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**RESEARCH PAPER**

# Ozone increases root respiration but decreases leaf $CO_2$ assimilation in cotton and melon

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## Abstract

**It is well established that exposure of plant foliage to tropospheric ozone ($O_3$) inhibits photosynthetic gas exchange in leaves and the translocation of current photosynthate to sink tissues. It is less clear what impact $O_3$-reduced source strength has on the physiological responses of sink tissue such as fine roots. The responses were investigated of carbon acquisition in leaves and carbon utilization in the respiration of fine roots, following chronic (weeks) and acute (hours) exposures to $O_3$ in open top chambers. Previous reports indicate increased, decreased, and unchanged rates of root respiration following exposure to $O_3$. A decline in source activity is confirmed, but an increase in sink respiration is reported in fine roots of Pima cotton (cv. S-6) and muskmelon (cv. Ambrosia hybrid). Leaf source strength and root sink activity changed in opposing directions, thus there was no positive correlation that might indicate direct substrate control of root function. Additional linkages between shoot and root following exposure to $O_3$ may be involved.**

Key words: Allocation, cotton, *Cucumis melo*, gas exchange, *Gossypium barbadense*, melon, ozone, photosynthesis, root respiration.

## Introduction

### *Carbon acquisition and allocation*

Chronic exposure to $O_3$ inhibits allocation of biomass to developing roots in Pima cotton (*Gossypium barbadense* L.; Grantz and Yang, 1996), muskmelon (*Cucumis melo* L.; Fernandez-Bayon *et al.*, 1993; DA Grantz and S Yang, unpublished data), and many other species (Cooley and Manning, 1987; Reiling and Davison, 1992; Darrall, 1989; Rennenberg *et al.*, 1996). In cotton (Grantz and Yang, 1996, 2000) the root/shoot biomass ratio decreased and leaf area specific root hydraulic conductance declined despite reduction of leaf area. Degraded root system function may contribute to $O_3$-induced inhibition of shoot gas exchange and carbon acquisition (Grantz *et al.*, 1999).

Numerous studies have demonstrated an $O_3$-induced reduction in photosynthetic carbon assimilation ($A_n$; Reich, 1983; Dann and Pell, 1989; Farage *et al.*, 1991) in Pima cotton (Grantz and Farrar, 1999, 2000), muskmelon (Fernandez-Bayon *et al.*, 1993) and other cucurbits (Castagna *et al.*, 2001; Fernandez-Bayon *et al.*, 1993). A parallel reduction of stomatal conductance ($g_s$) was often observed, confirming, and in some cases contributing, to the observed limitation of $A_n$. Both direct and indirect impacts of $O_3$ on $A_n$ reduce the quantity of carbohydrate (CHO) available for export from source leaves to sink tissues such as fine roots. In addition, the inhibition of export of recent photosynthate may further limit CHO supply to roots (Grantz and Farrar, 1999, 2000; Darrall, 1989).

### *Root respiration*

The reduced allocation of CHO to roots must eventually reduce substrate availability for root growth and maintenance respiration. In tomato, $O_3$ reduced substrate availability and the production of root exudates (McCool and Menge, 1983) which adversely affected mycorrhizal infection. $O_3$-induced increases in the translocation of photosynthate to roots have also been observed, particularly with low $O_3$ concentrations (Ponderosa pine: Scagel

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Abbreviations: $A_n$, net carbon assimilation; $R_r$, fine root respiration; $g_s$, stomatal conductance; OTC, open top exposure chamber; CF, charcoal-filtered air; MO3, HO3, medium or high ozone concentration; CHO, carbohydrate; $Q$, respiratory quotient.

 

and Andersen, 1997; *Trifolium repens*: Blum *et al.*, 1983; *Triticum aestivum:* McCrady and Andersen, 2000).

Responses of fine root respiration ($R_r$) to $O_3$ remain unclear, with previous studies providing an array of contrasting conclusions. $O_3$ decreased $R_r$ in some conifers (*Pinus taeda:* Edwards, 1991; *P. armandi:* Shan *et al.*, 1996; *Pseudotsuga menziesii:* Gorisson and van Veen, 1988), and in such annual crops as *Phaseolus vulgaris* (Hofstra *et al.*, 1981; Ito *et al.*, 1985). By contrast, $O_3$ increased $R_r$ in other conifers (*Pinus ponderosa*: Scagel and Andersen, 1997) and temperate broad-leafed trees such as deciduous red oak (*Quercus rubra*: Kelting *et al.*, 1995). The extent to which these conflicting observations reflect interspecific variability, contrasting experimental conditions, or inherent variability in measured values of $R_r$, remains unknown. These studies report chronic $O_3$ exposures which may have allowed the acclimation of allometry, root system morphology, and physiological function. Such a restoration of homeostasis confounds the interpretation of primary $O_3$ impacts on $R_r$.

As $R_r$ may consume over half of the net primary productivity (Lambers *et al.*, 1996) and up to 75% of CHO translocated to roots (Högberg *et al.*, 2002), the magnitude and direction of $O_3$ impacts on this below-ground carbon sink are of considerable interest. The diversity of $O_3$ effects on $R_r$ noted above has hindered the prediction of $O_3$ impacts on carbon sequestration as part of the overall effects of global change, and has prevented appropriate parameterization of long-term $O_3$ impacts on plant growth and development. $O_3$ effects on $R_r$ could also serve as early diagnostic signals of $O_3$ damage to vegetation (Richards, 1989; Taylor and Ferris, 1996).

### *Current investigation*

$O_3$ impacts on cotton and melon, species which use different mechanisms of phloem loading and contrasting primary transport sugars, were used to test three hypotheses: (1) chronic (long-term) exposure to $O_3$ reduces $R_r$; (2) chronic changes in $R_r$ are correlated with changes in $A_n$; (3) acute (short-term) exposure to $O_3$ reduces $R_r$ and $A_n$ in parallel or sequentially.

Hypotheses 1 and 2 are rejected, as chronic $O_3$ exposure significantly increased $R_r$ in both cotton and melon, while reducing leaf $A_n$. Over short exposures, the $O_3$ effects on $R_r$ were more variable and hence more difficult to identify, despite a high degree of replication. The acute effects were entirely consistent with chronic impacts, though temporal resolution was inadequate to evaluate Hypothesis 3 in a definitive manner. Leaf source strength and root sink activity changed in opposing directions over all time scales considered, with no suggestion of a positive correlation indicative of direct substrate control of $R_r$. Additional and previously unappreciated linkages between shoot and root may be involved in $O_3$ phytotoxicity.

## Materials and methods

### *$O_3$ and environmental exposure*

The Open Top Chamber (OTC; 3.1 m diameter × 2.4 m height; Heagle *et al.*, 1973) facility at the University of California Kearney Agricultural Center (103 m elevation, 36.598 N 119.503 W) was used for all experiments. Ten OTCs are available at this facility, with nine used in the present studies.

$O_3$ was generated by corona discharge (Model G22; Pacific Ozone Technology, Brentwood, CA) from oxygen (Model AS-12; AirSep Corporation, Buffalo, NY). The daily time-course of $O_3$ concentration was regulated in a single OTC using a dedicated $O_3$ monitor (Model 49C, Thermo Environmental Instruments, Franklin, MA) interfaced to a computer for feedback control. The other eight OTCs were controlled using manual proportioning valves to control the flow of $O_3$. $O_3$ concentration was determined in all OTCs approximately every 15 min. Air was sampled continuously from the centre of each chamber through a Teflon dust filter and teflon tubing attached to a multiport solenoid valve. All $O_3$ concentration data were archived electronically.

Three $O_3$ exposure regimes were imposed. The charcoal-filtered treatment (CF) was nominally $O_3$-free (Table 1), but exhibited slightly positive $O_3$ concentrations, nearly equivalent to global (pristine) background concentrations. This occurred due to imperfections in the charcoal filters and incursion of ambient air through the open top of each OTC, despite the installation of a conical frustrum. The medium $O_3$ treatment (MO3) reproduced the diurnal profile and maximal concentration observed on exceptionally polluted midsummer days at this location (Table 1). The high $O_3$ treatment (HO3) was 1.6-fold greater than MO3 at each time point. The $O_3$ concentrations achieved during the acute, short-term exposures are summarized as accumulated concentration with no threshold (SUM 00; Table 2) and as means of all (day and night) concentrations (Mean; Table 2). The exposures were the same each day.

### *Chronic exposure experiment*

Seeds of cotton (*Gossypium barbadense* L. cv. Pima S-6; JG Boswell Co., Corcoran CA) or melon (canteloupe/muskmelon; *Cucumis melo* cv. Ambrosia Hybrid; Burpee Seed Co., Warminster, PA) were sown approximately 2 cm deep in 6–40 mesh sintered clay (QuickSorb, A&M Products, Taft, CA) in 9.0 l tapered (45 cm deep, 18 cm diameter) pots (Treepot; Hummert International, Earth City, MO). Pots were washed with water prior to planting. Pots containing ungerminated seed were irrigated to run-through and randomized among the three $O_3$ exposure regimes. Seedlings were thinned to one per pot as the first true leaf began to expand.

Plants were grown for approximately 6 weeks (cotton) or 5 weeks (melon) until they had attained five leaves. A total of seven (cotton) or 10 (melon) independent experiments, each with at least two plants per treatment, were conducted. Experiments were randomly assigned to the replicate CF, MO3 and HO3 OTCs. Pots were automatically irrigated to run-through at least daily and up to several times per day as required by the weather. A complete fertilizer

**Table 1.** *Nominal exposure characteristics for three ozone exposure regimes (ppb)*

| Ozone treatment | Daily maximum | 12 h mean |
|---|---|---|
| CF | 0 | 0 |
| MO3 | 140 | 90 |
| HO3 | 224 | 143 |

**Table 2.** *Ozone exposure characteristics (sum with no threshold, mean for all hours including night) for the sample periods in the acute $O_3$ exposure regimes*

| Ozone treatment | Exposure duration | Sum 00 (ppb h) (Mean±SE) | 24 h mean (ppb) (Mean±SE) |
|---|---|---|---|
| CF | 1 h | 8.23±1.48 | 8.23±1.48 |
| CF | 3 h | 29.6±4.2 | 9.86±1.40 |
| CF | 5 h | 48.6±7.4 | 9.71±1.49 |
| CF | 29 h | 298±56 | 10.3±2.0 |
| CF | 53 h | 545±139 | 10.3±2.6 |
| HO3 | 1 h | 142±12 | 142±12 |
| HO3 | 3 h | 475±28 | 158±10 |
| HO3 | 5 h | 944±64 | 189±13 |
| HO3 | 29 h | 3602±382 | 124±13 |
| HO3 | 53 h | 6611±827 | 125±16 |

(Miracle Gro; Scotts Miracle-Gro Products Inc., Port Washington, NY; 1.3 g $l^{-1}$) was applied to run-through weekly.

Data were analysed by 2-way ANOVA, with measurements classified by $O_3$ treatment and date of measurement. The two species were analysed separately.

### *Acute exposure experiment*

Seeds of cotton and melon were planted in individual conical tubes (Ray Leach Single Cell Cone-Tainers; 4 cm diameter × 20 cm deep; Hummert International, Earth City, MO). Seeds were planted approximately 2 cm deep in 6-40 mesh sintered clay (QuickSorb). 35 tubes were spaced evenly in a cone tray (Hummert International, Earth City, MO). Tubes were sterilized with hypochlorite (10%; 3 min) and rinsed prior to planting.

Germination and growth were in a heated, whitewashed greenhouse. Each container was irrigated to run-through daily, twice daily as required by the weather, and fertilized weekly (Miracle-Gro; 3.1 g $l^{-1}$). Plants were grown for approximately 3 weeks until they had attained two fully expanded true leaves during the period 20 March–23 October, 2002. 48 h before experiments cotyledons were excised to limit carbohydrate supply to root tissue from stored photosynthate. Experiments were initiated at 09.00 h PDT when plants were transferred from the greenhouse to a CF OTC. Plants in OTCs were irrigated every 2 h with automated drip irrigation.

Plants were allowed to acclimate to the modified environment for 1 h. At 10.00 h exposure timing began when a randomly selected 50% of plants were transferred to a HO3 OTC, leaving 50% of plants in the CF OTC. Measurements on cotton and melon were conducted on different, often alternating, days, at 1, 3, 5, 29, and 53 h after initiation of $O_3$ exposure. Mean and accumulated $O_3$ exposures for each treatment are shown in Table 2. A total of 19 independent experiments were performed with cotton and 15 independent experiments with melon, each with at least two plants per treatment. All short-term exposures were conducted in the same CF, MO3 and HO3 chambers.

Data were analysed by paired sample *t*-tests (matched CF, HO3 samples) within individual exposure times (1–53 h) and over all measurements taken together. No consistent trends were observed over the time of exposure nor date of measurement. The two species were analysed separately

### *Gas exchange*

Measurements of $A_n$ and $g_s$ were obtained on the youngest fully expanded leaf, using a steady-state gas exchange system (Model LI-6400; Li-Cor Inc., Lincoln, NE). The LI-6400 was operated with an internal light source (1000 μmol $m^{-2}$ $s^{-1}$; blue (20%) and red (80%) light emitting diodes; Li-Cor) and with control of $CO_2$ concentration in the cuvette ($C_a$; 400 ppm), using small cylinders of pressurized $CO_2$, and a constant flow (500 μmol $s^{-1}$). $A_n$ and $g_s$ were expressed relative to projected leaf area of the measured leaf.

### *Root respiration*

Intact root systems were obtained from two plants in each treatment at each measurement time point. The container and the sintered clay potting medium were removed by immersion in cold water. The medium was removed (virtually quantitatively) from the roots by gentle agitation. The terminal 3–4 cm of fine root were excised and immediately transferred to a respirometer chamber.

Fine root respiration ($R_r$) was determined in liquid phase with a Clark-type oxygen electrode (Delieu and Walker, 1972). Four respirometer chambers (Oxygraph Oxygen Electrode System; PP Systems, Haverhill, MA) were run in parallel, interfaced with a computer for data acquisition and analysis. A magnetic stir bar was placed in each chamber, separated from the root material by a porous metal screen. Temperature control (25 °C) was maintained by the circulation of water through a precision water bath (Model 9100, Isotemp Pittsburgh, PA,) and through the plastic housing of each respirometer chamber.

Electrodes were calibrated using air-saturated water, and oxygen-free water obtained by adding a small amount of sodium dithionite to each chamber. Following several rinsings, 2 ml of water was placed in each chamber. When output had become stable (about 10 min), fine root samples were introduced. $R_r$ was expressed relative to the blotted wet mass of root in each chamber.

### *Dark/sunlit test of root respirometer measurements*

To confirm that differences in $R_r$ would be detected using these methods, two independent experiments were performed with sunlit and dark-adapted cotton. Plants were grown in the greenhouse in Cone-Tainers as for acute exposure experiments, and acclimated for 1 d outside, sheltered from direct sun and the night sky. In the early morning, plants were irrigated to run-through, randomly assigned to two groups, and placed either in an open location and irrigated hourly (sunlit) or shrouded in dark plastic and placed in a deeply shaded, outdoor location (dark adapted). $R_r$ was determined, as above, in mid-afternoon (15.00 h PDT). The experiments did not differ so data were pooled and analysed by unpaired *t*-test with 12 individual plants per treatment.

## Results and discussion

### *$O_3$ exposure*

Experiments were conducted under field exposure conditions, and over the range of environmental conditions observed during the commercial growing season for both cotton and melon in the San Joaquin Valley. Temperature and cloud cover were more variable early and late in the season than during midseason (not shown). Solar radiation was relatively constant from day to day in this semi-arid environment, although day length and maximum insolation changed seasonally (Fig. 1A). The same $O_3$ profile was imposed on all days, at three concentrations defined as CF, MO3, and HO3 (above). Mean time-courses and variability attained in the CF and HO3 treatments are shown in Fig. 1B. The modest seasonal shifts in alignment of instantaneous $O_3$ concentration (Fig. 1B), and environmental parameters such as solar radiation (Fig. 1A) were

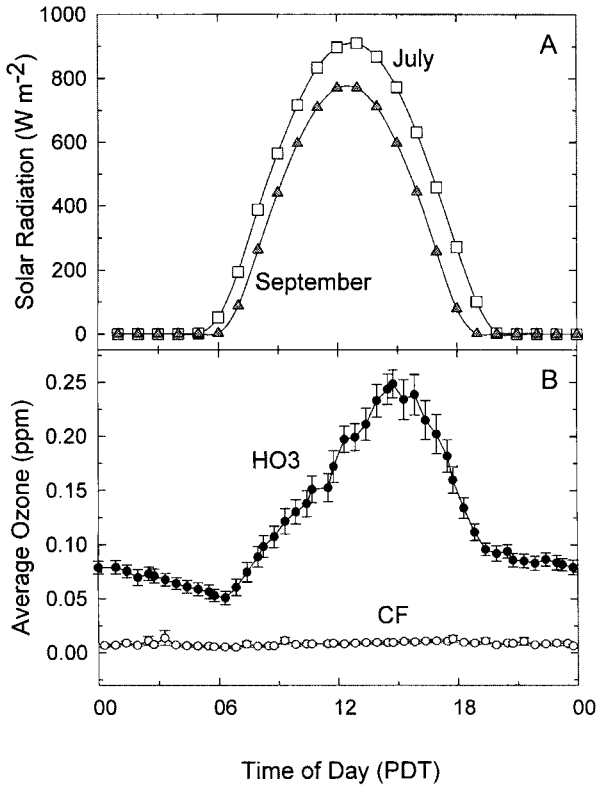


**Fig. 1.** Representative daily time-courses of (A) solar radiation measured above the OTCs during mid- (squares) and late- (triangles) growing season, 2002; and (B) representative $O_3$ concentrations (mean ±SE over all initial days of acute exposure experiment in $O_3$-enriched (solid line and filled symbols) and charcoal-filtered (broken line and open symbols) open top chambers.

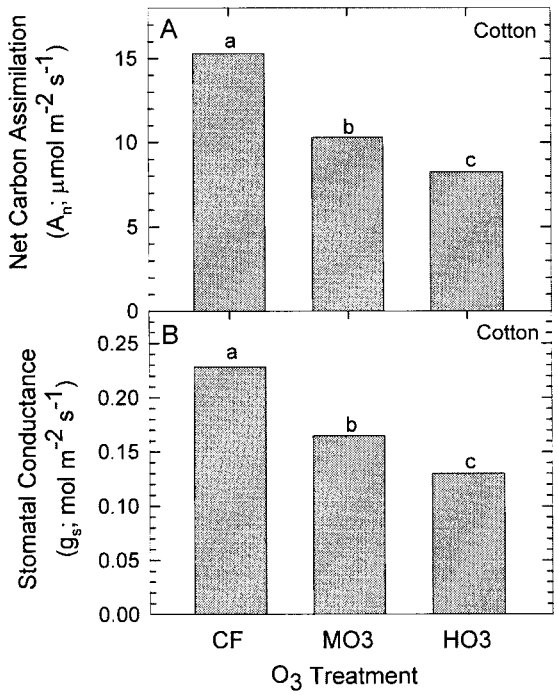


**Fig. 2.** Effect of chronic exposure to contrasting $O_3$ concentrations on gas exchange parameters (A, net carbon assimilation; B, stomatal conductance) of cotton. Mean ±SE. Bars with different letters are different at $P$ <0.05.

not associated with seasonal trends in physiological parameters nor sensitivity to $O_3$. The range of conditions introduced some variability, but increased the generality of conclusions to be drawn from these observations.

### *Leaf gas exchange*

Leaf gas exchange was adversely affected by chronic exposure to $O_3$. In the youngest fully expanded leaves of both cotton (Fig. 2) and melon (Fig. 3), mid-afternoon values of photosynthetic carbon assimilation ($A_n$; Figs 2A, 3A) were suppressed by $O_3$ exposure. The greatest impact was observed between the CF and MO3 treatments, with little further inhibition as the $O_3$ concentration increased 1.6-fold (Figs 2, 3; cf. MO3, HO3). These results mostly confirmed much of the previous research (Reich, 1983; Dann and Pell, 1989; Farage *et al.*, 1991). The mechanism of photosynthetic inhibition is unclear, and multiple sites of primary oxidant attack are possible, in addition to secondary effects possibly involving water relations (Grantz *et al.*, 1999) and end product inhibition (Darrall, 1989; Goldschmidt and Huber, 1992; Grantz and Farrar, 2000).

$A_n$ was lower in cotton than in melon under these conditions, and considerably more sensitive to $O_3$ (>30% reduction at MO3 in cotton compared with <25% in melon). The inhibition of $A_n$ per unit area of young, highly productive, leaves underestimates the actual impact of $O_3$ on whole plant productivity. Carbon assimilation of plants of both species (not shown) was further reduced by $O_3$-inhibited leaf area production and accelerated senescence and abscission of lower leaves.

Stomatal conductance ($g_s$) was also reduced by chronic exposure to $O_3$ in both cotton and melon (Figs 2B, 3B). $O_3$ often induces parallel declines in $g_s$ and $A_n$ (Grantz and Yang, 1996). The magnitude of inhibition of $g_s$ in the present experiments was similar to that of $A_n$ in cotton, but considerably greater than that of $A_n$ in melon. This stomatal sensitivity may reflect the greater baseline level of $g_s$ in melon than in cotton, although this larger leaf conductance for $O_3$ entry did not lead to increased sensitivity to $O_3$ of other processes (e.g. visible symptoms or $A_n$). The consistency of mesophyll and stomatal responses to chronic $O_3$ exposure, and the realistic values of calculated intercellular $CO_2$ concentration (not shown), provide additional support to the accuracy of the measurements of $A_n$ and responses to $O_3$ under these experimental conditions.

The acute exposure experiments, in which plants were transfered from $O_3$-free growth conditions to HO3 revealed that the onset of these photosynthetic responses to chronic $O_3$ exposure was relatively rapid. The time-course data were somewhat confounded by a steady

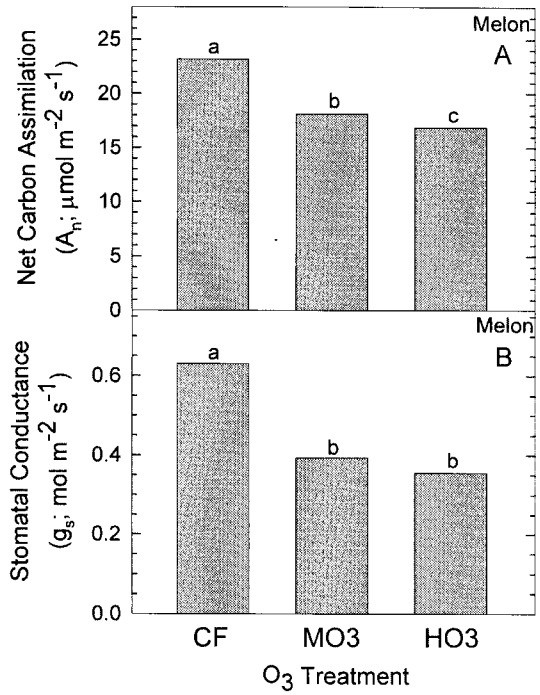


**Fig. 3.** Effect of chronic exposure to contrasting $O_3$ concentrations on gas exchange parameters (A, net carbon assimilation; B, stomatal conductance) of melon. Mean ±SE. Bars with different letters are different at $P$ <0.05.

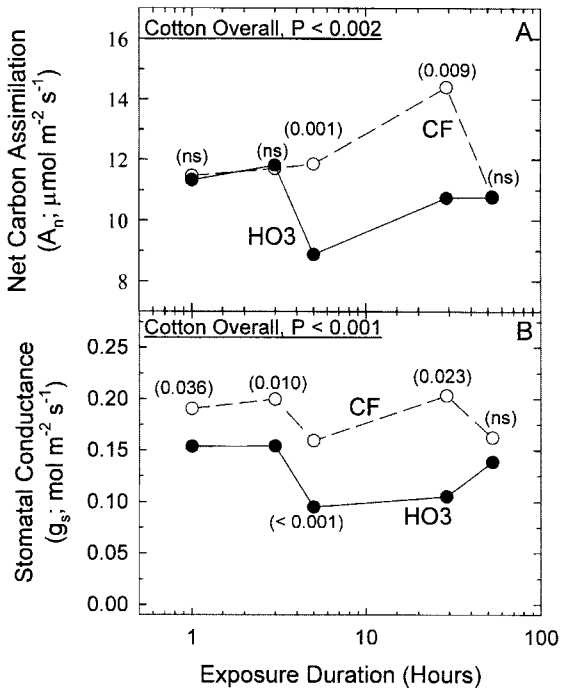


**Fig. 4.** Effect of acute (short-term) exposure to contrasting $O_3$ concentrations on gas exchange parameters (A, net carbon assimilation; B, stomatal conductance) of cotton. Mean ±SE. Note log scale for time axis.

increase in $A_n$ in the CF treatment over the first 29 h in cotton (Fig. 4A, open circles) and 5 h in melon (Fig. 5A). Under these conditions (i.e. transfer from one $O_3$-free environment to another), $A_n$ increased with increasing solar radiation from the first measurement at 11.00 h. $A_n$ may also have undergone acclimation to the higher radiation OTC environment, as suggested by the increase of $A_n$ of CF-treated cotton through mid-afternoon of day 2, before declining precipitously at mid-afternoon of day 3 (53 h; Fig. 4A). $A_n$ of CF-treated melon increased throughout day 1, but declined sharply at mid-afternoon of day 2 (Fig. 5A), before recovering somewhat on day 3. These temporal trends were consistent across many experiments under a range of seasonally variable conditions, but were not further investigated.

$A_n$ of cotton was not inhibited by HO3 during the first 3 h of exposure (Fig. 2A, solid circles), increasing in parallel with the CF-treated plants. By 5 h (15.00 h PDT), a period of high insolation (Fig. 1A) and high $O_3$ concentration (Fig. 1B), the HO3 plants exhibited a substantial decline in $A_n$ that was not evident in CF-treated plants, resulting in a highly significant difference between the $O_3$ treatments that was maintained throughout day 2 (29 h). HO3-treated plants remained depressed at mid-afternoon of day 3, but the unexpected decline in $A_n$ of CF-treated plants eliminated significant differences.

In acutely exposed cotton, $A_n$ of CF-treated plants was consistently greater than or equal to $A_n$ of HO3-treated plants. As no consistent diel nor seasonal patterns were observed, data were pooled for analysis over all times and dates. Paired sample $t$-test of $O_3$ effects indicated a significant reduction of $A_n$ due to $O_3$ exposure ($P$ <0.002; Fig. 4A), consistent with observations of the chronically exposed plants described above (Fig. 2A).

The time-courses of $A_n$ in CF- and in HO3-treated cotton were reflected in those of stomatal conductance ($g_s$; Fig. 4B). Responses of $g_s$ may have preceded those of $A_n$, This would be an important result, but the data for cotton are insufficient to allow confidence in this conclusion and this was clearly not the case in melon (below). In cotton, $g_s$ was significantly depressed by $O_3$ within the first hours of exposure, and remained consistently and generally significantly depressed below CF values throughout the 53 h exposure. By mid-afternoon of day 3 (53 h), $g_s$ in the CF-treated plants declined along with $A_n$, obscuring potential treatment differences. Over all observations, however, $O_3$ significantly reduced $g_s$ in acutely exposed cotton ($P$ <0.001; Fig. 4B).

In acutely exposed melon, $A_n$ was reduced by $O_3$ at the first observation following 1 h of exposure, and significantly suppressed at each subsequent timepoint except on day 2 (29 h) when a decline in CF along with HO3 values obscured treatment differences (Fig. 5A). On day 3 (53 h), both CF and HO3 plants recovered, though HO3 remained significantly depressed by $O_3$. Analysis of all measure-

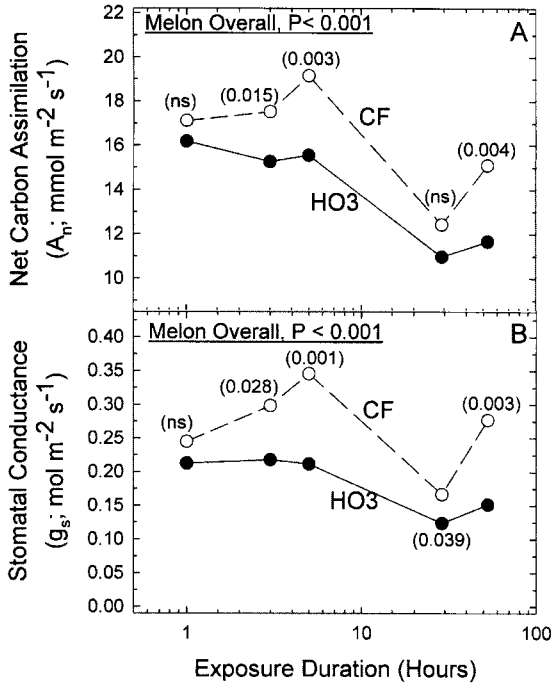


**Fig. 5.** Effect of acute (short-term) exposure to contrasting $O_3$ concentrations on gas exchange parameters (A, net carbon assimilation; B, stomatal conductance) of melon. Mean ±SE. Note log scale for time axis.

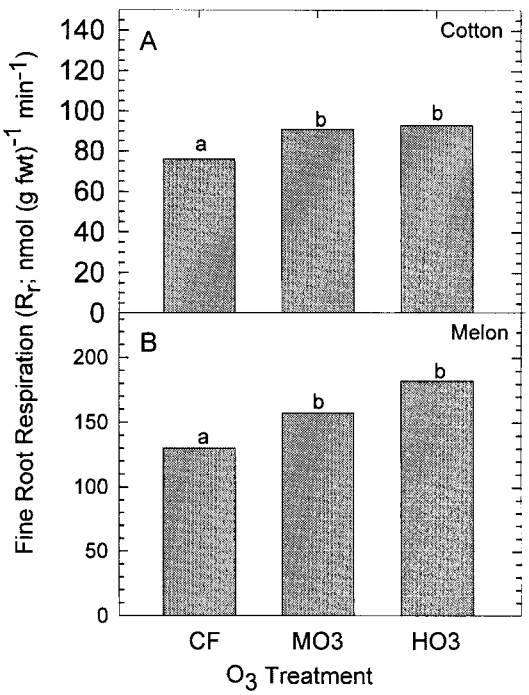


**Fig. 6.** Effect of chronic exposure to contrasting $O_3$ concentrations on respiration of fine roots on fresh weight basis (A, cotton; B, melon). Mean ±SE. Bars with different letters are different at $P$ <0.05.

ments demonstrated a significant reduction of $A_n$ by $O_3$ in acutely exposed melon ($P$ <0.001; Fig. 5A).

A consistent depression of $g_s$ in melon was observed in HO3 relative to CF plants. The impact of $O_3$ was first observed in parallel with changes in $A_n$, with a modest depression observed after 1 h of exposure and significant suppression at all subsequent measurements. The depression of gas exchange on the second day of exposure, observed in both $g_s$ and $A_n$, differed from observations in cotton (cf. Figs 4, 5) in which maximum values of $A_n$ and $g_s$ were observed at this time. $O_3$ significantly depressed $g_s$ of acutely exposed melon over the entire experiment ($P$ <0.001; Fig. 5B).

### *Root respiration*

Fine root respiration ($R_r$) was significantly affected by chronic exposure to $O_3$. Whereas leaf assimilation of substrate $CO_2$ ($A_n$) declined, consumption of substrate CHO in roots ($R_r$) increased, in both cotton (Fig. 6A) and melon (Fig. 6B). The greatest impact in both species was observed between the CF and MO3 treatments, with little further increase between MO3 and HO3.

The species differences in physiological activity observed in gas exchange parameters under CF conditions (1.5-fold greater $A_n$ in melon than cotton; cf. Figs 2A, 3A) were reflected in CF values of $R_r$ (1.7-fold greater in melon than cotton; cf. Fig. 6A, B). However, in contrast to the greater sensitivity of gas exchange in melon, the sensitivity of $R_r$ was similar in the two species. $R_r$ increased by about 1.2-fold in MO3 and only slightly more in HO3. Thus, $O_3$ caused an increase in $R_r$ of both species, by contrast with the decrease in $A_n$.

Acute responses of fine root respiration ($R_r$) to short-term exposures to $O_3$ were difficult to resolve. Following 1 h of exposure to $O_3$, $R_r$ of cotton was increased by about 15% ($P$=0.056; Fig. 7A). Measurements were consistently greater in HO3 than in CF-treated roots of cotton, but the differences at individual timepoints were not generally statistically significant. Over all measurements $R_r$ of cotton was significantly increased by the HO3 treatment ($P$=0.029). There was no diurnal pattern in values of $R_r$, consistent with the observations of Walters *et al.* (1993) in a variety of temperate tree species.

Short-term responses of $R_r$ to $O_3$ in melon (Fig. 7B) were less clear than those in cotton. At three out of five measurement timepoints, $R_r$ was increased by $O_3$ exposure. At the first (1 h) observation, enhancement of $R_r$ was similar to that observed in cotton (about 15%) though not statistically significant due to considerable variability. At the last measurement timepoint (53 h) the enhancement of $R_r$ was significant ($P$=0.043), but attributed largely to the unexplained decline in mean CF values. Over all measurements in melon, the experiment did not resolve a significant effect of $O_3$ on $R_r$.

The rates of $R_r$ observed in both species are consistent with many similar measurements (Reich *et al.*, 1998;

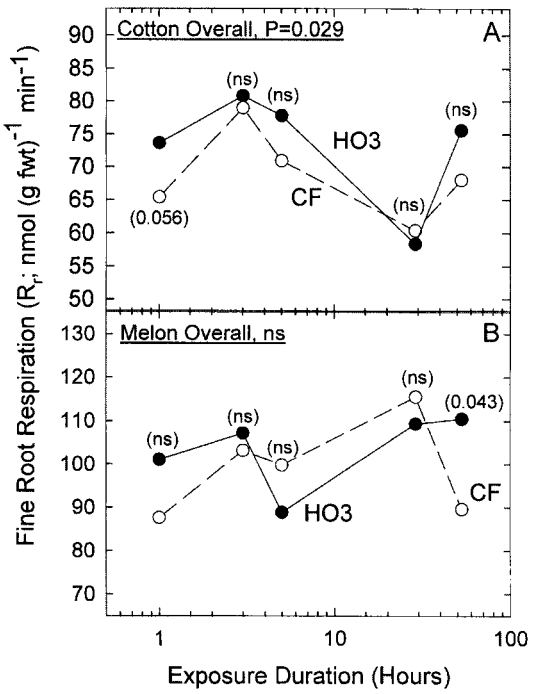


**Fig. 7.** Effect of acute (short-term) exposure to contrasting $O_3$ concentrations on respiration of fine roots on a fresh weight basis (A, cotton; B, melon). Mean ±SE. Note log scale for time axis.

Edwards, 1991; Gunn and Farrar, 1999; Singh and Blanke, 2000). These rates, measured in the terminal 3–4 cm of the root system, are representative of the whole fine root system (i.e. most of the roots in these young experimental plants) as suggested by the longitudinal mapping of $R_r$ along roots of peach (Bidel *et al.*, 2001).

The difficulty in resolving acute impacts of $O_3$ on $R_r$ was further explored through a test of the respirometry methodology. Responses of $R_r$ in cotton were contrasted following exposure to dark or sunlight. By contrast with the variable and modest increase in $R_r$ following $O_3$ exposure (Figs 6A, 7A), darkness clearly suppressed $R_r$ ($P$=0.04; Table 3), using the same techniques with the same species and a much smaller sample size ($n$=12 plants).

This reduction in $R_r$ was expected as a consequence of short-term reduction in export of CHO to the root system in darkened plants, and resulting substrate limitation of $R_r$ (Begna *et al.*, 2002; Dwivedi, 2000; Lambers *et al.*, 1996; Walters *et al.*, 1993). The contrast between these results and the opposing changes in $A_n$ and $R_r$ following $O_3$-exposure suggests that linkages other than substrate availability may operate at the whole plant level to mediate $O_3$ phytotoxicity. For example, effects of altered irradiance on $A_n$ may precede effects on $R_r$ (e.g. in *Lolium multiflorum*; Hansen and Jensen, 1977), suggesting a lag due to transport and temporal buffering due to utilization of stored CHO in sink respiration. In the present studies no consistent lag was observed between $O_3$ impacts on $A_n$ and

**Table 3.** *Effect of dark adaptation on fine root respiration of cotton* (n=*12*; *mean* ±*SE*)

| | Respiration (nmol $O_2$ $g^{-1}$ FW $s^{-1}$) | Significant difference |
|---|---|---|
| Sunlit | 128±12 | $P$=0.040 |
| Dark | 96±8 | |

on $R_r$, and the effects were in opposing directions. The hypothesized impact of substrate limitation caused by $O_3$ was not observed. No direct role of limitation of $R_r$ by substrate CHO is suggested.

The acute, short-term responses of $R_r$ are consistent with the conclusions from the longer term, chronic exposure experiments. This is particularly so in cotton, in which significant differences were observed within individual time-points and experiment-wise comparisons. These results are also supported by those of Scagel and Andersen (1997), who found consistent, though generally non-significant, increases in fine root respiration in Ponderosa pine growing in both high and low fertility media, particularly late in the growing season. Kelting *et al.* (1995) also observed a 50% enhancement of fine root respiration in $O_3$-exposed red oak. These effects derived from opposing non-significant effects of $O_3$ on respiration and biomass of roots. The current results are also consistent with the unpublished observations with rooted single leaves of Pima cotton (S Gunn and DA Grantz, unpublished data), in which acute exposure to $O_3$ (as described in Grantz and Farrar, 1999, 2000) consistently, but non-significantly, increased $R_r$.

### *Coupling of source strength and sink respiration*

The quantity of carbohydrate (CHO) available for export from source leaves to sink tissues such as fine roots is reduced by the direct and indirect impacts of $O_3$ on $A_n$, as well as by $O_3$-inhibited export of recent photosynthate from source leaves (Grantz and Farrar, 1999, 2000; Darrall, 1989). This suggests that $O_3$ could reduce $R_r$ in response to reduced substrate availability.

$O_3$ has been observed to reduce $R_r$ in some cases (Edwards, 1991; Hofstra *et al.*, 1981; Ito *et al.*, 1985; Gorisson and van Veen, 1988; Shan *et al.*, 1996). This might be attributed to reduced import of current photosynthate into respiring fine roots and resultant substrate limitation of $R_r$. Clearly, darkening cotton plants in the present study (Table 3) resulted in the rapid down-regulation of fine root respiration. Reich *et al.* (1998) found a strong correlation between $A_n$ and $R_r$ in a number of northern tree species, particularly when $A_n$ was expressed on a leaf mass basis. However, the addition of exogenous sucrose to roots did not reliably increase $R_r$ in several grass species (Gunn and Farrar, 1999), suggesting

that $R_r$ does not operate at the margin of substrate availability, except perhaps following a reduction in irradiance (Begna *et al.*, 2002).

In the present studies, $A_n$, a primary measure of current photosynthate available for export from source leaves to sink tissues such as fine roots, was significantly reduced by $O_3$ at 5 h and 29 h of exposure in cotton and at 3, 5, and 53 h in melon. It is clear that neither the time-course nor magnitude of $O_3$-induced reduction of current photosynthate ($A_n$) is correlated with any similar reduction in $R_r$ in either cotton or melon. Indeed, regression analysis (not shown) demonstrates a pronounced (non-significant) negative trend between $A_n$ and $R_r$ in both cotton and melon. The negative relationships describe both the control and $O_3$-treated plants. Inhibition of $A_n$ and the potential reduction in CHO available to roots does not appear to control $R_r$ over the initial period of exposure to $O_3$ (1–53 h) nor over the longer time scales of the chronic exposure experiments (5–6 weeks).

No mechanistic explanation is yet available for these opposing trends in $A_n$ and $R_r$ (i.e. in the present experiments and those of Scagel and Andersen, 1997; Kelting *et al.*, 1995). The simultaneous occurrence of increased $R_r$ and inhibited root growth in all these studies suggests that $O_3$ impacts on below-ground carbon storage and root system development could reflect diversion of CHO to $R_r$ at the expense of root growth. However, this conclusion raises the question of the initial mechanism of $O_3$-enhanced root respiratory activity.

The lack of positive correlation between $O_3$ effects on $A_n$ and $R_r$ suggests a non-substrate linkage between these two carbon fluxes, both of which are clearly affected by $O_3$. Following $O_3$ exposure, shoot-sourced phytohormones could signal leaf demand for nutrients to support foliar repair processes (Kelting *et al.*, 1995), potentially stimulating accelerated uptake of minerals for export to the foliage in xylem fluid. Similarly, the depletion of nutrients from the root tissues, reflecting enhanced utilization in the shoot, could stimulate nutrient uptake by roots. Both would increase $R_r$ (Singh and Blanke, 2000). An analogous response was observed following chronic (6 week) deficiency of $K^+$ in *Brassica oleracea* (Singh and Blanke, 2000), in which $R_r$ increased as root growth declined. However, in the case of $K^+$ deficiency the root:shoot biomass ratio increased, in contrast to the reduction observed following exposure to $O_3$ (Grantz and Yang, 1996).

Increased $R_r$ could reflect damage to root tissues associated with $O_3$ exposure of the shoot. No mechanism for such destructive linkage has been suggested. Nevertheless, a potential toxic response is suggested in at least one species (*Picea abies*) by observations of cytological damage in root meristems (Muller and Grill, 1994; Muller *et al.*, 1994*a*, *b*). The respiratory quotient ($Q$) may also reflect tissue damage. Scagel and Andersen (1997) observed an increase in $R_r$ following $O_3$ exposure in Ponderosa pine, accompanied by an increase in ($Q$). Carbon starvation of roots of *Zea mays* led to proteolysis, release of N, and increases of specific amino acids, particularly asparagine following dark-treatment (Brouquisse *et al.*, 1992, 1998), indicative of metabolic derangement. The extent to which this occurs following $O_3$ exposure is unknown.

Alternatively, the increase in $Q$ may indicate respiratory utilization of increasingly complex CHO substrates following $O_3$ exposure. It is noteworthy that in the present experiments $R_r$ increased following $O_3$ exposure in both the sucrose-transporting cotton and the predominant stachyose-transporting melon. Changes in the sugars involved in translocation from source leaves to respiring roots following exposure to $O_3$ have not been adequately addressed. Preliminary indications suggest that $O_3$ exposure shifts the soluble sugar pool in fine roots, by reducing the relative concentration of sucrose and increasing that of stachyose, in both cotton and melon (DA Grantz, unpublished observations). More mechanistic investigation of potential $O_3$ impacts on phloem loading and the resulting profiles of CHO in the phloem sap and in sink tissues may warrant further investigation.

## Conclusions

Chronic exposure to $O_3$ reduces carbon assimilation while increasing respiration in fine roots. Acute responses of plants transferred to $O_3$-containing environments demonstrate that these physiological responses occur within hours, and roughly in parallel in leaf and shoot. As these carbon fluxes ($A_n$ and $R_r$) are in opposing directions, a simple substrate linkage is unlikely. Reduced root growth and $O_3$-induced reductions in root:shoot biomass ratio are consistent with $O_3$-limitation of carbohydrate transport to developing roots. The increased respiratory activity may reflect linkage between shoot and root involving demand for nutrients, although this generally results in increased root:shoot ratio. Alternatively, the linkage may reflect changes in the sugar profiles available to support root respiration, possibly attributed to changes in phloem loading in source leaves. Yet another alternative is the transfer of phytotoxic materials from $O_3$-damaged leaves to roots, inducing damage and enhancing $R_r$ associated with repair mechanisms in roots. At present data are insufficient to resolve these possibilities.

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