

# Effects of Plant Water Status on Stomatal Activity, Photosynthesis, and Nitrate Reductase Activity of Field Grown Cotton<sup>1</sup>

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## ABSTRACT

The effects of varying degrees of water stress on stomatal activity, photosynthesis, and nitrate reductase activity were examined in field grown cotton (*Gossypium hirsutum* L., cv 'Dunn 56C'). A relationship between plant water status and activity of each measured physiological parameter was established.

Slight increases in leaf diffusive resistance were observed as leaf water potentials decreased although complete stomatal closure due to water stress was not generally observed. In many cases, visibly wilted leaves with zero turgor potentials exhibited minimal diffusive resistances. Morning and afternoon values of leaf diffusive resistance were distinctly different even though no correlation between leaf water potential and diffusive resistance was evident.

Water stress substantially reduced photosynthesis in both vegetative and reproductive leaves of cotton. Photosynthetic rates of each leaf type responded differently to declining leaf water potentials. The data suggest that the photosynthetic reduction could not be attributed to stomatal closure.

The activity of nitrate reductase was adversely affected by declining leaf water potentials. The nocturnal activity of nitrate reductase (respiratory linked) was also reduced by severe water stress. However, the reduction from maximum daily activity to minimum night time activity was similar in both stressed and non-stressed plants. These data suggest that inhibition of nitrate reductase activity could be due to long term water stress effects rather than temporal changes in plant water status.

The data presented indicate that stomata of field grown cotton are relatively insensitive to water stress, at least within the range of leaf water potentials observed in this study. Measurement of stomatal activity may not be a good criterion for assessing plant water status of cotton. The measurement of one or more physiological processes may prove a better index of plant water status as well as providing sensitive selection criteria for breeding more drought tolerant varieties.

**Additional index words:** Nitrate concentration, Nocturnal NRA.

**S**TUDIES on the effects of water stress on various physiological processes in cotton (*Gossypium hirsutum* L.) appear to be somewhat limited. Since stomatal activity, photosynthesis, and nitrate reductase activity are considered to be important in regulating yield, information regarding the effects of water stress on these processes would be beneficial.

Simultaneous reductions in photosynthesis and stomatal conductance with increased leaf water deficits have been reported for cotton (4, 9, 11, 27). Recent evidence (4) suggest that photosynthesis and transpiration of cotton were substantially reduced by induced water stress, and leaf diffusive resistances increased rapidly when leaf water potentials decreased to approximately -14 bars. Jordan and Ritchie (13) found that the stomata of field grown cotton failed to close at -27 bars leaf water potential, whereas, stomata of greenhouse grown plants closed at -16 bars water potential. Boyer (5) failed to observe increase diffusive resistance in cotton leaves when water stress was induced by salinity, but did observe an apparent reduction in photosynthesis.

Water stress is apparently responsible for reduced activity of nitrate reductase in sorghum (*Sorghum bicolor* L. Moench); (26), corn (*Zea mays* L.); (3, 18, 19), and barley (*Hordeum vulgare* L.) (12). Morilla et al. (19) suggested that inhibition of nitrate reductase activity by water stress may be due in part to reduced enzyme synthesis. Regardless of whether enzyme activity or synthesis is reduced by water stress, the inhibitory effects of drought on nitrate reductase need further investigation in view of the evidence suggesting close correlation between yield and nitrate reductase activity (7, 8). Although nitrate reductase activity has been partially characterized in cotton (23), the effects of water stress on nitrate reductase activity of cotton are unknown.

The scarcity of information regarding stomatal activity, photosynthesis, and nitrate reductase activity of field grown cotton prompted the investigations reported in this paper. Comparative data relating these physiological processes to water stress may prove beneficial toward understanding the drought tolerance and productivity of plants adapted to arid or semi-arid conditions.

## MATERIALS AND METHODS

Information regarding plant material, experimental designs, and methodology for leaf water, osmotic and turgor potential determinations are described in a preceding paper (1). Abaxial and adaxial stomatal resistances were determined with a Lambda LI-60 diffusive resistance meter and a LI-15S or LI-20S sensor calibrated according to the methods of Kanemasu et al. (15). Leaf diffusive resistances were subsequently calculated by assuming that individual surface resistance act in parallel with respect to total leaf resistance. Stomatal closure was considered to occur when diffusive resistance values exceeded 30 sec cm<sup>-1</sup>, which corresponded to values obtained during the night. Light intensities were measured using a Lambda LI-70 Quantum Sensor and are reported as photosynthetically active radiation (PAR). Measurements were made perpendicular to the leaf surface.

Photosynthetic rates were determined using the <sup>14</sup>CO<sub>2</sub> labeling technique described by Naylor and Teare (20). Nitrate reductase activity (NRA) was assayed by the in vivo technique described by Klepper et al. (16). Nitrate concentrations of the leaf tissue were determined utilizing a specific ion electrode.

Values for photosynthesis, NRA, and leaf diffusive resistances were obtained from the uppermost fully expanded vegetative leaves and leaves subtending flowers or 20- to 25-day old bolls

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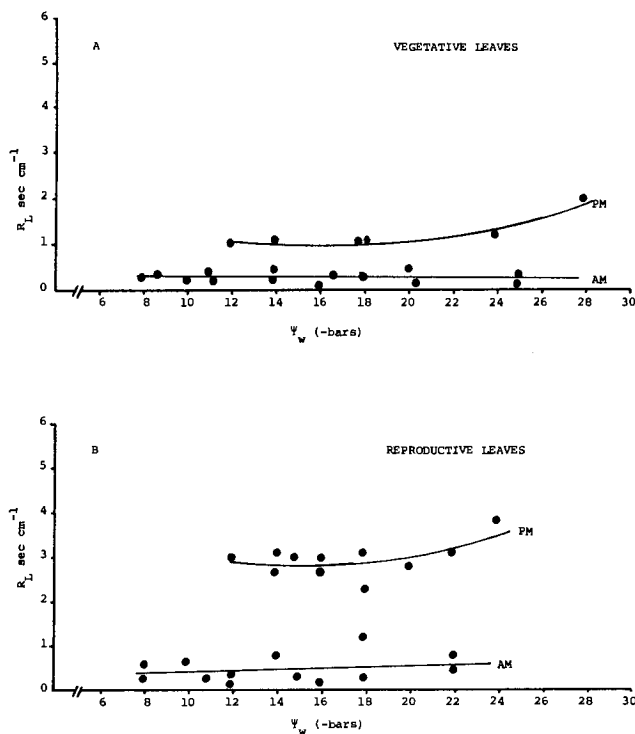


Fig. 1. Relationship between leaf diffusive resistance ( $R_L$ ) and leaf water potential ( $\Psi_w$ ) of vegetative (A) and reproductive (B) cotton leaves. Data for vegetative and reproductive leaves are at equivalent radiation (PAR) levels.

depending on the stage of growth. All values represent the mean of three to four samples of different leaves for each data point.

## RESULTS AND DISCUSSION

Leaf diffusive resistances of field grown cotton appear to be virtually independent of leaf water potential. Morning (prior to 1300, CDT) resistances of vegetative leaves remain relatively constant over a broad range of leaf water potentials (Fig. 1A). Afternoon (after 1400, CDT) values were greater than morning values irrespective of leaf water potential, although a slight increase in afternoon resistances was observed when leaf water potentials decreased to  $-28$  bars. A similar response was observed with respect to reproductive leaves, although diffusive resistances were greater in the reproductive leaves as compared with vegetative leaves particularly in the afternoon (Fig. 1B). Recent evidence suggests that leaf age is a strong determinant influencing stomatal response of cotton plants grown in growth chambers (14). Since the reproductive leaves and vegetative leaves are of different chronological and presumably physiological age, the present data support this observation, but only with respect to afternoon leaf diffusive resistances. Leaf age did not appear to influence resistances observed in the morning. The data do support previous evidence indicating that stomata of field grown cotton do not close at leaf water potentials approaching  $-27$  bars (13). Jordan and Ritchie (13) suggested that stomata of field grown cotton do not close in order to maintain water flux through the

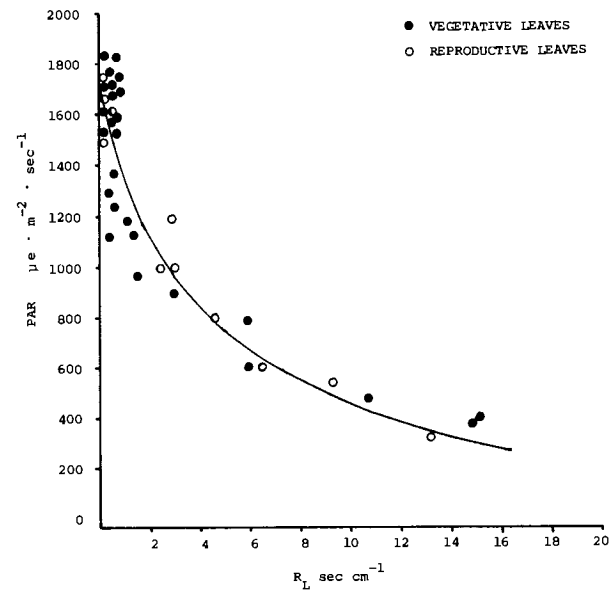


Fig. 2. Relationship between photosynthetically active radiation (PAR) and leaf diffusive resistance ( $R_L$ ) of vegetative and reproductive cotton leaves.

plant. Data reported here, indicating that stomata of vegetative leaves are insensitive to leaf water potential, would support this hypothesis. Since stomata of reproductive or lower canopy leaves generally exhibit considerably greater diffusive resistances in the afternoon than upper canopy leaves, stomata of upper leaves may remain open in order to sustain water flux through the plant as water potentials decline during the course of the day.

Vegetative and reproductive leaves exhibited a similar pattern with respect to stomatal response to radiation (Fig. 2). Thus, the major factor controlling stomatal activity of field grown cotton appears to be light rather than leaf water potentials.

The differences between morning and afternoon diffusive resistances of vegetative leaves is particularly interesting. Recent data (10, 17) have emphasized the relationship between stomatal activity and vapor pressure gradients. Thus, the differences in leaf diffusive resistances observed in this study could be due to changes in relative humidity during the day as has been observed in cotton grown in growth chambers (22). The role of vapor pressure gradients in controlling stomatal activity needs further clarification, particularly in field grown plants.

Decreased leaf water potentials adversely affected photosynthesis in vegetative leaves of cotton within the range of potentials observed in this study (Fig. 3A). Maximum photosynthetic rates ( $35$  to  $40$   $\text{mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ ) were attained when leaf water potentials were  $-12$  to  $-14$  bars. As leaf water potentials decreased to  $-20$  to  $-28$  bars, a corresponding decline in photosynthetic rates was observed, reaching a minimum value of  $5$  to  $6$   $\text{mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ . Since this reduction could not be attributed to stomatal closure, some other aspect of the photosynthetic process was presumably affected. Additionally, photosynthetic rates did not recover from water stress

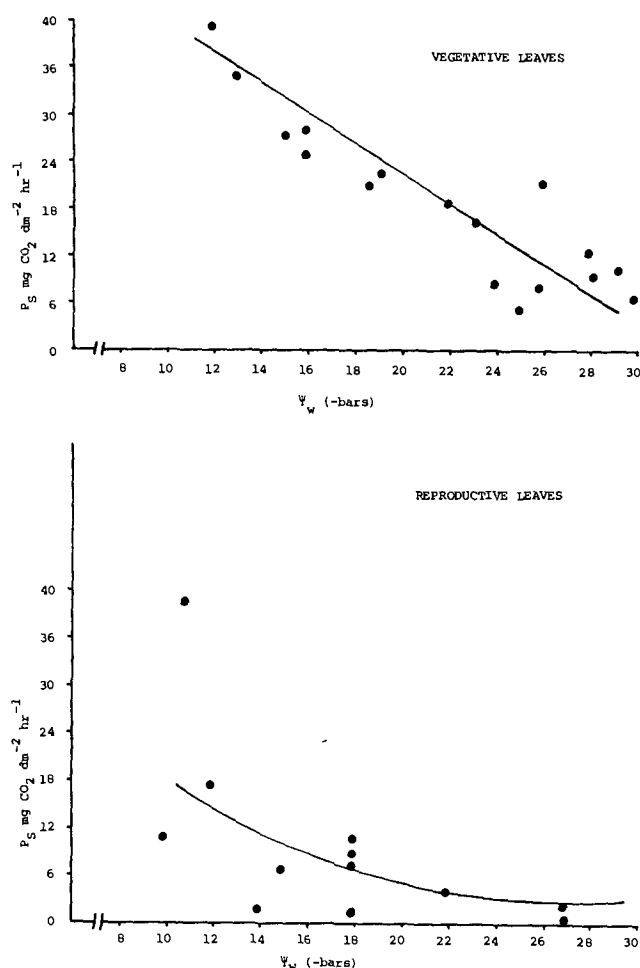


Fig. 3. Relationship between photosynthesis ( $P_s$ ) and leaf water potential ( $\Psi_w$ ) of vegetative (A) and reproductive (B) cotton leaves. Data for vegetative and reproductive leaves are at equivalent radiation (PAR) levels.

Table 1. Nitrate reductase activity and nitrate concentration of non-stressed vegetative (V) and reproductive (R) cotton leaves. Data were obtained at approximately 1100 (CDT).

Leaf type	30 July			4 Aug.		
	$\Psi_w$ †	NRA‡	$\text{NO}_3^- \cdot \text{N}$ §	$\Psi_w$	NRA	$\text{NO}_3^- \cdot \text{N}$
V	$-14.0 \pm 0.8$	$6.89 \pm 0.15$	155	$-16.0 \pm 0.7$	$6.45 \pm 1.50$	105
R	$-11.0 \pm 1.1$	$7.83 \pm 1.75$	235	$-16.0 \pm 0.8$	$6.68 \pm 2.00$	87

†  $\Psi_w$  = bars.  
 ‡ NRA =  $\mu\text{m NO}_2^-$  produced  $\cdot \text{g fresh wt}^{-1} \cdot \text{hr}^{-1}$ .  
 §  $\text{NO}_3^- \cdot \text{N}$  concentration =  $\mu\text{g NO}_3^- \cdot \text{N} \cdot \text{g fresh wt}^{-1}$  of leaf blade.

(data not shown), thereby supporting recent evidence indicating that photosynthesis in cotton is unable to fully recover following stress (4). Water stress is known to decrease Hill reaction activity (6, 21, 25) and translocation (28, 29). These factors could be responsible for inhibiting photosynthesis of cotton in this study.

A satisfactory relation between photosynthesis and leaf water potential of reproductive leaves was difficult to establish (Fig. 3B). The confounding influence of light intensity would be a major factor in controlling photosynthesis of leaves in the lower

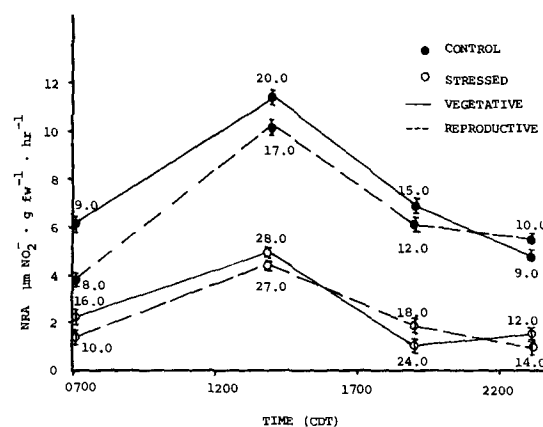


Fig. 4. Diurnal changes (20 to 21 August) in nitrate reductase activity of cotton leaves. Vertical bars represent  $\pm$  one standard deviation. Numbers adjacent to data points indicate leaf water potentials in bars. The standard deviation for leaf water potentials was consistently less than 1 bar.

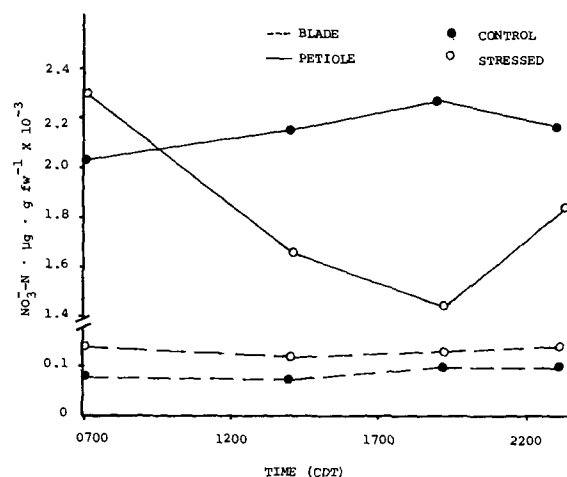


Fig. 5. Diurnal changes (20 to 21 August) in nitrate-nitrogen concentration of petioles and blades of vegetative cotton leaves. Leaf water potentials are the same as in Fig. 4 at the equivalent data points.

canopy. Thus, photosynthesis in reproductive leaves would be expected to be considerably lower than vegetative leaves irrespective of leaf water potential as was observed. Even when light intensities were equivalent to those received by the vegetative leaves ( $> 1,750 \mu\text{e} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$ ), photosynthetic rates of reproductive leaves were considerably less at any specific leaf water potential.

Nitrate reductase activity (NRA) and leaf blade nitrate concentrations of non-stressed cotton leaves on two dates are reported in Table 1. The rates of  $\text{NO}_3^-$  reduction compare very favorably with those reported by Radin (23) for greenhouse grown plants. On 20 August, coinciding with severe water stress, NRA was evaluated on a diurnal basis (Fig. 4). Nitrate reductase activity decreased in the stressed plants, while activity in nonstressed plants was similar to that observed on 30 July and 4 August when compared at the same time of day (1100 CDT).

Leaf water potentials of the stressed plants were 8 to 9 bars lower at midday compared with the non-stressed plants. This substantial difference could account for the reduced NRA. The apparent implication might be that threshold value of leaf water potential must be reached prior to inhibition of enzyme activity. Since NRA of cotton seems to be affected by water stress, it would prove beneficial to ascertain the approximate level of water potential required to inhibit activity. Previous data indicate that NRA is substantially reduced when leaf water potential approaches —15 bars in sorghum (26). Inhibition of activity was observed when leaf water potentials were anywhere between —2 and —12 bars in barley (12). Nitrate reductase activity continues during the night and the degree of activity is apparently dependent on the degree of plant water stress (Fig. 4). Night-time activity, apparently coupled to respiration (2, 24) is substantially less in the water stressed plants. However, the percent reduction from maximum to minimum daily activity is approximately equivalent in both treatments. This suggests that reduced enzyme activity may be the result of long term stress effects rather than temporal changes in plant water status or changes due to respiration.

Nitrate-N concentrations in the leaves were also affected by water stress but not to the extent of NRA (Fig. 5). Although petiole nitrate-N concentrations were similar in plants of both treatments, blade concentrations were higher in non-stressed plants during the course of the day, except at 0700 when no apparent differences existed between treatments. Thereafter, nitrate-N levels declined in the stressed plants while remaining relatively constant in the control plants. The data suggest a possible effect of water stress on nitrate uptake or perhaps, compartmentalization.

The data presented here provide evidence of water stressed induced metabolic disorders in cotton. There is little evidence, if any, to suggest that water stress induced stomatal closure in field grown cotton. It should be noted that in many cases, very low diffusive resistances were obtained on leaves that were visibly wilted. Therefore, measurement of leaf diffusive resistances may not be a reliable estimate of plant water stress and related physiological activity.

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