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Ozone impacts on allometry and root hydraulic conductance are not mediated by source limitation nor developmental age

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Abstract

O_3 could reduce growth and carbohydrate allocation to roots by direct inhibition of photosynthesis and source strength. Alternatively, O_3 could reduce growth indirectly by inhibition of root hydraulic development through a primary lesion in carbohydrate translocation. Another alternative is that O_3 could slow the rate of plant development, only apparently altering carbohydrate allocation at a given plant age. Pima cotton (*Gossypium barbadense* L.) is used to address these possibilities, and four hypotheses were tested: (1) O_3 exposure reduces leaf pools of soluble sugars; (2) pruning leaf area and reducing source strength to match that of O_3-treated plants reproduces O_3-effects; (3) pruning lower leaf area more closely reproduces O_3 effects than pruning upper leaf area; and (4) manipulating plant age and thereby plant size to match O_3-treated plants reproduces O_3-effects. All were falsified. Soluble sugars did not decline. Pruning upper and lower leaves and manipulating plant age all reduced biomass and leaf area similarly to O_3-exposure, but neither reproduced O_3 effects on biomass allocation nor root function. It is concluded that O_3 induces an allometric shift in carbohydrate allocation that is not mediated by photosynthetic inhibition nor by alteration of developmental age. Effects of O_3 could be mediated by direct effects on phloem loading, with consequent inhibition of translocation to roots and root system development.

Key words: Carbon allocation, oxidant, air pollution, root:-shoot communication, translocation, ozone, cotton.

Introduction

Ozone (O_3) inhibits growth and induces shifts in the root biomass ratio (fraction of total biomass in root tissue, R) in cotton (Grantz and Yang, 1996; Olszyk *et al.*, 1993; Oshima *et al.*, 1979; Temple, 1990) and other species (Barnes *et al.*, 1998; Cooley and Manning, 1987; Laurence *et al.*, 1994; Reiling and Davison, 1992). The mechanism of this and other oxidant impacts on plants and ecosystems remains poorly characterized (Alscher *et al.*, 1997).

O_3 could alter plant development through direct limitation of source strength. Some evidence supports this concept. Visual symptoms of O_3 injury develop on photosynthetically active leaves. Stomatal conductance, activity of photosynthetic enzymes, rates of electron transport, and carbon assimilation per unit leaf area all decline with increasing exposure (Farage *et al.*, 1991; Pell *et al.*, 1994). O_3 further decreases carbon gain by reducing plant leaf area (L) through accelerated senescence, and by increasing respiratory demand for antioxidant and repair metabolism.

Other evidence is less supportive. Reduction of photosynthetic capacity through mechanisms not associated with O_3 does not consistently alter carbon allocation to roots (Stitt and Schulze, 1994). R was not altered by reducing PPFD (Laurer *et al.*, 1993), CO_2 concentration (Stitt, 1991), nor Rubisco activity using molecular techniques (Fichtner *et al.*, 1993), though all reduced carbon assimilation and source strength.

O_3 could retard plant growth and development, uncoupling chronological from developmental age. In this case, apparent changes in allocation following O_3-exposure could represent plant size-specific values of R, rather than changes in the allometric coefficient (Farrar and Gunn, 1996; Farrar and Williams, 1991).

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Data from Grantz and Yang (Grantz and Yang, 1996) and Oshima *et al.* (Oshima *et al.*, 1979) suggest that O_3 alters allocation in Pima and upland cottons, respectively. O_3 generally decreases R, though increases have been observed in various species (Reiling and Davison, 1992). O_3-induced alteration of the allometric coefficient has been documented in a few cases.

O_3 could directly inhibit carbohydrate translocation to developing roots. Inhibition of longitudinal transport would alter carbohydrate allocation much like other types of source limitation, as considered in the allocation model of Minchin *et al.* (Minchin *et al.*, 1993). Phloem transport has been suggested as a possible site of ozone action (Mortensen and Engvild, 1995).

Recent evidence in Pima cotton indicates that acute exposure to O_3 inhibits export of current assimilate from source leaves (Grantz and Farrar, 1999). O_3 also inhibited export of recent assimilate from the older leaves of aspen (*Populus* spp.) and bean seedlings (*Phaseolus vulgaris* L.) that provide carbohydrate to the roots (Coleman *et al.*, 1995; Ito *et al.*, 1985; McLaughlin and McConathy, 1983).

This study attempts to distinguish between the three potential mechanisms of O_3-phytotoxicity identified above, all operating at the level of the whole plant (Miller, 1988). O_3-inhibited photosynthetic carbon assimilation could induce a source limitation of carbohydrate translocation to roots (the *source strength* hypothesis). O_3-reduced plant size and retarded development could alter instantaneous values of R that resemble changes in biomass allocation (the *developmental age* hypothesis). O_3-disrupted translocation of recent assimilate from source leaves could alter allocation patterns with secondary consequences for root function and growth (the *translocation* hypothesis). In the latter case the primary O_3-induced lesion might be oxidation of a sensitive protein involved in phloem loading.

Whole plant techniques with Pima cotton were utilized to investigate whether manipulation of source strength or plant age reproduces O_3-altered biomass allocation and root function. A direct O_3 effect on translocation is not directly tested in this study, but emerges through a process of elimination as a likely hypothesis for further experimentation.

Materials and methods

Plant material

Pima cotton (*Gossypium barbadense* L.; cv. S-6) was sown in 12 l pots containing plaster sand:peat moss:bark shavings (2:1:1, by vol.; U.C. Mix No. 2 amended with 3.01 kg m^{-3} lime, 1.43 kg m^{-3} single superphosphate, 0.14 kg m^{-3} each of KNO_3 and K_2SO_4, and 0.06 kg m^{-3} micronutrients (Cu:Zn:Mn:Fe, 3:1:1.5:1.5, by vol.). Pots were randomly distributed among 10 greenhouse fumigation chambers (Continuously Stirred Tank Reactors, CSTRs) as described previously (Grantz and Yang, 1996) at the Air Pollution Research Center, University of California, Riverside. About 10 d after emergence, plants were thinned to a uniform population of one seedling per pot.

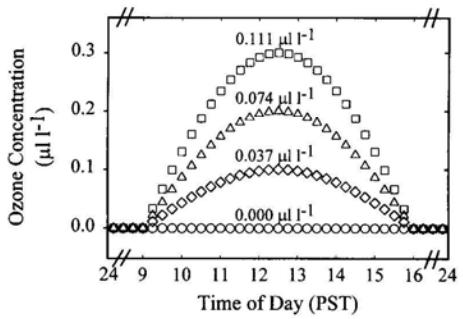

Fig. 1. Daily ozone exposure dynamics (Pacific Standard Time, PST) for the four treatments in the Continuously Stirred Tank Reactors (CSTRs). Values shown for each curve are 12 h means (07.00 h to 19.00 h).

Pots were irrigated to run through with H_2O on alternate days, and with half-strength Hoagland's solution weekly. Plants were grown under natural sunlight (80% through glass) during the summer, when greenhouse temperature ranged from 22–28/18–22 °C (day/night) and RH was 25/50%.

Ozone fumigation

Each CSTR was exposed to one of four O_3 treatments applied 7 d $week^{-1}$, 7 h d^{-1}. O_3 was produced from O_2 (GEC-1A, Griffin Technic Corp., Lodi, NJ, USA) as described previously (Grantz and Yang, 1996). Air within each CSTR was circulated with a fan and sampled sequentially from the centre of each chamber through teflon tubing to a multiport solenoid valve for assay with a single ozone monitor (1003AH, Dasibi Environmental Corp., Glendale, CA) interfaced to a microcomputer for feedback control of $[O_3]$.

The $[O_3]$ in each chamber was increased sinusoidally from 0.0 µl l^{-1} at 09.00 h to a maximum at 12.30 h, and then decreased to 0.0 µl l^{-1} at 16.00 h. Peak $[O_3]$ were 0.0 µl l^{-1}, 0.1 µl l^{-1}, 0.2 µl l^{-1}, and 0.3 µl l^{-1} (Fig. 1). Corresponding 12 h mean $[O_3]$ (07.00 h to 19.00 h) were 0.0 µl l^{-1}, 0.037 µl l^{-1}, 0.074 µl l^{-1}, and 0.111 µl l^{-1}, respectively. These protocols provided quasi-realistic exposure dynamics, and have yielded highly reproducible responses to O_3-exposure (Grantz and Yang, 1996). The experiment has been replicated a large number of times, with a single representative experiment ($n=4$ plants per treatment) presented here for growth and hydraulic measurements, and a separate experiment ($n=4$) presented for carbohydrate analysis to allow for required differences in drying of plant tissue. Plants were harvested 8 weeks after planting.

Source strength

Plants in six CSTRs were exposed to control $[O_3]$ of 0.0 µl l^{-1}. In three of these, leaf pruning was imposed. L was evaluated daily in a non-destructive manner (length × width) in these chambers and in the various O_3-treatments. Entire expanding leaves were excised at mid-petiole in the pruning treatment, whenever L exceeded that of the plants in the target O_3 treatment. Thus the pruned plants periodically attained somewhat greater L than the target plants, were pruned to somewhat smaller L, and then again exceeded L of the O_3-treated plants. On average, and at final harvest (8 weeks after planting), the

leaf areas were comparable. The experiment was repeated twice with representative data from a single experiment ($n=4$) presented here.

Developmental age

In three CSTRs exposed to the control [O_3] of 0.0 μl l^{-1}, plants were harvested at 3, 4, 5, and 6 weeks after planting. This provided a similar range of plant size as was observed over the range of O_3-exposures. The experiment was repeated twice with representative data from a single experiment ($n=4$) presented here.

Translocation

No direct manipulation of carbohydrate translocation was attempted in this study. A direct effect of O_3 on phloem loading or longitudinal transport of carbohydrate is suggested as an alternative to the source strength and developmental age hypotheses tested here. Direct tests of the translocation hypothesis are considered elsewhere (Grantz and Farrar, 1999), as are the indirect consequences of O_3-reduced root development on source strength and plant growth (Grantz *et al.*, 1999).

Physiological measurements

Root hydraulic conductance: Root hydraulic conductance (K_R; kg s^{-1} MPa^{-1}) on a per plant basis was determined in each experiment by the transpiration method (Yang and Grantz, 1996) according to the relationship

$$K_R = T_R/(\psi_{RS}-\psi_{BX}) \quad (1)$$

in which T_R is transpiration rate (kg s^{-1}) from the entire plant with soil evaporation excluded, and ψ_{RS} and ψ_{BX} are water potentials (MPa) at the root–soil interface and in the xylem at the base of the shoot, respectively. K_R reflects root hydraulic conductance, the limiting segment of the water transport pathway in the whole plant of Pima cotton (Grantz and Yang, 1996). The leaf area specific root hydraulic conductance was calculated as

$$K'_R = K_R/L \quad (2)$$

where L is attached leaf area of the whole plant at the time of the measurement.

Plant size and biomass allocation: L at harvest was measured with a leaf area meter (3100, LI-COR Inc., Lincoln, NE), and the basal diameter of the plant was determined with digital calipers.

For biomass determination soil was removed from the roots in running water. Fine and coarse roots, stems and leaves of three age classes (lamina plus petiole) were placed in separate paper bags, and dried at 75 °C in a drying oven to constant weight. Dry weights were determined with an electronic balance (PM200; Mettler, Inc.; capacity 200 g; precision 0.0001 g). Excised leaves from the pruned plants were discarded and not considered part of leaf area nor mass at harvest.

Carbohydrate analysis: For analysis of non-structural carbohydrates the tissues were separated and freeze-dried to constant weight (48 h). Dry weights were determined with the electronic balance (PM200, Mettler, Inc.). The dried tissue was ground (Wiley Mill; 40 mesh), and subsampled (0.10 g). Subsamples were extracted in 4.0 ml of 80% EtOH at 80 °C for 30 min, and centrifuged (5 min, 6000 rpm; Model 5403; Eppendorf, Hamburg, FRG). This was repeated four times.

For determination of soluble sugars, 2.0 ml of the pooled ethanolic supernatant was evaporated to dryness (SpeedVac Concentrator; Savant; Farmingdale, NY, USA) and resuspended in 1.0 ml H_2O. This was passed through 1 ml of anion exchange resin (Rexyn 300; formate form; Fisher Scientific Inc.) followed by 1 ml of cation exchange resin (AG50W-X8; protonated form; BIO-RAD, Hercules, CA) to remove contaminant ions, followed by 6 ml H_2O to elute fully the sugars. This solution was evaporated to driness (SpeedVac) and the residue dissolved in 150 μl H_2O, filtered (0.45 μm pore size) and separated by HPLC with a Sugar-Pak column (Waters; Milford, MA) and 156 Refractive Index Detector (Altex; Fullerton, CA) with a peak integrator (4290 Integrator; Varian; Sugarland, TX). Peaks were quantified against authentic standards (Sigma Diagnostics, Inc.; St Louis, MO, USA) by co-elution and summed for total soluble sugars. The major sugar was sucrose (about 50%) with stachyose and many others constituting the remainder.

For starch (Madore, 1990; Hendrix, 1993) the pellet was dried to constant weight (55 °C), resuspended in 2.0 ml of 2 N KOH and subjected to partial alkaline hydrolysis and tissue disintegration at 100 °C for 1 h. At room temperature the suspension was adjusted with 2.0 ml of 2 N CH_3COOH to pH 4.5. Starch was fully hydrolysed to glucose residues using amyloglucosidase (Fluka 10115; Rankonkoma, NY, USA) dissolved in 50 mM Na acetate buffer, pH 4.5. Glucose was assayed colorimetrically with hexose kinase (Sigma Diagnostics, Inc.; St Louis, MO, USA; Procedure 16-UV) in a microplate reader (3550-UV; Bio-Rad) at 340 nm. Authentic glucose served as the standard. Glucose determinations were back-calculated to starch concentrations as [0.9 × glucose] to reflect the addition of 1 H_2O $glucose^{-1}$ during hydrolysis. Carbohydrate contents are expressed as dry weight ratios (mg carbohydrate g^{-1} plant material).

Results

Source limitation

Ozone impacts on leaf carbohydrate status: Ozone could reduce the pool of labile transport sugars in source leaves, indicating a possible source strength limitation to carbohydrate translocation. However, the pool of soluble sugars in young, fully expanded, photosynthetically active leaves of Pima cotton (Fig. 2; squares) did not decline with increasing leaf exposure to O_3, but rather trended

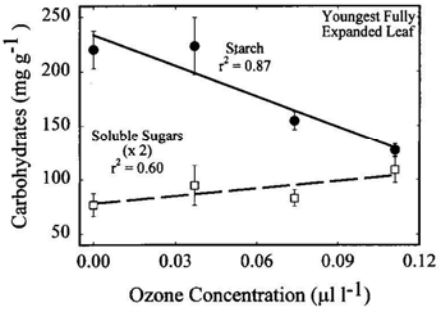

Fig. 2. Relationship between starch (solid line, closed circles) and total soluble sugar (broken line, open squares) contents of youngest fully expanded leaves of Pima cotton and the 12 h mean [O_3] during growth. For soluble sugars values plotted are twice observed values.

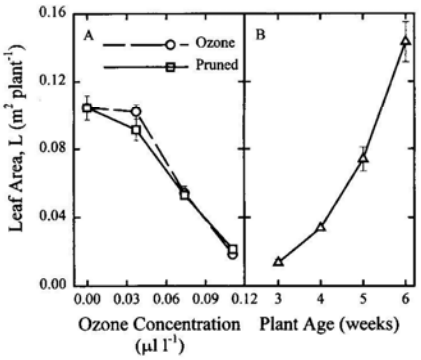

Fig. 3. Relationship between leaf area (L) of Pima cotton at 5 weeks after planting and [O_3] (A; circles). Similar relationship for plants not exposed to O_3 but whose leaf areas were repeatedly pruned during plant development to simulate O_3-treated plants (A; squares—plotted against simulated O_3 exposure). Relationship between leaf area (L) and plant age in the absence of O_3 fumigation (B; triangles).

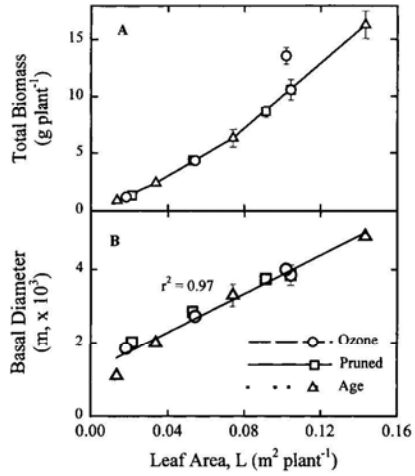

Fig. 4. Relationship between plant biomass (A) and stem diameter (B) and leaf area (L) for the three ranges of plant sizes achieved as in Fig. 1. Lines are fit by eye (A) or represent a single combined linear regression fit to all treatments (B).

upwards on a dry weight basis. The translocation process was thus not limited by substrate availability in individual source leaves to support phloem loading. As total leaf area (L) declined with increasing exposure to O_3 (Fig. 3A; circles), the calculated total content of transport sugars on a per plant basis declined with increasing [O_3] (not shown).

The major non-structural carbohydrate in these leaves was starch (Fig. 2; circles). Starch content was unaffected by moderate exposure to O_3, but declined substantially at higher [O_3], yielding a significant decline over the entire range of O_3-exposures.

Total biomass: Exposure to O_3 substantially inhibited plant growth, reducing areas of individual leaves (not shown) and total plant L (Fig. 3A; circles). Total plant biomass (Fig. 4A; circles) and basal diameter (Fig. 4B; circles), also declined with increasing [O_3].

The O_3-induced reduction of L and thus of photosynthetic source strength was simulated by frequent pruning of young leaf area from plants grown under O_3-free conditions. Throughout the growth period and at harvest (Fig. 3A; squares) the leaf-pruned plants displayed values of L similar to those exhibited by the corresponding O_3-treated plants (cf. Fig. 3A; circles, squares). The rate of leaf appearance was somewhat accelerated in the O_3-treated plants (not shown).

L of plants exposed to 0.037 μl l^{-1} O_3 was somewhat higher than expected (Fig. 3A; circles), similar to values in O_3-free air. Biomass production at 0.037 μl l^{-1} O_3 was also relatively large, exceeding that observed at 0.0 μl l^{-1} O_3 (Fig. 4A; circles). With the exception of this anomaly, the reduction of L by pruning resulted in a similar decline in plant biomass to that observed in plants exposed to various [O_3], with a similar relationship between biomass and L (Fig. 4A; cf. circles, squares). Basal stem diameter also exhibited the same relationship with L regardless of the factor limiting leaf area development and plant size (Fig. 4B).

Biomass allocation: Total biomass (Fig. 4A), and biomass in leaves, stems and roots (not shown) all decreased consistently with decreasing L whether reduced by O_3-exposure or leaf-pruning. However, the allocation of this biomass differed between the treatments.

Following exposure to a range of [O_3], the ratio of biomass allocated to leaves was unchanged (about 0.60 not shown), while allocation to stem tissue increased from about 0.25 to 0.35 of total biomass. The change in allocation to roots (root biomass ratio, R) was most significant. R declined from about 0.14 to about 0.06 (Fig. 5; circles) as L declined from about 0.11 to 0.02 m^2 $plant^{-1}$ and [O_3] increased from 0.0 to 0.111 μl l^{-1}. This

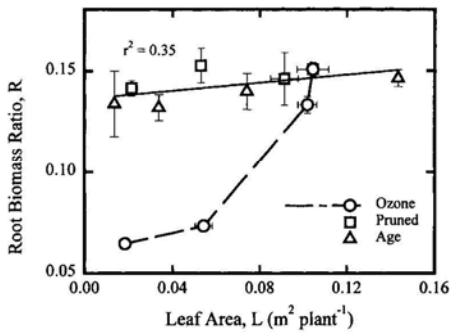

Fig. 5. Relationship between biomass allocation to root tissue and leaf area (L) for the three ranges of plant sizes achieved as in Fig. 1. Lines are combined regressions for leaf-pruned and age treatments (solid) or fit by eye to the O_3 data (broken).

is a reduction by half in resource allocation to the developing root system over this range of $[O_3]$.

Across the imposed range of severity of leaf pruning, biomass allocation to leaves and stems remained relatively unchanged at about 0.5 to 0.6 and 0.4 to 0.3, respectively (not shown), over a range of plant sizes and L (Fig. 3A, 4A). Allocation to the shoot (leaves plus stem) increased slightly, while allocation to roots declined slightly with decreasing L and plant size (Fig. 5; squares). R in the pruned plants remained about 0.14–0.15. This relationship between allocation and L was not similar to that observed following O_3-exposure, despite the similar relationship between L and biomass production following the two treatments.

Source leaf insertion level: Accelerated senescence following O_3-exposure typically impacts lower, older leaves. The leaf pruning protocol involved removal of young, developing leaf area. Because the lower leaves may preferentially export carbohydrate to the root system, the possibility that lower leaf pruning could more adequately simulate the effect of O_3 on allocation was evaluated.

Pruning of upper or lower non-senescing leaves induced similar effects on total plant productivity and L (Fig. 6A) and on allocation patterns (Fig. 6B). In both cases leaf pruning (decreasing L) led to greater biomass allocation to leaves relative to stems, but increased only slightly allocation to shoots relative to roots. The resulting allocation pattern with decreasing L (Fig. 6B; circles and squares) was similar to that observed in the main leaf pruning treatment (cf. Fig. 5; squares). Removal of upper or lower leaf area caused similar effects on whole plant development.

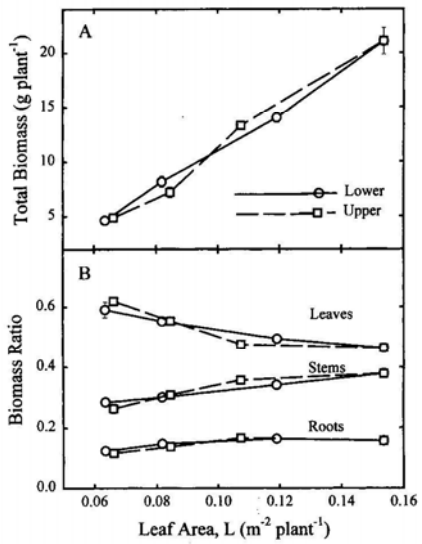

Fig. 6. Relationship between plant biomass (A) and biomass allocation (B) and leaf area (L) for plants pruned of apical (upper; circles) or basal (lower; squares) leaf area in the absence of exposure to O_3.

Developmental age

Total biomass: The plant sizes obtained by exposure to a range of $[O_3]$ were reproduced using control plants of different chronological ages. Plants harvested at 3–6-weeks-old displayed L (Fig. 3B; triangles) and plant biomass (Fig. 4A; triangles) that overlapped those observed in plants exposed to O_3 or subjected to leaf pruning.

The relationship between biomass and L during development of these young plants was consistent with relationships observed across the range of severity of both O_3-exposure and leaf pruning (Fig. 4A). The relationship between stem diameter and L was also very similar for all three treatments (Fig. 4B).

Biomass allocation: Allocation of biomass in the plants of different ages exhibited somewhat more complex dynamics than in the plants subjected to leaf pruning, though equally dissimilar to that observed in O_3-exposed plants. Biomass allocated to leaves (about 0.65) and stems (about 0.24) was relatively constant through about 5–6 weeks after planting, when L (Fig. 3B) and plant size (Fig. 4A; triangles) were similar to those in the other treatments. Between 6 and 8 weeks, allocation to leaves declined relative to stems (not shown) though total biomass allocation to the shoot (leaves plus stem) remained nearly constant. This resulted in constant allocation to the roots (R; Fig. 5; triangles) over the range of plant sizes and L measured. This is similar to the pattern observed following leaf pruning (Fig. 5; squares) and unlike that observed following O_3-exposure (Fig. 5; circles). Thus the change in allocation patterns observed following plant exposure to O_3 does not reflect O_3-impacts on plant size or developmental age.

Hydraulic properties

Ozone exposure: Exposure to O_3 not only reduced total root biomass (not shown), R (Fig. 5; circles), and stem diameter (Fig. 4B; circles), but also severely impacted total root and plant hydraulic conductance on a whole plant basis (K_R; Fig. 7; circles). On a leaf area basis (K'_R; Fig. 8; circles), O_3 also substantially reduced hydraulic efficiency. The decline in K'_R implies that O_3-impacts on K_R, and the capacity to provide water and nutrients to the transpiring shoot, were greater than impacts on the development and maintenance of transpiring leaf area.

Source strength: The impact of O_3 on K_R (whole plant basis) as a function of L (Fig. 7; squares) was fully reproduced by leaf pruning. This is consistent with the similar effects of O_3-exposure and leaf pruning on plant size and L. However, K'_R (leaf area basis) responded very

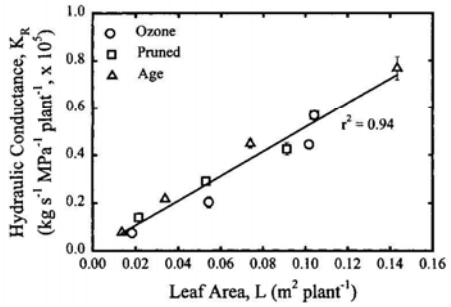

Fig. 7. Relationship between hydraulic conductance on a per plant basis (K_R) and leaf area (L) for the three ranges of plant sizes achieved as in Fig. 1. The line represents the combined linear regression fit to all treatments.

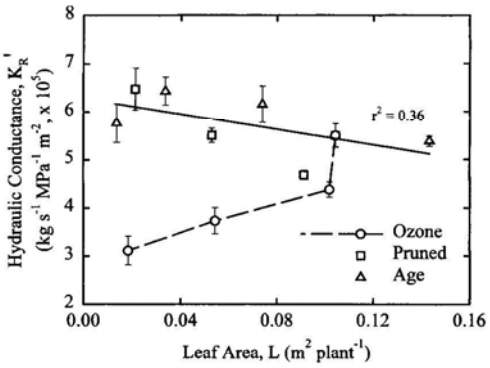

Fig. 8. Relationship between hydraulic conductance on a unit leaf area basis (K_R') and leaf area (L) for the three ranges of plant sizes achieved as in Fig. 1. The solid line represents the combined linear regression fit to the leaf-pruned and age treatments. The broken line is fit by eye to the O_3 data.

differently to O_3 and leaf pruning. K_R' increased with decreasing L resulting from leaf-pruning (Fig. 8; squares), but declined markedly with decreasing L following exposure to O_3 (Fig. 8; circles).

Developmental age: Altering plant size by manipulating chronological age revealed a similar pattern. K_R decreased with decreasing plant size and L (Fig. 7; triangles), along the same relationship observed in leaf-pruned and O_3-treated plants. In contrast, K_R' increased with decreasing L resulting from decreasing plant age (Fig. 8; triangles), similar to the effect of leaf pruning (Fig. 8; squares). This contrasted markedly with the decline in K_R' with decreasing L observed following exposure to increasing [O_3] (Fig. 8; circles).

Translocation

No data are presented here on the direct impact of O_3 on phloem loading or translocation. As the source strength and developmental age hypotheses were falsified (above), the possibility that O_3 impacts carbohydrate transport directly becomes increasingly viable.

Discussion

This study questioned whether O_3 acts on carbohydrate allocation and root development through reduced source strength, retarded growth and development, or inhibited translocation. Three hypotheses related to source strength and a fourth related to developmental age were tested: (1) O_3-exposure reduces the pool of soluble transport carbohydrates in source leaves; (2) leaf pruning to reduce photosynthetic source strength reproduces effects of O_3-exposure; (3) pruning lower source leaf area reproduces O_3-effects more closely than pruning upper leaf area; (4) manipulating chronological age and thus plant size reproduces the O_3 effects observed in similar sized plants exposed to O_3. All four hypotheses were falsified.

Source limitation?

O_3 effects on biomass allocation and root hydraulic conductance were not reproduced by reducing either upper or lower source leaf area. Direct O_3-impacts on photosynthesis are neither sufficient (wheat; Meyer *et al.*, 1997) nor necessary (cotton; Grantz *et al.*, 1999) to explain O_3 impacts on whole plants. On a whole shoot basis, O_3 impacts on source strength may be minimized by compensatory photosynthetic activity in the young upper leaves (Pell *et al.*, 1994). Direct effects of O_3 on photosynthesis occur over short time periods (Farage *et al.*, 1991), but chronic reduction of gas exchange may also result from reduced carbohydrate translocation and root development (Meyer *et al.*, 1997). Reducing whole plant source strength by pruning of leaf area over an extended period did not reproduce O_3 impacts on allometry or root function.

Transport sugars: O_3 did not consistently reduce the pool sizes of soluble, transport sugars in source leaves in the present study. In seedlings and mature branches of Douglas fir (Gorissen *et al.*, 1994; Gorissen and Van Veen, 1998; Smeulders *et al.*, 1995) retention of recent photosynthate in a non-soluble fraction increased in current year needles following chronic exposure to O_3. Starch content declined as in the present study. In Scots pine and Norway spruce (Peace *et al.*, 1995) starch reserves were unaffected by O_3, though carbon assimilation, foliar sucrose content, and activities of sucrose phosphate synthase and sucrose-6-phosphatase, both active in sucrose synthesis, declined. In these cases sugar contents declined, possibly limiting translocation as in brown rust-infected barley leaves (Tetlow and Farrar, 1993), in which apoplastic and symplastic sugar concentrations were reduced as export declined. In contrast, O_3 reduced carbon assimilation, but increased foliar concen-

trations of starch and fructans in wheat (Barnes *et al.*, 1995) and other species (Rennenberg *et al.*, 1996).

In poplar (*Populus* spp.) prior to visible senescence, soluble sugar contents increased with [O_3] (Fialho and Bucker, 1996; Landolt *et al.*, 1994), while starch was unaffected. With the onset of O_3-accelerated senescence, total starch concentration declined and concentrated along the minor veins in bundle sheath cells, the sites of phloem loading. In wheat (Meyer *et al.*, 1997) O_3-impacts on carbon assimilation were attributed to feedback inhibition by end-product accumulation. In Pima cotton, leaf contents of soluble sugars increased with increasing acute (Grantz and Farrar, 1999; and unpublished results) and chronic (above) exposure to O_3. O_3 did not limit allocation through substrate limitation of phloem loading.

Biomass production: Ozone reduced total plant leaf area (L) by reducing leaf expansion, consistent with previous studies in cotton (Temple, 1990). The leaf-pruning treatment also decreased L and yielded similar relationships between biomass and L and basal stem diameter and L. The leaf area and mass excised by pruning were not added to totals determined at harvest. The biomass ratios after plant acclimation to the pruning manipulations were of primary interest.

Plants grown under 0.037 $\mu l\, l^{-1}$ [O_3] accumulated more biomass than those grown in O_3-free air, though they displayed slightly less L. This led to a discontinuity in the relationship between biomass and L that was not observed in the leaf-pruning treatment. Enhanced growth at moderate [O_3], relative to lower or higher [O_3], is often observed (e.g. in bean, *Phaseolus vulgaris* L.; Sanders *et al.*, 1992). Similar excursions yielded anomalies in the relationship between hydraulic conductance and L at moderate levels of O_3-exposure and leaf-pruning.

Biomass allocation: O_3 reduced R of cotton similar to previous observations (Grantz and Yang, 1996; Temple, 1990; Oshima *et al.*, 1979). The reduction of R with declining L was not observed in the leaf pruning treatments, despite similar effects of leaf-pruning and O_3 on L and total biomass. These data do not support the hypothesis that the O_3-induced reduction of R is mediated by O_3-reduced L or photosynthetic source strength. This conclusion is valid to the extent that similar leaf areas imply similar photosynthetic capacities on a whole plant basis, an assumption supported by the consistent relationships between L and biomass observed in all treatments.

Hydraulic conductance: K_R (whole plant basis) generally scales with plant size (Fiscus and Markhart, 1979; Rüdinger *et al.*, 1994). This was observed in O_3-treated spruce (Lee *et al.*, 1990) and in Pima cotton (Grantz and Yang, 1996). This is consistent with the single linear relationship observed in this study between K_R and L exhibited by plants exposed to O_3 and to leaf-pruning as K_R and L declined by more than 80%.

K'_R (leaf area basis) generally scales inversely with plant size (Fiscus and Markhart, 1979; Rüdinger *et al.*, 1994). This was the case for plants subjected to leaf-pruning in the present experiment, but not for O_3-treated plants. K'_R was reduced by about 50% as L declined from 0.11 to 0.02 m^2 following O_3-fumigation, but increased by about 20% over the same range of L when plant size was manipulated by leaf-pruning.

Root development dominated the hydraulic efficiency of these plants since root resistance resistance ($1/K'_R$) constituted about 80% of whole-plant resistance in similar plants of Pima cotton (Grantz and Yang, 1996). The unique O_3-effect on K'_R reflected the much larger O_3 impact on allocation to root biomass than on allocation to L, indicating a clear difference between K'_R modified by O_3-reduced allocation to roots and K'_R modified by leaf pruning and reduced source strength.

Altered developmental age?

Biomass production: O_3 retarded plant development, as represented by biomass or L at a given harvest date, clearly altering the relationship between developmental age and chronological age. Total plant biomass was similarly related to L as both decreased with increasing [O_3] and with declining plant age.

Biomass allocation: The changes in allocation observed following O_3-exposure, i.e. instantaneous values of R, could be associated with this altered age–size relationship (Farrar and Gunn, 1996) rather than with O_3-induced changes in the allometric coefficient (Farrar and Williams, 1991). Similar changes in R observed following CO_2 enrichment may reflect such impacts on developmental age (Farrar and Gunn, 1996). This possibility was investigated by comparing plants with similar biomass and L achieved either by exposure to a range of [O_3] or by varying plant age.

In contrast to biomass productivity, O_3 effects were not reproduced by reducing plant age and size. A significant decline in R was only observed in the O_3-treated plants. This reflected an allometric shift as demonstrated by the altered relationship between R and age-independent measures of plant development such as total biomasss and L. The allometric shift observed in O_3-treated plants indicates that retardation of plant development does not mediate the impact of O_3 on carbohydrate allocation.

Hydraulic conductance: All three treatments reduced K_R and L by over 80%. K'_R, however, increased by 20% over the same range of L when plant size varied with plant age or leaf-pruning, but declined by 50% following O_3-exposure. Simulated impacts on shoot gas exchange

(Grantz *et al.*, 1999) were therefore considerably greater following O_3-exposure than following a similar reduction in productivity related to leaf removal or retarded development. The O_3 effect appears to be distinct.

Inhibition of translocation

The *translocation* hypothesis emerged as a viable alternative for the mechanism of O_3 impacts on whole plants. The regulation of carbohydrate allocation and of translocation remain poorly characterized. An O_3 effect on translocation is consistent with recent modeling (Grantz *et al.*, 1999) and experimental (Grantz and Farrar, 1999) results. The model of Minchin *et al.* (Minchin *et al.*, 1993) predicted that a reduction in source strength, whether due to inhibited assimilation or translocation, would reduce import by distant roots more than proximal shoot sinks.

O_3 could affect phloem loading in source leaves or the energetics of phloem transport along the translocation pathway. Either is consistent with O_3-impacts on membrane integrity (Alscher *et al.*, 1997) and on the kinetics of sugar export from source leaves of Pima cotton (Grantz and Farrar, 1999). As O_3 did not reduce the linear velocity of translocation in wheat, but reduced the amount of carbohydrate translocated per unit time (Mortensen and Engvild, 1995; Fangmeier *et al.*, 1994) a source limitation due to impaired phloem loading is suggested.

SO_2, another oxidant species, also inhibited translocation (McLaughlin and McConathy, 1983) in whole plants. Sulphite in solution inhibited sucrose uptake by membrane vesicles (Maurousset *et al.*, 1992), without disrupting either the pH gradient nor the membrane potential that drive sucrose transport. Sucrose uptake was similarly sensitive to O_3 and to the sulphydryl reagent, PCMBS (*p*-chloromercuribenzene-sulphonic acid; Madore, 1990). It was speculated whether the myriad effects of O_3 on plant development could occur through oxidation of sulphydryl groups in phloem-associated proteins such as the sucrose translocator.

Conclusion

O_3 reduced biomass production, leaf area development and biomass allocation to roots. The first two effects were completely reproduced by harvest of plants at different ages, or by artificially reducing photosynthetic leaf area during development. In contrast, relative allocation of biomass to developing root systems and consequent root functional properties were reduced by O_3 but not by the other treatments. O_3-induced an allometric shift in root versus shoot partitioning that was unrelated to plant size, and was not mediated by O_3-inhibited photosynthetic capacity. The metabolic regulation of this allometric shift, along with other aspects of translocation in general, remain to be elucidated. A direct O_3 impact on phloem loading is suggested as a focus of future study. Altered shoot to root allocation, plant hydraulic efficiency, and shoot gas exchange may be indirect consequences of this putative primary lesion. Studies of O_3 effects on apoplastic and symplastic phloem loading species might prove useful in further elaborating the mechanism of O_3 action.

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