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BRIEF COMMUNICATION

Decline in photosynthesis as related to alterations in chloroplast ultrastructure of a cotton leaf during ontogeny

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Abstract

Field studies were conducted to investigate ontogenic changes in leaf photosynthesis and chloroplast ultrastructure of a single cotton (*Gossypium hirsutum* L.) leaf subtending the fruit. A 20-d old leaf was the most physiologically active with net photosynthetic rate (P_N) of 16.5 μmol m^{-2} s^{-1} and nitrogen (N) concentration of 168 mmol m^{-2}. These values declined with leaf age and a close relationship existed between them. Concurrent with declines in P_N, ultrastructural alterations occurred in the chloroplast: the 20-d old leaf had increased grana number and thylakoids per granum and a few plastoglobuli. Afterwards, the grana number and thylakoids per granum declined with leaf age indicating disintegrated grana and stroma lamellae. Concomitant with disintegrated membrane system was the presence of numerous large plastoglobuli. The P_N was closely related to grana number and thylakoids per granum suggesting that the decline in P_N with leaf age was associated with ultrastructural changes in the chloroplast.

Additional key words: *Gossypium hirsutum;* grana; plastoglobuli; stroma lamellae.

In cotton, the major suppliers of carbon for the development of fruit are the leaf subtending the fruit, the leaf subtending the adjacent fruiting position, and the leaf subtending the sympodia (Wullschleger and Oosterhuis 1990a). However, the subtending leaves generally senesce at a fast rate due to the indeterminate growth

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habit of the cotton plant and are thus incapable of providing adequate assimilates and N required by the developing fruit (Constable and Rawson 1980, Bondada *et al.* 1997).

In general, the photosynthetic capacity, *i.e.*, the maximum rate of carbon assimilation by a single leaf at saturating irradiance and optimal conditions, declines with leaf age (Chapin *et al.* 1987) or declines after reaching maximum activity (Sobrado 1996), *i.e.*, after reaching photosynthetic maturity (Šesták 1985). Since chloroplast is the site of photosynthesis, the decline in photosynthesis is expected to correspond with ultrastructural alterations in the chloroplast as the leaf progresses towards senescence. In this paper, we report the concurrent changes in photosynthesis and chloroplast ultrastructure during leaf ontogeny.

Cotton (*Gossypium hirsutum* L.) seeds of cv. Stoneville 506 were planted on May 15, 1990 and May 20, 1991, at the Agricultural Experiment Station, University of Arkansas, Fayetteville, AR, USA. Plots consisted of six 5-m rows spaced 0.95 m apart in a moderately well-drained Captina silt loam soil (fine-silty, siliceous, mesic Typic Fragiudult). All the plots were hand-thinned for a stand density of *ca.* 7.2 plants per m^2 (72 000 plants per ha). Fertilizer consisted of 3.2-1.4-2.6 g m^{-2} of N-P-K incorporated before planting and an additional side-dressing of 3.0 g(N) m^{-2} at pinhead square stage. The plots received supplemental furrow irrigation throughout the season to provide a well-watered soil environment. Cultural practices pertaining to herbicides and insecticides were applied according to recommendations. The trials were done with 20, 30, 40, and 60 d-old leaves in a completely randomized design with three replications in each year. Leaf age was recorded by tagging 50 plants in each plot with white jewelers tags at main-stem node 10, fruiting position 1 over a period of one week. The day when the leaves first unfolded was considered as day 1.

Photosynthesis (P_N) of leaves from each leaf-age group was measured in a closed system using a *LI-6000* portable photosynthesis system (*LI-COR*, Lincoln, NE, USA) equipped with either a 250 or 1000 cm^3 stirred cuvette. Ten consecutive measurements at 3-s intervals were taken for the rate of CO_2 depletion from the chamber. Six leaves from each leaf age, two in each replication ($n = 6$), were used.

After determining P_N, the same leaves were harvested to study chloroplast ultrastructure using transmission electron microscopy (TEM). Leaf segments (4-6, <2 mm^2) from two leaves per replication in each leaf-age group were fixed at room temperature under weak vacuum for 2 h in a modified Karnovsky's fixative consisting of 2 % paraformaldehyde and 2 % glutaraldehyde, buffered in 0.05 M cacodylate buffer at pH 7.2. Further fixation details are described by Bondada *et al.* (1994). Fifteen chloroplasts in one representative section from two leaves per replication in each leaf age were used to determine the number of plastoglobuli, grana, and thylakoids per granum by directly counting in TEM (×30 000). Transmission electron micrographs were used to determine the size of the plastoglobuli.

Entire leaves minus the segments for electron microscopy were oven dried for at least 72 h at 60 °C, and ground to a powder for total nitrogen analysis by the $KMNO_4$-Fe^+ modification of the semi-micro-Kjeldahl procedure (Bremner and Mulvaney 1982).

All values were subjected to analysis of variance (*SAS Institute*, Cary, N.C., USA). Regression analysis was done to assess the relationships between photosynthesis, leaf N concentration, and chloroplast ultrastructure.

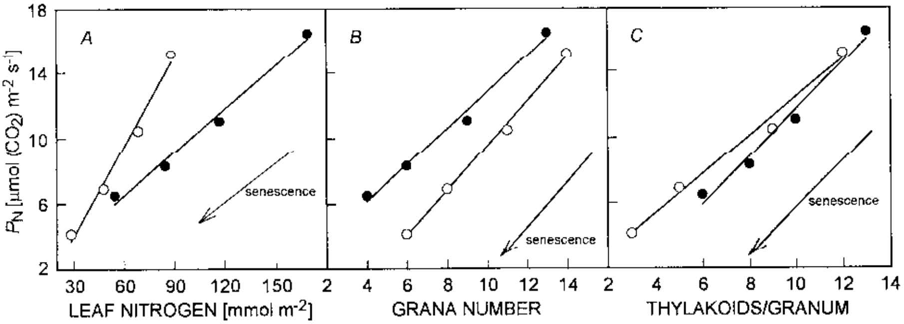

Fig. 1. Relationship between net photosynthetic rate (P_N) and leaf nitrogen concentration (*A*), grana number (*B*), and thylakoids/granum (*C*) during leaf ontogeny of field-grown cotton in the year 1990 (○) and 1991 (●). Each point represents mean of six leaves.

Leaf expansion was completed in less than 20 d during which maximum physiological activity was observed. High values for P_N (16.5 µmol m^{-2} s^{-1}) and leaf N concentration (168 mmol m^{-2}) were observed by the 20 d-old leaf, thereafter, photosynthesis and leaf N concentration declined with advancing leaf age (Fig. 1*A*) in full accord with previous study on cotton (Wullschleger and Oosterhuis 1990b) and other crops (Šesták 1985). In cotton, the decline in leaf photosynthesis with increasing leaf age is the major contributor to declining canopy photosynthesis as the season progresses (Oosterhuis and Wullschleger 1992). The contribution of leaf senescence to declining canopy photosynthesis and to carbon availability for yield is a potential limitation in crop production (Wullschleger and Oosterhuis 1992).

Table 1. Ontogenic changes in chloroplast ultrastructure of cotton leaves. Plastoglobuli size in [µm]. Mean values from 15 chloroplasts in one representative section from each replication in each leaf age. Means within a column followed by the same letter are not significantly different (LSD at $p = 0.05$).

Leaf age	Grana number		Thylakoids/granum		Plastoglobuli number		Plastoglobuli size	
[d]	1990	1991	1990	1991	1990	1991	1990	1991
20	14^a	13^a	12^a	13^a	4^c	3^c	0.55^c	0.60^c
30	11^b	9^b	9^b	10^b	8^b	9^b	0.85^b	0.90^b
40	8^c	6^c	5^c	8^c	12^a	13^a	1.25^a	1.29^a
60	6^c	4^c	3^c	6^c	14^a	14^a	1.28^a	1.31^a

The relationship between leaf N and P_N suggests a redistribution of leaf N to younger leaves for optimization of the whole photosynthetic income (Field and Mooney 1986). Since 75 % of the leaf N is invested in the chloroplast and most of that is used in photosynthesis (Chapin *et al.* 1987), the removal of N from the chloroplast with leaf age would cause ultrastructural changes in the chloroplast.

TEM showed that the young 20-d old leaf chloroplast had high number of grana and thylakoids per granum (membrane system) with a few small plastoglobuli indicating a well developed membrane system (Table 1). Similar results were reported in young spinach leaves (Lichtenthaler 1968). After 20 d, the membrane system disintegrated as indicated by a decrease in grana number and thylakoids per granum coupled with increased number and size of plastoglobuli (Table 1). Large plastoglobuli within the chloroplast are the most conspicuous indicator of leaf senescence (Butler and Simon 1971) and these large plastoglobuli appear in the chloroplast whenever thylakoids start breaking down (Lichtenthaler 1968). We confirmed this connection between plastoglobuli content and the degradation of thylakoids.

Both grana number and thylakoids per granum number were closely related to P_N as the leaf progressed towards senescence (Fig. 1*B*, *C*). Since the granum is the site of photosystems and chlorophyll (Šesták 1985), a close relationship between photosynthesis and the membrane system was expected. Thus, the decline in photosynthesis was associated with changes in the chloroplast ultrastructure during leaf ontogeny.

References

Bondada, B.R., Oosterhuis, D.M., Norman, R.J.: Cotton leaf age, epicuticular wax, and ^{15}N absorption. - Crop Sci. **37**: 807-811, 1997.

Bondada, B.R., Oosterhuis, D.M., Wullschleger, S.D., Kim, K.S., Harris, W.M.: Anatomical considerations related to photosynthesis in cotton (*Gossypium hirsutum* L.) leaves, bracts, and the capsule wall. - J. exp. Bot. **45**: 111-118, 1994.

Bremner, J.M., Mulvaney, C.S.: Nitrogen-total. - In: Page, A.L. (ed.): Methods of Soil Analysis. Part 2. 2nd Ed. Pp. 595-624. American Society of Agronomy and Soil Science Society of America, Madison 1982.

Butler, R.D., Simon, E.W.: Ultrastructural aspects of senescence. - Adv. gerontol. Res. **3**: 73-129, 1971.

Chapin, F.S., III, Bloom, A.J., Field, C.B., Waring, R.H.: Plant repsonses to multiple environmental factors. - Biol. Sci. **37**: 49-57, 1987.

Constable, G.A., Rawson, H.M.: Carbon production and utilization in cotton: Inferences from a carbon budget. - Aust. J. Plant Physiol. **7**: 539-553, 1980.

Field, C., Mooney, H.A.: The photosynthetic-nitrogen relationships in wild plants. - In: Givinish, T.J. (ed.): On the Economy of Plant Form and Function. Pp. 25-55. Cambridge University Press, New York 1986.

Lichtenthaler, H.K.: Plastoglobuli and fine structure of the plastids. - Endeavour **27**: 144-149, 1968.

Oosterhuis, D.M., Wullschleger, S.D.: Physiological implications of leaf aging in cotton productivity. - Arkansas Farm Res. **41**: 12-14, 1992.

Šesták, Z. (ed.): Photosynthesis during Leaf Development. - Academia, Praha; Dr W Junk, Dordrecht - Boston - Lancaster 1985.

Sobrado, M.A.: Leaf photosynthesis and water loss as influenced by leaf age and seasonal drought in an evergreen tree. - Photosynthetica **32**: 563-568, 1996.

Wullschleger, S.D., Oosterhuis, D.M.: Photosynthetic and respiratory activity of fruiting forms within the cotton canopy. - Plant Physiol. **94**: 463-469, 1990a.

Wullschleger, S.D., Oosterhuis, D.M.: Photosynthesis of individual field-grown cotton leaves during ontogeny. - Photosynth. Res. **23**: 163-170, 1990b.

Wullschleger, S.D., Oosterhuis, D.M.: Canopy leaf area development and age-class dynamics in cotton. - Crop Sci. **32**: 1141-1154, 1992.