron transport during senescence of the primary leaves of *Phaseolus vulgaris* L. III. Kinetics of chlorophyll fluorescence emission from intact leaves. J Exp Bot **32:** 999–1008
17. **Jenkins GI, Baker NR, Woolhouse HW** (1981) Changes in chlorophyll content and organization during senescence of the primary leaves of *Phaseolus vulgaris* L. in relation to photosynthetic electron transport. J Exp Bot **32:** 1009–1020
18. **Kitajima M, Butler WL** (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochim Biophys Acta **376:** 105–115
19. **Kura-Hotta M, Satoh K, Katoh S** (1987) Relationship between photosynthesis and chlorophyll content during leaf senescence of rice seedlings. Plant Cell Physiol **28:** 1321–1329
20. **Marschner H** (1986) Mineral Nutrition of Higher Plants. Academic Press, London
21. **Mengel K, Kirkby EA** (1987) Principles of Plant Nutrition, Ed 4. International Potash Institute, Bern
22. **Nilsen ET, Stetler DA, Gassman CA** (1988) Influence of age and microclimate on the photochemistry of *Rhododendron maximum* leaves II. Chloroplast structure and photosynthetic light response. Am J Bot **75:** 1526–1534
23. **Röbbelen G** (1957) Untersuchen an strahleninduzierten Blattfarbmutanten von *Arabidopsis thaliana* (L.) Heynh. Z Indukt Abstammungs-Vererbungsl **88:** 189–252
24. **Sanger JE** (1971) Quantitative investigations of leaf pigments from their inception in buds through autumn coloration to decomposition in falling leaves. Ecology **52:** 1075–1089
25. **Schramel P** (1988) ICP and DCP spectrometry for trace element analysis in biomedical and environmental samples. Spectrochimica Acta **43B:** 881–896
26. **Schramel P, Wolf A, Seif R, Klose B-J** (1980) Eine neue Apparatur zur Druckveraschung von biologischem Material. Fresenius Z Anal Chem **302:** 62–64
27. **Steffan I, Schramel P** (1988) Bestimmung von Stickstoff in Fichennadeln. Laborpraxis **12:** 1354–1361
28. **Schreiber U, Schliwa U, Bilger W** (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res **10:** 51–62
29. **Sesták Z** ed (1985) Photosynthesis During Leaf Development. Dr W. Junk, Dordrecht
30. **Suzuki S, Nakamoto H, Ku MSB, Edwards GE** (1987) Influence of leaf age on photosynthesis, enzyme activity, and metabolite levels in wheat. Plant Physiol **84:** 1244–1248
31. **Thomson WW, Nothnagel EA, Huffaker RC** eds (1987) Plant Senescence: Its Biochemistry and Physiology. American Society of Plant Physiologists, Rockville, MD
32. **Virgin HI** (1954) The distortion of fluorescence spectra in leaves by light scattering and its reduction by infiltration. Physiol Plant **7:** 560–570
33. **Weis E** (1985) Chlorophyll fluorescence at 77K in intact leaves: characterization of a technique to eliminate artifacts related to self-absorption. Photosynth Res **6:** 73–86
34. **Wolf FT** (1956) Changes in chlorophylls a and b in autumn leaves. Am J Bot **43:** 714–718