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**Communication**

# Responses of Transpiration and Hydraulic Conductance to Root Temperature in Nitrogen- and Phosphorus-Deficient Cotton Seedlings

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## ABSTRACT

**Suboptimal N or P availability and cool temperatures all decrease apparent hydraulic conductance (*L*) of cotton (*Gossypium hirsutum* L.) roots. The interaction between nutrient status and root temperature was tested in seedlings grown in nutrient solutions. The depression of *L* (calculated as the ratio of transpiration rate to absolute value of leaf water potential [$\Psi_w$]) by nutrient stress depended strongly on root temperature, and was minimized at high temperatures. In fully nourished plants, *L* was high at all temperatures ≥20°C, but it decreased greatly as root temperature approached the chilling threshold of 15°C. Decreasing temperature lowered $\Psi_w$ first, followed by transpiration rate. In N- or P-deficient plants, *L* approached the value for fully nourished plants at root temperatures ≥30°C, but it decreased almost linearly with temperature as roots were cooled. Nutrient effects on *L* were mediated only by differences in transpiration, and $\Psi_w$ was unaffected. The responses of $\Psi_w$ and transpiration to root cooling and nutrient stress imply that if a messenger is transmitted from cooled roots to stomata, the messenger is effective only in nutrient-stressed plants.**

One of the earliest symptoms of growth limitation by N or P supply is a specific inhibition of leaf expansion (12, 13). In cotton and some other species, N or P deficiency decreases root *L*[1] (9, 12, 13, 18). Radin and coworkers (12, 13) presented evidence that the decreased *L* can limit the delivery of water to the growing leaves and thus curtail cell expansion. Both N and P stress decrease the *L* of individual cells in the root cortex (17), implying that the shift in root properties results from changes in properties of cell membranes.

Root *L* is sensitive also to chilling temperatures in plants of tropical or subtropical origin such as cotton (4, 6). Experiments reported here were designed to test for interactions of nutrient status and root temperature on *L*.

## MATERIALS AND METHODS

Cotton seeds (*Gossypium hirsutum* L. cv Deltapine 70) were germinated in moist vermiculite and transferred to continuously aerated nutrient solutions after 3 d as described earlier (13). Seedlings were grown in groups of four with their roots suspended in 16-L containers of solution. The composition of the modified half-strength Hoagland solution is reported elsewhere (16). The complete solution contained 5 mM $NO_3^-$ and 0.5 mM $H_2PO_4^-$; in N- and P-deficient solutions $Cl^-$ was substituted for 96% of the N or 100% of the P. After transfer to nutrient solutions, plants were grown in a growth chamber with a 14-h daylength, day/night temperatures of 30/21°C, and PPFD of 450 to 500 $\mu$mol $m^{-2}$ $s^{-1}$ at plant height (determined with a LiCor LI-190 quantum sensor; LiCor Instruments, Lincoln, NE, USA).[2] Humidity was uncontrolled, but the daytime RH remained at 45 ± 5%. Nutrient depletion and pH changes were very small at this early stage of growth, and the solutions were not renewed.

One week after seedlings were transferred to nutrient solutions, the seedlings and their containers were placed in a second growth chamber maintained at 25°C, with PPFD of 450 $\mu$mol $m^{-2}$ $s^{-1}$. The cotyledons were fully unfolded and mostly expanded at this stage of growth, but the first true leaf was still very small. In the second chamber each container with nutrient solution was immersed in one of five water baths that were chilled or heated to different constant temperatures. In each experiment, the lowest solution temperature was between 7 and 13°C, and the highest temperature was ≥30°C. Data from six successive experiments were combined, and measurements obtained at the same root temperatures in different experiments were treated as replicates. As a test of consistency, all experiments included a root temperature of 25°C. Plants were transferred to the second growth chamber approximately 2 h after the beginning of the light period, and transpiration rates of the cotyledons were determined 4 h later, when root temperatures had equilibrated with the baths and fluxes had stabilized at new steady-state values. The LiCor LI-1600 steady-state porometer (set to maintain ambient RH) was used to obtain transpiration rates from each surface, and the values were added. Contributions of other tissues to whole-plant transpiration were ignored. Measurements were made on all seedlings, which were then

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[1] Abbreviations and symbols: *L*, apparent hydraulic conductance; $\Psi_w$, water potential.

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that might also be suitable.

individually tagged for identification. After determination of transpiration, each seedling shoot was enclosed in a plastic bag and severed at the hypocotyl, and $\Psi_w$ was immediately determined with a pressure chamber (13). The values of $\Psi_w$ thus obtained closely corresponded to values from excised cotyledons. Areas of cotyledons of each seedling were determined with a LiCor LI-3100 leaf area meter, and root systems were individually oven-dried and weighed.

The transpiration rate was converted to flux per unit root length using leaf area: root length ratios of 4.7, 4.1, and 3.8 $cm^2/m$ for fully nourished and N- and P-stressed seedlings, respectively. These age- and nutrient-specific ratios were determined as described earlier (13). Hydraulic conductance was then approximated as the ratio of transpiration rate to the absolute value of $\Psi_w$. Relationships of transpiration, $\Psi_w$, and $L$ to root temperature were approximated by cubic polynomial regressions. Correlation coefficients ranged from 0.83 to 0.98.

## RESULTS AND DISCUSSION

In the fully nourished controls, the transpiration rate was high and little affected by root temperatures from 13 to 35°C (Fig. 1A). Cooling the roots below 13°C, however, caused a sharp decline in transpiration. The leaf $\Psi_w$ began to decrease as root temperature fell below about 20°C (Fig. 1B), and at the coolest temperatures the cotyledons were wilted. This response is typical of chilling-sensitive plants in general and of cotton in particular (6). At 13°C, the leaf $\Psi_w$ was near −1.0 MPa, which corresponded approximately to the value for zero turgor in the cotyledons (not shown; data obtained by pressure-volume procedures).

Nutrient stress markedly altered the effect of root temperature on transpiration. In both N- and P-stressed plants, the transpiration rate approached that of the fully nourished seedlings at the warmest root temperatures tested, but it decreased almost linearly with decreasing temperature down to about 10°C (Fig. 1A). At chilling temperatures (<12°C), nutrient stress had little or no effect upon transpiration rate. These data show that the expression of nutrient effects on transcription was strongly temperature-dependent (and vice versa). Notably, nutrient stress did not affect leaf $\Psi_w$ at any root temperature (Fig. 1B). Thus, N or P deficiency greatly altered the relationship between transpiration rate and $\Psi_w$. The increased stomatal responsiveness to declining leaf $\Psi_w$ is similar to earlier demonstrations that nutrient deficiency enhances stomatal responses to water stress (10, 11).

The $L$ of intact plants was estimated as the ratio of transpiration rate to absolute value of $\Psi_w$. Transpiration is expressed here as volume flux per unit root length, as the root provides the major resistance to water flow, especially in chilled seedlings (1, 5, 6). In fully-nourished plants, $L$ was high at root temperatures ≥20°C, but it decreased sharply in a narrow temperature range centered on about 15°C (Fig. 1C). In N- and P-stressed plants, $L$ approached that of the fully nourished controls at the highest temperatures tested. However, $L$ of nutrient-stressed plants declined almost linearly with decreasing temperature, again approaching the control value at temperatures <12°C (Fig. 1C). Nutrient stress substantially decreased $L$ at intermediate root temperatures (15–25°C). This intermediate range includes the temperature at which all previous experiments were carried out (12–14, 17).

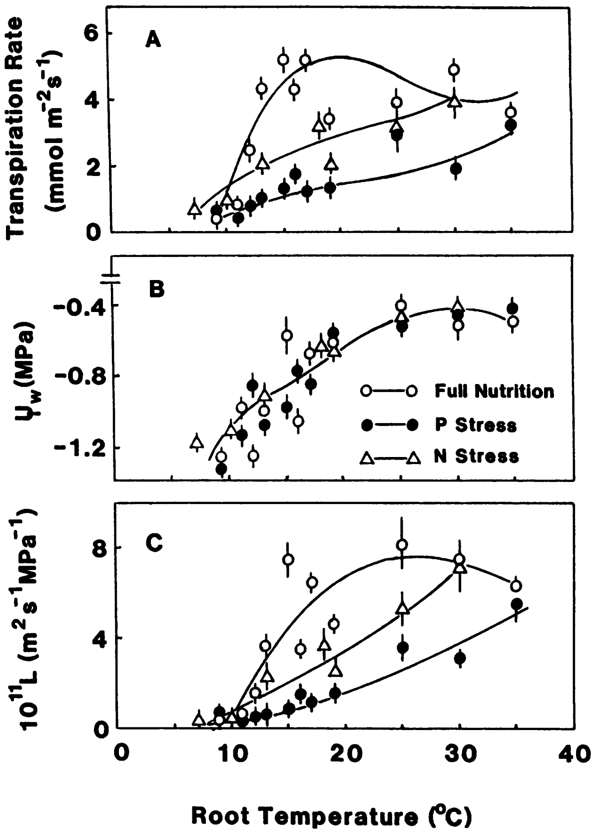

**Figure 1.** Transpiration rates, water potentials, and hydraulic conductances of cotton seedlings. Roots of fully nourished, N-deficient, or P-deficient plants were maintained at various temperatures in a growth chamber with a constant air temperature of 25°C. Hydraulic conductance was calculated as the ratio of transpirational flux per unit root length to the absolute value of $\Psi_w$. All values shown as means ± SE. Regressions were calculated separately for each nutrient treatment except in the case of $\Psi_w$, for which all data were combined.

The values of $L$ reported here are about half those previously reported for cotton seedlings (13). Most of this discrepancy arises from the implicit assumption in the approximation of $L$ that flux is linearly related to $\Delta P$. In the previous study, $L$ was determined as the slope of the flux-$\Delta P$ relationship specifically at high fluxes, where it considerably exceeds the slope at low fluxes (13).

The simplest interpretation of these results is that nutrient stress affects radial water flow rather than axial flow. The axial resistance depends upon physical properties of the xylem (20), and thus should be nearly independent of temperature. At 30 to 35°C, though, the effect of nutrients was almost completely abolished (Fig. 1C). This evidence is inconsistent with large effects of nutrients on axial resistance (17). However, it does not prove that axial resistance is negligible. Some evidence implies that axial resistance may be important in cotton roots, especially in the segment of the tip distal to fully mature xylem (8).

Cooling roots of bean (*Phaseolus vulgaris* L.) decreased $L$, transpiration, and growth rate, but did not affect $\Psi_w$ (7).

Smith and Dale (19) argued that this syndrome resulted from temperature-dependent production of ABA by roots and its transfer to the leaves. In cotton, cooling roots does decrease $\Psi_w$, but the nutrient stresses do not (Fig. 1B). Thus, root cooling *per se* does not elicit behavior that implies a messenger role for ABA or any other compound. If ABA is a messenger from the roots, it is effective only in nutrient-stressed plants. Earlier studies (10, 16) showed that N or P deficiency increases stomatal responsiveness to ABA, perhaps through changes in cytokinins. Nutrient stress can decrease by 90% the ABA concentration causing incipient closure, from 0.1 $\mu$M to as low as 0.01 $\mu$M (16). This decrease may allow ABA from the roots to affect transpiration, as the lower concentration is well within the range typically observed in the xylem sap (2, 3). Although cool temperature enhances ABA accumulation in cotton leaves (15), and may also enhance its accumulation and release from cotton roots, poor stomatal responsiveness to ABA in fully nourished plants would preclude large effects on transpiration. It seems likely that in cotton, root cooling (below 30°C) and nutrient stress are both required for stomatal closure because the former may affect the level of ABA and the latter may affect responsiveness to ABA. This hypothesis transfers emphasis from the ABA concentration in the xylem sap to whatever factor controls stomatal responsiveness to root-derived ABA.

Finally, one should note that the interaction of temperature with nutrient status to control $L$ is specific to the root. Shoot temperatures from 19 to 38°C had no effect on $L$ of N- and P-stressed plants when root temperature was constant (14). Thus, root and shoot temperatures must be considered separate variables exerting independent effects on the plants. In most studies of plant water relations, root temperature is uncontrolled and may not even be measured. Especially in free-standing pots, diurnal temperature fluctuations and gradients can be substantial. Consistent results in studies of plant water relations may require more attention to the complex effects of these nonconstant or nonuniform root temperatures.

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