

Chronic vs. Short-Term Acute O₃ Exposure Effects on Nocturnal Transpiration in Two Californian Oaks

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We tested the effect of daytime chronic moderate ozone (O₃) exposure, short-term acute exposure, and both chronic and acute O₃ exposure combined on nocturnal transpiration in California black oak and blue oak seedlings. Chronic O₃ exposure (70 ppb for 8 h/day) was implemented in open-top chambers for either 1 month (California black oak) or 2 months (blue oak). Acute O₃ exposure (~1 h in duration during the day, 120–220 ppb) was implemented in a novel gas exchange system that supplied and maintained known O₃ concentrations to a leaf cuvette. When exposed to chronic daytime O₃ exposure, both oaks exhibited increased nocturnal transpiration (without concurrent O₃ exposure) relative to unexposed control leaves (1.8× and 1.6×, black and blue oak, respectively). Short-term acute and chronic O₃ exposure did not further increase nocturnal transpiration in either species. In blue oak previously unexposed to O₃, short-term acute O₃ exposure significantly enhanced nocturnal transpiration (2.0×) relative to leaves unexposed to O₃. California black oak was unresponsive to (only) short-term acute O₃ exposure. Daytime chronic and/or acute O₃ exposures can increase foliar water loss at night in deciduous oak seedlings.

KEYWORDS: ozone exposure, nighttime transpiration, *Quercus kelloggii*, *Quercus douglasii*

INTRODUCTION

The occurrence of nocturnal transpiration has been cited as an adaptation in both nutrient- and water-limited environments. Nocturnal transpiration under drought conditions helps to redistribute water from hydrologically deep sources to near-surface roots. Root water loss in near-surface soil horizons increases nutrient availability and potential for uptake in soil immediately adjacent to fine roots[1]. Nocturnal transpiration has also been found in plants drought stressed the previous day. In this case, nocturnal transpiration is proportional to the level of daytime drought stress and is thought to promote rehydration of foliage[2].

Nocturnal transpiration has also been reported previously for three species experimentally exposed to moderate daytime ozone (O₃) exposures[3,4]. In these studies, nighttime transpirational losses were 12–

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50% that of daytime transpirational rates. In a fourth study, moderately high to high exposure of both O₃ and NO_x was correlated to nocturnal transpiration averaging 11% that of daytime values[5]. In these cases, loss of stomatal control at night is likely to be the result of mechanical failure of the guard cells in the presence of strong oxides. Ozone exposure is known to modify cell membrane permeability to K⁺[6], to modify Ca⁺⁺-based signaling[7], and to alter the chemical structure of cell walls such that water loss is buffered and slows guard cell response to changing conditions of water potential (e.g., delignification)[8].

In this paper, we report the effects of daytime O₃ exposure on nocturnal transpiration in seedlings of two deciduous trees: California black oak and blue oak. Chronic medium-term O₃ exposure, acute short-term O₃ exposure, and the combined effects of both were employed to test effects on nocturnal transpiration relative to leaves unexposed to O₃.

MATERIALS AND METHODS

Plant Material

Two broadleaf, deciduous oak species were chosen for flux measurements: California black oak (*Quercus kelloggii* Newb.) and blue oak (*Q. douglasii* Hook & Arn). Although botanical references state that blue oak is deciduous, blue oak retains up to 3 years of leaves in the foothills of central and the southern Sierra Nevada (N. Grulke, field observations). Blue oak seedlings in this experiment retained 3 years of leaves, but response of only current-year leaves was measured. Oaks were germinated from acorns and grown in greenhouses until 3–5 years old. There was no evidence that these seedlings were hybrids with any other oak species.

Experimental Design

In mid-March 2005, seedlings were placed in open-top exposure chambers and subjected to a chronic O₃ exposure of 70 ppb for 8 h/day for 1 (California black oak) or 2 months (blue oak). One or two plants of each oak species were placed in three charcoal-filtered and three elevated open-top chambers. Oaks exposed to chronic O₃ exposure or to activated charcoal-filtered air were transferred to the greenhouse in the early morning of experimental acute O₃ exposure. The maximum O₃ concentration observed in the greenhouse was 10 ppb. Adjacent leaves of oaks on the same plant were exposed to either no O₃ exposure (using a commercial gas exchange system) or ~1 h of known O₃ exposure (120–220 ppb) using a novel gas exchange system[9] briefly described below. The whole plant was exposed to daylight within the greenhouse, and leaves in the cuvettes were exposed to constant light (850 μmol m⁻² sec⁻¹, photon flux density), ambient temperature (22–28°C), and relative humidity (22–60%). The following night, gas exchange of the paired leaves (with and without prior O₃ exposure) was measured with a carefully calibrated, open gas exchange system (Model 6400, LiCor Instruments, Lincoln, NE) with no exposure to light at 390 ppm CO₂. During gas exchange measurements at night, ambient conditions in the cuvette were matched with that of the greenhouse: leaf VPD averaged 1.3 kPa, relative humidity averaged 34%, and leaf temperature averaged 18°C.

O₃ Exposure Gas Exchange System

We designed a novel gas exchange system to test the effects of O₃ exposure alone on foliar gas exchange[9] and to measure O₃ flux to leaves directly. We were unable to use commercial gas exchange systems for this purpose because 95% of the O₃ supplied to commercial cuvettes is adsorbed by the internal surfaces. In brief, the source of the air flow was premixed CO₂ (in air), humidified and cooled prior to the cuvette. A low flow of high concentration of O₃ was metered into the cooled, humidified air

stream with a mass flow controller. Matched custom O₃ monitors, designed for both low flow (120 sccm) and fast response (20 sec), were used to measure O₃ concentration, one before (reference) and one after (sample) the cuvette. After O₃ measurements, air was passed to either the reference or sample CO₂ and H₂O infrared gas analyzers, respectively, then exhausted. Ozone concentration was measured in an empty cuvette with stable temperature and humidity, before and after leaf gas exchange measurements. Although covered with Teflon film, cuvette surfaces adsorbed 10–30% of O₃ delivered, and null cuvette O₃ adsorption was accounted for in all measurements of foliar O₃ uptake. After O₃ flux was measured in the null cuvette, one leaf on a plant was inserted into the O₃-exposure cuvette, and an adjacent leaf on the same plant was simultaneously measured using an O₃-free commercially available LiCor (model 6400) gas exchange system. Light level, leaf temperature, air flow, and cuvette fan speed were matched in the two systems.

RESULTS

California Black Oak

One month of chronic O₃ exposure during the daytime increased foliar nocturnal transpiration (without concurrent O₃ exposure or exposure to light) by 1.8× relative to foliage not exposed to O₃ (Fig. 1). Nocturnal transpiration resulting from chronic O₃ exposure was 30% of daytime values (based on a maximum daytime value observed of ~1.8 mmol m⁻² sec⁻¹). Although there was an increase in nocturnal transpiration in response to short-term acute O₃ exposure, differences were not statistically significant.

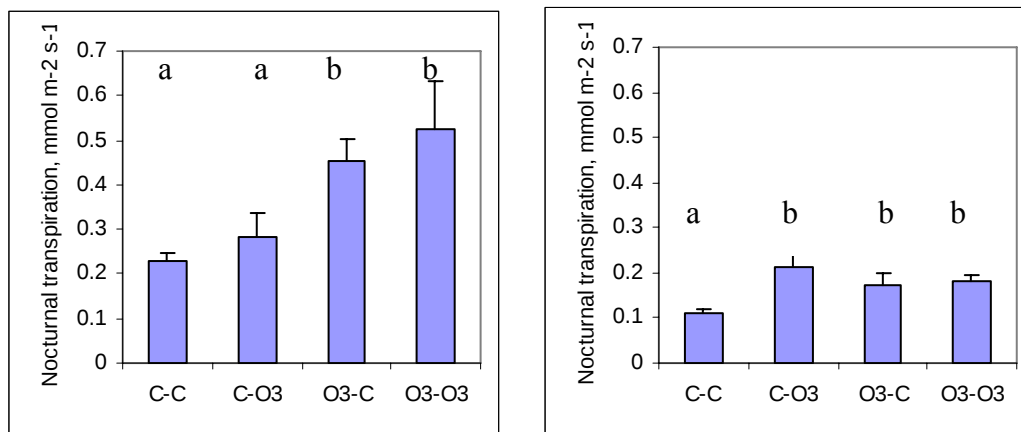


FIGURE 1. Nocturnal transpiration of California black oak leaves (on left) and blue oak leaves (on right) measured after daytime exposure, with no ambient light or pre-exposure to light. C-C: plants exposed to activated charcoal-filtered (CFA) air with no short-term exposure to O₃; C-O₃: plants exposed to CFA with short-term acute O₃ exposure; O₃-C: plants exposed to chronic O₃ exposure with no short-term exposure to O₃; O₃-O₃: plants exposed to both chronic and acute O₃ exposure. Error bars indicate ± 1 S.E. for four plants in each group. Different letters indicate statistical differences at the $p = 0.05$ level between bars.

To display the relationship between nocturnal transpiration (T) and O₃ dose, we plotted the ratio of T with short-term acute O₃ to T without O₃ vs. O₃ dose experienced in the short-term acute O₃ exposures (Fig. 2, left). Because the numerator and denominator represent gas exchange of different leaves (at night), and the duration of exposure and O₃ concentration varied with each short-term exposure, there was significant variability in response in the graphed response. For California black oak, O₃ doses <~200 ppb

h had a relatively small effect on nocturnal transpiration and the regression line was not statistically significant.

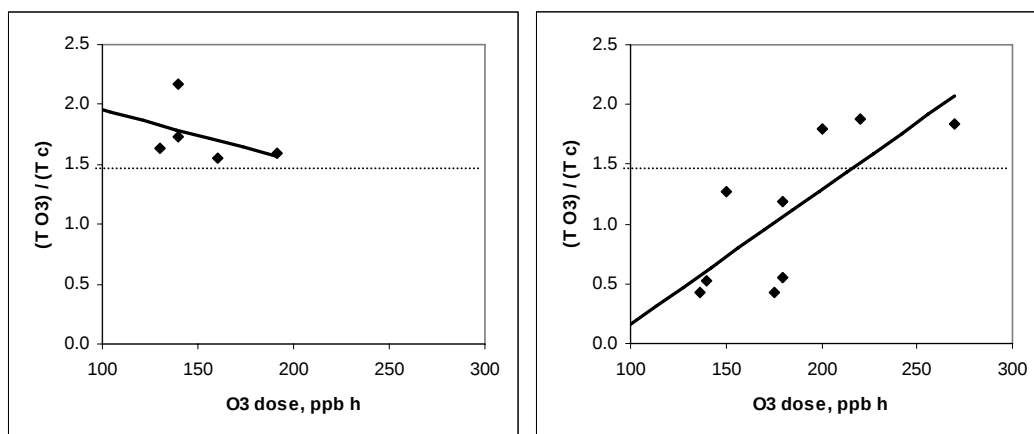


FIGURE 2. The ratio of nocturnal transpiration (T) with acute O₃ exposure divided by the transpiration of an adjacent leaf with no O₃ exposure, plotted as a function of the daytime short-term acute O₃ exposure. The dashed line indicates a 1:1 ratio of T which would be expected if there were no effect of short-term, acute O₃ exposure. There was no statistically significant regression fit for California black oak (left). A linear regression was significant for blue oak (right).

Blue Oak

Two months of chronic O₃ exposure during the daytime increased foliar nocturnal transpiration (without concurrent O₃ exposure or exposure to light) by 1.6× relative to foliage not exposed to O₃ (Fig. 1). Nocturnal transpiration resulting from this chronic O₃ exposure was 16% of daytime values (based on a maximum daytime value observed of ~1.5 mmol m⁻² sec⁻¹). For plants with no prior O₃ exposure (controls grown in activated charcoal-filtered, open-top chambers), short-term (1 h) acute O₃ exposure significantly increased nocturnal transpiration 2.0× that of control leaves. Nocturnal transpiration resulting from this acute O₃ exposure was 24% of daytime values. There were no synergistic effects of both chronic moderately high and short-term acute O₃ exposure.

The ratio of T with short-term acute O₃ to T without O₃ was plotted against O₃ dose experienced in the short-term acute O₃ exposures (Fig. 2, right). The ratio increased with increasing O₃ dose and the regression was statistically significant ($p < 0.05$). Ozone doses exceeding 180 ppb h significantly increased nocturnal transpiration in leaves exposed to short-term acute O₃ exposure.

The response of both deciduous oaks was combined to illustrate the effect of both chronic and acute O₃ exposure on nocturnal transpiration (Fig. 3). The lower line represents the transpirational response to short-term acute exposure for leaves with no prior O₃ exposure (plotted vs. dose). The upper line represents the transpirational response to short-term acute exposure for leaves chronically exposed to O₃. In both datasets, the values on the y-axis (representing 0 dose) are transpiration of the control leaves in each treatment (CFA or chronic O₃ exposure, respectively) without short-term acute O₃ exposure. The lower regression line is a statistically significant fit ($p < 0.05$), but there were no differences in y-intercept or slope between the two lines. The response lines suggest that (1) short-term acute O₃ exposure increases nocturnal transpiration, (2) chronic O₃ exposure increases overall nocturnal transpiration, and (3) the interaction between chronic and acute O₃ exposures was not synergistic.

DISCUSSION

Stomates may remain open at night and there is an apparent net efflux of water from leaves, after daytime long-term chronic or short-term acute O₃ exposure. Foliage of California black oak and blue oak seedlings exhibited elevated nocturnal transpiration when exposed to chronic daytime O₃ exposure. Short-term acute O₃ exposure during the day did not significantly (additionally) enhance nocturnal transpiration in

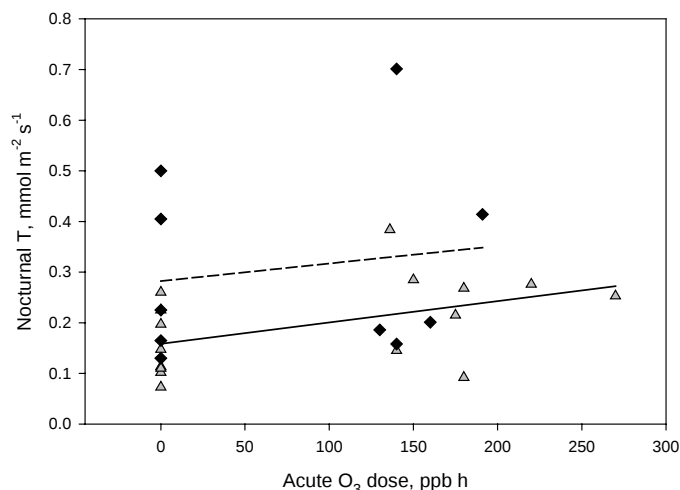


FIGURE 3. Nocturnal transpiration (T) of both oaks plotted together for saplings grown in charcoal-filtered air (grey triangles, lower solid line) or grown with chronic O₃ exposure (black diamonds, upper dotted line). The effect of short-term acute O₃ exposure is suggested by the change from measurements in the control cuvette (on y-axis) vs. the values plotted for the (nonzero) acute O₃ dose. The regression lines did not differ significantly.

either species when previously conditioned to chronic daytime O₃ exposure. However, in blue oak with no prior O₃ exposure, short-term (1 h) acute O₃ exposure significantly enhanced nocturnal transpiration. California black oak with no prior O₃ exposure was unresponsive to short-term acute O₃ exposure.

California black oak had higher daytime gas exchange rates and relatively high nocturnal T (30% that of maximum daytime values) in response to only 1 month of chronic O₃ exposure. Chronic O₃ exposure over 2 months in blue oak also resulted in increased nocturnal transpiration, but not as much as for California black oak (16% that of maximum daytime values). Although blue oak apparently was more tolerant to chronic O₃ exposure, it was more sensitive than California black oak to short-term acute O₃ exposure. At night after short-term acute O₃ exposure, blue oak had 24% of the maximum daytime T observed. Stimulation of nocturnal transpiration was ephemeral in blue oak; nocturnal transpiration was similar in unexposed and exposed leaves 2 days after acute O₃ exposure (data not shown). In *Arbutus unedo*, a Mediterranean tree, a 90-day O₃ exposure induced prolonged aberrations in stomatal behavior for up to 10 days[10].

Species of oak are known to emit isoprene and other volatile organic compounds[11] that degrade O₃, thus potentially modifying cuvette O₃ concentrations. The relative emission of these two oaks is unknown. Because the O₃ concentration of the cuvette was measured (average of the O₃ concentration entering and exiting the cuvette) and boundary layer was minimized by high fan speeds, the potential for relative differences in isoprene emission between the two species were not expected to play a role in these observations.

Prior exposure of plants to chronic daytime O₃ exposure may have preconditioned the antioxidant system[12] such that a short duration, high-O₃ exposure was limited in effect. Apoplastic ascorbate is believed to be the first line of defense against O₃ uptake, and perhaps such a defense was already in place in this species to minimize the effects of such short-term acute O₃ exposure[13]. Because short-term (1 h, acute) O₃ exposure elicited little response of foliar gas exchange of California black oak seedlings (without prior O₃ exposure), this species may have endogenously more antioxidant defenses relative to blue oak. These responses underscore the need for additional studies of such systems in native species.

Although O₃ exposure[3,4] and both O₃ and NO_x exposure[5] have been implicated in aberrant, elevated nocturnal transpiration in conifers, the results presented here provide strong experimental evidence for the role of O₃ in the foliar loss of water at night in seedlings of two deciduous oaks.

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