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Comparison of calculated and measured foliar O₃ flux in crop and forest species

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Using a new system to concurrently measure H₂O, O₃, and CO₂ flux, the conventional method of calculating O₃ flux generally overestimated direct measures by 25–50%.

Abstract

We designed a new gas exchange system that concurrently measures foliar H₂O, O₃, and CO₂ flux (HOC flux system) while delivering known O₃ concentrations. Stomatal responses of three species were tested: snapbean, and seedlings of California black oak (deciduous broadleaf) and blue oak (evergreen broadleaf). Acute O₃ exposure (120–250 ppb over an hour) was applied under moderate light and low vapor pressure deficits during near steady state conditions. The rate of stomatal closure was measured when the whole plant was placed in the dark. An adjacent leaf on each plant was also concurrently measured in an O₃-free cuvette. Under some conditions, direct measurements and calculated foliar O₃ flux were within the same order of magnitude; however, endogenously low gs or O₃ exposure-induced depression of gs resulted in an overestimation of calculated O₃ fluxes compared with measured O₃ fluxes. Sluggish stomata in response to light extinction with concurrent O₃ exposure, and incomplete stomatal closure likewise underestimated measured O₃ flux.

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Keywords: Ozone uptake; Ozone flux; *Phaseolus vulgaris*; *Quercus kelloggii*; *Quercus douglasii*

1. Introduction

In the level II approach of the critical levels concept of the UN-ECE (Fuhrer et al., 1997), a flux-based measurement rather than a foliar O₃ exposure metric has been proposed to set regulatory limits for ambient O₃ concentrations. Leaf-level O₃ flux is calculated using leaf conductance (gs) and hourly O₃ concentration, with a multiplying constant to accommodate the difference in diffusivity between water vapor and O₃. O₃ flux, expressed on a leaf surface area or an individual stoma value, is the regulatory entity. For flux calculation, the bulk air O₃ concentration is used as that concentration next to the

stomata, assuming that the internal O₃ concentration is zero. This approach ignores the leaf boundary layer, and the possibility that O₃ breaks down within the boundary layer.

The question of which leaf conductance to use in the calculation is debatable: gs measured before or after exposure, an average over the day, or an hourly gs value multiplied by an hourly average O₃ concentration in the air could be used. In fact, the Emberson et al. (2000) model uses maximum assimilation (A) data, and the idealized relationship between A and gs (Farquhar and Sharkey, 1982) to estimate maximum gs. In the model, this constructed gs is modified by light levels, temperature, vapor pressure deficit, soil moisture deficit, and phenology, which is then used to calculate flux (as the regulatory entity) using hourly average O₃ concentrations. Although the model helps establish regions at risk, our concern is that known effects of O₃ exposure on stomatal behavior will result

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in significant error in the regulatory entity, and the predictive capability of the model with increases in global O₃ concentrations (Hough and Derwent, 1990) will deteriorate through time.

Ozone exposure modifies stomatal conductance. Aberrant stomatal behavior with O₃ exposure was first reported two decades ago, when both Reich and Lassoie (1984 for poplar, *Populus deltoides* × *trichocarpa*) and Keller and Häslér (1984 for Norway spruce, *Picea abies* (L.) Karst, and white fir, *Abies alba* Miller) described ‘sluggish’ stomatal responses to changes in light. Several authors have continued to report on this response as well as O₃-induced sluggish response to changes in vapor pressure deficits (sugar maple, *Acer saccharum* Marsh, Tjoelker et al., 1995; Scots pine, *Pinus sylvestris* L., Kellomäki and Wang, 1997). Stomatal aberrations can last up to 10 days after chronic O₃ exposure has ceased (Paoletti, 2005).

Sluggish stomatal responses with O₃ exposure suggests an uncoupling of the normally tight relationship between A and gs (Paoletti and Grulke, 2005) that is assumed in most physiologically based modeling. Sluggish stomata are probably very slowly coming to (theoretical) equilibrium with A under steady-state conditions. However, such ‘steady state’ conditions are not common in the field and functionally, A cannot be considered to be tightly coupled with gs with chronic O₃ exposure or acute events. Incomplete stomatal closure can occur with moderate and above O₃ exposures (Wieser and Havranek, 1995; Matyssek et al., 1995), and can have a significant effect on total O₃ flux into the leaf. If the stomatal response is altered by exposure to O₃, then there may be large discrepancies in the regulatory entity as calculated, due to stomatal uptake of O₃.

There have been only three publications that report direct measurements of foliar O₃ flux (Laisk et al., 1989; Moldau and Bichele, 2002; Moldau et al., 1990). In their experiments, the high flow requirement of commercially available O₃ monitors was skirted by injecting the lower flow, exiting sample air into a higher flow ‘carrier’ gas to the O₃ monitor, much like one would use a gas chromatograph. In order to accurately detect the diluted sample air by the high flow O₃ monitor, a high O₃ concentration is required (0.2–2.0 ppm). In other publications, known O₃ concentrations have been supplied to the leaf cuvette, but the O₃ concentration of the exiting cuvette air was not measured (Pasqualini et al., 2002; Grulke and Paoletti, 2005): flux was only calculated under concurrent O₃ exposure, and not measured.

Commercial gas exchange systems adsorb almost all of O₃ supplied directly to the cuvette, and the majority of conductance measurements reported in the literature have not been measured concurrently with O₃ exposure. We designed and demonstrate here the use of a new gas exchange system that delivers known O₃ concentrations to leaves and concurrently measures foliar H₂O, O₃, and CO₂ flux (HOC flux system). Two custom, low-flow, fast-response O₃ monitors were designed for the purpose of maintaining O₃ at biologically realistic moderate (50 ppb) to acute (200+ ppb) O₃ levels in a leaf cuvette. The HOC system allows a comparison of calculated

and directly measured foliar O₃ flux. We have conducted measurements with concurrent O₃ exposure for three species: snapbean, California black oak, and blue oak—as a proof-of-concept. In this paper, the HOC system performance was evaluated, and stomatal behavior response to changes in light level with and without concurrent O₃ exposure are presented. The HOC system was used to elucidate some of conditions under which calculated O₃ flux (the regulatory entity) may not be representative, and differ from that of direct measures of foliar uptake.

2. Materials and methods

2.1. Choice of species

Three species in different physiognomic classes were chosen for flux measurements: an annual crop (an O₃-sensitive and an O₃-insensitive variety of snapbean, *Phaseolus vulgaris*), broadleaf deciduous tree seedlings (California black oak, *Quercus kelloggii*), and broadleaf evergreen tree seedlings (blue oak, *Quercus douglasii*). The snapbean O₃-sensitivity types were developed at the Raleigh USDA-ARS by Drs. Fitz Booker, Ed Fiscus, and Kent Burkley. In their studies, the sensitive phenotype had greater gs in low O₃ levels, but lower total plant water use due to lower total leaf area. The insensitive phenotype had greater A and gs when exposed chronically to moderate O₃ levels (70 ppb), and higher total plant water use (E.L. Fiscus, pers. commun.).

California black oak is a drought-adapted species common throughout California in the transition between chaparral and the montane, mixed conifer forest (McDonald, 1990a). California black oak was symptomatic in southern California during the high O₃ exposures of the mid 1970s (Miller et al., 1980), but foliar injury under ambient field conditions has not been reported recently. Earlier onset of senescence and stomatal aberrations with elevated nitrogen deposition and high background O₃ exposure has been reported in this species in the field (Grulke et al., 2005). Blue oak is found in the dry, interior foothills throughout California (McDonald, 1990b), but little physiological data are available for this species.

2.2. Plant propagation and experimental design

All three species were grown in a temperature- and humidity-controlled greenhouse in Riverside, California. Gas exchange measurements were conducted on snapbean that were of an age between six to nine weeks from time of germination in a greenhouse. Direct measures of foliar O₃ flux were conducted on four O₃-sensitive, and four O₃-insensitive snapbean plants. Oaks were germinated from acorns collected locally, and grown in greenhouses until 3–5 years old. In mid-March, 2005, plants were placed in open-top exposure chambers and subjected to a chronic O₃ exposure of 70 ppb for 8 h per day for 1 (California black oak) or 2 months (blue oak). One plant of each oak species were placed three charcoal-filtered or three elevated open top chambers.

In the greenhouse, paired measurements of gas exchange with and without cuvette O₃ were conducted on adjacent leaves on each snapbean and oak seedling. The O₃-free leaf gas exchange measurements were made with a LiCor Instr. Model 6400 open system (Lincoln, NE), referred to here as the HC system (only H₂O and CO₂ flux). The leaf gas exchange measurements with elevated O₃ were made with the HOC system described below, which used two custom O₃ monitors (one for the reference air and one for the sample air) for the O₃ flux, and a second LiCor, Instr. Model 6400 (or 6262) for the H₂O and CO₂ flux from the reference and sample gases (respectively) for the HOC system.

2.3. HOC system description

A custom-designed gas exchange system was used for this study because of the inability to experimentally maintain elevated O₃ in the cuvette of conventional gas exchange systems. A prototype of this system that supplied

elevated O_3 to a small cuvette, but that did not measure O_3 flux, was described previously in Grulke and Paoletti (2005). That system has been modified here (Fig. 1) to directly measure flux using two custom-made, low flow and volume, fast response O_3 monitors (specially designed by 2B Tech Inc., Boulder, CO), one for the reference (air entering the cuvette), and one for the sample air (air exiting the cuvette). Air was supplied to the system from a pressurized tank of 435 ppm CO_2 in breathing air. The CO_2 concentration was high so that when the stream of O_3 was added and when CO_2 was removed due to photosynthesis, the cuvette concentration averaged ~ 390 ppm). The dry tank air was humidified with a dewpoint generator (model 610, LiCor Instr.), passed to a custom refrigerator with a large peltier cooling block, and then to a tee where a small flow of concentrated O_3 ($5\text{--}25\text{ ml min}^{-1}$) was added to the air stream using a mass flow controller (MFC, GFC171 Aalborg Instr., Orangeburg, NY).

Ozone was generated from dry O_2 in a custom made O_3 generator, consisting of a UV light and a glass tube with a sliding aluminum sleeve, which modified the amount of UV light absorbed in the stream of O_2 . The flow of the combined O_3 and humidified and cooled reference air was metered using another MFC. Just prior to the cuvette, 120 ml min^{-1} was passed to the reference O_3 monitor. A small, precision pump pulled the air through the O_3 analyzer tube and this outgoing air was then pushed through another MFC (reading, not controlling flow) to the reference CO_2 and H_2O infra-red gas analyzers (IRGAs; open flow).

The O_3 monitor was modified from standard models (Dasibi, TECO, or Monitor Instr.) for low flow appropriate for biological applications. Because of the reduced size of the analyzer tube, the air flow was shifted between the sample line and a bypass through a scrubber to provide the zero using a short timing cycle (2 s). A small pressure transducer was installed between these two lines to reduce oscillations in the CO_2 and H_2O IRGAs. The bulk of the air flowed to the cuvette (total gas flow into the cuvette was 180 ml min^{-1}), and then was passed to a second, sample air O_3 monitor. This provided an over-pressure to the cuvette. The operational range of O_3 concentration in the HOC system cuvette was $50\text{--}250\text{ ppb (+)}$.

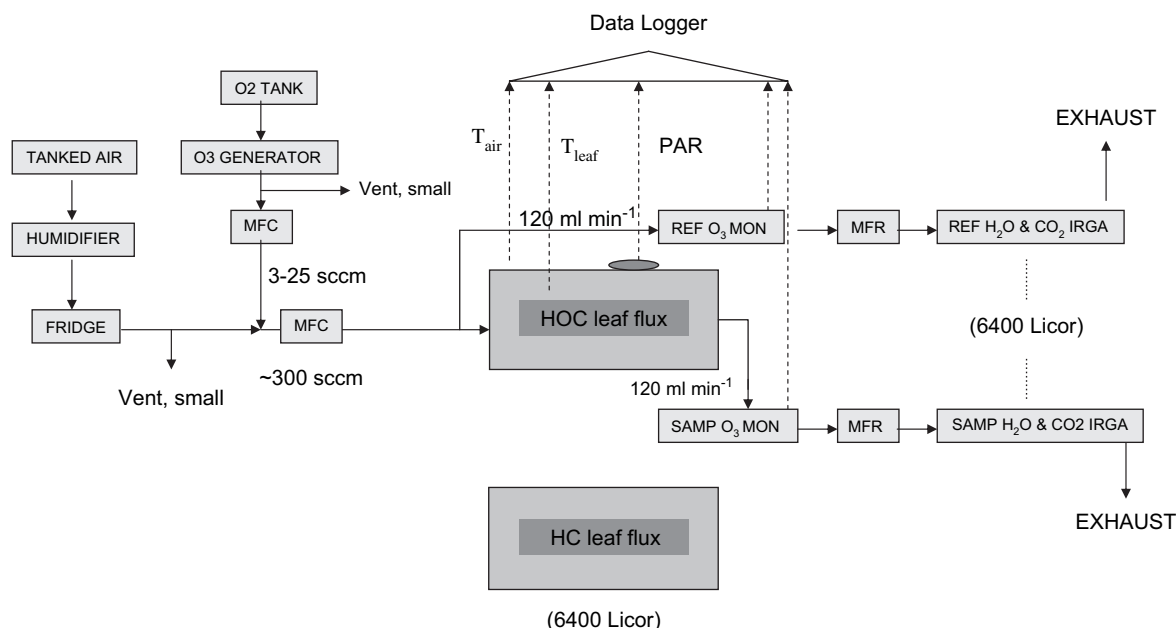
The cuvette was lined with Teflon film and contained two fine-wire thermocouples (leaf and air), nylon filament to hold the leaf in place, and a Teflon-covered miniature fan. The fan, to minimize the boundary layer, was identical to that used in the model 6400 leaf cuvette (LiCor Instr.) and was set to the same high speed controlled by a custom, miniature voltage regulator. The closed-cell foam gasket for the cuvette was obtained from LiCor Instr.). The exhausted air from the sample O_3 monitor was passed to another MFC

(read only, not to control the flow), and then to the sample side of the CO_2 and H_2O IRGAs.

For the snapbeans, reference and sample air exiting from the O_3 monitors was monitored with a LiCor model 6262 in differential mode. For the oaks, the flow from the reference and sample O_3 monitor was passed to the reference and sample sides, respectively, of the CO_2 and H_2O IRGAs of a closed, LiCor model 6400 cuvette. The flows to the reference and sample sides of the CO_2 and H_2O IRGAs were monitored, matched, and were very stable. The model 6400 was more stable with respect to the small changes in pressure caused by the shift from the zero to the sample air in the O_3 monitors. The temperature of the system cuvette and the two LiCor cuvettes were carefully matched, as was the light in the HC and HOC cuvettes. Gas exchange measurements were made during the day in a greenhouse so the whole plant received natural light. In the HC system cuvette, the red + blue light (provided by LiCor) was used to drive gas exchange. In the HOC system cuvette, the ambient light in the greenhouse was augmented using a multimirror projection lamp (GE-ESD) and socket (#2CX-30, both from Kennedy-Webster, Chicago, IL). An acrylic diffuser was used between the light source and the cuvette.

The system was difficult to fully hydrate in the low humidity conditions of California. To solve this problem, the dewpoint generator and cooler were run overnight prior to experimentation with greenhouse air to maximize air moisture. Once the air from the tank and O_3 were added to the system in the morning, it took a minimum of 45 min to reach O_3 flux equilibrium under illumination in the whole system. Other authors have also reported such a lag in system stability (Moldau et al., 1990; Moldau and Bichele, 2002). Once stability was reached, a “null” cuvette (without the leaf) measurement was recorded for 15 min. After the null cuvette measurement, the leaf was inserted into the cuvette, making contact with the fine wire thermocouple. After the leaf gas exchange reached equilibrium, the cuvette and whole plant was covered with an opaque cloth, generally for 30–40 min. This rapid shift from moderate light to complete dark was used to both reduce and measure the rate of decrease of the stomatal aperture between the O_3 -free cuvette (HC) and the elevated O_3 cuvette (HOC system). After dark equilibration (about 30–45 min), when the percent O_3 flux achieved a level similar to that of the initial null cuvette flux, the leaf was removed from the cuvette, and another null cuvette measurement was made.

Null cuvette measurements were incorporated into the calculations for flux in the HOC system cuvette. For the HC system, the signals for H_2O and CO_2 were matched both for the null cuvette as well as during high gas exchange



rates at equilibrium. Although O_3 flux in the null cuvette was correlated to cuvette relative humidity (greater O_3 adsorption and/or degradation with higher cuvette relative humidity), measured foliar O_3 flux was not correlated to cuvette relative humidity because null cuvette gas exchange (H_2O , CO_2 , and O_3) was subtracted from foliar gas exchange rates. Therefore, the null cuvette measures were an effective correction.

Leaf and cuvette air temperature, light level, and signals from the O_3 monitors (and LiCor model 6262 IRGA when in use) were monitored with a data logger (model 21x, Campbell Inc., Logan, UT), and graphed continuously on a laptop screen. Temperatures in the cuvette with illumination ranged from 22 to 27 °C (but changed slowly over the day). Light level was constant during measurements, but varied between 800 and 900 $\mu mol\ m^{-2}\ s^{-1}$ (Q) between plants. Relative humidity of the air stream ranged from 22 to 60%, and changed little (2%) for plants with low gs, but changed more (5%) with higher leaf area and higher gs over the course of the illuminated measurements.

Data were merged into a single Excel spreadsheet (from the control leaf with no O_3 exposure in the HC system, the HOC system leaf with acute, short-term O_3 exposure, and the data logger). To calculate O_3 flux, gs was multiplied by O_3 concentration and the constant 0.612 to account for the differences in diffusivity between water and O_3 . The internal foliar O_3 concentration was assumed to be zero (Laissak et al., 1989). Comparable to the regulatory entity, foliar O_3 uptake in O_3 -free air was calculated from HC system gs, and the O_3 concentration of the reference air supplied to the HOC cuvette (HC_flux(E); see Table 1 for a list of terms). In our experiments, we reported two calculated O_3 fluxes and one measured O_3 flux from the HOC system. The former O_3 fluxes were calculated from gs measured with concurrent O_3 exposure using (1) O_3 concentration of the entering air (HOC_flux(E)), and (2) the average O_3 concentration in the cuvette ((reference air + sample air)/2, HOC_flux(A)). Direct measure of foliar O_3 flux was also determined (HOC_flux(M)) from the difference between the reference and sample air O_3 concentration, the molar flow rate, and the leaf area. If cuticular O_3 uptake were small, the HOC_flux(M) was expected to be most comparable to HOC_flux(A). Foliar O_3 flux was reported on a one leaf surface area basis: the majority of the stomata are on the bottom leaf surface in all three species.

3. Results

3.1. Comparison of calculated and measured O_3 flux

Although measured and calculated O_3 fluxes were within the same order of magnitude, the O_3 flux measured in the HOC system (HOC_flux(M); see Table 1 for definition of

terms) was much lower ($5\ nmol\ m^{-2}\ s^{-1}$) than the regulatory entity ($13\ nmol\ m^{-2}\ s^{-1}$, the calculated O_3 flux using the gs from the O_3 -free HC cuvette (HC_flux(E)) (Fig. 2). As expected, calculated O_3 flux using the average O_3 concentration in the HOC cuvette (HOC_flux(A)) was the most similar to measured O_3 flux (HOC_flux(M)). Calculated O_3 flux using entering air (HOC_flux(E)) and average cuvette O_3

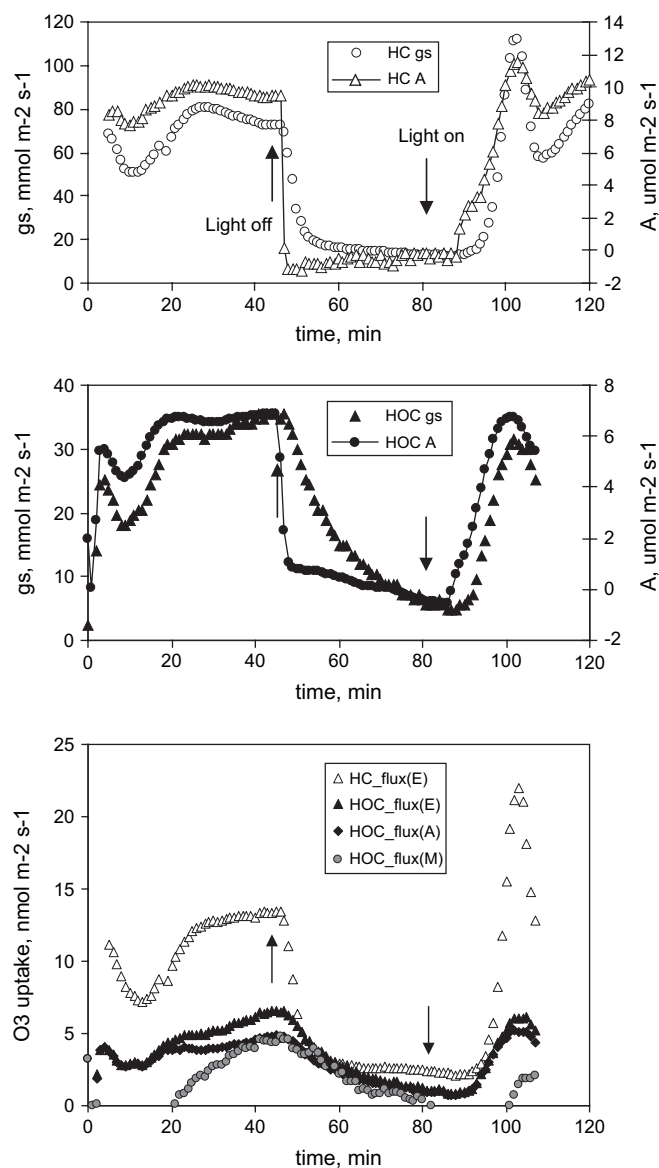


Fig. 2. Response of an O_3 -insensitive snapbean. Stomatal conductance (gs) and assimilation (A) measured in the HC system (top) and in the HOC system (middle). Calculated and measured foliar O_3 fluxes are given in the bottom graph. O_3 flux in O_3 -free air (HC_flux(E)) was calculated using O_3 concentration of air entering the HOC cuvette. O_3 flux with concurrent O_3 exposure was calculated using the O_3 concentration of air entering the HOC cuvette (HOC_flux(E)) and the average O_3 concentration in the cuvette (HOC_flux(A)). Direct measures of O_3 flux (HOC_flux(M)) were determined from the difference between entering and exiting O_3 concentration in the HOC cuvette, the molar flow rate, and the leaf area in the cuvette. Delay in stabilization of HOC_flux(M) was due to pressure changes in the sample O_3 monitor when the cuvette was opened to insert the leaf, then closed. Arrows indicate when the external light source was turned off, or on. Terms are defined in Table 1.

Table 1
Definition of terms

gs	Stomatal conductance in $mmol\ H_2O\ m^{-2}\ s^{-1}$
A	Net assimilation in $\mu mol\ CO_2\ m^{-2}\ s^{-1}$
O_3 flux	Foliar O_3 flux in $nmol\ O_3\ m^{-2}\ s^{-1}$
HC gs, A	Leaf gas exchange in Licor 6400 cuvette without concurrent O_3 exposure
HOC gs, A	Leaf gas exchange in HOC cuvette, with concurrent, short-term, acute O_3 exposure
HC_flux(E)	Regulatory entity: calculated foliar O_3 flux in Licor 6400 cuvette without O_3 exposure, using the O_3 concentration of the reference air (O_3 entering (E) the HOC system chamber)
HOC_flux(E)	Calculated foliar O_3 flux using HOC gs and O_3 concentration of the entering (E) air stream
HOC_flux(A)	Calculated foliar O_3 flux using HOC gs and the average (A) cuvette O_3 concentration
HOC_flux(M)	Direct measure of foliar (+ cuticle) O_3 flux using the difference between entering and exiting air O_3 concentration, molar flow rate, and leaf area in the HOC cuvette

concentration (HOC_flux(A)) also differed because of leaf uptake (6 vs. 5 $\text{nmol m}^{-2} \text{s}^{-1}$), but the two fluxes changed in tandem. If cuvette O_3 concentration had not been known (e.g., if only entering or reference O_3 concentration were known), calculated O_3 flux would have been overestimated by 20% (see Pasqualini et al., 2002).

In all measures of HOC_flux(M) , there was an apparent gap in data after the null cuvette measure lasting 20–35 min. One might suspect that this was due to a large volume in the chamber. Instead, the lag before positive fluxes appeared was due to pressure changes from opening and closing the HOC cuvette when the leaf was inserted. The pressure change resulted in a rapid increase in the sample, but not the reference O_3 monitor, and thus a temporary ‘negative’ O_3 flux (only positive O_3 flux values graphed, Fig. 2). When the light was extinguished (at 50 min), stomatal aperture declined quickly and all measures of O_3 flux declined at a more or less similar rate. Because g_s in both the HC and HOC systems did not reach zero, the calculated O_3 flux remained above zero, but the measured O_3 flux ultimately became very close to zero (at 75 min). When the light was turned back on (at 80 min), a temperature-induced pressure change again resulted in a negative O_3 flux, which then began to recover by 100 min but never fully recovered before the experiment was terminated. Such transitory pressure changes also occur in commercial systems with large changes in light level. Aside from this lag period, calculated O_3 flux based on g_s with concurrent O_3 exposure are good, but not perfect, approximations of foliar O_3 flux for an insensitive snapbean.

Similar comparisons between calculated and measured O_3 flux were obtained for California black oak grown in an O_3 -free environment (Fig. 3). Calculated O_3 flux based on the average cuvette O_3 concentration was nearly identical to measured O_3 flux (after equilibration). If only the entering air O_3 concentration had been known, O_3 flux would have been overestimated by 25%. If only g_s of a leaf in an O_3 -free cuvette were known (HC system), calculated O_3 flux would have been overestimated by 50% relative to directly measured flux. For a California black oak under chronic O_3 exposure, the added stress of concurrent acute O_3 exposure depressed g_s by 80% (Fig. 4). Measured O_3 flux was again very similar to that calculated using system g_s and average cuvette O_3 concentration. However, when the black oak seedling was placed in the greenhouse and measured with an O_3 -free cuvette (HC system with the O_3 -free greenhouse air), there was evidence of a short-term increase in both A (not shown) and g_s . Calculated O_3 flux based on the O_3 -free cuvette (HC system) measurements greatly exceeded that measured in the HOC system.

If cuticular uptake were detectable with this system, then measured O_3 flux (HOC_flux(M)) would always be greater than that calculated (HOC_flux(A)). However, measured O_3 flux was greater than calculated O_3 flux in the HOC system with concurrent O_3 exposure only half the time. The median range of differences was $\pm 25\%$. At equilibrium, the differences between calculated system O_3 flux and measured system O_3 flux could not be accounted for by either varying the

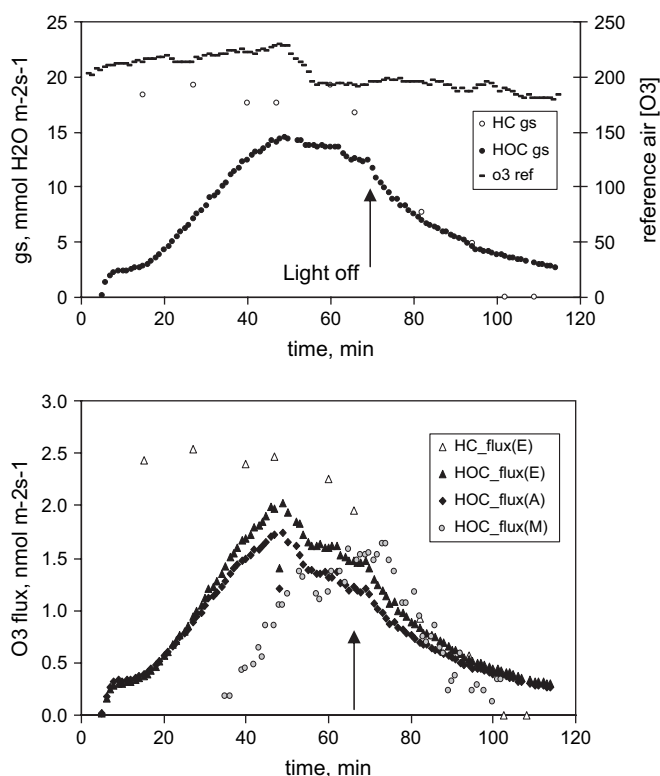


Fig. 3. Response of California black oak grown in O_3 -free air with and without acute, short-term O_3 exposure. Time course of reference air $[\text{O}_3]$, g_s measured in the O_3 -free cuvette (HC system), and g_s measured with concurrent O_3 exposure in the HOC system (top graph). Time course of calculated (HC_flux(E) , HOC_flux(E) , and HOC_flux(A)) and measured foliar O_3 flux (HOC_flux(M)) given in the bottom graph. Arrows indicate when the external light source was turned off.

cuvette relative humidity (22–60%) or O_3 concentration (0.1–0.25 ppm). However, there was a species difference: blue oak had, on average, 30% lower measured O_3 flux than calculated flux. The leaves of this species are hairy, and measured O_3 flux would have been expected to be greater, not lower than that calculated. In California black oak, measured and calculated O_3 flux were within 2% on average, and measured O_3 flux was slightly greater than that calculated. In snapbean, measured O_3 flux was 40% greater than calculated O_3 flux based on average O_3 concentration in the cuvette. There were no differences between sensitive or insensitive phenotypes.

O_3 flux based on reference air O_3 concentration (HC_flux(E)) was always greater than HOC_flux(E) . The ratio of HC to HOC O_3 flux (E) was significantly correlated to the ratio of lf to sys g_s ($r = 0.76$), but less correlated to cuvette relative humidity ($r = 0.43$) and reference O_3 concentration ($r = -0.30$: the higher the O_3 concentration, the lower the g_s and O_3 flux in the HOC system).

3.2. Stomatal aberrations with direct O_3 exposure

In snapbean, approximately one hour of acute O_3 exposure decreased maximum g_s and A under moderate light (Table 2). Maximum g_s was depressed by 50% in the O_3 -sensitive

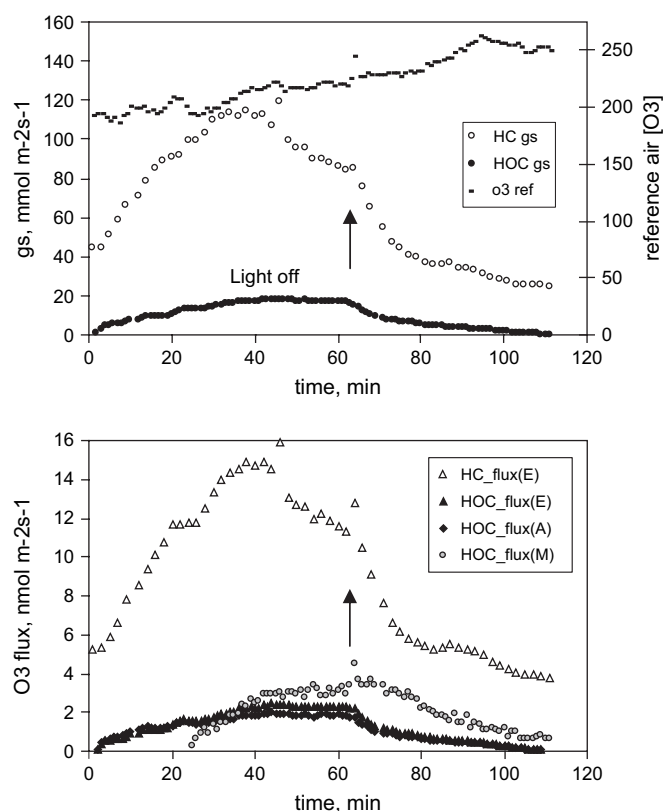


Fig. 4. Response of California black oak grown in chronic, month long O_3 exposure in OTCs, then exposed to either O_3 -free air or acute O_3 exposure. See Fig. 3 for further description.

phenotype and by 60% in the O_3 -insensitive phenotype. Maximum gs in blue oak was similarly depressed by short-term, acute O_3 exposure in both activated-charcoal filtered OTCs, but differences were not significant. The maximum A at equilibrium followed the same patterns in blue oak and snapbean: short-term acute O_3 exposure depressed A significantly in both species. Because California black oak seedlings exposed to chronic O_3 exposure rapidly increased gas exchange when transferred to the greenhouse (see Fig. 4), there were no significant effects of chronic or acute O_3 exposure on either A or gs, or the rate of stomatal closure in this study.

When the cuvette (and whole plant) was placed in the dark, stomatal closure was sluggish when concurrently exposed to O_3 (HOC system) relative to gs measured in O_3 -free air (HC system). For insensitive snapbean (Fig. 2), reduction in stomatal aperture with light extinction was twice as slow with acute O_3 exposure as in O_3 -free air. Stomatal response to increasing light was slightly different in the HC and HOC systems, but this difference did not wholly account for sluggish response with concurrent O_3 exposure. For blue oak, acute O_3 exposure significantly reduced the rate of stomatal closure in response to extinguishing the light, whether the seedlings were grown in charcoal-filtered air or elevated O_3 . California black oak had a similar pattern, but differences were not significant. O_3 -sensitive snapbean had the fastest stomatal closure in an O_3 -free cuvette when the plant was placed in the dark.

Table 2

Summary of maximum gs and A under moderate light at equilibrium with and without concurrent, short-term, acute O_3 exposure, and rate of stomatal closure (change in gs per min) in response to the whole plant being placed in the dark

Species	Chronic	Acute	Max gs	gs reduction rate	Max A
QD	C	C	121 (47)	2.89 (0.65)a	7.68 (0.48)a
QD	C	O_3	50 (15)	0.84 (0.20)b	3.85 (0.46)b
QD	O_3	C	104 (25)	2.47 (0.25)a	9.28 (0.32)a
QD	O_3	O_3	57 (18)	1.14 (0.31)b	4.81 (0.71)b
<i>p</i>			—	0.008	<0.001
QK	C	C	34 (10)	1.06 (0.39)	3.69 (0.99)
QK	C	O_3	34 (13)	0.64 (0.41)	3.57 (1.99)
QK	O_3	C	69 (9)	3.56 (2.68)	4.62 (1.4)
QK	O_3	O_3	30 (7)	0.29 (0.04)	2.10 (0.79)
<i>p</i>			—	—	—
PV	Sensitive	C	96 (14)a	4.82 (1.61)a	12.04 (0.86)a
PV	Sensitive	O_3	45 (6)b	1.00 (0.25)b	7.13 (0.63)b
PV	Insensitive	C	104 (19)a	3.11 (0.24)c	11.17 (1.01)a
PV	Insensitive	O_3	34 (4)b	0.74 (0.04)b	6.44 (0.97)b
<i>p</i>			0.019	<0.001	0.003

Plants were exposed to activated charcoal filtered air or chronic O_3 exposure in open top chambers for 1 month (QK, *Quercus kelloggii*) or 2 months (QD, *Quercus douglasii*). Snapbean (PV, *Phaseolus vulgaris*) was grown in a filtered greenhouse and had only short-term acute O_3 exposure. The significance (*p*) is reported only where significant (<0.05) for a 1-way ANOVA (SPSS 2000), treating each combination as a separate entity. Numbers in parentheses are mean $\pm$ 1 S.E.

In two California black oaks, one chronically exposed to O_3 and one grown in activated charcoal filtered air, gs after 40 min in the dark was $>30 \text{ mmol m}^{-2} \text{ s}^{-1}$ when measured concurrently with acute O_3 exposure. Gs measured on an adjacent leaf on the same plant, in the dark, in an O_3 -free cuvette, was undetectable. In two blue oaks, also one chronically exposed to O_3 and one grown in activated charcoal filtered air, gs measured concurrently with acute O_3 exposure was 30% greater than that measured in an O_3 -free cuvette. In these plants, calculated O_3 flux based on the HC system gs (the regulatory entity) greatly underestimated flux.

Several O_3 metrics were tested for the significance of correlation with maximum gs and A, and rate of stomatal closure (Table 3), including the average O_3 concentration, dose, O_3 -flux(A), and O_3 -flux(M). For California black oak, no correlation coefficient was significant. For snapbean, all O_3 metrics tested had a significant, negative correlation with maximum gs and A, and the rate of stomatal closure. For blue oak, the average O_3 concentration had the highest correlation coefficient with maximum gs and A, and stomatal closure. Dose, O_3 -flux(A), and O_3 -flux(M) were not correlated with maximum gs, but were significantly correlated to rate of stomatal closure and maximum A. The average O_3 concentration had the highest correlation coefficient.

4. Discussion

Calculated foliar O_3 flux from leaves measured in O_3 -free cuvettes (HC_flux(E), the regulatory entity) overestimated measured foliar O_3 flux with concurrent O_3 exposure

Table 3
Physiological response to acute O₃ exposure

Species	Max gs	Stomatal closure rate	Max A
QD			
Ave [O ₃]	−0.599	−0.807	−0.915
Dose	—	−0.724	−0.795
O ₃ flux (A)	—	−0.610	−0.567
O ₃ flux (M)	—	−0.699	−0.784
PV			
Ave [O ₃]	−0.693	−0.836	−0.838
Dose	−0.631	−0.775	−0.804
O ₃ flux (A)	−0.564	−0.702	−0.760
O ₃ flux (M)	−0.646	−0.729	−0.772

Physiological response (maximum gas exchange at equilibrium in moderate light, and rate of change of gs to extinguishing the light) to acute O₃ exposure in a HOC cuvette was tested for correlation with average O₃ concentration in the cuvette (ave [O₃]), dose (ppb h), calculated foliar flux (HOC_flux(A)), and measured foliar flux (HOC_flux(M)). See Table 2 for acronyms. The sign and correlation coefficient (*r*) is given only for significant values (*p* < 0.05) (SPPlus 2000). There were no significant relationships for California black oak.

(HOC_flux(M)). Reductions in gs as a result of concurrent O₃ exposure further reduced gs and measured foliar O₃ flux, and resulted in greater overestimation by the regulatory entity. Sluggish stomatal responses to changes in environmental conditions (demonstrated here for light), or incomplete stomatal closure, resulted in underestimation by the regulatory entity relative to direct measurements. In a few plants of both California black oak and blue oak, there was a lack of stomatal closure when the plant was placed in the dark. Under these conditions, the regulatory entity underestimated direct measures of foliar O₃ flux by 80%. Concurrent short-term, acute O₃ exposure depressed calculated O₃ flux (HOC_flux(E)) by >20% relative to calculated O₃ flux for foliage in an O₃-free cuvette (HC_flux(E)). Under concurrent acute O₃ exposure, calculated O₃ flux using reference air O₃ concentration (E) was 15% greater than calculated O₃ flux using the average cuvette O₃ concentration (A). Calculated O₃ flux using the average cuvette O₃ concentration with concurrent O₃ exposure was very similar to that obtained by direct measurement of foliar O₃ flux ((HOC_flux(M)). When exposure, dose, and calculated and measured O₃ flux were tested, the average cuvette O₃ concentration had the highest correlation coefficient with maximum gs, A, and rate of stomatal closure (Table 3).

Stomatal conductance and foliar O₃ flux was greatest in snapbean, followed by blue oak, followed by California black oak. Variation in endogenous rates of gs within a species also significantly modified O₃ flux. California black oak and blue oak seedlings chronically exposed to O₃ had relatively low gas exchange, and had little response to short-term, acute O₃ exposure, because gs and O₃ flux into the leaf was also low. In these individuals, acute O₃ exposure did not result in sluggish stomatal closure when placed in the dark (Fig. 4): the rate of stomatal closure was nearly the same in both charcoal-filtered and chronic O₃ exposure.

Besides cuticular adsorption, biogenic hydrocarbons emitted from foliage react with O₃ (Fehsenfeld et al., 1992). *Phaseolus vulgaris* does not emit either isoprene or monoterpenes, the most

common biogenic VOCs (Arey et al., 1991). Black oaks emit 2–3 times more isoprene than blue oak (Geron et al., 2001). Monoterpene emission was not found in blue oak (Tanner and Zielinska, 1994). Our data suggest that non-stomatal flux of O₃ was low in all three species. The best empirical support for lack of significant, non-stomatal flux was that there was little difference in O₃ flux between a null cuvette measurement and leaves in the cuvette in the dark. California black oak had nearly identical measured and calculated O₃ flux (based on the average concentration). In wheat, non-stomatal deposition was small compared to the stomatal uptake (Pleijel et al., 2004).

Models developed (Emberson et al., 2000; Grunhage et al., 2001) to estimate crop or forest species use steady-state parameters. However, even in an open-grown tree, on the southern aspect, light varied >2 standard deviations from the mean in low (30–200 Q), medium (600–900 Q), and high (1400–1800 Q) average background light levels two thirds of the time (Grulke, unpubl. data for mature *Pinus ponderosa*). Despite the errors associated with estimating non-foliar O₃ flux in forest or crop stands, whole ecosystem flux data (Krupius et al., 2002; Matyssek et al., 2004) is perhaps the best check on modeled O₃ flux because of the concurrent O₃ canopy exposure, and varying environmental conditions.

Despite two decades of research, stomatal aberrations in response to O₃ exposure have been largely ignored. Current modeling efforts predict O₃ effects on plants, but the modeling approach for the EU-ECE level II (Emberson et al., 2000; Grunhage et al., 2001) uses assimilation models (Farquhar and Sharkey, 1982) with calculated gs (and calculated O₃ flux from calculated gs). In this environment, the potential for incorporating future improvements in our understanding of stomatal behavior into the flux-based O₃ metric are limited. The consequence for using an assimilation-derived modeling approach to estimate O₃ flux is to minimize the importance of direct O₃ effects on plant water balance and the lack of predictive capabilities of models to establish the link between O₃ exposure, increased susceptibility to drought stress, and deterioration of forest health.

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