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BRIEF COMMUNICATION

Relationships between nitrogen content and net gas exchange components of a cotton leaf during ontogeny

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Abstract

Relationships between leaf nitrogen (N) content and leaf gas exchange components of a single cotton (*Gossypium hirsutum* L.) leaf subtending the fruit during ontogeny were investigated under field conditions. A 20-d old leaf exhibited the highest physiological activity characterized by net photosynthetic (P_N) and transpiration (E) rates, stomatal conductances to CO_2 exchange (g_{sCO2}) and water vapor transfer (g_{sH2O}), and nitrogen (N) content. With the advent of leaf senescence, the gas exchange rates declined as exhibited by the 30-, 40-, and 60-d old leaves. Regression analysis indicated close relationships between g_{sCO2} and P_N, and g_{sH2O} and E as the leaves advanced towards senescence. Both P_N and g_{sCO2} were related to N as they declined with leaf age. Thus, the declines in P_N were associated with stomatal closure and removal of N during leaf ontogeny.

Additional key words: *Gossypium hirsutum*; leaf age; photosynthesis; stomatal conductance; transpiration.

The gas exchange activities, chloroplast ultrastructure, and leaf N concentration as a function of leaf age have been well documented in cotton (Constable and Rawson 1980, Wullschleger and Oosterhuis 1990, Bondada *et al.* 1997, Bondada and Oosterhuis 1998) and other crops (Frank 1981, Šesták 1985, Čatský and Šesták 1997). Little is known, however, about the changes in relationships between N

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content and gas exchange components during the life span of a single cotton leaf subtending the fruit. Information in this regard is necessary since the assimilate supplied from the subtending leaf determines the productivity of the fruit it subtends. Therefore, an investigation was undertaken in which leaf N content and gas exchange characteristics of known leaf ages were measured to determine relationships between P_N, g_{sCO2}, g_{sH2O}, and leaf N content.

Seeds of *Gossypium hirsutum* L., 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 m^{-2} (72 000 plants ha^{-1}). 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 conducted using four leaf-age treatments, 20-, 30-, 40-, and 60-d old leaves in a completely randomized design with three replications in each year. For the determination of leaf age, approximately 50 plants in each plot were tagged 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. Six leaves from each leaf age in each replication ($n = 6$) were used for gas exchange measurements.

P_N measurements of leaves from each leaf-age group were made in a closed system using a model *LI-6000* portable photosynthesis system (*LI-COR*, Lincoln, NE, USA) equipped with either a 250 cm^3 or 1000 cm^3 stirred cuvette. The CO_2 concentration at the point of gas exchange measurements was 345-350 cm^3 m^{-3}. During measurement, cuvette CO_2 concentrations declined by approximately 10-15 cm^3 m^{-3}. Ten consecutive measurements at 3-s intervals were taken for the rate of CO_2 depletion from the chamber. Calibration of the CO_2 sensor was checked at the beginning of each measurement period and corrections made when necessary. g_{sH2O} was measured using a *LI-1600* (*LI-COR*, Lincoln, NE, USA) steady-state porometer. The values of g_{sH2O} were divided by 1.56 to determine g_{sCO2} (Bugbee 1992). All measurements were recorded on three replications (2 leaves per replication, $n = 6$) in each leaf-age group. All readings were made between 12:30 and 14:30 h on cloudless days when ambient PPFD exceeded 1700 µmol m^{-2}. After measuring gas exchange activities, all leaves from each leaf age-group were oven-dried for at least 72 h at 60 °C, and ground to a powder for total N analysis by the $KMnO_4$-Fe^{2+} modification of the semi-micro-Kjeldahl procedure (Bremner and Mulvaney 1982). All values were subjected to analysis of variance (*SAS Institute*, Cary, NC, USA). Regression analyses were made to assess the relationships between gas exchange components and leaf N content.

Relationships between gas exchange components: The gas exchange activities declined with advancing leaf age in accord with previous studies on cotton (Constable and Rawson 1980, Wullschleger and Oosterhuis 1990) and other crops (Frank 1981, Šesták 1985, Čatský and Šesták 1997). The present study showed that as P_N and g_{sCO2} declined with leaf age, a close relationship existed between them (Fig. 1*A*). The strong relationship between g_{sCO2} and P_N is similar to that observed by Aslam *et al.* (1977) in cassava and by Davis and McCree (1978) in bush beans. The decrease in P_N associated with reduced g_{sCO2} was proclaimed as the principal limitation to photosynthetic CO_2 assimilation leading to a decrease in total productivity (Farquhar and Sharkey 1982). Although stomatal conductance is believed to be a major factor limiting photosynthetic capacity (Farquhar and Sharkey 1982), there is not a causal relationship (Pearcy *et al.* 1987). This relationship just reflects the sensitivity of stomatal guard cells to some metabolite of mesophyll cell photosynthesis (Wong *et al.* 1979).

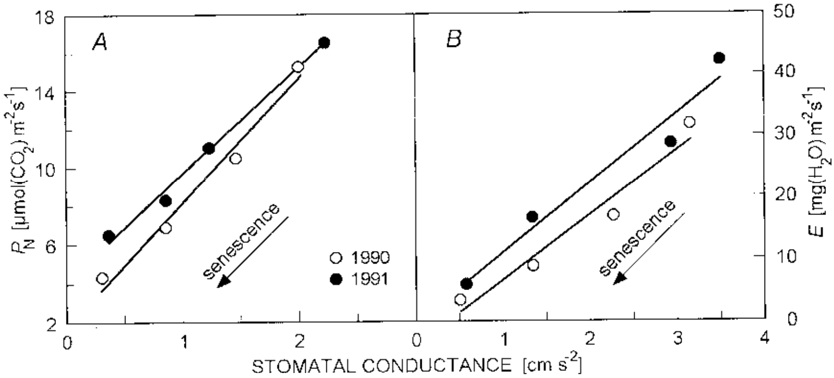

Fig. 1. Relationships between net photosynthetic rate (P_N) and stomatal conductance to CO_2 exchange (g_{sCO2}) (*A*), and between transpiration rate (*E*) and stomatal conductance to water vapor transfer (g_{sH2O}) (*B*) during leaf ontogeny of field-grown cotton in the year 1990 (○) and 1991 (●). Each data point represents mean of six leaves.

Similar to relationship between P_N and g_{sCO2}, *E* and g_{sH2O} were closely related (Fig. 1*B*) suggesting that water vapor and CO_2 exchange rates were affected similarly by leaf aging. Since the water vapor and CO_2 exchanges take place through stomatal pores, similar response would be expected. Analogous results were obtained with other crops (Frank 1981). The increase in water vapor and CO_2 exchange rates of young leaves was attributed to a high level of activity in the mesophyll tissue and wide stomatal opening (DeMichele and Sharpe 1972).

Relationships between leaf N content and gas exchange components: Analogous to gas exchange components, the leaf N content also declined with leaf age (Fig. 2). In general, leaf N declines with increasing leaf age in essentially all plants and provides a completely natural source of intraspecies (and intraplant) variation (Field and Mooney 1986). The decrease in N with advancing leaf age may be a genetically programmed process (Buchanan-Wollaston 1997) which results in translocation of N from old leaves to new leaves and reproductive structures (Field and Mooney 1986). Furthermore, the continuous withdrawal of N from the vegetative organs to meet the requirements of fruit development is responsible for leaf senescence (Sinclair and de Wit 1975).

As the leaf N content declined, g_{sCO2} also declined with leaf age and a close relationship existed between them (Fig. 2*A*). Reduction in g_{sCO2} with declining leaf N content with leaf age causes a decline in the ability of stomates to stay open resulting in reduction in stomatal aperture (Davis and McCree 1978). Thus, stomatal aperture size is linked to N content of the leaves with stomatal conductance decreasing with decreasing content of N in the leaves (Field and Mooney 1986).

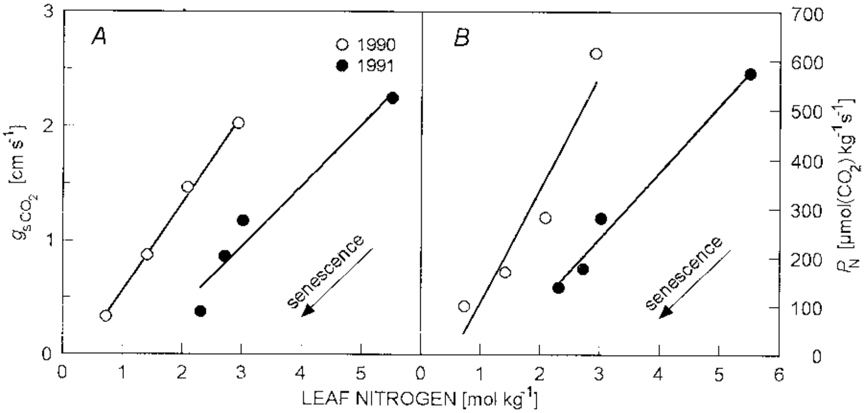

Fig. 2. Relationships between stomatal conductance to CO_2 exchange (g_{sCO2}) and leaf N (*A*), and between net photosynthetic rate (P_N) and leaf N (*B*) during leaf ontogeny of field-grown cotton in the year 1990 (○) and 1991 (●). Each data point represents mean of six leaves.

A close relationship also existed between P_N and leaf N content as the leaf progressed towards senescence (Fig. 2*B*). Leaf photosynthetic capacity has often been highly correlated with leaf N (Field and Mooney 1986, Evans 1989) regardless of whether variation in leaf N was due to micro-environment, leaf age, or N availability (Reich and Schoettle 1988). Moreover, this relationship is relatively insensitive to differences among species or growth conditions (Field and Mooney 1986) and should be considered universal when expressed on leaf mass basis (Reich *et al.* 1992). The reason for such a strong tie between N and photosynthesis includes the paramount role of N on light harvesting and CO_2 fixation (Evans 1989). Thus, the age-related changes in P_N were associated with changes in g_{sCO2} and leaf N content. This implied that, in addition to alterations in the chloroplast ultrastructure (Bondada and Oosterhuis 1998), the declines in P_N were also associated with N removal and stomatal closure indicating that an ensemble of factors contributed to the decline in P_N during leaf ontogeny. Other factors which have been reported to regulate P_N are intercellular conductances, chlorophyll content, and enzyme activities (Čatský and Šesták 1997).

References

Aslam, M., Lowe, S.B., Hunt, L.A.: Effect of leaf age on photosynthesis and transpiration of cassava (*Manihot esculenta*). - Can. J. Bot. **45**: 2288-2295, 1977.

Bondada, B.R., Oosterhuis, D.M.: Decline in photosynthesis as related to alterations in the chloroplast ultrastructure during ontogeny of a cotton leaf. - Photosynthetica **35**: 467-471, 1998.

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

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.

Buchanan-Wollaston, V.: The molecular biology of leaf senescence. - J. exp. Bot. **48**: 181-199, 1997.

Bugbee, B.G.: Calculating fluxes into leaves. - In: Salisbury, F.B., Ross, C.W. (ed.): Plant Physiology. Pp. 64-65. Wadsworth Publishing Company, Belmont 1992.

Čatský, J., Šesták, Z.: Photosynthesis during leaf development. - In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 633-660. Marcel Dekker, New York - Basel - Hong Kong 1997.

Constable, G.A., Rawson, H.M.: Effect of leaf position, expansion and age on photosynthesis, transpiration and water use efficiency of cotton. - Aust. J. Plant Physiol. **7**: 89-100, 1980.

Davis, S.D., McCree, K.J.: Photosynthetic rate and diffusion conductance as a function of age in leaves of bean plants. - Crop Sci. **18**: 280-282, 1978.

DeMichele, D.W., Sharpe, P.J.H.: A morphological and physiological model of the stomata. - In: Murphy, C., Hesketh, J.D., Strain, B.R. (ed.): Workshop on Tree Growth Dynamics and Modeling. Pp. 69-85. Durham 1972.

Evans, J.R.: Photosynthesis and nitrogen relationships in leaves of C_3 plants. - Oecologia **78**: 9-19, 1989.

Farquhar, G.D., Sharkey, T.D.: Stomatal conductance and photosynthesis. - Annu. Rev. Plant Physiol. **33**: 317-345, 1982.

Field, C., Mooney, H.A.: The photosynthesis-nitrogen relationship in wild plants. - In: Givnish, T.J. (ed.): On the Economy of Plant Form and Function. Pp. 25-55. Cambridge University Press, Cambridge - London - New York - New Rochelle - Melbourne - Sydney 1986.

Frank, A.B.: Effect of leaf age and position on photosynthesis and stomatal conductance of forage grasses. - Agron. J. **81**: 70-74, 1981.

Pearcy, R.W., Björkman, O., Caldwell, M.M., Keeley, J.E., Monson, R.K., Strain, B.R.: Carbon gain by plants in natural environments. - BioScience **37**: 21-29, 1987.

Reich, P.B., Schoettle, A.W.: Role of phosphorus and nitrogen in photosynthetic and whole plant carbon gain and nutrient use efficiency in eastern white pine. - Oecologia **77**: 25-33, 1988.

Reich, P.B., Walters, M.B., Ellsworth, D.S.: Leaf life span in relation to leaf, plant, and stand characteristics among diverse ecosystems. - Ecol. Monogr. **62**: 365-392, 1992.

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

Sinclair, T.R., de Wit, C.T.: Photosynthate and nitrogen requirements for seed production in various crops. - Science **189**: 565-567, 1975.

Wong, S.C., Cowan, I.R., Farquhar, G.D.: Stomatal conductance correlates with photosynthetic capacity. - Nature **282**: 424-426, 1979.

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