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## A Field System to Deliver Desired O<sub>3</sub> Concentrations in Leaf-level Gas Exchange Measurements: Results for Holm Oak Near a CO<sub>2</sub> 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 O<sub>3</sub> concentrations in leaf-level gas exchange measurements: results for Holm oak near a CO<sub>2</sub> spring. – *Phyton* (Horn, Austria) 45 (1): 21–31, 3 figures. – English with German summary.

Conventional gas exchange systems adsorb ozone (O<sub>3</sub>) 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 CO<sub>2</sub> concentrations, humidified, cooled) and augmented with either background or elevated O<sub>3</sub>. 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) O<sub>3</sub> monitor. We tested whether elevated CO<sub>2</sub> would alter stomatal response to short term, steady state elevated O<sub>3</sub>. Holm oak (*Quercus ilex* L., *Fagaceae*), an evergreen broadleaf tree growing near a geothermal CO<sub>2</sub> vent, has been exposed over its lifetime to a gradient of CO<sub>2</sub> concentrations. We chose trees in areas averaging 450 µl l<sup>-1</sup> (low background CO<sub>2</sub>) and 1500 µl l<sup>-1</sup> (super-elevated CO<sub>2</sub>). Background O<sub>3</sub> exposure at this site is moderate (10 am to 5 pm averages of 62 nl l<sup>-1</sup> in June). We measured gas exchange at the growth CO<sub>2</sub> levels, and at ambient O<sub>3</sub> or 1.7x ambient O<sub>3</sub> concentrations. At low

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background CO<sub>2</sub>, short term elevated O<sub>3</sub> depressed foliar transpiration. Because there was little concurrent change in net assimilation, instantaneous transpiration efficiency was increased. At super-elevated CO<sub>2</sub>, short term elevated O<sub>3</sub> did not affect foliar transpiration. Because there was a concurrent decrease in net assimilation, instantaneous transpiration efficiency was decreased at elevated CO<sub>2</sub> and O<sub>3</sub>.

### Zusammenfassung

GRULKE N. E. & PAOLETTI E. 2005. Ein Freilandssystem, das gewünschte O<sub>3</sub> Konzentrationen für Gaswechsellmessungen an Blättern einstellt: Ergebnisse für Steineichen an einer CO<sub>2</sub> Quelle. – *Phyton* (Horn, Austria) 45 (1): 21–31, 3 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Konventionelle Gaswechsellmesssysteme absorbieren Ozon (O<sub>3</sub>) trotz Versuchen das System vor den Messungen abzusättigen. Das hier getestete offene Photosynthesemesssystem versorgt eine kleine Blattküvette mit Zuluft, die es entweder mit Außenkonzentrationen von O<sub>3</sub> oder erhöhtem O<sub>3</sub> anreichert. CO<sub>2</sub> Konzentrationen, Feuchtigkeit und Temperatur dieses Luftstroms werden kontrolliert. Zwei Neuerungen führten zum Erfolg: 1) Die Versorgung der Küvette mit vorgekühlter Zuluft statt der Verwendung von Peltierkühlern direkt in der Küvette; und 2) die Verwendung eines selbstentworfenen Ozonmessgerätes, das mit niedrigen Durchflussraten auskommt (100 cm<sup>3</sup> s<sup>-1</sup>) und kurze Ansprechzeiten hat (20 s). Wir testeten, ob erhöhtes CO<sub>2</sub> die Spaltöffnungsreaktionen der Blätter auf kurzzeitig, aber konstant erhöhtes O<sub>3</sub> ändert. Die neben einer geothermalen CO<sub>2</sub> Quelle wachsenden, immergrünen Steineichen (*Quercus ilex* L., *Fagaceae*) waren Zeit ihres Lebens einem Gradienten von CO<sub>2</sub> Konzentrationen ausgesetzt. Wir wählten Bäume an Standorten, wo die CO<sub>2</sub> Konzentrationen im Durchschnitt 450 µl l<sup>-1</sup> (niedrige Hintergrundkonzentration an CO<sub>2</sub>) und 1500 µl l<sup>-1</sup> (stark erhöhtes CO<sub>2</sub>) betragen. Die O<sub>3</sub> Hintergrundkonzentration an diesem Ort ist moderat (ein 10:00 bis 17:00 h Tagesmittelwert von 62 nl l<sup>-1</sup> im Juni). Wir bestimmten die Gaswechselraten bei den entsprechenden Wachstums-CO<sub>2</sub>-Konzentrationen kombiniert mit Hintergrundozon sowie 1.7x erhöhter Ozonkonzentration. Unter niedrigem Hintergrund-CO<sub>2</sub> führte kurzzeitig erhöhtes Ozon zu einer Verringerung der Transpiration der Blätter. Da gleichzeitig die Nettphotosynthese kaum variierte, war der momentane Wassernutzungskoeffizient erhöht. Bei stark erhöhtem CO<sub>2</sub> beeinflusste erhöhtes Ozon die Transpiration nicht. Da sich gleichzeitig die Nettphotosynthese verringert, war der momentane Wassernutzungskoeffizient unter erhöhtem O<sub>3</sub> und CO<sub>2</sub> niedriger.

### Introduction

Considerable interest has been generated in understanding plant response to CO<sub>2</sub> enriched environments. However, little attention has been paid to understanding the effects of O<sub>3</sub> within the context of elevated CO<sub>2</sub> 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 CO<sub>2</sub> 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 O<sub>3</sub>

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  $\text{CO}_2$  and  $\text{O}_3$  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  $\text{CO}_2$ -enriched environment,  $\text{O}_3$  may delay photosynthetic downregulation, or photosynthetic downregulation may be accelerated via limitation of sucrose export (GRANTZ & FARRAR 1999, 2000). If background  $\text{O}_3$  is low to moderate, then we can expect a concomitant change in stomatal conductance with photosynthesis (WEBER & al. 1993). If background  $\text{O}_3$  is higher, aberrations in stomatal behavior may make predictions of water balance difficult.

Conventional photosynthetic systems inadvertently scrub  $\text{O}_3$  from the cuvette, so that during measurements there is little  $\text{O}_3$  in the cuvette. Gas exchange measurements under these conditions describe long-term cumulative effects of  $\text{O}_3$ , 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 ( $\sim 78 \text{ nl l}^{-1}$ )  $\text{O}_3$  concentrations. Two areas near a natural  $\text{CO}_2$  vent near Lajatico, Italy were chosen for study of Holm oak (*Quercus ilex* L.) with lifetime exposure to super-elevated  $\text{CO}_2$  ( $1500 \mu\text{l l}^{-1}$ ) as well as lower  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{SO}_2$  as reported for some  $\text{CO}_2$  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).  $\text{H}_2\text{S}$  and  $\text{SO}_2$  concentrations reach maximum values of 60 and  $4 \text{ nl l}^{-1}$ , respectively, which are low in comparison with other  $\text{CO}_2$  springs.

Background  $\text{CO}_2$  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  $\text{O}_3$  monitor (Dasibi Model 1108, Glendale, CA) positioned at the canopy level. Two locations were se-

lected where the mean  $\text{CO}_2$  concentrations ranged around 450 (low-background  $\text{CO}_2$ ) and 1500 (super-elevated  $\text{CO}_2$ )  $\mu\text{l l}^{-1}$ , 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  $\text{nl l}^{-1}$   $\text{O}_3$  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  $\text{O}_3$  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  $\text{O}_3$ . When  $\text{O}_3$  was supplied at high levels (600  $\text{nl l}^{-1}$ ), only 5% of the original value was measured in the air from the cuvette. Another problem encountered with Peltier cooling blocks was scavenging of  $\text{O}_3$  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  $\text{O}_3$  concentration of outgoing air was  $66 \pm 1\%$  (S.D.) at the elevated  $\text{CO}_2$  concentration and  $68 \pm 3\%$  at the ambient  $\text{O}_3$  concentration that of incoming air with no leaf in the cuvette using two carefully calibrated, low flow, fast response  $\text{O}_3$  monitors. Other custom-designed leaf gas exchange systems to maximize cuvette  $\text{O}_3$  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  $\mu\text{l l}^{-1}$   $\text{CO}_2$  – i.e. the average ambient  $\text{CO}_2$  concentrations at the two locations – at approximately 1  $\text{l min}^{-1}$ . Air was humidified in a dewpoint generator (model 610, LiCOR Instruments, Inc., Lincoln, NE), and a small flow of  $\text{O}_3$  (20–60  $\text{ml min}^{-1}$ ) was added to the air stream using a mass flow meter (GFC171, Aalborg Instruments, Orangeburg, NY) to maintain desired  $\text{O}_3$  concentrations in the reference airline. Ozone was generated (Model Heliozon, Helios Italquartz, Milano, Italy) from ambient air and did

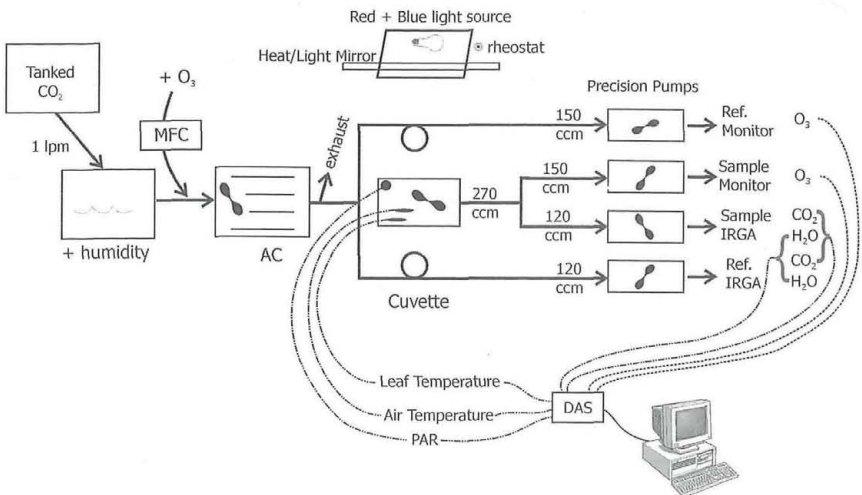


Fig. 1. Diagram of gas exchange system with  $\text{O}_3$  control.

not have detectable production of  $\text{NO}_x$  (Environment AC 31M). The  $\text{O}_3$ -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  $\text{O}_3$  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  $\text{CO}_2$ -grown foliage was measured at low  $\text{CO}_2$  concentrations in the cuvette, with either low or elevated  $\text{O}_3$ . All leaves were measured at low  $\text{O}_3$  first,  $\text{O}_3$  was adjusted to the elevated level, flows balanced, and the same leaves were re-measured at elevated  $\text{O}_3$ . Super-elevated  $\text{CO}_2$ -grown foliage was measured at high  $\text{CO}_2$  concentrations in the cuvette, with either low or elevated  $\text{O}_3$ . 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  $22^\circ$  to  $27^\circ \text{C}$ . Leaf to air vapor pressure deficits were maintained at 2.2 to 2.6 kPa. Ambient  $\text{O}_3$  concentrations were supplied at  $68 \text{ nl l}^{-1}$  and the cuvette outgoing air concentration averaged  $46 \text{ nl l}^{-1}$ . Elevated  $\text{O}_3$  concentrations were supplied at  $119 \text{ nl l}^{-1}$  and cuvette outgoing air concentration averaged  $78 \text{ nl l}^{-1}$ .

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  $\text{CO}_2$  and  $\text{O}_3$  levels were tested with a 2-way analysis of variance (S-Plus 2000). The effects of short term elevated  $\text{O}_3$  on foliar instantaneous transpiration efficiency (assimilation versus transpiration) were tested between lines for colinearity (DRAFER & SMITH 1981). Significance is reported at the  $P < 0.05$  level.

### Results & Discussion

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

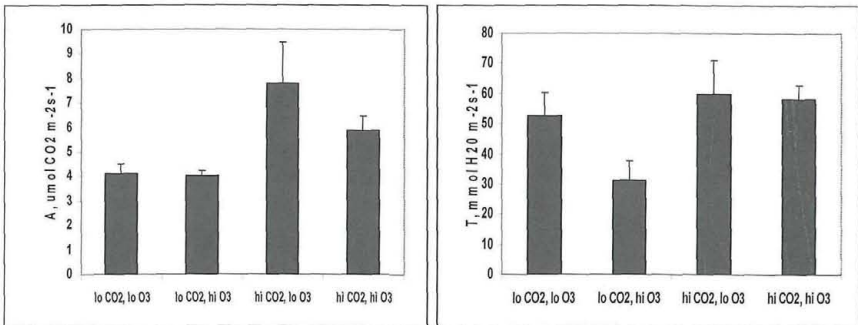


Fig. 2. Net assimilation (left) and foliar transpiration (right) in growth  $\text{CO}_2$  concentration (lo =  $450$  or hi =  $1500 \mu\text{l l}^{-1}$ ) and at ambient (lo =  $46 \text{ nl l}^{-1}$ ) or elevated (hi =  $78 \text{ nl l}^{-1}$ )  $\text{O}_3$  concentrations.

In the Aspen-FACE study, the elevated  $\text{CO}_2$  and  $\text{O}_3$  treatment had the highest photosynthetic rate, growth, and stomatal conductance of all treatments despite that the two gases singly enhanced ( $\text{CO}_2$ ) and reduced ( $\text{O}_3$ ) gas exchange (NOORMETS & al. 2001). Their experiment combined both sensitive and tolerant aspen clones. The tolerant clone was not more sensitive to  $\text{O}_3$  at elevated  $\text{CO}_2$  than the sensitive clone. Their elevated  $\text{CO}_2$  concentrations were 24% higher than our low background  $\text{CO}_2$  level, and their elevated  $\text{O}_3$  concentrations were 30% lower than ours. Noormets' results were supported in another study of aspen: high photosynthetic response in combined elevated  $\text{CO}_2$  and  $\text{O}_3$  (VOLIN & al. 1998).

Although the high CO<sub>2</sub> 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 CO<sub>2</sub> could respond to short term elevated O<sub>3</sub> exposure. Besides CO<sub>2</sub> vents, super-elevated CO<sub>2</sub> 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 CO<sub>2</sub> beyond 1000 µl l<sup>-1</sup> 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 O<sub>3</sub> at low background CO<sub>2</sub> (450 µl l<sup>-1</sup>) (p = 0.083), but was unaffected by elevated O<sub>3</sub> at super-elevated CO<sub>2</sub> concentrations (Fig. 2). In this study, lower transpiration in elevated O<sub>3</sub> was not accompanied by reduced A, so that instantaneous transpirational efficiency was increased (p = 0.016). At super-elevated CO<sub>2</sub> concentrations, foliar transpiration was similar to that measured at background CO<sub>2</sub> and O<sub>3</sub>. There was a strong increase in instantaneous transpiration efficiency from low to elevated CO<sub>2</sub> at low background O<sub>3</sub> levels (slope of the regression line, A/T, differed significantly (p = 0.03); Fig. 3), as expected. Elevated O<sub>3</sub> at low CO<sub>2</sub> 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 CO<sub>2</sub> between background and elevated O<sub>3</sub>, 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 CO<sub>2</sub> 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 CO<sub>2</sub> site.

Elevated O<sub>3</sub> generally reduces *g<sub>s</sub>* 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 CO<sub>2</sub> enrichment alone, decreased WUE in elevated O<sub>3</sub> alone, and a loss of WUE with both elevated O<sub>3</sub> and CO<sub>2</sub> (OLSZYK & al. 2002). The CO<sub>2</sub>-enrichment in their study was approximately 710 µl l<sup>-1</sup>, 60% greater than our low background CO<sub>2</sub> concentrations, and their high O<sub>3</sub> treatment was 30% greater than our elevated O<sub>3</sub> treatment. In our experiment, the opposite effect was found at low CO<sub>2</sub> with elevated O<sub>3</sub>. Due to reduced foliar transpiration combined with no net change in assimilation, instantaneous transpiration efficiency was increased by elevated O<sub>3</sub> at low (450 µl l<sup>-1</sup>) background CO<sub>2</sub>. Elevated O<sub>3</sub> in our super-elevated CO<sub>2</sub> growth environment responded similarly to OLSZYK & al.'s 2002 elevated CO<sub>2</sub> × O<sub>3</sub> responses despite the difference in concentrations of CO<sub>2</sub> 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.

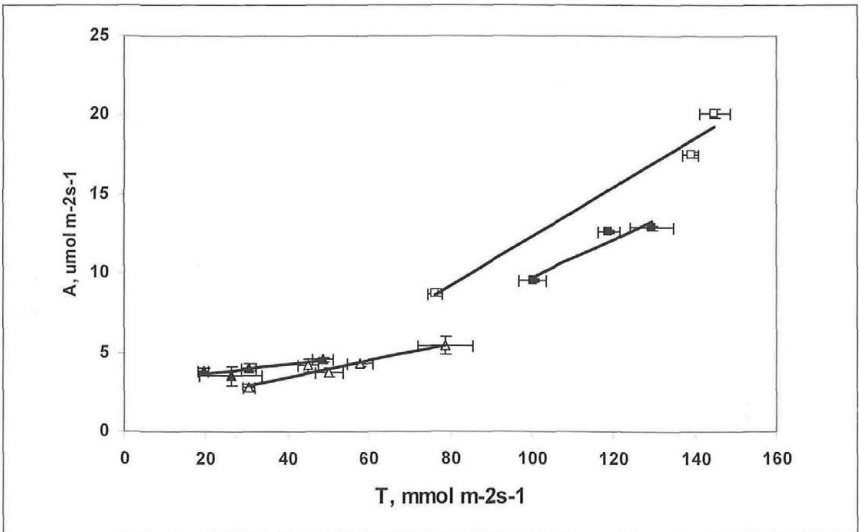


Fig. 3. Instantaneous transpiration efficiency of foliage growth  $CO_2$  concentrations and at ambient or elevated  $O_3$  concentrations. Squares: high  $CO_2$  ( $1500 \mu l l^{-1}$ ); Triangles: low  $CO_2$  ( $450 \mu l l^{-1}$ ); Open symbols: low  $O_3$  ( $48 \text{ nl l}^{-1}$ ); Closed symbols: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

### Conclusion

For this oak forest stand, growing over its lifetime to elevated  $CO_2$  comparable to many  $1.5x$   $CO_2$  scenarios ( $500 \mu l l^{-1}$ ),  $O_3$  concentrations over the range of 46 to  $78 \text{ nl l}^{-1}$  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



CO<sub>2</sub> vent also had widely fluctuating CO<sub>2</sub> concentrations. Lack of stomatal responsiveness to elevated O<sub>3</sub> in super-elevated CO<sub>2</sub> environment, however, is consistent with doubled-CO<sub>2</sub> enrichment response to other environmental stressors such as leaf to air vapor pressure deficits and drought (HEATH 1998). Elevated O<sub>3</sub> exposure for plants acclimated to super-elevated CO<sub>2</sub> appeared to reduce leaf-level instantaneous transpiration efficiency.

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