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A Field System to Deliver Desired O₃ Concentrations in Leaf-level Gas Exchange Measurements: Results for Holm Oak Near a CO₂ Spring

By

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With 3 Figures

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Summary

GRULKE N. E. & PAOLETTI E. 2005. A field system to deliver desired O₃ concentrations in leaf-level gas exchange measurements: results for Holm oak near a CO₂ spring. – *Phyton* (Horn, Austria) 45 (1): 21–31, 3 figures. – English with German summary.

Conventional gas exchange systems adsorb ozone (O₃) despite attempts to saturate the system prior to measurements. A steady-state, open photosynthetic system was designed and used in the field to supply a small leaf cuvette with conditioned air stream (growth CO₂ concentrations, humidified, cooled) and augmented with either background or elevated O₃. Two innovations led to success: 1) supplying the cuvette with cooled air instead of Peltier cooling within the cuvette; and 2) using a custom-designed, low flow (capable of 100 sccm), fast response (20 s) O₃ monitor. We tested whether elevated CO₂ would alter stomatal response to short term, steady state elevated O₃. Holm oak (*Quercus ilex* L., *Fagaceae*), an evergreen broadleaf tree growing near a geothermal CO₂ vent, has been exposed over its lifetime to a gradient of CO₂ concentrations. We chose trees in areas averaging 450 µl l⁻¹ (low background CO₂) and 1500 µl l⁻¹ (super-elevated CO₂). Background O₃ exposure at this site is moderate (10 am to 5 pm averages of 62 nl l⁻¹ in June). We measured gas exchange at the growth CO₂ levels, and at ambient O₃ or 1.7x ambient O₃ concentrations. At low

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background CO₂, short term elevated O₃ depressed foliar transpiration. Because there was little concurrent change in net assimilation, instantaneous transpiration efficiency was increased. At super-elevated CO₂, short term elevated O₃ did not affect foliar transpiration. Because there was a concurrent decrease in net assimilation, instantaneous transpiration efficiency was decreased at elevated CO₂ and O₃.

Zusammenfassung

GRULKE N. E. & PAOLETTI E. 2005. Ein Freilandssystem, das gewünschte O₃ Konzentrationen für Gaswechsellmessungen an Blättern einstellt: Ergebnisse für Steineichen an einer CO₂ Quelle. – *Phyton* (Horn, Austria) 45 (1): 21–31, 3 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Konventionelle Gaswechsellmesssysteme absorbieren Ozon (O₃) trotz Versuchen das System vor den Messungen abzusättigen. Das hier getestete offene Photosynthesemesssystem versorgt eine kleine Blattkuvette mit Zuluft, die es entweder mit Außenkonzentrationen von O₃ oder erhöhtem O₃ anreichert. CO₂ Konzentrationen, Feuchtigkeit und Temperatur dieses Luftstroms werden kontrolliert. Zwei Neuerungen führten zum Erfolg: 1) Die Versorgung der Kuvette mit vorgekühlter Zuluft statt der Verwendung von Peltierkühlern direkt in der Kuvette; und 2) die Verwendung eines selbstentworfenen Ozonmessgerätes, das mit niedrigen Durchflussraten auskommt (100 cm³ s⁻¹) und kurze Ansprechzeiten hat (20 s). Wir testeten, ob erhöhtes CO₂ die Spaltöffnungsreaktionen der Blätter auf kurzzeitig, aber konstant erhöhtes O₃ ändert. Die neben einer geothermalen CO₂ Quelle wachsenden, immergrünen Steineichen (*Quercus ilex* L., *Fagaceae*) waren Zeit ihres Lebens einem Gradienten von CO₂ Konzentrationen ausgesetzt. Wir wählten Bäume an Standorten, wo die CO₂ Konzentrationen im Durchschnitt 450 µl l⁻¹ (niedrige Hintergrundkonzentration an CO₂) und 1500 µl l⁻¹ (stark erhöhtes CO₂) betrugen. Die O₃ Hintergrundkonzentration an diesem Ort ist moderat (ein 10:00 bis 17:00 h Tagesmittelwert von 62 nl l⁻¹ im Juni). Wir bestimmten die Gaswechselraten bei den entsprechenden Wachstums-CO₂-Konzentrationen kombiniert mit Hintergrundozon sowie 1.7x erhöhter Ozonkonzentration. Unter niedrigem Hintergrund-CO₂ führte kurzzeitig erhöhtes Ozon zu einer Verringerung der Transpiration der Blätter. Da gleichzeitig die Nettophotosynthese kaum variierte, war der momentane Wassernutzungskoeffizient erhöht. Bei stark erhöhtem CO₂ beeinflusste erhöhtes Ozon die Transpiration nicht. Da sich gleichzeitig die Nettophotosynthese verringert, war der momentane Wassernutzungskoeffizient unter erhöhtem O₃ und CO₂ niedriger.

Introduction

Considerable interest has been generated in understanding plant response to CO₂ enriched environments. However, little attention has been paid to understanding the effects of O₃ within the context of elevated CO₂ environments. Ozone has already doubled since pre-industrial times (HOUGHTON & al. 1996), and is expected to further increase 20 to 50% over the next 15 years (HOUGH & DERWENT 1990). The singular effects of CO₂ enrichment include increases in carbon acquisition and storage, decreases in water use, and altered within-plant nutrient (especially nitrogen) dynamics (HAILE-MARIAM & al. 2000). The singular effects of elevated O₃

include decreases in carbon acquisition and storage, decreases in water use, and altered within-plant carbon and nutrient dynamics (HEATH & TAYLOR 1997). The combined effects of elevated CO_2 and O_3 in general result in reduced stomatal conductance, with increased water use efficiency (WUE) in most cases (KELLOMÄKI & WANG 1997, LIPPERT & al. 1997, KELLOMÄKI & al. 1998, MANES & al. 1998, BROADMEADOW & al. 1999). Further generalities of their combined effects are difficult to make. Within the context of a CO_2 -enriched environment, O_3 may delay photosynthetic downregulation, or photosynthetic downregulation may be accelerated via limitation of sucrose export (GRANTZ & FARRAR 1999, 2000). If background O_3 is low to moderate, then we can expect a concomitant change in stomatal conductance with photosynthesis (WEBER & al. 1993). If background O_3 is higher, aberrations in stomatal behavior may make predictions of water balance difficult.

Conventional photosynthetic systems inadvertently scrub O_3 from the cuvette, so that during measurements there is little O_3 in the cuvette. Gas exchange measurements under these conditions describe long-term cumulative effects of O_3 , but in the temporary absence of the oxidant. A system was designed and employed in the field to measure leaf-level gas exchange characteristics under ambient ($\sim 46 \text{ nl l}^{-1}$) and elevated ($\sim 78 \text{ nl l}^{-1}$) O_3 concentrations. Two areas near a natural CO_2 vent near Lajatico, Italy were chosen for study of Holm oak (*Quercus ilex* L.) with lifetime exposure to super-elevated CO_2 ($1500 \mu\text{l l}^{-1}$) as well as lower CO_2 concentrations ($450 \mu\text{l l}^{-1}$).

Materials and Methods

Research was conducted in June 2003 in a 40–60 yr old coppiced Holm oak stand, growing over their lifetime to elevated CO_2 levels at Lajatico, Italy (lat. $43^\circ 26' \text{N}$, long. $10^\circ 42' \text{E}$; located 20 km SSW of Volterra and 60 km ESE of Pisa). The enriched area extends over 0.7 ha, with homogeneous pedo-climatic and canopy conditions (MIGLIETTA & al. 1993, TOGNETTI & al. 1998). Soils are non-calcareous, brown, loamy, clayey, developed from calcareous marl (pH 6–7), and similar across the area (RAIESI 1998). Associated species include *Quercus cerris*, *Q. pubescens*, *Fraxinus ornus*, *Arbutus unedo*, *Myrtus communis*, among others. The CO_2 vent is in the bed of a small river in the northwestern slope (20%) of a hill at about 200 m asl. Carbon dioxide concentrations decline uphill from the vent. Significant nocturnal accumulation of SO_2 as reported for some CO_2 springs do not occur at Lajatico (POLLE & al. 2001). The emitted gas is contaminated with sulphur-containing gases but do not appear to influence S metabolism significantly (SCHULTE & al. 2002). H_2S and SO_2 concentrations reach maximum values of 60 and 4 nl l^{-1} , respectively, which are low in comparison with other CO_2 springs.

Background CO_2 concentrations were established by TOGNETTI & al. 2000 and were supported by two weeks of continuous measurements one month prior to this experiment at the same scaffolding locations by means of an O_3 monitor (Dasibi Model 1108, Glendale, CA) positioned at the canopy level. Two locations were se-

lected where the mean CO₂ concentrations ranged around 450 (low-background CO₂) and 1500 (super-elevated CO₂) µl l⁻¹, at 130 and 5 m from the vent, respectively. Ozone concentrations recorded at the closest rural measurement station (Gabbro, located in a forest dominated by *Q. ilex* 23 km W of Lajatico) summed as an M8 value (mean of the hourly concentrations from 10 am to 5 pm) of 62 nl l⁻¹ O₃ in 1999–2003 (Chini, pers. comm.)

A custom-designed system was developed (Fig. 1) for this study because of the inability to experimentally maintain elevated O₃ in the cuvette of conventional gas exchange systems (models 6400 and 6200, LiCOR Instruments, Inc.; model 1, Ciras photosynthetic system, PP Systems, Herts, UK). Even in custom-made cuvettes, Peltier cooling blocks within the cuvette were particularly absorptive of O₃. When O₃ was supplied at high levels (600 nl l⁻¹), only 5% of the original value was measured in the air from the cuvette. Another problem encountered with Peltier cooling blocks was scavenging of O₃ by water vapor condensing onto the cooling surfaces, although surfaces were Teflon coated. This problem was solved by air conditioning the air stream just prior to the cuvette. In the new system, the O₃ concentration of outgoing air was 66 ± 1% (S.D.) at the elevated CO₂ concentration and 68 ± 3% at the ambient O₃ concentration that of incoming air with no leaf in the cuvette using two carefully calibrated, low flow, fast response O₃ monitors. Other custom-designed leaf gas exchange systems to maximize cuvette O₃ concentrations have been designed and utilized for laboratory conditions (PASQUALINI & al. 2002).

Air was supplied to the system from a pressurized tank of either 450 or 1500 µl l⁻¹ CO₂ – i.e. the average ambient CO₂ concentrations at the two locations – at approximately 1 l min⁻¹. Air was humidified in a dewpoint generator (model 610, LiCOR Instruments, Inc., Lincoln, NE), and a small flow of O₃ (20–60 ml min⁻¹) was added to the air stream using a mass flow meter (GFC171, Aalborg Instruments, Orangeburg, NY) to maintain desired O₃ concentrations in the reference airline. Ozone was generated (Model Heliozon, Helios Italquartz, Milano, Italy) from ambient air and did

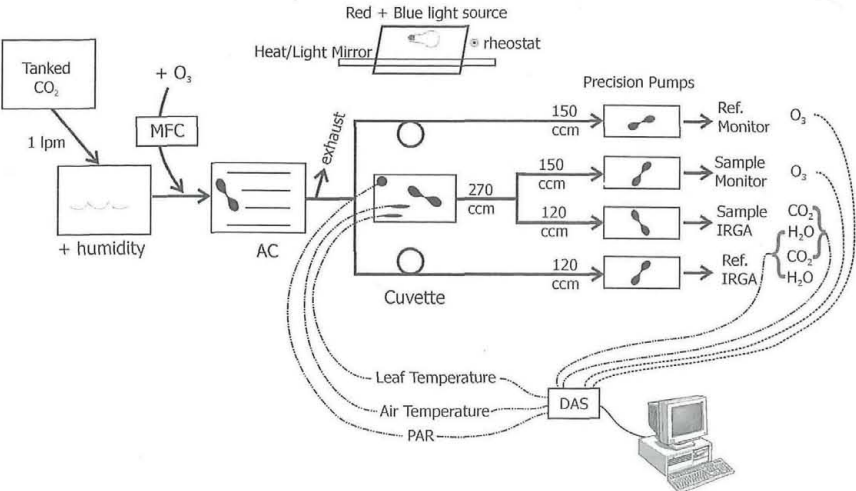


Fig. 1. Diagram of gas exchange system with O₃ control.

not have detectable production of NO_x (Environement AC 31M). The O_3 -enriched, humidified air was passed to a custom-built, Teflon-lined, sealed box made of acrylite, cooled with an electronically regulated Peltier block, insulated with reflective bubble-pack. Mixing of the air within the air conditioner was ensured using a small fan downstream from the inlet and parallel in flow to Peltier fins.

From the air conditioner, air was split into three flows: 150 sccm to a reference O_3 monitor (modified for low flow rate, and fast response time (20 s), 2B Technology, Boulder, CO); 200 sccm to the reference side of a $\text{CO}_2/\text{H}_2\text{O}$ infrared gas analyzer (IRGA, model 6262, LiCOR Instruments); and 200 sccm to the cuvette, which then passed to the sample side of the $\text{CO}_2/\text{H}_2\text{O}$ IRGA. The flows were kept constant between the reference and sample side of the IRGA using matched, calibrated, miniature precision pumps (Sensidyne Instruments, Clearwater, FL) controlled with a custom-made voltage regulator. A 75 cc, Teflon-lined acrylite cuvette had a small, low vibration, variable speed fan for mixing the air, and both leaf and air thermocouples (type T, copper-constantan). Because there was only one cuvette, the sample air was delayed by 25 s relative to the reference line; however, the responses reported here were steady state and in equilibrium for at least 0.5 hr at $450 \mu\text{l l}^{-1} \text{CO}_2$ and at least 1.0 h at $1500 \mu\text{l l}^{-1} \text{CO}_2$ prior to recording, so the time lag was not considered important. A miniature light sensor (Hamamatsu Corp., Bridgewater, NJ) with heat sink was mounted just outside the cuvette, calibrated against a calibrated light sensor (model 190s, LiCOR Instruments). Teflon tubing and Teflon or stainless steel swagelock connectors (San Diego Pipe and Fitting, CA) were used throughout the system.

Low background CO_2 -grown foliage was measured at low CO_2 concentrations in the cuvette, with either low or elevated O_3 . All leaves were measured at low O_3 first, O_3 was adjusted to the elevated level, flows balanced, and the same leaves were re-measured at elevated O_3 . Super-elevated CO_2 -grown foliage was measured at high CO_2 concentrations in the cuvette, with either low or elevated O_3 . All gas exchange measurements were made on 1 yr old sun leaves, near the top of the canopy, accessed with a 7 m scaffold. Because the light environment of adjacent branches can affect stomatal conductance of a branch even in full shade, all gas exchange measurements were made at night between 11 pm and 5 am. Making measurements at night also minimized the effects of endogenous changes in foliar respiration through the day. Foliar respiration rates of Holm oak did not change significantly from late night to pre-dawn. Light was supplied by a multimirror projection lamp (GE ESD, Chicago, IL), and passed through a parallel heat/light separation mirror (OCLI, Santa Rosa, CA) and acrylite diffuser before reaching the cuvette. Photosynthetic photon flux density was maintained at $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$, and leaves were allowed to equilibrate for 1 hr at this light level prior to insertion into the cuvette. Leaf temperature ranged from 22° to 27°C . Leaf to air vapor pressure deficits were maintained at 2.2 to 2.6 kPa. Ambient O_3 concentrations were supplied at 68 nl l^{-1} and the cuvette outgoing air concentration averaged 46 nl l^{-1} . Elevated O_3 concentrations were supplied at 119 nl l^{-1} and cuvette outgoing air concentration averaged 78 nl l^{-1} .

Analog signals from the ozone monitor, $\text{CO}_2/\text{H}_2\text{O}$ IRGA, thermocouples, and light sensors were connected to a data logger (model 21x, Campbell Scientific, Inc., Logan, UT), printed to the screen, and stored every 10 s on a laptop computer. Ambient relative humidity and temperature (external to the cuvette, within the canopy) were also monitored within the canopy with the data logger.

Differences between CO_2 and O_3 levels were tested with a 2-way analysis of variance (S-Plus 2000). The effects of short term elevated O_3 on foliar instantaneous transpiration efficiency (assimilation versus transpiration) were tested between lines for colinearity (DRAPER & SMITH 1981). Significance is reported at the $P < 0.05$ level.

Results & Discussion

Net assimilation (A) was unaffected by elevated O_3 (78 nl l^{-1}) at the low background CO_2 concentration ($450 \mu\text{l l}^{-1}$) (Fig. 2): there was no statistical significance in assimilation measured at the two O_3 levels. Relative to the lower CO_2 concentration, net assimilation was significantly greater ($p = 0.0001$) when measured at super-elevated CO_2 ($1500 \mu\text{l l}^{-1}$) and ambient O_3 (46 nl l^{-1}). The stimulatory effects of high CO_2 was not as great when combined with elevated O_3 (2 way analysis of variance, $\text{CO}_2 \times \text{O}_3$; $p = 0.006$). The canopy foliage was exposed to super-elevated CO_2 on average, but concentrations were highly variable. If the foliage had been in equilibrium with $1500 \mu\text{l l}^{-1}$, then assimilation would have been identical to that taken at $450 \mu\text{l l}^{-1}$ with low O_3 .

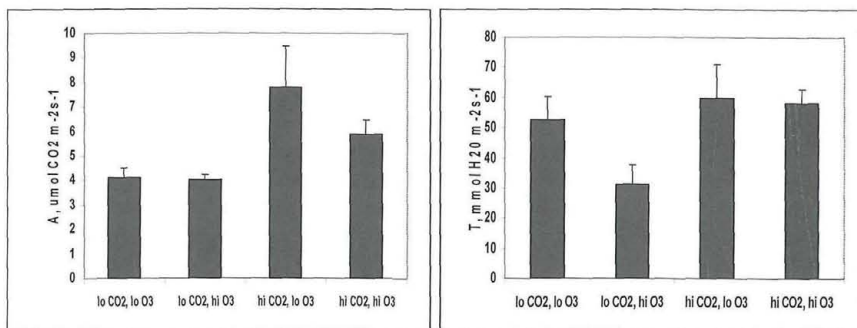


Fig. 2. Net assimilation (left) and foliar transpiration (right) in growth CO_2 concentration (lo = 450 or hi = $1500 \mu\text{l l}^{-1}$) and at ambient (lo = 46 nl l^{-1}) or elevated (hi = 78 nl l^{-1}) O_3 concentrations.

In the Aspen-FACE study, the elevated CO_2 and O_3 treatment had the highest photosynthetic rate, growth, and stomatal conductance of all treatments despite that the two gases singly enhanced (CO_2) and reduced (O_3) gas exchange (NOORMETS & al. 2001). Their experiment combined both sensitive and tolerant aspen clones. The tolerant clone was not more sensitive to O_3 at elevated CO_2 than the sensitive clone. Their elevated CO_2 concentrations were 24% higher than our low background CO_2 level, and their elevated O_3 concentrations were 30% lower than ours. Noormets' results were supported in another study of aspen: high photosynthetic response in combined elevated CO_2 and O_3 (VOLIN & al. 1998).

Although the high CO₂ concentrations do not represent any expected in the next two centuries, we intended the comparisons only to provide insight on how plants equilibrated to differing background levels of CO₂ could respond to short term elevated O₃ exposure. Besides CO₂ vents, super-elevated CO₂ concentrations can be found in tightly sealed growth chambers or greenhouses at the end of dark cycles, near disturbed landfill sites with buried refuse, and in space shuttle cabins (as summarized in WHEELER & al. 1999). However, elevating CO₂ beyond 1000 µl l⁻¹ has been found to promote little further carbon gain, and its overall effect detrimental (VAN BERKAL 1984).

Foliar transpiration (T) was reduced 20% by elevated O₃ at low background CO₂ (450 µl l⁻¹) (p = 0.083), but was unaffected by elevated O₃ at super-elevated CO₂ concentrations (Fig. 2). In this study, lower transpiration in elevated O₃ was not accompanied by reduced A, so that instantaneous transpirational efficiency was increased (p = 0.016). At super-elevated CO₂ concentrations, foliar transpiration was similar to that measured at background CO₂ and O₃. There was a strong increase in instantaneous transpiration efficiency from low to elevated CO₂ at low background O₃ levels (slope of the regression line, A/T, differed significantly (p = 0.03); Fig. 3), as expected. Elevated O₃ at low CO₂ concentration significantly altered y-intercept of the regression line, but slopes did not significantly differ. Despite low variance about the regression lines in super-elevated CO₂ between background and elevated O₃, too few leaves were measured to statistically distinguish between the two lines (test of coincidence, p = 0.14), but differed significantly when a 2-way analysis of variance was applied (see above). Despite constant CO₂ in the supply air stream, and using the same leaf age, a wide range in absolute rates of assimilation and foliar transpiration were obtained in the super-elevated CO₂ site.

Elevated O₃ generally reduces *gs* due to concomitant decrease in A (WEBER & al. 1993). *Pinus ponderosa* seedlings grown in null-balance mesocosms for 3 years had a net response of increased instantaneous WUE with CO₂ enrichment alone, decreased WUE in elevated O₃ alone, and a loss of WUE with both elevated O₃ and CO₂ (OLSZYK & al. 2002). The CO₂-enrichment in their study was approximately 710 µl l⁻¹, 60% greater than our low background CO₂ concentrations, and their high O₃ treatment was 30% greater than our elevated O₃ treatment. In our experiment, the opposite effect was found at low CO₂ with elevated O₃. Due to reduced foliar transpiration combined with no net change in assimilation, instantaneous transpiration efficiency was increased by elevated O₃ at low (450 µl l⁻¹) background CO₂. Elevated O₃ in our super-elevated CO₂ growth environment responded similarly to OLSZYK & al.'s 2002 elevated CO₂ × O₃ responses despite the difference in concentrations of CO₂ between the two

experiments: elevated O_3 reduced the increase in WUE with elevated CO_2 . Ellsworth 1999 reported no significant change in WUE for a successional, mixed conifer/hardwood forest. Elevated CO_2 levels at the Duke-FACE site were 25% higher than our low background CO_2 level and background O_3 is approximately 30% greater than those at Lajatico, Italy (based on regional O_3 concentrations, E.H. Lee, U.S. Environmental Protection Agency, unpubl. data). Three lines of evidence were used: leaf-level g_s , pre-dawn and mid-day leaf water potentials and components, and soil moisture content. This result held for both drought and non-drought periods. However, their stand was still stimulated by elevated CO_2 and our stand, at least at the background lower CO_2 level ($450 \mu l l^{-1}$) was likely equilibrated on the long-term.

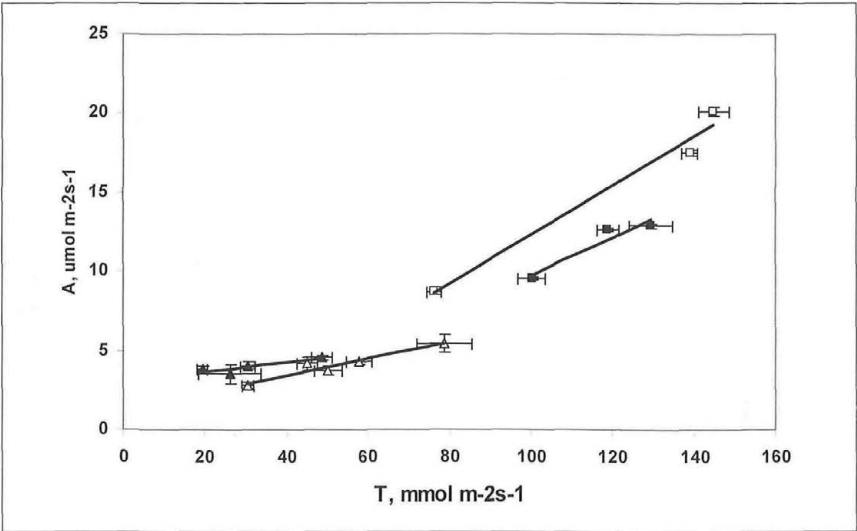


Fig. 3. Instantaneous transpiration efficiency of foliage growth CO_2 concentrations and at ambient or elevated O_3 concentrations. Squares: high CO_2 ($1500 \mu l l^{-1}$); Triangles: low CO_2 ($450 \mu l l^{-1}$); Open symbols: low O_3 (48 nl l^{-1}); Closed symbols: high O_3 (78 nl l^{-1})

Conclusion

For this oak forest stand, growing over its lifetime to elevated CO_2 comparable to many $1.5\times$ CO_2 scenarios ($500 \mu l l^{-1}$), O_3 concentrations over the range of 46 to 78 nl l^{-1} did not affect assimilation rate. Due to depressed foliar transpiration, however, instantaneous transpiration efficiency increased, not decreased, in response to concurrent elevated O_3 . Although differing in magnitude from fluctuations in CO_2 control in free-air CO_2 enrichment systems, the super-elevated CO_2 growth environment of the

CO₂ vent also had widely fluctuating CO₂ concentrations. Lack of stomatal responsiveness to elevated O₃ in super-elevated CO₂ environment, however, is consistent with doubled-CO₂ enrichment response to other environmental stressors such as leaf to air vapor pressure deficits and drought (HEATH 1998). Elevated O₃ exposure for plants acclimated to super-elevated CO₂ appeared to reduce leaf-level instantaneous transpiration efficiency.

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