Water Relations of Cotton Plants under Nitrogen Deficiency

II. ENVIRONMENTAL INTERACTIONS ON STOMATA

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ABSTRACT

Nitrogen deficiency in cotton plants (*Gossypium hirsutum* L.) considerably increased the sensitivity of stomata to water stress. At air temperatures of 27, 35, and ≥40 C, threshold potentials for complete stomatal closure were −10, −15, and −26 bars in N-deficient plants and −20, −20, and −30 bars in high-N plants, respectively. This three-way interaction among N supply, water potential, and air temperature was similar to that exerted on leaf expansion. The effects of N supply on stomatal behavior could not be explained on the basis of either osmotic or structural considerations. Rather, effects of N deficiency on mesophyll and stomata were independent and divergent. Stomatal behavior may impart a stress avoidance type of drought resistance to N-deficient plants.

In a companion paper (10), we reported that N deficiency in cotton plants is accompanied by substantial effects upon leaf structure, and that such leaves have some of the characteristics normally associated with adaptation to drought. The similarities are limited to structural adaptations, however, because N-deficient plants show only a slight degree of osmotic adjustment (10). Cutler *et al.* (4), on the basis of a simple leaf model, proposed that the reduced sensitivity of drought-adapted plants to stress largely resulted from structural rather than osmotic adaptations. This argument is also implicit in many other studies (*e.g.* 2, 14) in which the change in RWC[1] per unit change in Ψ is considered paramount. The question arises whether N deficiency imitates drought adaptation physiologically as well as structurally, *i.e.* does N deficiency decrease the sensitivity of growth to water stress? Because the structural alterations of N-deficient plants are not accompanied by large-scale changes in osmotic concentrations, this question bears directly upon the hypothesis of Cutler *et al.* (4). Here, we report that N deficiency greatly increases the sensitivity of stomata and of growth to water stress. This interaction between N nutrition and water stress is apparently independent of both structural and osmotic considerations.

MATERIALS AND METHODS

Plant Growth in the Greenhouse. Cotton (*Gossypium hirsutum* L. cv. Deltapine 16) plants were germinated and grown as described in a companion paper (10), except that the low-N nutrient solution contained 1 mM NO_3^- instead of 0.2 mM. The high-N concentration remained at 5 mM. The relative growth rate (leaf area basis) of the N-deficient plants was typically about one-half

of the high-N rate. Leaf N concentrations (per cent of dry weight) were as follows: nitrate-N, 0.17 and 0.53% in low-N and high-N plants, respectively; reduced N, 2.2 and 3.1% in low-N and high-N plants, respectively. There were no visible differences in leaf color.

Imposition of Drought. High-N and low-N plants were grown until the fifth leaf of the low-N plants was fully expanded, at which time watering was discontinued. On separate occasions, drying was followed in three different temperature regimes: cool (maximum T_a 27 C), warm (identical to the growth regime, with maximum T_a 35 C), and hot (maximum T_a = 40–42 C). After watering was discontinued, resistances to water vapor diffusion were followed with a Li-Cor[2] LI-60 diffusive resistance meter fitted with a LI-20S sensor (Lambda Instruments Corp., Lincoln, Nebr.). Total leaf resistances were calculated from the parallel resistances of abaxial and adaxial surfaces. Readings were taken out of direct sunlight. Resistances were measured only in early afternoon, at the time of maximum T_a and minimum Ψ. All measurements were on the fifth true leaf, counting acropetally. Leaf Ψ was determined in a pressure chamber (10) immediately after measurement of diffusive resistance.

Growth during Drought. The growth of plants was followed in the cool and warm regimes after watering was discontinued. The 24-h increase in length of the youngest completely unfolded lamina was determined (to the nearest mm) along the midrib. All measurements were taken in the early afternoon. Immediately after the second measurement, leaves were excised and their potentials determined.

Osmotic Shock. Plants were grown in a growth chamber in aerated nutrient solutions containing either 1 mM or 5 mM NO_3^-. Conditions were as described earlier (11). When the fifth leaf of the low-N plants was fully expanded, plants were subjected to sudden osmotic stress by adding NaCl to the nutrient solutions (final concentrations 0, 50, 100, and 150 mM). Diffusive resistance (both surfaces considered in parallel) and Ψ of the fifth leaf were measured after establishment of a new steady-state, or about 30 min after initiation of stress. All procedures were carried out about 8 h after the beginning of the 14-h light period.

RESULTS

Stomatal Sensitivity to Osmotic Shock. Plants were grown in the growth chamber in nutrient solutions containing high N or low N, and diffusive resistances were measured after addition of NaCl to the solution. Salt caused considerably greater increases in resistance in low-N plants than in high-N plants (Fig. 1, lower
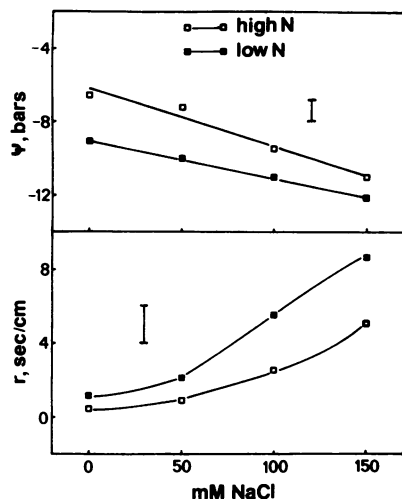
FIG. 1. Diffusive resistances (lower half) and Ψ (upper half) of leaves of low-N and high-N plants stressed by addition of NaCl to the nutrient solutions. Vertical bars indicate LSD$_{0.05}$.

half). As reported for greenhouse-grown plants (10), these low-N plants maintained a lower Ψ than high-N plants. Because of the greater restriction of water loss in salinized low-N plants than in salinized high-N plants, this difference in Ψ decreased with increasing concentrations of salt (Fig. 1, upper half).

Diffusive Resistance during Drying. High-N and low-N plants were allowed to deplete their water supplies, and diffusive resistances were followed during drying (Fig. 2). In high-N plants measured at $T_a = 35$ C, stomatal closure began at about -10 bars and proceeded gradually to -20 bars, at which point resistance greatly increased with further decreases in Ψ. There was no apparent change from this pattern at the cool temperature ($T_a = 27$ C). When plants were subjected to the hot temperature ($T_a = 40$–42 C), however, stomatal sensitivity was largely lost and resistance remained low to potentials as low as -30 bars. Because the wilting point of these plants was above -20 bars (10), turgor was lost long before stomatal closure was complete.

Diffusive resistance behaved entirely differently in low-N plants (Fig. 2). At 27 C, stomatal closure was complete at -10 bars, considerably above the wilting point (10). The Ψ for stomatal closure was decreased to -15 bars at 35 C and to about -26 bars at $\geq$40 C. At all temperatures tested, stomata of low-N plants were more sensitive to water stress than stomata of high-N plants. In both nutritional treatments, stomatal response to water stress was largely lost at the highest temperature except at very severe stress.

Growth during Drying. The daily increment in length of terminal leaves was followed during drying in the cool and the warm regimes. Growth of high-N plants virtually ceased when the minimum daily Ψ (early afternoon) reached -23 bars (Fig. 3). This threshold was unaffected by temperature regime, although the maximum growth rate at high potentials was clearly dependent upon temperature. Predawn recovery potentials for both temperature treatments were about -8 bars when growth ceased. With the low-N plants, growth ceased when the minimum daily Ψ declined to about -14 bars in the cool regime and -17 bars in the warm regime (Fig. 3). Again, predawn recovery potentials were not noticeably different (-4 to -5 bars). It is apparent that Ψ, temperature, and N nutrition exerted an interaction on leaf elongation similar to the three-way interaction on diffusive resistance.

Survival Value of Early Stomatal Closure. Figure 4 shows the effects of 4 days of drought on plants grown under normal greenhouse conditions, but in sand culture (the sand providing a rapid depletion of available moisture). The low-N and high-N plants gained 0.65 ± 0.18 and 1.07 ± 0.32 g dry weight, respectively, with total water loss (evapotranspiration) of 450 ± 7 and 417 ± 28 g/plant, respectively. Leaf water potentials for the low-

N and high-N plants were -19.4 ± 0.1 and <-40 bars, respectively, after 6 days of drought. The high-N plants wilted beyond recovery in spite of using slightly less water than the low-N plants. On the other hand, the low-N plants did not wilt. Presumably, the stomatal closure at relatively high Ψ in low-N plants prevented excessively rapid depletion of water, allowing a better balance between uptake and loss.

DISCUSSION

These data show that stomatal and whole-plant responses to water stress differ between high-N and low-N plants. Both diffusive resistance and growth were much more sensitive to decreasing Ψ in N-deficient plants than in high-N plants. At a cool T_a (27 C), the sensitivity of low-N plants was so great that stomata closed at a Ψ well above the point of zero turgor. This effect of N deficiency on stomatal behavior resembled that reported by Shimshi (15) in
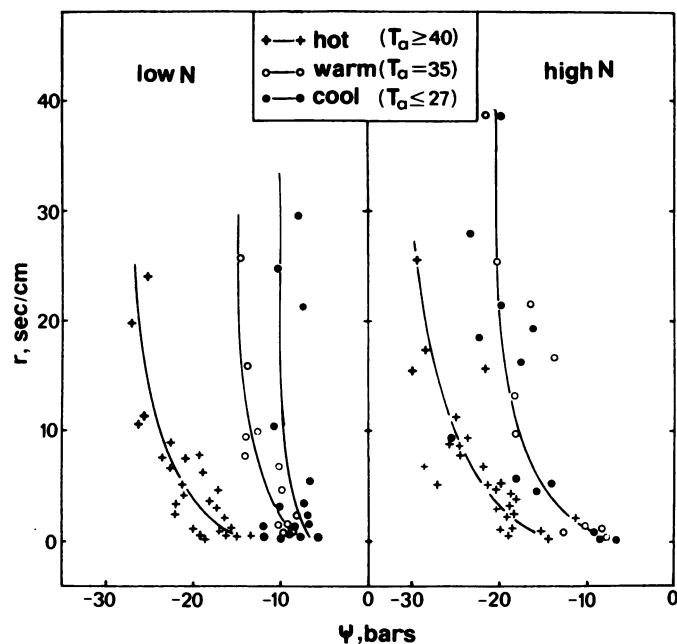

FIG. 2. Relationship between diffusive resistance and leaf Ψ during drying of greenhouse-grown low-N and high-N plants. Plants were all grown in a warm regime (maximum air temperature 35 C) until watering was discontinued.
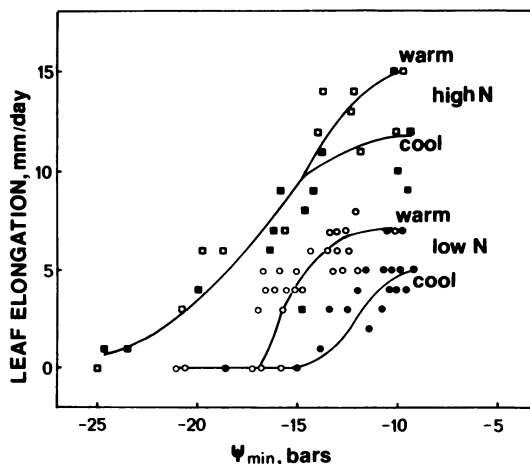

FIG. 3. Growth rate of terminal leaves of low-N and high-N plants as a function of minimum daily leaf Ψ (in early afternoon). Growth conditions as in Figure 2. (O, □): Warm temperature regime; (●, ■): cool temperature regime.

FIG. 4. Low-N (left) and high-N (right) plants greenhouse-grown in sand culture and allowed to dry for 4 days. See text for details. Bar indicates 20 cm.

bean plants, although he did not measure Ψ. At T_a = 40 to 42 C, stomatal sensitivity to tissue ψ was largely lost, and transpiration continued well past the wilting point. Although Ψ, temperature (12), and N deficiency (9, 13, 15) are known to influence diffusive resistance, to our knowledge this is the first report of the interactions among these three parameters on stomatal behavior.

The interaction of Ψ and N nutrition on leaf growth, although similar to that exerted on diffusive resistance, is not likely to be mediated by stomatal control of photosynthesis, because leaf growth is more sensitive than photosynthesis to water stress (6). Because N deficiency apparently alters cell wall structure (10), the interaction may be based on an altered minimum turgor requirement for growth. Causes of the temperature $-$ Ψ interaction on growth remain obscure.

In field-grown or otherwise drought-conditioned cotton, diffusive resistance is largely independent of Ψ down to about -30 bars (1, 7). The present results show that high temperature and (to a lesser extent) high N can simulate this type of stomatal behavior. Because the plants did not undergo any drought-conditioning cycles, apparently adaptation *per se* was unnecessary for the response to high temperature. Drying occurred over several days (high-N, high temperature) to several weeks (low-N, cool temperature), though, and the possibility of adaptive (or deadaptive) changes during this interval cannot be excluded. Because of the profound differences in rate of drying and degree of stress achieved, the effects of drought-conditioning cycles on low-N and high-N plants could not be compared without hopeless confounding.

The behavior of low-N plants at cool temperatures is remarkable. Plants in such a regime remained turgid up to 6 weeks after last watering, because transpiration was greatly reduced below a Ψ of -10 bars. In Levitt's (8) terminology, such plants are "water-savers." This water-saving behavior, by slowing water depletion

and prolonging the period of turgor maintenance and growth, actually resulted in slightly greater water use during a 4-day test in sand culture (Fig. 4).

The hypothesis upon which these experiments were based was that N-deficient plants, because of their xeromorphism, would respond to water stress as if they were drought-conditioned. This response did not occur. Instead, N deficiency considerably increased stomatal sensitivity to stress. Although the xeromorphic structure (and the slight osmotic adjustment) under N deficiency may have produced a degree of stress tolerance in the leaf mesophyll, stomata of low-N plants displayed stress avoidance rather than tolerance. This behavior is quite different from the apparent stomatal stress tolerance typically seen after drought conditioning (3, 16). In the latter case, it seems likely that apparent tolerance results simply from turgor maintenance due to other adaptive changes.

Our results make clear that stomatal sensitivity to stress is an independent characteristic which can be environmentally controlled, and which can be separated from osmotic adjustment or other means of turgor regulation. The independence of mesophyll and stomatal responses to stress can be rationalized by the demonstration of multiple feedback loops for stomatal control (5). Presumably, the stress sensitization by N deficiency is mediated by a loop other than the water loop.

Note Added in Proof. Ishihara *et al.* (1978 Jap J Crop Sci 47: 664–673) clearly showed that nitrogen fertilization increased stomatal aperture in field-grown rice.

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