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Effect of O_3 on Hydraulic Architecture in Pima Cotton[1]

Biomass Allocation and Water Transport Capacity of Roots and Shoots

David A. Grantz* and Shudong Yang

Department of Botany and Plant Sciences, and Statewide Air Pollution Research Center, University of California, Riverside, Kearney Agricultural Center, 9240 South Riverbend Avenue, Parlier, California 93648

Pima cotton (*Gossypium barbadense* L. cv S-6) exhibits foliar injury and yield reduction at ambient concentrations of O_3. We tested the hypotheses that O_3 reduces the allocation of biomass to the root system, and that this disrupted carbohydrate allocation impairs root hydraulic capacity relative to transpiring leaf area. Both hypotheses are supported, even though leaf area development is itself reduced by O_3. Seedlings were grown in pots in greenhouse fumigation chambers and exposed from planting to sinusoidal O_3 profiles with peak concentrations of 0, 0.1, 0.2, and 0.3 $\mu L\ L^{-1}$ (12-h averages of 0, 0.037, 0.074, and 0.111 $\mu L\ L^{-1}$). At 8 weeks after planting, stem basal diameter, leaf area, and total plant dry weight decreased by 61, 83, and 88%, whereas root/shoot dry weight ratio declined from 0.16 to 0.09 g/g. Hydraulic conductance decreased per plant by 85%, and per unit leaf area by 35%. Conductance of all organs declined per plant, but only root conductance declined per leaf area by 41%. Root resistance increased from 69 to 82% of whole plant resistance, a functional consequence of reduced carbon allocation to roots. Stomatal conductance declined with root hydraulic conductance, protecting short-term leaf water status. Reduced root hydraulic efficiency may mediate O_3 injury to whole plants by reducing shoot gas exchange and biomass productivity through the inhibition of water and nutrient acquisition.

Plants growing in polluted air basins are exposed to anthropogenic O_3 at concentrations that suppress agricultural yields (Heck et al., 1982; Lefohn et al., 1988), damage native vegetation (Skelly et al., 1983; Materna, 1984), and lead to changes in plant growth and structure (Heggestad et al., 1988; Miller, 1988; Heggestad and Lee, 1990; Temple et al., 1993). The mechanism by which tropospheric O_3 causes these deleterious effects on vegetation is poorly understood. Visual symptoms of O_3 damage typically appear on the leaves and are associated with the suppression of photosynthesis (Koziol and Whatley, 1984; Reich and Amundson, 1984; Heath, 1988) and reduction of Rubisco activity (Pell and Pearson, 1983). Exposure to O_3 typically reduces total biomass accumulation, and typically to a greater extent in roots than in shoots (e.g. Tingey et al., 1971; Miller, 1988; Kostka-Rick et al., 1993). Of 20 diverse plant species surveyed by Cooley and Manning (1987), 17 exhibited a decline in root-to-shoot dry weight ratio. This altered plant morphology, with associated disruptions of integrated plant function, could represent the principal effect of O_3 exposure on whole plants (McLaughlin et al., 1982).

Reduced root growth in response to O_3 could reduce soil exploration and root hydraulic conductance and might confer increased sensitivity to soil water or nutrient deficits in plants exposed to O_3. Reduced root hydraulic conductance could reduce shoot water status, increase the tension on the xylem water column, and potentially increase drought susceptibility (Heggestad et al., 1985), particularly in plants growing on stored soil moisture or in variable field environments. Reduced hydraulic conductance relative to transpiring leaf area could enhance cavitation in the xylem vessels and induce systemic vascular failure due to embolism (Tyree and Sperry, 1988). Desiccation and accelerated abscission of leaves, a commonly observed response to O_3 exposure, could then follow.

Little is known about the effect of O_3 on the hydraulic conductance of plants. Lee et al. (1990) demonstrated an increase in root hydraulic conductance per unit root dry weight in red spruce (*Picea rubens* Sarg.) seedlings exposed to O_3, but did not present conductance per unit of transpiring leaf area. This latter parameter expresses the functional balance between the water transport capacity of the root system and the water demand of the shoot. Information on the effects of O_3 on the hydraulic conductance of roots, stems, petioles, and leaves, separately and integrated as whole plants, is required to fully understand root-shoot interactions and whole plant responses during exposure to O_3, particularly in environments with a high evaporative

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* Corresponding author; e-mail david@uckac.edu; fax 1–209–891–2593.

Abbreviations: CSTR, continuously stirred tank reactor; F_T, sap flow rate through an intact plant (kg s^{-1}); F_O, F_{SH}, F_P, F_S, solution flow rate through an excised shoot, excised shoot with leaf margins removed, with leaves removed, and with petioles removed (kg s^{-1}); g_s, stomatal conductance (cm s^{-1}); K_L, K_P, K_S, K_{SH}, K_R, K_T, total hydraulic conductance (plant basis, kg s^{-1} MPa^{-1}) of all leaves, all petioles, all stems, shoot, root, and whole plant; K^*_L, K^*_P, K^*_S, K^*_{SH}, K^*_R, K^*_T, hydraulic conductance (leaf area basis, kg s^{-1} MPa^{-1} m^{-2}), same subscripts; *P*, hydrostatic pressure (MPa); Ψ_S, Ψ_L, water potential of soil and leaf, respectively (MPa); r, root radius; R_L, R_P, R_S, R_{SH}, R_R, R_T, hydraulic resistance (plant basis, MPa s kg^{-1}), same subscripts.

demand. Hydraulic conductance may also serve as a surrogate for other root functions—nutrient acquisition, phytohormone production—that may provide integrating mechanisms for O_3 effects on whole plant growth and development.

Pima cotton (*Gossypium barbadense* L. cv S-6) was selected for agronomic traits under conditions of low O_3 concentrations, and exhibits substantial sensitivity to ambient O_3 at concentrations currently prevailing in commercial production areas (Grantz and McCool, 1992). Under typical conditions in these areas with a high evaporative demand and mineral nutrient limitations, root proliferation and hydraulic conductance may be positively associated with agronomic and biological productivity. Tropospheric O_3 represents a substantial limitation to the productivity of cultivated and native plants in these arid regions.

In the present study we investigate the effect of exposure to realistic concentrations of O_3 on biomass allocation among roots and above-ground organs, and on potential functional consequences for root and shoot hydraulic conductance and resulting whole plant hydraulic architecture. We analyze dry weights and hydraulic properties of leaves, petioles, stems, and roots, and we express the hydraulic properties on per plant and per unit leaf area bases. The studies are performed with vegetative plants, since reproductive structures are strong sinks for current and stored photoassimilate and for mineral nutrients, which further depresses the root-to-shoot dry weight ratio (Cooley and Manning, 1987).

MATERIALS AND METHODS

Seeds of Pima cotton (*Gossypium barbadense* L. cv S-6) were planted in 3.7-L pots containing a mixture of sand: peat moss:bark shavings (2:1:1, v/v). At approximately 10 d after emergence plants were thinned to one seedling per pot. Pots were irrigated with tap water daily and fertilized with one-half-strength Hoagland solution (0.3 L per pot) weekly. Plants were used for measurements at about 8 weeks after planting, when they were 0.3 to 0.6 m tall, depending on O_3 exposure.

Pots were irrigated on the afternoon before and in the early morning of the day the plants were to be used for measurements. All measurements were performed between 10 AM and 2 PM (Pacific Standard Time) on sunny, spring days.

Growth Conditions

Plants were grown in greenhouse O_3 exposure chambers (CSTR) as described by Heck et al. (1978) and located in a greenhouse that was ventilated with charcoal-filtered air. Each CSTR was 1.8 m high × 1.5 m in diameter, was constructed with Teflon walls, and had a continuously rotating mixing paddle at the top to enhance air circulation. The greenhouse was located at the Statewide Air Pollution Research Center (University of California, Riverside). Day/night air temperatures were 25 to 30/17 to 22°C, RH was 25/60%, and PPFD was approximately 80% of full sun.

Plants were exposed to O_3 from the time of planting. Natural diurnal courses of O_3 exposure were approximated by initiating the generation of O_3 at 9 AM and ending at 4 PM. O_3 concentration was increased sinusoidally from zero to the maximum for each chamber at 12:30 PM, and then decreased sinusoidally. The maximal values of these half-sine wave exposures were 0 (occasionally up to 0.01), 0.1, 0.2, and 0.3 $\mu L\ L^{-1}$. This resulted in 12-h average exposures (7 AM to 7 PM) of 0, 0.037, 0.074, and 0.111 $\mu L\ L^{-1}$, respectively.

O_3 was generated from O_2 with an O_3 generator (model GEC-1A, Griffin Technic, Lodi, NJ) and delivered to the CSTRs through a computerized system of mass flow controllers (model 5850, Brooks Instrument Division, Emerson Electric, Hatfield, PA). Air was sampled near the center of each CSTR and the O_3 concentration was analyzed with an UV absorption O_3 monitor (model 1003 AH, Dasibi Environmental, Glendale, CA). The measured O_3 concentration was used as feedback to the computerized distribution system.

Experimental Design

Eight CSTRs were used for O_3 exposures, with single O_3 concentrations assigned to pairs of chambers at random. Plants were treated as experimental units in a completely random design (Steel and Torrie, 1960).

Mean separations using the protected LSD (Steel and Torrie, 1960) and regression analyses were performed using SAS (Cary, NC) software. Symbols in figures represent mean data ± SE, with significant differences ($P < 0.05$) within a single O_3 exposure level indicated by different lowercase letters associated with pairs of points.

All phases of the experiment have been repeated several times in various seasons of different years, with highly reproducible results. The present communication relates data pertaining to plants grown in spring 1994: dry weight and biomass ratios ($n = 12$ plants), whole plant hydraulic conductance (transpiration method; $n = 4$), root hydraulic conductance ($n = 4$), and shoot hydraulic conductance ($n = 3$). Variability in hydraulic measurements was relatively low for plants grown under the same conditions. Hydraulic measurements on shoot components are reported only for cases in which all of the shoot organs were carried through all phases of the experiment without damage. A typical and not infrequent failure mode involved the breakage of a petiole or branch stem, preventing further hydraulic measurements. Shoot conductance did not dominate plant conductance under any conditions.

Measurement of Hydraulic Conductance

Transpirational Method for Whole Plants

Prior to each measurement of K_T, the pot and soil surface were enclosed in a plastic bag that was covered with aluminum foil and sealed at the base of the stem. The pot was placed on an electronic balance (PM 4000, Mettler, Hightstown, NJ; capacity 4000 g, precision 1×10^{-2} g) located outside of the greenhouse and sheltered from the wind.

PPFD, air temperature, and RH were consistently 1600 to 2100 μmol m^{-2} s^{-1}, 27 to 33°C, and 26 to 33%, respectively.

The balance was interfaced to a microcomputer so that water loss (transpiration, F_T) was calculated automatically every 3 s, and averaged every 150 s. When F_T was constant (±10% for 30 min) it was accepted as a valid estimate of F_T (Eq. 1).

Based on the conventional Ohm's law analogy, whole plant hydraulic conductance (K_T, kg s^{-1} MPa^{-1}) was calculated using intact plants as

$$K_T = F_T / (\Psi_s - \Psi_L), \quad (1)$$

where F_T is the rate of water flow through the whole plant from the root system to transpiring leaves and then lost to the atmosphere (kg s^{-1}), and Ψ_S and Ψ_L are the water potentials (MPa) prevailing in the soil near the roots and in the transpiring leaves during the measurement, respectively.

Following each whole plant measurement all leaves were excised from the plant into aluminum foil-covered ziplock bags, which were immediately sealed. Water potentials of at least four representative upper, exposed leaves per plant were determined as xylem pressure potentials with a pressure chamber (precision 0.01 MPa) within a few minutes of excision and the average was taken as Ψ_L.

After determination of F_T and Ψ_L, the pot containing the leafless plant remained on the balance and was shaded. Water potential was allowed to reach equilibrium between the shoot and soil for about 5 h. Stem water loss was always near zero during this period, suggesting that the previously measured F_T represented only transpirational water flux. The stem was then excised and its water potential determined using the pressure chamber. Stem water potential determined in this fashion was always near 0 MPa, the expected value for recently irrigated soil, and was taken as Ψ_S.

Hydrostatic Pressure-Induced Flow Method for Root Systems

Pots with intact plants were removed from the greenhouse to the laboratory and the shoots were excised approximately 0.1 m above the soil surface early in the morning. The pot with undisturbed soil and root system was sealed inside of a laboratory-designed pressure vessel (0.3 m high and 0.3 m in diameter) with the cut stem protruding through a gasket in the top of the vessel, similar to the method of Passioura (1988). Pressure was applied using compressed air. The cut stem was connected by clamped tubing to a syringe (5 × 10^{-3} L). Root exudation increased the level of solution in the syringe. A pipette was positioned with one end immersed in the syringe and the other end in a plastic receiving bottle (0.25 L) resting on an electronic balance (model AE200, Mettler; capacity 200 g, precision 1 × 10^{-4} g). Liquid exuding from the cut stem was siphoned through the tubing to the receiving bottle on the balance.

Root hydraulic conductance (K_R, kg s^{-1} MPa^{-1}) was determined as

$$K_R = \Delta F / \Delta P, \quad (2)$$

where ΔF is the difference between the two rates of sap flow through the root system and ΔP is the difference between the two pressures (approximately 0.3 and 0.8 MPa) applied to the roots that induced the flows. Sap flow (F) was calculated automatically by the microcomputer, as above, and accepted when constant (±10% for 30 min).

Hydrostatic Pressure-Induced Flow Method for Shoot Components

Pots with intact plants were removed from the greenhouse to the laboratory and immersed in tap water to approximately 0.15 m above the soil surface. The shoot was excised at the soil surface under water, recut with a new razor blade, and attached to tubing containing a deionized, degassed, and filtered (0.1 μm) aqueous solution of oxalic acid (10 mol m^{-3}; added to inhibit microbial growth). The shoot was then completely immersed in water. The tubing was connected to a plastic reservoir of solution (0.25 L) placed on an electronic balance (model AE200, Mettler; capacity 200 g, precision 1 × 10^{-4} g). The reservoir level on the balance was 2.1 m above the water level immersing the shoot, yielding P = 0.02 MPa. Initial flow rate into the shoot (F_0) was often relatively high, reflecting tissue water deficits. Rehydration was generally complete after approximately 2 h as F_0 (kg s^{-1}) decreased to near zero and became constant. When F_0 became constant, the margins of all leaf laminae were excised with sharp scissors. The solution entering the shoot at the cut stem now exuded through the open xylem vessels at the leaf margins and the rate of flow (F_{SH}, kg s^{-1}) increased substantially.

When F_{SH} became constant (±10% for 30 min), the leaf laminae were excised distal to the petioles, and the rate of exudation through the cut petioles (F_P, kg s^{-1}) was determined. When F_P became constant and was recorded, the petioles were excised and exudation directly from the stems (F_S, kg s^{-1}) was determined.

Component hydraulic conductances (kg s^{-1} MPa^{-1}) were converted to the resistances of stems, petioles, and leaves (R_S, R_P, and R_L; MPa s^{-1} kg^{-1}) and assumed to be in series. Whole shoot resistance (R_{SH}) was expressed as

$$R_{SH} = R_L + R_P + R_S. \quad (3)$$

The resistance of each shoot component, i.e. leaves, petioles, and stems, was successively determined by difference. R_{SH} (per plant basis) was determined directly as

$$R_{SH} = P / (F_{SH} - F_0) \quad (4)$$

and of the stem directly as

$$R_S = P / (F_S - F_0) \quad (5)$$

and of the petiole by difference as

$$R_P = P / (F_P - F_0) - R_S = P / (F_P - F_0) - P / (F_S - F_0) \quad (6)$$

and of the leaves by difference as

$$R_L = P/(F_{SH} - F_0) - R_S - R_P = P/(F_{SH} - F_0) - P/(F_P - F_0). \quad (7)$$

The hydraulic conductances of the leaves, petioles, stems, shoot, and whole plant (K_L, K_P, K_S, K_{SH}, and K_T, kg s^{-1} MPa^{-1}) on a per plant basis were then determined as the reciprocals of R_L, R_P, R_S, R_{SH}, and R_T. Conductances were also expressed on a per unit leaf area basis (K^*_L, K^*_P, K^*_S, K^*_{SH}, K^*R, and K^*_T, kg s^{-1} MPa^{-1} m^{-2}) by normalizing K per individual plant by its leaf area, including laminae, excised margins, and petioles, determined with a leaf area meter (model LI 3100, Li-Cor, Lincoln, NE).

g_s

g_s was measured on abaxial leaf surfaces of the youngest, fully expanded leaf of 18 plants, grown near the same time as those that were used for whole plant hydraulic measurements with the transpiration method. Measurements were obtained in full sun, sheltered from the wind, using a steady-state porometer (model LI-1600, Li-Cor).

Measurement of Dry Weight and Leaf Area

Plants used for growth measurements were separated into leaves (including the petioles), stems, and roots. Leaf area was determined on pooled leaves from individual plants. Dry weights of plant components were determined using an electronic balance (model 200, Mettler; capacity 200 g, precision 1×10^{-4} g) after drying at 75°C to constant weight (about 1 week).

Stem basal diameter was measured at 5 cm above the soil using electronic digital calipers (model 500, Mitutoyo, Tokyo). The average of two measurements per stem, taken perpendicular to each other, was calculated to be the stem diameter.

RESULTS

Growth and Biomass Allocation

Whole plant size, including leaf area (Fig. 1A), stem diameter (Fig. 1B), and biomass of all plant components (Fig. 1C) decreased with increasing exposure to O_3 during seedling development. Leaf area was reduced by 83% (Fig. 1A), stem diameter was reduced by 61% (Fig. 1B), and whole plant dry weight was reduced by 88% (Fig. 1C) when exposed to 0.111 $\mu L\ L^{-1}$ O_3. The reductions in biomass were nearly proportional among all plant components (Fig. 1C), with root system biomass reduced to very low levels.

The relative allocation of whole plant dry weight to leaves increased only about 5% with increasing O_3 exposure (Fig. 2A), whereas allocation to stems increased by about 11%. The relative allocation of biomass to roots, however, decreased by 40%, from 13% of plant dry weight in charcoal-filtered air to only about 8% at 0.111 $\mu L\ L^{-1}$ O_3.

This large change in proportional biomass in roots exerted substantial effects on the relationship between the root system and the whole plant. Dry weight ratios of root

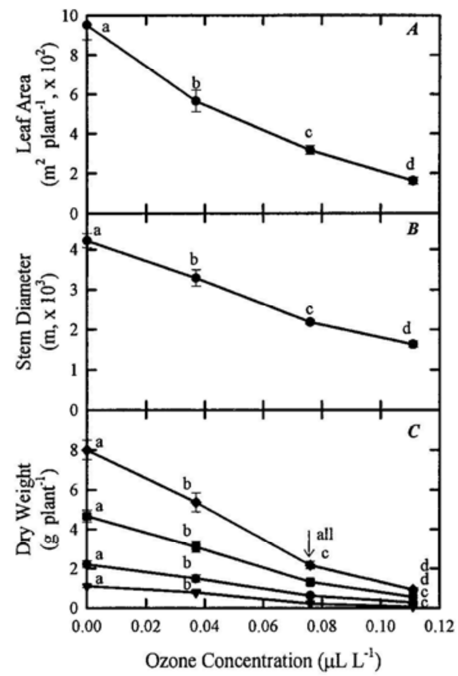

Figure 1. Relationship between leaf area (A), stem basal diameter (B), and dry weight components (C), and 12-h mean O_3 exposure. Data points (mean ± SE; n = 12 plants per point) associated with different letters within a line are different at $P < 0.05$. ●, Stems; ■, leaves; ▼, roots; and ♦, whole plant.

to shoot (Fig. 2B, ●) and root to leaf (Fig. 2B, ■) declined substantially with increasing O_3 concentration above approximately 0.037 $\mu L\ L^{-1}$. The ratio of root biomass to transpiring leaf area (not shown) also decreased substantially. Moderate exposure to O_3 (0.037 $\mu L\ L^{-1}$) slightly increased (+7%) the relative biomass allocation to the root system (Fig. 2B). A similar increase in plant growth at this O_3 concentration has been observed in some experiments. A further increase in O_3 concentration always severely reduced below-ground allocation and whole plant biomass accumulation.

Hydraulic Conductance

The hydraulic conductances on a per plant basis of petioles (K_P; Fig. 3A, ▲), leaves (K_L; Fig. 3A, ■), stems (K_S; Fig. 3A, ●), and roots (K_R; Fig. 3C, ▽) were reduced by 66, 90, 92, and 94%, respectively, by exposure to 0.111 $\mu L\ L^{-1}$ O_3. Whole plant conductance was reduced by about 85%.

Conductance did not decline as sharply on a per unit leaf area basis as it did on a per plant basis, since transpiring leaf area was also reduced by exposure to O_3. Stem conductance (K^*_S; Fig. 3B, ●) declined by 13%, whereas leaf conductance (K^*_L; Fig. 3B, ■) was essentially unchanged and petiole conductance (K^*_P; Fig. 3B, ▲) increased by about 270%. Only the hydraulic conductance of roots was reduced substantially on a per unit leaf area basis by 41% (K^*_R; Fig. 3C, ▼). Whole plant conductance per unit leaf

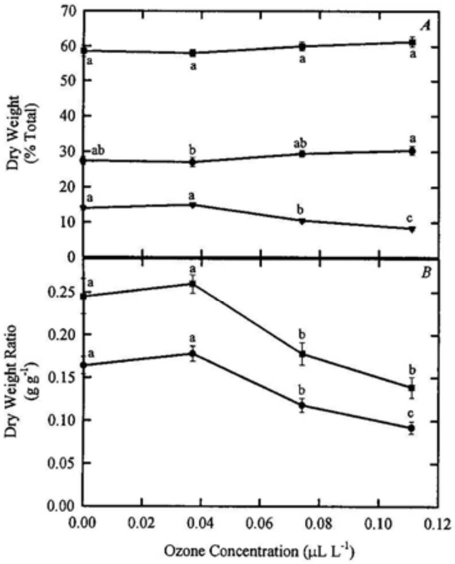

Figure 2. Relationship between dry weight allocation (A) and root-to-leaf (■) and root-to-shoot (●) dry weight ratios (B), and 12-h mean O_3 exposure. Symbols in (A) are as in Figure 1; n = 12.

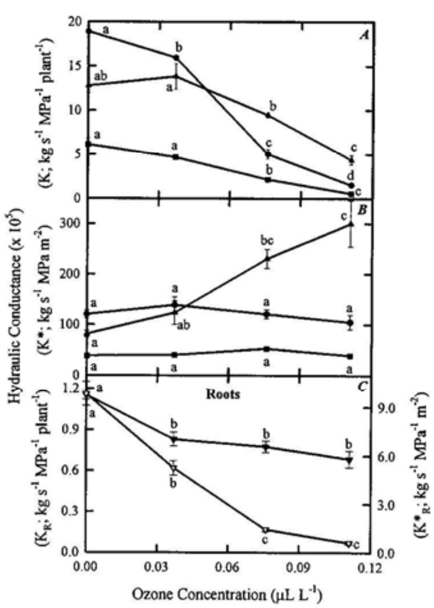

Figure 3. Relationship between hydraulic conductances of leaves, stems, and petioles (n = 3) on whole plant (A) and unit leaf area (B) basis, and of roots (C) (n = 4), and 12-h mean O_3 exposure. Symbols are as in Figure 1. ▲, Petioles. In C, ▼, Leaf area basis; ▽, plant basis.

area was reduced by about 35%, an effect clearly dominated by O_3 effects on the root system, since total conductance of the shoot increased by about 20% (Fig. 3B).

There was no evidence of an increase in whole plant conductance per unit leaf area (Fig. 4, ●) at 0.037 $\mu L\ L^{-1}$ O_3, despite the increase in root-to-shoot and root-to-leaf dry weight ratios (cf. Fig. 2B) at this concentration.

The whole plant hydraulic conductances (K^*_T) that were determined with intact plants using the transpiration method (Fig. 4, ●) were confirmed (Fig. 4, ■) with the values calculated from the component hydraulic resistances that were determined independently on excised roots (K^*_R), stems (K^*_S), petioles (K^*_P), and leaves (K^*_L). The two methods of determining whole plant conductance gave similar results, with little difference observed in the largest plants (exposed to charcoal-filtered air) and somewhat greater variability in the smallest plants (exposed to 0.111 $\mu L\ L^{-1}$ O_3), which were more fragile and difficult to manipulate. All values from both methods were within the 95% confidence intervals of the same regression relationship (Fig. 4).

The O_3-induced decline in root conductance on both per plant and per leaf area bases is particularly important. The roots represent the limiting conductance to liquid water transport in these cotton plants, even under control conditions without exposure to O_3. Hydraulic resistance (1/conductance) of the root system accounted for 69% of the whole plant resistance in charcoal-filtered air (not shown, but see Fig. 3). The shoot accounted for less than one-third of the resistance, with stem, petioles, and leaves accounting for about 6, 9, and 17%, respectively, of the whole plant resistance. With increasing O_3 concentration from 0 to 0.111 $\mu L\ L^{-1}$, the percentage of whole plant resistance increased to 82% in roots, but declined to 2% in petioles and 13% in leaves; the relative contribution of the stem was unchanged. The major effect of O_3 on the hydraulic architec-

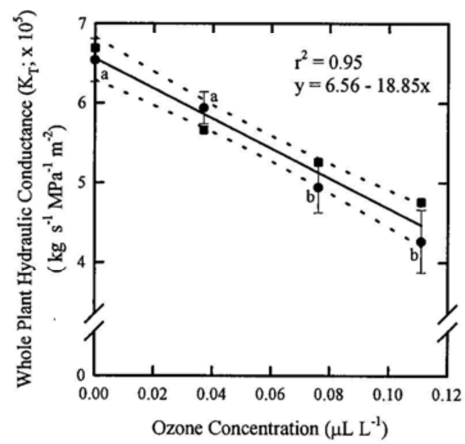

Figure 4. Whole plant hydraulic conductance on a leaf area basis measured directly on intact plants using the transpiration method (●; n = 4) and calculated from average conductances of roots, stems, petioles, and leaves (■), as a function of 12-h mean O_3 exposure. Solid line represents the least-squares regression of form $Y(\times 10^5) = a + bX$; n = 8. Dashed lines represent the 95% confidence intervals.

Hydraulic Conductance

The reduction of root mass available to support the transpiring leaf area displayed by the O_3-treated plants suggested a substantial shift in the balance of hydraulic supply and demand. In cotton most root biomass is in the tap root, whereas most water and nutrient uptake occurs in root tips and hairs. Allocation to roots provided an indirect indication that O_3 may impair plant hydraulic efficiency. To obtain a more direct and quantitative measure of this effect, we determined the hydraulic conductance of the intact plants and of their component organs.

Although O_3-induced effects on acquisition and allocation of biomass are well established, few studies have considered their consequences for whole plant or root hydraulic properties (e.g. Lee et al., 1990). In the present study we demonstrate that chronic exposure to O_3 induced substantial reductions in whole plant and root hydraulic conductance on a per plant basis (K_T and K_R). The relationships between K_T and plant size, represented by root biomass or stem diameter ($r^2 = 0.97$ and 0.96, respectively), are consistent with relationships between shoot hydraulic properties, also on a per plant basis, and the basal diameter of maple trees (*Acer saccharum* L. and *Acer rubrum* L.; Yang and Tyree, 1993, 1994). Similar positive relationships on a conductance per plant basis are generally observed from a variety of plant systems (e.g. red oak [*Quercus rubra* L.; Ren and Sucoff, 1995], bean [*Phaseolus vulgaris* L.; Fiscus and Markhart, 1979], Norway spruce [*Picea abies* L.; Rudinger et al., 1994], and sugarcane [*Saccharum* spp. L.; Meinzer and Grantz, 1990]).

The hydraulic impacts of O_3 in Pima cotton could reflect the same allometric relationships and associated vascular development that have been observed in other systems. The O_3 effect, however, appears to differ in significant ways from these size-associated differences. Hydraulic conductance of whole plants or roots per unit root dry weight (not shown, but see Figs. 1C and 3A) increased with O_3 exposure in Pima cotton. Similar results have been reported from oxidant studies with red spruce (Lee et al., 1990). This apparently reflects a greater O_3-induced reduction of tap root biomass (a function of r^2) than of absorbing fibrous root surface area (a linear function of r), despite substantial effects on fibrous root development (e.g. in beet; Ogata and Maas, 1973).

A significant result of the present study, distinct from other systems, is the decline in whole plant or root hydraulic conductance on a leaf area basis (K^*_T and K^*_R) with plant size, following chronic exposure to O_3. In Pima cotton K^*_T was as closely and positively related to plant size ($r^2 > 0.99$) as was K_T. To our knowledge, this positive association of K^*_T with plant size has not been previously reported. In the maple, bean, spruce, and oak systems (see refs. cited above) hydraulic properties expressed on a leaf area basis were independent of, or negatively related to, plant size. This reflects a typical developmental balance between leaf area and hydraulic efficiency, which tends to maintain a relatively constant shoot water status as leaves, petioles, and stems become longer and roots become thicker and more suberized with age, reducing their conductance per unit leaf area (e.g. Fiscus and Markhart, 1979). Variability in plant size in these other studies was obtained by sampling plants of different ages, whereas in the present study variability was induced by oxidant injury.

Hydraulic conductance on a unit leaf area basis represents a functional normalization that relates transpiring leaf area to the capacity of the hydraulic supply system (Yang and Tyree, 1993). O_3 exposure induced an unusual reduction in development of root hydraulic capacity that exceeded the accompanying reduction in development of leaf area. An important impact of exposure of plants to O_3 is to increase the already dominant role of the root system in limiting the transport of liquid water through plants to transpiring leaves. Under these conditions only the integration of root and shoot function, resulting in concomitant stomatal closure, could prevent shoot water deficits.

Implications for Plant Response to the Environment

The reduction of hydraulic conductance on a leaf area basis (K^*_T) suggests that O_3 could enhance the susceptibility of plants to water deficits by degrading shoot water status during periods of high evaporative demand; in some studies this prediction has been realized. O_3 increased the impact of water deficit in soybeans (*Glycine max* L.; Heggestad et al., 1985) and red spruce (*Picea rubens* L.; Roberts and Cannon, 1992). Water deficit caused a greater loss of biomass productivity and a lower (more negative) Ψ_L in the presence of O_3 than under clean-air conditions. However, the reverse situation has also been observed, sometimes in the same species. In the present study Ψ_L of O_3-exposed seedlings of Pima cotton was similar to or greater than Ψ_L of O_3-free controls. Similar results have been reported for upland cotton (Temple, 1986, 1990a), alfalfa (*Medicago sativa* L.; Temple et al., 1988a), and red spruce (Lee et al., 1990). Shoot water status in the absence of edaphic drought is a function of both hydraulic conductance and transpiration rate.

O_3 causes substantial reductions in stomatal conductance in general (Mansfield, 1973), and in Pima (Grantz and McCool, 1992; present study) and upland (Temple, 1986) cottons, specifically. In the present study g_s of Pima cotton was reduced by 41%, whereas K^*_T decreased by only about 35% over a range of O_3 concentrations from 0 to 0.111 μL L^{-1}. Transpiration may be reduced somewhat less than g_s (Jarvis and McNaughton, 1986; Meinzer and Grantz, 1989, 1990). The integrated plant response to O_3 in the present case led to an increase in Ψ_L of 13%. In field exposure chambers g_s and K^*_T of Pima cotton (cv S-6) were both reduced over a range of O_3 concentrations, resulting in nearly constant Ψ_L (D.A. Grantz and P.M. McCool, unpublished observations). Also in field chambers, g_s of upland cotton was similarly reduced by about 40% (Temple, 1986, 1990a, 1990b; Temple et al., 1988b), and Ψ_L increased in most cases. Improved shoot water status, despite the reduction of hydraulic supply efficiency, reflects an apparent integration of root and shoot functions at the level of the whole plant.

In variable field environments, this conservation of shoot water status may not prevail. Reduction of g_s by O_3 reverses more quickly than similar reductions of K^*_T when O_3 is removed, e.g. by changing weather patterns. In the plants used in the present study, g_s recovered substantially within 24 h after removal of O_3 (0.111 μL L^{-1}; not shown). The contrasting response times of vapor-phase and liquid-phase conductances may explain the variability often observed in leaf water relations of plants exposed to O_3. For example, orange trees exposed to high O_3 concentrations exhibited substantially lower Ψ_L than control leaves on some days, but similar water potentials on most days (Olszyk et al., 1991).

The effect of O_3 on drought resistance may depend on species differences in the balance of O_3 effects on K^*_T and g_s, as well as on locally prevailing soil moisture, evaporative demand, and ambient O_3 concentrations (see also Heagle et al., 1988). Further research on the factors mediating altered biomass allocation in plants exposed to tropospheric O_3 and on the consequences of these alterations for integrated root and shoot function may contribute substantially to the elucidation of the mechanism of O_3 effects on whole plants and plant communities.

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