Water Relations of Cotton Plants under Nitrogen Deficiency

III. STOMATAL CONDUCTANCE, PHOTOSYNTHESIS, AND ABSCISIC ACID ACCUMULATION DURING DROUGHT

Received for publication April 8, 1980 and in revised form August 21, 1980

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ABSTRACT

Nitrogen nutrition exerted a strong effect on stomatal sensitivity to water stress in cotton. In well-watered plants grown with 0.31 millimolar N in the nutrient solution, stomata closed at a water potential of -9 bars even though the wilting point was below -15 bars. For each doubling of nutrient N level, the water potential for stomatal closure decreased by about 2 bars. Elevated intercellular CO₂ concentrations caused only slight stomatal closure regardless of N nutrition. Exogenous abscisic acid (ABA) greatly increased stomatal sensitivity to elevated CO₂ concentrations.

Plants subjected to water stress gave the following responses: (a) decreased stomatal conductance at ambient external CO₂ concentration; (b) increased stomatal sensitivity to elevated CO₂ concentrations; (c) decreased mesophyll conductance to CO₂; and (d) increased endogenous ABA content. All of these responses to stress occurred at a higher water potential in N-deficient plants than in normal plants. The results show that N nutrition and water stress interact to control ABA accumulation and the events regulated by that accumulation.

Stomatal movement and its regulation have been studied since the early days of plant physiology. It is now known that both irradiance and Ci can strongly affect stomatal conductance (13). In addition, the phytohormone ABA causes stomatal closure, in part by sensitizing stomata to Ci (7, 20). The synthesis of ABA in leaves during water stress is believed to account for drought-induced stomatal closure (11).

Recently Radin and Parker (19) reported that stomata of N-deficient cotton plants were much more sensitive to water stress than those of normal plants. Stomata of N-deficient plants closed at ψ as high as -10 bars, whereas those of normal plants closed at about -20 bars. Stomatal closure in N-deficient plants was not a result of leaf senescence (17). The effects of N deficiency were consistent with the finding that N-deficient plants have increased levels of ABA (6, 10, 14). Because ABA increases stomatal sensitivity to Ci (7, 20), any effects of this endogenous ABA on stomata should be manifested as differences in the plots of stomatal conductance versus Ci. Here, we examine the effects of N nutrition and water stress on ABA accumulation, photosynthesis, and stomatal responses to Ci in cotton leaves.

MATERIALS AND METHODS

Plant Growth Conditions. Seeds of cotton (Gossypium hirsutum L. cv. Deltapine 61) were germinated and grown in a greenhouse in 14-liter pots containing a mixture of peat moss, vermiculite, and sand. The greenhouse was cooled by refrigeration, with temperatures ranging from about 35 C during early afternoon to 22 C at night. Peak PAR was generally 1,800 to 2,000 μE m⁻² s⁻¹. After establishment, plants were thinned to two/pot. Three times weekly, enough nutrient solution was added to each pot to ensure substantial leaching. The nutrient solutions were a modified half-strength Hoagland solution containing up to 5 mM N as KNO₃ + Ca(NO₃)₂, but no reduced N. When N concentrations were less than 5 mM, K⁺ and Ca²⁺ concentrations were maintained by substituting KCl + CaCl₂ for KNO₃ + Ca(NO₃)₂.

Imposition of Water Stress. Watering was discontinued after the fifth leaf above the cotyledons had fully expanded. Stomatal and photosynthetic parameters were followed in the fifth leaf as stress progressed. For diffusive resistance measurements in the greenhouse, ψ was determined in early afternoon with a pressure chamber. For gas-exchange work, ψ was determined (on a leaf other than the fifth) at approximately 9:00 AM, just before the plants were transferred to the growth chamber (see below). The progression of stress was considerably faster in high-N plants than in N-deficient plants because of treatment effects on both leaf area and stomatal behavior. Typically, plants grown on 5 mM N showed incipient afternoon wilting 4 to 7 days after watering. Plants grown on 0.62 mM N reached the same point in twice that time.

Treatment with ABA. A leaf at the fifth node on a well-watered plant was sprayed to runoff with a solution of 0.1 mM (±)-ABA (Sigma) containing 0.1% (v/v) Tween 20 or Triton X-100. Gas-exchange characteristics were determined the following day. Leaves of high-N plants received three sprays. Leaves of N-deficient plants were generally more sensitive to ABA and received only one spray.

Diffusive Resistances. As drying progressed in the greenhouse,
diffusive resistances to water vapor transport were followed with a transit-time diffusion porometer as described earlier (19). All measurements were in the early afternoon, at the time of minimum ψ. Total leaf resistance was calculated from the parallel resistances of abaxial and adaxial surfaces. Immediately following this measurement, the leaf was excised and ψ was determined in a pressure chamber.

These results are presented as resistances rather than conductances because the method of calibration and use of the porometer produced considerable scatter at high conductances. At conductances less than the maximum (the most useful range of the porometer), treatment effects were most easily visualized in terms of resistances.

Gas Exchange in Controlled CO2. Plants were placed in a controlled environment chamber to provide constant temperature (27°C) and ambient lighting (250 μE m⁻² s⁻¹ PAR). A small clip-on cuvette was attached to the appropriate leaf, enclosing a circular area of 5.9 cm². The cuvette was constructed of acrylic plastic and had provisions for dividing an airstream equally between abaxial and adaxial chambers. Normal airflow was 605 ml/min. Reflective film was placed on the face of the cuvette with a cutout for the assimilation chamber to prevent excessive heating of the leaf.

The airstream was made up by mixing commercial compressed air with N₂ containing 3% CO₂ (v/v) in a gas-mixing manifold. Flows from each cylinder, and the flow rate of the mixture, were monitored with float-type glass flowmeters. The compressed air contained very little CO₂ and no detectable moisture.

Leaf temperature was monitored with a 0.1-mm (4-mil) copper-constantan thermocouple junction (Omega Engineering, Stamford, CT) appressed to the underside of the leaf inside the cuvette and connected to a Wescor TH-65 thermocouple thermometer (Wescor Instruments, Logan, UT). Because the air was dry, transpiration in the cuvette was great enough to keep leaf temperatures at or below 30°C, except at very low stomatal conductances.

The photosynthetic light source was a 500-w tungsten halogen lamp with a fan for forced-air cooling, a focusing lens to provide high irradiance over a small area, and a built-in heat filter. This system was commercially available as a Kodak Carousel slide projector. PAR was measured with a Li-Cor LI-185 meter equipped with an LI-190S quantum sensor (Lambda Instruments, Lincoln, NE). The cuvette was positioned in the light beam to receive 2000 μE m⁻² s⁻¹. This level saturated both stomatal opening and photosynthesis under all conditions.

Transpiration and Photosynthesis. The effluent airstreams from each side of the leaf were combined and passed into a psychrometer calibrated at the appropriate flow rate and temperature. Wet- and dry-bulb thermocouple temperatures were monitored with the Wescor thermocouple thermometer. Whenever the composition of the incoming mixture was altered, the stomata were allowed to equilibrate for at least 30 min before measurements were recorded. Transpiration rates were calculated from the steady-state increases in humidity across the leaf. Conductances were calculated by assuming (a) that the air in the substomatal cavity was saturated with water vapor at the temperature of the leaf and (b) that the effective humidity gradient was the arithmetic mean of the initial and final gradients. With the transpiration rates actually achieved, this second assumption led to errors of 5% or less when compared to Gastra's (9) equations. Reported conductances are the unseparated stomatal + boundary layer conductances. Boundary-layer conductance, determined from evaporation from wet blotting paper, was about four times greater than the maximum leaf conductance.

Photosynthetic rates were followed by a modification of the technique of Clegg et al. (4). Three 10-ml gas samples were slowly withdrawn from the effluent airstream, and then three samples were taken from the incoming airstream. All samples were stored until analysis in disposable syringes in which the plungers had rubber tips for a tight seal. The needles were sealed to the barrels with cyanoacrylate adhesive, and the needle tips were inserted into rubber stoppers to prevent leakage. Gas samples did not change composition even when stored several hours in this fashion. Samples were injected into an N₂ stream that was passed through a Beckman 315A IR gas analyzer equipped with a Perkin-Elmer M-2 integrator set to operate in the peak height mode. Peak heights were converted to CO₂ concentrations from a standard curve. Photosynthetic rates were calculated from the differences in CO₂ concentrations.

The cᵢ were calculated according to Farquhar et al. (8). gᵢ values were determined from the slopes of curves relating photosynthetic rate to cᵢ (15). This method prevented errors resulting from changes in compensation point. However, because all measurements were made in air of normal O₂ content, photospiration could still have affected the results.

Endogenous ABA Levels. The ABA levels of plants were followed for 7 days after discontinuation of watering. Discs 18 mm in diameter were cut from the fifth leaf of eight high-N and eight low-N plants in the early afternoon, and each group was combined into two replicate samples. The discs were immediately frozen on dry ice and stored at −40°C until lyophilization. ψs were measured on separate plants in the same pots.

ABA was extracted and determined as described earlier (1). Results are expressed as ng ABA/cm². Compared to concentrations on a dry weight basis, the units chosen tend to underestimate the ABA levels of low-N leaves relative to high-N leaves, because low-N leaves contain less dry weight per unit area, and they undergo less shrinkage during stress (18).

RESULTS

Diffusive Resistance in Normal Air. Greenhouse-grown plants showed stomatal responses to ψ in normal air (about 350 μl/l) which clearly depended upon N nutrition. With 0.31 mM N in the nutrient solution, resistance increased rapidly at a ψ of −8 bars and reached 10 s/cm at −9 bars (Fig. 1). As available N was increased to 5 mM (four successive doublings), the ψ for a resistance of 10 s/cm was decreased to −18 bars. These results are similar to data reported earlier (19). For further study of stomatal regulation, plants were grown at only two N levels: 5 mM (high-N) and 0.62 mM (low-N). These two treatments afforded the best combinations of growth rate, water use, leaf size, and stomatal differences for
Responses to Increasing CO₂. gₛ in well-watered plants declined, but only slightly, in response to increasing cᵢ (Fig. 2). There was little or no difference between high-N and low-N leaves in the response to increasing cᵢ. In contrast, leaves sprayed with 0.1 mM ABA responded very strongly to cᵢ. In such leaves, gₛ decreased from about 0.2 to less than 0.05 mol m⁻² s⁻¹ when cᵢ was increased from 100 to 300 μl/l (Fig. 2). The effect of exogenous ABA was independent of N nutrition, although the low N stomata seemed to respond to lesser quantities of applied ABA (data not shown).

Photosynthetic rate was strongly affected by cᵢ and by N nutrition (Fig. 3). Typically, high-N leaves had a slightly lower compensation point, a slightly greater cᵢ for saturation, and a greater maximum rate of CO₂ uptake than low-N leaves. At saturating cᵢ, photosynthesis in the high-N leaves was about 50% greater than in the low-N leaves, reaching rates of almost 40 μmol m⁻² s⁻¹ (about 60 mg CO₂ dm⁻² h⁻¹). Although treatment with ABA greatly modified stomatal behavior, it had less effect upon the other aspects of photosynthesis. In high-N leaves, ABA did not affect CO₂-limited photosynthesis at all (cᵢ ≤ 200 μl/l) but, in low-N leaves, the gₛ at limiting CO₂ (cᵢ ≤ 175 μl/l) apparently decreased by a small amount (Fig. 3). The loss of photosynthetic capacity in low-N leaves continued slowly for several days (data not shown) and presumably was an indirect effect of ABA rather than a direct effect (21).

Responses to Water Stress. The relationship between gₛ and cᵢ changed as ψ declined; also, the effect of water stress upon this relationship depended upon N status. In high-N leaves, increasing cᵢ had little effect upon gₛ until ψ had declined to −15.8 bars (Fig. 4, bottom). Although gₛ decreased somewhat in these leaves in response to stress, the response was almost independent of cᵢ at −12.2 bars, and was only partially dependent upon cᵢ at −15.8 bars. In contrast, any decline in ψ of low-N leaves caused an immediate sensitization to cᵢ (Fig. 4, top). In these plants, most or all of the stomatal response to stress appeared to result from this induction of sensitivity to CO₂.

When photosynthesis is plotted against cᵢ, the slope of the curve at limiting CO₂ is proportional to gₛᵢ. Before the onset of stress, low-N plants had a gₛᵢ (slope) about 20% less than that of high-N plants (Fig. 3). (In terms of resistances, these values were 2.5 and 2.1 s/cm for low- and high-N leaves, respectively). As ψ declined, gₛᵢ also decreased in both high-N and low-N leaves. Again, the effect of water stress on this relationship depended upon N status. In high-N leaves, gₛᵢ declined very slowly until ψ reached −12 bars, at which point it began to drop rapidly (Fig. 5). Conductance continued to decrease with declining ψ until it reached a very small value. In low-N leaves, gₛᵢ began to drop at about −7 bars and had decreased 50% at −10 bars (Fig. 5). Because of these changes in photosynthetic capacity, cᵢ tended to increase as ψ decreased, especially in the high-N leaves (Fig. 6). The cᵢ was slightly higher in low-N leaves above −15 bars, but this relationship was reversed below −15 bars (Fig. 6).

The increased sensitivity of stomata to cᵢ during stress (Fig. 4) presumably was tied to ABA accumulation in the leaf, because
exogenous ABA had a similar effect (Fig. 2). Analyses of leaf tissue confirmed that ABA levels increased in both high-N and low-N plants as $\psi$ decreased, and the increases occurred at higher $\psi$ in low-N plants (Fig. 7). At any comparable $\psi$, ABA levels were greater in the low-N leaves than in the high-N leaves. During the 7 days without water, $\psi$ decreased only to $\pm 17$ bars in the low-N plants but reached $-26$ bars in the high-N plants. Despite this difference in $\psi$, the two groups of plants reached similar maximum ABA levels (Fig. 7).

**DISCUSSION**

Our results document a strong interaction of N nutrition and water stress on the physiology of the cotton leaf. In N-deficient leaves, both $g_s$ and $g_m$ were much more sensitive to declining $\psi$ than in normal leaves (Figs. 1, 4, and 5). The differences in stomatal behavior could not be explained by any effects of N deficiency on endogenous prestress ABA contents because N deficiency by itself did not cause stomata to imitate behavior seen in ABA-sprayed leaves (Fig. 2). Although differences in photosynthesis at high $\psi$ (Fig. 3) caused a slightly greater steady-state $c_i$ in low-N leaves than in high-N leaves (Fig. 6), the effect of that increased $c_i$ was extremely small and, again, could not account for the differences in stomatal behavior. Further work showed that the differences in stomatal behavior arose only after the onset of water stress (Fig. 4). The induction of stomatal sensitivity to $c_i$ by stress strongly resembled the effect of applied ABA (Fig. 2) and was correlated with increases in endogenous ABA during stress (Fig. 7). This correlation is weakened by the absence of ABA determinations at $\psi$ values above $-10$ bars, when stomatal differences were evident at about $-8$ bars (Figs. 4 and 7). Part of this discrepancy resulted from sampling times: samples for ABA analysis were taken in early afternoon at the time of minimum $\psi$, but samples in the gas-exchange experiments were necessarily taken in the morning. Taken as a whole, the data provide strong evidence that ABA regulates stress-induced stomatal closure in both high-N and low-N plants.

From the ABA-like responses to stress (Fig. 4), it seems likely that low-N leaves began to accumulate ABA at a $\psi$ as high as $-8.4$ bars. Earlier work (18) established that the wilting point of low-N plants was below $-15$ bars. Thus, the threshold for ABA accumulation was apparently well above zero. In high-N leaves, ABA-like responses appeared at a $\psi$ much closer to the reported wilting point (18). In this respect, the behavior of high-N plants was consistent with the observations of Pierce and Raschke (16), who reported a threshold turgor of zero for ABA synthesis in several plants including cotton.

It has frequently been reported that N deficiency causes increased ABA levels in plants (6, 10, 14). Because N deficiency raises the threshold $\psi$ for ABA accumulation, normal diurnal changes in $\psi$ could initiate ABA accumulation, even in well-watered plants that do not approach wilting. This mechanism could produce elevated ABA levels independently of, or instead of, any direct effects of N deficiency on ABA metabolism. It seems likely that stomatal closure in N-deficient plants was regulated by ABA synthesized during stress (Fig. 4), but a possible role for ABA synthesized as a direct result of N deficiency remains unclear.

In both high-N and low-N leaves, the $g_m$ declined under water stress more or less in concert with the decline in $g_s$ and increase in ABA content. Because of this decrease in photosynthetic capacity, $c_i$ increased slowly in both treatments as the stomata closed (Fig. 6). These results differ widely from some previous work with cotton (22, 23; see also Hsiao (11) for discussion). A recent field study of cotton (2) also showed that water stress caused substantial nonstomatal inhibition of photosynthesis. The key to these findings may be the relatively slow development of stress. Similarly, osmotic adjustment in sorghum in response to stress occurred primarily under conditions of slow drying (12).

Boyer (3) has described the extensive work documenting nonstomatal inhibition of photosynthesis by water stress. Collatz (5), working with jojoba, also reported stress-induced decreases in $g_m$ which paralleled stomatal closure and stabilized $c_i$. The jojoba responses closely resembled those reported here and may be related to the concept that $g_s$ and $g_m$ remain "balanced" (24). In cotton, nonstomatal inhibition of photosynthesis was unrelated to senescence, which occurred at much lower $\psi$ (17). No further breakdown on the $g_m$ into its photochemical, biochemical, and transport components is possible from these gas-exchange experiments.

Acknowledgments—We thank J. S. Boyer, G. Guinan, and J. R. Maune for helpful discussions and advice. L. L. Parker provided excellent technical assistance, and D. Brummel helped with equipment construction and maintenance.

**LITERATURE CITED**

6. DAIR JD, SD SEELEY, WT CAMPBELL 1979 Nitrogen deficiency influence on abscisic acid in tomato. Hortscience 14: 261-262
7. DURBE DR, GD FARQUHAR, K RASCHKE 1978 Effects of abscisic acid on the gain of the feedback loop involving carbon dioxide and stomata. Plant Physiol 62: 413-417
9. GAASTRA P 1959 Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature, and stomatal diffusion resistance. Meded Landbouwhogesch Wageningen 59: 1-68