

Stomata open at night in pole-sized and mature ponderosa pine: implications for O₃ exposure metrics

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Summary Ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) is widely distributed in the western USA. We report the lack of stomatal closure at night in early summer for ponderosa pine at two of three sites investigated. Trees at a third site with lower nitrogen dioxide and nitric acid exposure, but greater drought stress, had slightly open stomata at night in early summer but closed stomata at night for the rest of the summer. The three sites had similar background ozone exposure during the summer of measurement (2001). Nighttime stomatal conductance (g_s) ranged from one tenth to one fifth that of maximum daytime values. In general, pole-sized trees (< 40 years old) had greater nighttime g_s than mature trees (> 250 years old). In late summer, nighttime g_s was low (< 3.0 mmol H₂O m⁻² s⁻¹) for both tree size classes at all sites. Measurable nighttime g_s has also been reported in other conifers, but the values we observed were higher. In June, nighttime ozone (O₃) uptake accounted for 9, 5 and 3% of the total daily O₃ uptake of pole-sized trees from west to east across the San Bernardino Mountains. In late summer, O₃ uptake at night was < 2% of diel uptake at all sites. Nocturnal O₃ uptake may contribute to greater oxidant injury development, especially in pole-sized trees in early summer.

Keywords: ozone exposure, nighttime opening, stomatal conductance.

Introduction

Stomatal opening is under tight biological control to maximize carbon uptake while minimizing water loss (Cowan 1977). It is influenced by light, substomatal CO₂ concentrations (Aphalo and Jarvis 1993), vapor pressure deficit (Assmann and Gershenson 1991), foliar temperature, the whole-plant response of the soil water-to-air continuum including endogenous limitations (Ryan and Yoder 1997) and hormonal changes in response to drought stress (Christmann et al. 1995). Stomatal function can also be disturbed by oxidant air pollutants (Matyssek et al. 1995, Heath and Taylor 1997, Mansfield 1998, Robinson et al. 1998).

Stomata are not always totally closed during the night in C₃ plants (Musselman and Minnick 2000). Open stomata at night

have been reported in some tree species exposed to moderate and higher oxidant exposure (*Picea abies* (L.), Wieser and Havranek 1993; *Betula pendula* Roth., Matyssek et al. 1995; *Larix decidua* Mill., Wieser and Havranek 1995; *B. pendula*, *Populus euramericana* (Dode) Guinier and *Alnus glutinosa* (L.) Gaertner, Günthardt-Goerg et al. 1997). During the day at elevated oxidant concentrations, sluggish responses of stomata have also been observed in response to changes in light and vapor pressure deficits in coniferous (Tobiessen 1982, Reich and Lassoie 1984, Patterson and Rundel 1989, Grulke 1999) and herbaceous species (Younglove et al. 1988). Significant stomatal conductance (g_s) during the night could be related to compromised cuticle or stomatal occlusions that limit stomatal closure. Exposure to high concentrations of both nitrogen oxides and nitric acid vapor are known to alter cuticular and epistomatal wax structures and occlude stomata (Norby et al. 1989, Bytnerowicz et al. 1998, 1999). Ozone exposure can also degrade cuticular waxes (Barnes et al. 1988, Kerstiens and Lendzian 1989, Percy et al. 1992).

Although stomatal uptake of O₃ is the best metric for evaluating plant response to O₃ (Musselman and Minnick 2000, Grulke et al. 2002), both the USA (Stockwell et al. 1997) and the E.U. (Führer et al. 1997) use O₃ exposure, accumulated approximately from dawn to dusk over various thresholds (e.g., 40 ppb, 60 ppb), over the growing season. These metrics assume that stomata are closed and that O₃ concentrations decrease significantly at night. In mountainous terrain, however, O₃ concentrations can remain high during the night (Aneja et al. 1994, Loibl et al. 1994, Van Ooy and Carroll 1995).

In this paper, we describe nighttime stomatal behavior in two tree size classes (40-year-old and 250-year-old) of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.), one of the most O₃-sensitive conifers in western North America (Miller et al. 1983). In order to describe sluggish stomatal responses, we measured the time to reach equilibrium in response to small increases in low irradiances at sites differing in environmental stressors (ozone exposure, nitrogenous deposition and drought). We also estimated the contribution of nighttime O₃ uptake to total diel O₃ uptake in early and late summer.

Materials and methods

Research sites

Pole-sized (40 years old) and mature (> 250 years old) ponderosa pine were sampled in pine-dominated, mixed conifer forests (Barbour 1988). Three study sites were selected across the San Bernardino Mountains east of Los Angeles, California: two western sites (Crestline (CR): 34°14'05" N, 117°19'12" W; Strawberry Peak (SP): 34°14'00" N, 117° 08'12" W) and an eastern site (Barton Flats (BF): 34°09'42" N, 116°51'00" W) at 1800, 2240 and 1820 m in elevation, respectively. Historically, trees at the study sites were exposed to a gradient of O₃ exposure and nitrogen deposition already well described (Miller et al. 1989, Grulke et al. 1998, Grulke and Balduman 1999). However, pollutant deposition patterns in the San Bernardino Mountains have changed recently (see Table 1, Figure 1).

Soil water and plant water relations

Both gravimetric soil water content and predawn needle xylem water potentials (Ψ_L) were determined monthly during the same week as the intensive gas exchange measurements. Soil samples were collected within 1 m of the bole of the study trees, at a depth of 15 cm in mineral soil for pole-sized trees and at 15 and 50 cm for mature trees. Soil samples were passed through a 2-mm sieve, immediately placed in soil tins, sealed, weighed, oven-dried at 65 °C, and then reweighed. Water content was reported as percent of dry mass. Predawn needle xylem water potentials of previous-year fascicles from lower canopy foliage were determined with a pressure chamber (PMS, Corvallis, OR) for both tree size classes (Pallardy et al. 1991).

Canopy health assessments

At the end of September, chlorotic mottle of each needle age class and the number of needle age classes retained were assessed following excision of three mid-canopy branches of each study tree with a pole pruner. These attributes were aver-

aged for each tree size class at each site, and were used to calculate a canopy health score according to the Forest Pest Management method (FPM; Pronos et al. 1978). The FPM score is based on the first needle age class with more than 10% chlorotic mottling per needle on more than a third of the needles of that whorl.

Gas exchange measurements

Gas exchange of six trees per tree size class at each site was measured monthly from June through September 2001 with an open photosynthesis monitoring system (Model LI-6400, Li-Cor, Lincoln, NE). Three previous-year fascicles on primary branches in the lower third of the canopy were measured. Gas exchange measurements were conducted at night between 2300 h and predawn (0500–0600 h, depending on the month), with CO₂ concentration fixed at 385 ppm. Because the instrument was to operate near its detection limits, particular care was taken in the nightly calibration, and the instrument was allowed to reach equilibrium under ambient conditions for 1 h before measurements. The same CO₂ span gas was used throughout the summer, and the H₂O signal was calibrated with a dewpoint generator (Li-Cor Model 610) at stable temperatures at 1900 m (an elevation intermediate to the range at the sites). Before each light response, the sample and reference infrared gas analyzers were electronically matched. Null cuvette measurements were made before and after each light response curve and at no time exceeded 0.2 mmol H₂O m⁻² s⁻¹. To test for temporal delays in stomatal opening in response to dawn, needles were exposed to successive increases in irradiance (at night) to simulate the *g_s* response to dawn (blue + red light at 30, 60, 90 and 120 μmol m⁻² s⁻¹). At each irradiance, the time required for equilibration and the value of *g_s* at equilibrium were recorded. The instantaneous graphics program associated with the photosynthesis monitoring system assisted in equilibrium determination. Light response curves for mature trees in June at Barton Flats and for both tree size classes during the summer were not performed because of the excessive time required to open stomata (> 2 h). Stomatal conductance values were reported on a needle surface area basis. The needle surface area was determined from a geometric model and measurements of fascicle diameter and needle length enclosed in the cuvette.

Needle surface microscopy

The surfaces of needles from each site and tree size class were examined by scanning electron microscopy as described by Bytnerowicz et al. (1999). Without touching the cuticle, six previous-year needles from different primary branches were collected from each tree size class at each site in mid-August, placed in cryovials, and transported to the laboratory.

Atmospheric chemistry monitoring

Ozone concentrations were measured with an ozone monitor (Model 1003 AH, Dasibi, Glendale, CA) every 5 min and averaged and recorded at 1-h intervals at BF. Hourly ozone concentrations were adjusted based on a daily two-point calibration (O₃-free air and 100 ppb). The ozone monitor was calibrated against a tertiary transfer standard at the University of Califor-

Table 1. Summary of gaseous pollutant exposure from passive samplers (NO₂, HNO₃⁻, NH₃) at three sites over the summer of 2001. Replicate samples of NO₂ had a mean SD of 6%; HNO₃⁻ samplers had a mean 9% error; and quadruplicate samples had a mean 3% error (Bytnerowicz et al. 2002).

Site	Month	NO ₂ (ppb)	HNO ₃ ⁻ (μg m ⁻³)	NH ₃ (μg m ⁻³)
Crestline	June	6	5	4
	July	7	4	4
	August	7	6	4
Strawberry Peak	June	6	3	4
	July	8	3	3
	August	7	5	4
Barton Flats	June	2	2	2
	July	3	2	4
	August	3	2	3

nia, Riverside, and passed audit by South Coast Air Quality Air Management District on May 23, 2001. The light source of the O_3 monitor was adjusted on August 14 to bring the sample and control frequencies within the appropriate range. This resulted in a 5 ppb drop in the zero offset and a 6% decrease in the slope of the calibration curve. On September 21, the calibration was changed back to the same range as it had been before August 14. Because the sample and control frequencies were out of range before both adjustments, the calibration and resulting values were retained. Despite these problems, the error in the readings of the O_3 monitor had a mean daily value of only 2% (over the 87 days) and a range from -9 to 8%. The closest active O_3 monitoring station to the SP field site was 2 km east, and 80 m lower in elevation at the Rim Forest High School (maintained by the California Air Resources Board). This station has direct exposure to the pollution plume transported into the mountains. The SP field site was located on the north side of Strawberry Peak, 0.5 km into the forest from the forest–chaparral interface. Ozone concentrations at the field site are likely to be at least 2 ppb lower than those reported by the monitoring station, based on similar site comparisons in the Sierra Nevada (Bytnerowicz et al. 2002). For the Crestline site, O_3 data was obtained from the Lake Gregory station of the South Coast Air Quality Management District, 4 km east of the Crestline site. Their hourly values at 0400, 0500 and 0600 h were increased by 3 ppb based on statistical comparisons between active monitors stationed at Camp Paivika (on site) and Lake Gregory during 1993 and 1994 (Grunke 1999).

Atmospheric concentrations of nitrogen dioxide (NO_2), nitric acid vapor (HNO_3), and ammonia (NH_3) were measured from deposition on passive surfaces that were collected and analyzed every 2 weeks. Values given in Table 1 are thus the mean of the first and second 2-week exposure periods each month. Nitrogen dioxide was collected on Ogawa passive samplers in triplicate (Koutrakis et al. 1993) using triethanolamine (TEA) as an absorbent and determined colorimetrically with a Beckman DU-65 spectrophotometer (Ogawa 1998). Nitric acid vapor was collected in triplicate on Nylasorb nylon filters (Nylasorb, Pall Corporation) hung under polyethylene caps protecting the filters from wind and rain. Ammonia was determined with diffusion tubes (Gradco International, Winchester, U.K.) in quadruplicate, with 1% (w/v) sulfuric acid as a collection reagent. Air-borne NH_3 was converted to ammonium on collecting pads of the sampler. Ammonium was extracted with nanopure water and analyzed by colorimetry (Technicon TRAACS Autoanalyzer). Cumulative NH_3 exposure was calculated based on results of a calibration curve made against a honeycomb denuder system (Alonso et al. 2002).

Nighttime versus daytime O_3 uptake calculations

Daily O_3 uptake was calculated from diurnal measurements of g_s of six pole-sized trees at each site in each month (Grunke et al. 2002) during a year of average precipitation, and hourly O_3 concentrations measured in 2001 at each site, averaged for each hour over the month to match the conductance data. To calculate O_3 uptake, g_s was multiplied by a constant (0.612,

Laisk 1989) to adjust for the difference in diffusivity between water vapor and O_3 . Nighttime g_s was assumed to be constant over the whole night at the values measured. Daytime hours for each month varied (June, 0500–2000 h; July and August, 0500–1900 h; September, 0600–1800 h) based on photosynthetic photon flux (PPF) measurements made on site in 1993 and 1994 (Grunke et al. 2002). Stomatal conductance values $< 2.0 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ were considered within the measurement error of the gas exchange system and uptake was calculated, but not considered significant at this value.

Results

Environmental stressors

During the summer of 2001, all three sites had similar daytime O_3 concentrations (Figure 1). Ozone concentrations in July were depressed because of frequent morning fogs. Nighttime O_3 concentrations were similar at CR and BF, but were higher at Rim Forest (the closest O_3 monitoring station to SP) by 4 ppb (both sites) in June, by 14 and 12 ppb (CR and BF, respectively) in July, by 7 and 9 ppb in August, and by 5 and 16 ppb in September. The potential differences (2 ppb) in O_3 concentrations between the monitoring station and the field station were small relative to site-to-site differences. Nitrogen dioxide was twice as high (6–8 ppb) at the two western sites (CR, SP) as at the eastern site (BF) (Table 1). Nitric acid deposition averaged 40% higher ($4\text{--}6 \mu\text{g m}^{-3}$) at the westernmost site (CR) relative to SP; HNO_3 deposition was 80% higher at SP than at the eastern site (BF). Ammonia deposition was similar across all three sites ($3\text{--}4 \mu\text{g m}^{-3}$) and slightly less at the eastern site ($2 \mu\text{g m}^{-3}$) in early summer.

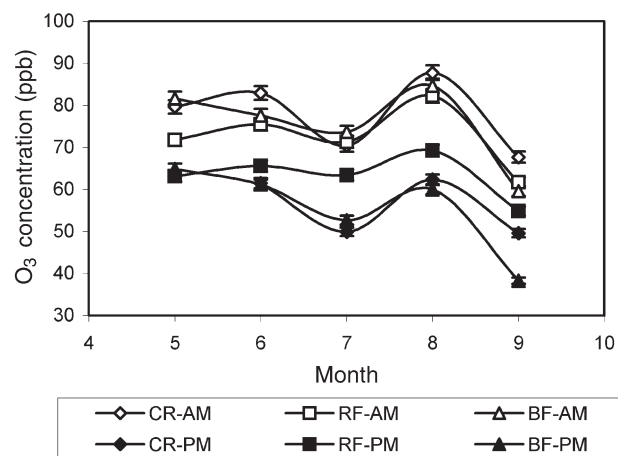


Figure 1. Mean monthly daytime (AM) and nighttime (PM) ozone concentrations for the three sites. The Barton Flats (BF) O_3 monitor had an error with a mean daily value of 2% and a range from -9% to 8% from June through September. Rim Forest High School monitoring station (RF) was the closest site to Strawberry Peak for measurements on an active monitor (data for this site courtesy of California Air Resources Board). Data for Crestline (CR) were from Lake Gregory, courtesy of South Coast Air Quality Resource Division. Error bars indicate ± 1 SE.

There were significant differences in soil water availability at the three sites (data not presented). Soil water content over the summer (June 22 through September 27) was low (2–5%) at both soil depths (15 and 50 cm) near trees at the eastern site, whereas soil water content ranged from 12% in June to 7% in September at the westernmost site (CR). The SP site had intermediate soil water content values, ranging from 8% in June to 7% in September. Within a site, the two tree size classes had similar predawn needle xylem potentials (Figure 2). Trees at the eastern site had lower predawn needle xylem potentials (–1.3 MPa) than at the other sites in June, and values declined to –1.9 MPa by September. At the western sites (CR, SP), predawn needle xylem potentials were relatively high (–1.0 MPa) in June, July and August. In September, predawn needle xylem potentials ranged between –1.3 and –1.7 MPa, but differed significantly between sites only for pole-sized trees.

Foliar injury and needle retention are visible symptoms of oxidant injury (Miller et al. 1996) and, to some extent, drought stress (Grulke 2003, Grulke et al. 2003). Among sites, SP had lower canopy health as reflected in the FPM score, especially for the pole-sized trees (Table 2). High background nitrogen deposition at CR may have mitigated chlorotic mottle symptoms, but would have also reduced foliar retention. The lower chlorotic mottle of foliage and the higher needle retention at BF indicates lower exposure to atmospheric pollution at this site, despite greater drought stress. Lower canopy health at SP supports greater total oxidant exposures, and could reflect

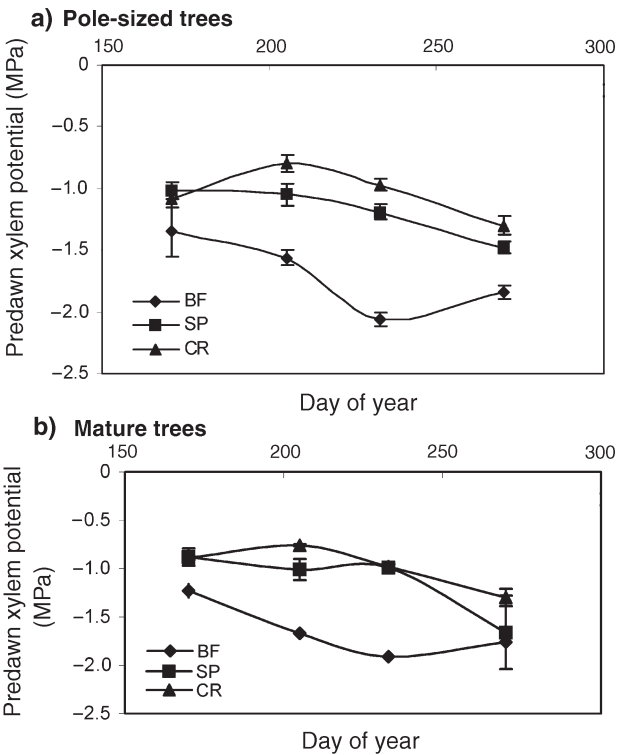


Figure 2. Seasonal course of predawn needle xylem potentials at the three sites. Symbols represent the mean $\pm$ 1 SE of six trees of each tree size class at the three sites monitored. Abbreviations: BF = Barton Flats; SP = Strawberry Peak; and CR = Crestline.

Table 2. Comparison of the Forest Pest Management (FPM) scores for historic versus current canopy health. Data are means with 1 SD. The FPM score is the whorl number of the youngest needle size class with 10% chlorotic mottle per needle on more than a third of the needles in that whorl.

Site	Tree size	1988 ¹	1994 ²	2001 ³
Barton Flats	Pole			2.5 (0.2)
	Mature	2.4	1.4	3.0 (0.3)
Strawberry Peak	Pole			1.3 (0.2)
	Mature	1.1	n.d.	1.6 (0.3)
Crestline	Pole			2.3 (0.4)
	Mature	0.3	0.4	2.6 (0.3)

¹ Miller et al. (1989); data taken 3 km east of Strawberry Peak.

² Miller and Rechel (1998); no data for mature trees.

³ This study.

greater late-summer drought stress.

Nighttime stomatal conductance

In June, nighttime g_s ranged from 8 to 19 mmol H₂O m⁻² s⁻¹ at the two western sites (CR, SP; Figure 3). At the eastern site (BF) in June, g_s was low (3 to 5 mmol H₂O m⁻² s⁻¹). Nighttime g_s decreased from early to late summer at all sites, from 19 to < 2 mmol H₂O m⁻² s⁻¹. Nighttime g_s of both tree size classes at CR was relatively high in June and was similar to that of mature trees at SP. The pole-sized trees at SP had lower nighttime g_s than mature trees in June. In July, nighttime g_s of the mature trees at the two western sites was lower than that of pole-sized trees. In late summer, nighttime g_s was < 2 mmol H₂O m⁻² s⁻¹ for both tree size classes at all sites.

No apparent cuticular lesions were detected on the needle surfaces of foliage of either tree size class that would have contributed significantly to needle conductance earlier in the summer (shown here only for mature foliage in August, Figure 4).

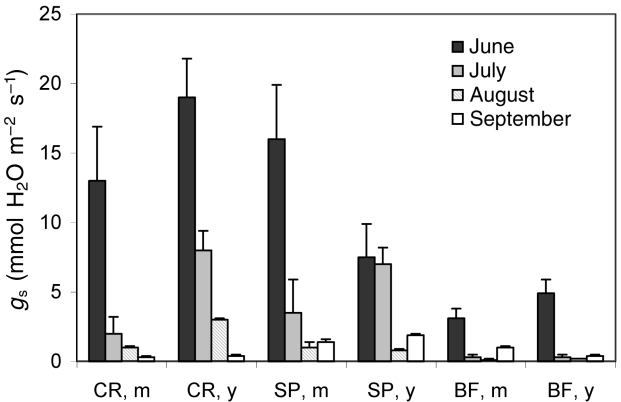


Figure 3. Nighttime stomatal conductance (g_s) at three sites over the growing season. Abbreviations: CR = Crestline with high pollution; SP = Strawberry Peak with moderately high pollution; BF = Barton Flats with moderate pollution; m = mature trees; and y = pole-sized trees.

There was also no evidence of stomatal occlusions that might have physically limited stomatal closure.

Stomatal conductance response to simulated dawn

The slope of the response curve of g_s to PPF for pole-sized trees did not differ significantly at the three sites in June ($P = 0.155$; Figure 5). The largest difference between the responses was the increasing y-intercept from BF ($2 \pm 0 \mu\text{mol m}^{-2} \text{s}^{-1}$) to SP ($6 \pm 2 \mu\text{mol m}^{-2} \text{s}^{-1}$) to CR ($19 \pm 3 \mu\text{mol m}^{-2} \text{s}^{-1}$) (CR statis-

tically significant based on a one-way ANOVA at $P = 0.05$). The maximum stomatal opening obtained at night at $120 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ declined from June to August at the two western sites (CR, SP) for both tree size classes (Figure 6). Mature trees had lower g_s at all irradiances than pole-sized trees at both CR and SP in July and August.

Seasonal increase in stomatal response to low light

The time required for stomata to open fully at $120 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ was similar between the two tree age classes at SP, and it took successively longer to reach equilibrium through the growing season (Figure 7). At CR, the time required to open stomata fully increased from June to July, but decreased from July to August for both tree age classes. In June, about the same time (10–15 min) was required to open the stomata from dark to $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ as was required to reach equilibrium at each higher irradiance. In July, 25–30 min was required to open stomata fully at $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ for both tree size classes at both sites. Stomata of pole-sized trees at Crestline were fully induced at $90 \mu\text{mol m}^{-2} \text{s}^{-1}$ and did not open further with additional light in July. In August, slightly more time (30–35 min) was required to open stomata fully at $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ at SP for both tree size classes, but less time (15–20 min) was required at CR for both tree size classes. Few differences were found between the two tree size classes in the speed of stomatal opening, but when there were differences, mature trees generally took less time to equilibrate than pole-sized trees.

Seasonal changes in daytime and nighttime O_3 uptake

Estimates of nighttime foliar O_3 uptake ranged from 11 to $<1\%$ of total daily uptake (Figure 8). In June, nighttime O_3 uptake was 3% of estimated 24-h uptake at BF, 5% at SP and 9% at CR. For the rest of the growing season at BF, nighttime g_s was low, and foliar O_3 uptake was negligible. In July, nighttime O_3 uptake accounted for 5% of diel uptake at both western sites. In August, nighttime O_3 uptake accounted for 4% of diel

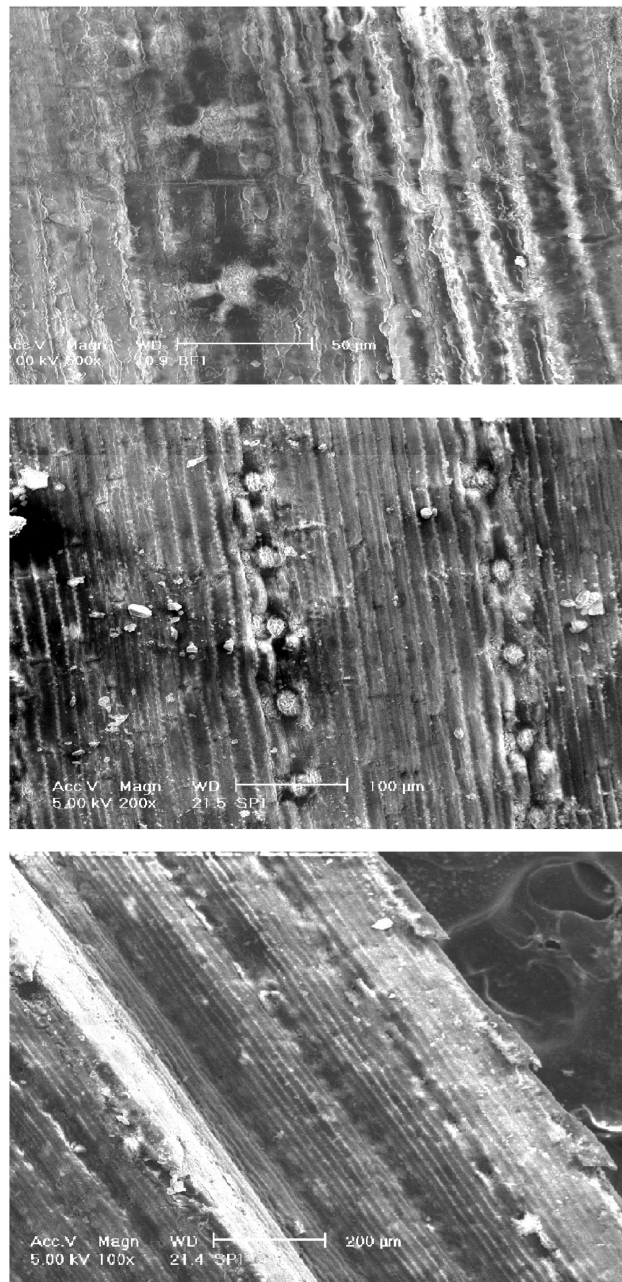


Figure 4. Micrographs of needle surfaces from pole-sized trees at three sites in August (from top to bottom): Barton Flats, Strawberry Peak and Crestline. Micrographs courtesy of P. Padgett (USDA Forest Service, Pacific Southwest Research Station, USA).

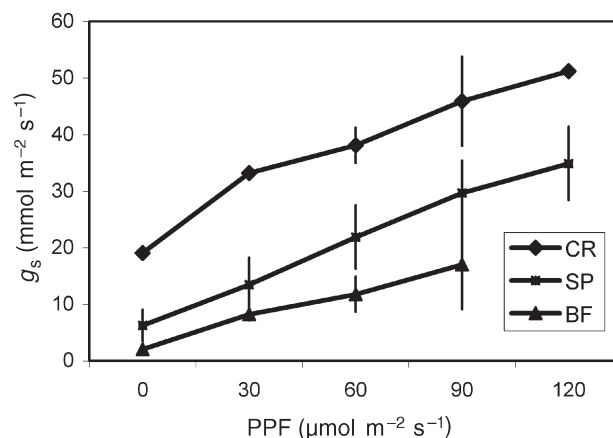


Figure 5. Stomatal response of pole-sized trees to simulated dawn (blue + red) at all sites in June. Abbreviations: g_s = stomatal conductance; PPF = photosynthetic photon flux; BF = Barton Flats; SP = Strawberry Peak; and CR = Crestline. Error bars indicate ± 1 SE about the mean.

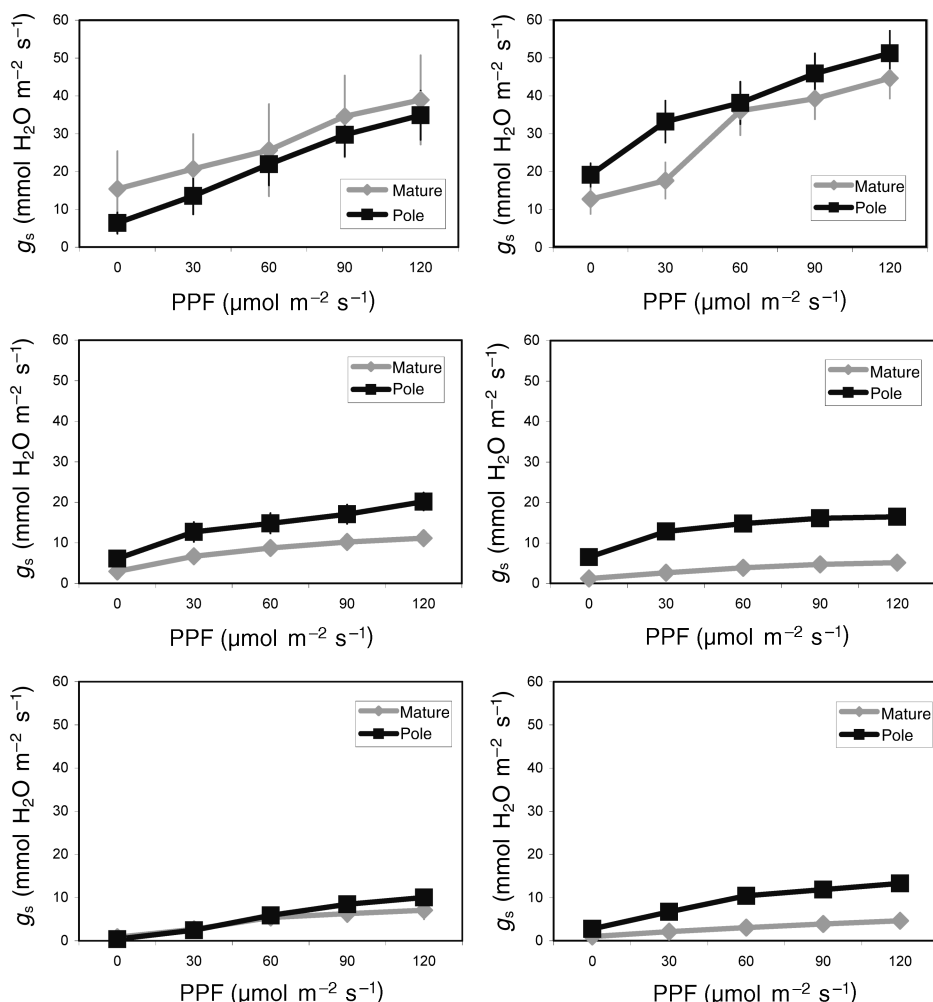


Figure 6. Seasonal course of stomatal response to simulated dawn (blue + red) at night at Strawberry Peak (left column) and Crestline (right column) in June (top), July (middle) and August (bottom). Abbreviations: g_s = stomatal conductance; and PPF = photo-synthetic photon flux. Error bars indicate ± 1 SE about the mean.

uptake at CR, but was negligible at SP. Nighttime O_3 uptake was negligible at all sites in September. Despite higher nighttime O_3 concentrations at SP, the proportion of nighttime O_3 uptake was lower than that at CR. The proportion of O_3 uptake attributable to nighttime uptake was significantly affected by time of dawn and dusk, sluggishness of stomata to close at dusk, and the resolution of g_s measurements near the detection limit of the instrument.

Discussion

In early summer, both pole-sized and mature trees had open stomata at night. Values of nighttime g_s for ponderosa pine ranged from 3 to 19 $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ in June, two to three times higher than nighttime values previously reported for other conifers (Keller and Häslér 1984, Skärby et al. 1987, Wieser and Havranek 1993). Typical values for daytime maximum g_s ranged from 60 to 90 $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ in early sum-

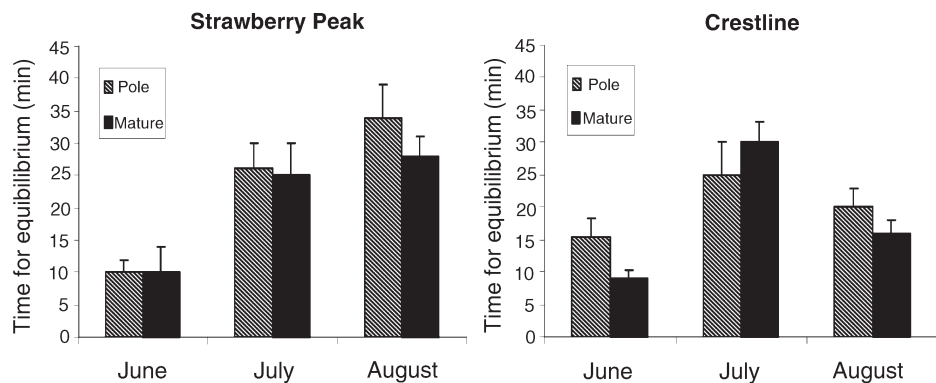


Figure 7. Time required to achieve a stable response to low irradiance ($30 \mu\text{mol m}^{-2} \text{s}^{-1}$) following darkness over the course of the growing season at Strawberry Peak and Crestline. Error bars indicate ± 1 SE about the mean.

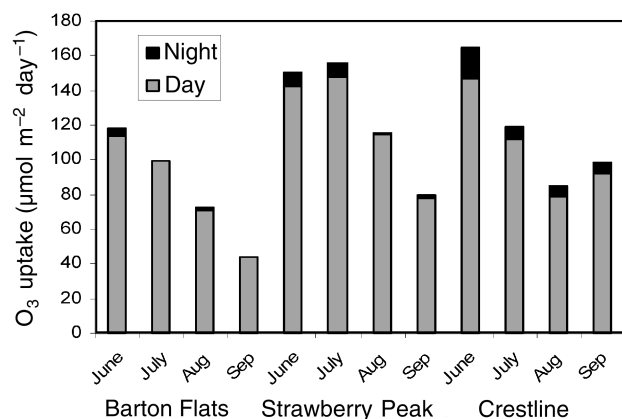


Figure 8. Estimated nighttime versus total daily O₃ uptake for ponderosa pine at monthly time steps at three sites in southern California.

mer and 35 to 70 mmol H₂O m⁻² s⁻¹ in late summer for pole-sized trees at the same sites (Grulke et al. 2002). No damage to needle cuticles were found that could explain the values of nighttime foliar transpiration observed, nor were stomatal occlusions apparent. Nighttime g_s decreased with accumulating pollutant exposure.

Pollutant exposure was higher at the western sites (CR: NO₂, HNO₃; SP: NO₂, nighttime O₃) relative to the eastern site (BF). Canopy health at SP was poor, especially for pole-sized trees. Canopy health at CR was slightly better than at SP, and high nitrogen deposition at CR may have helped mitigate foliar injury due to oxidant exposure (Grulke 2003). Although drought stress probably reduced pollutant uptake at the eastern site (BF), better canopy health supported overall lower pollutant exposure (Table 2). It is unknown how higher historical pollutant exposures affected our results. As late as 1994, O₃ concentrations were significantly greater at CR (80 ppb, 24-h mean over 6 months) than at SP (69 ppb) and BF (64 ppb) (Grulke 1999). Comparable values in 2001 were significantly lower at CR (62 ppb, 24 h average over 5 months), but similar at SP (65 ppb) and BF (62 ppb). Mature tree canopy health has improved at all sites since 1988 and at BF and CR since 1994.

Super-elevated CO₂ concentrations (e.g., > 5000 ppm) can open stomata at night (Wheeler et al. 1999), but in this study, background concentrations were not high (< 450 ppm at night) and were constantly monitored. Other air pollutants can disturb stomatal control mechanisms (Matyssek et al. 1995, Heath and Taylor 1997, Mansfield 1998, Robinson et al. 1998), but the responses to these pollutants have been studied mainly during the daytime. Under controlled experimental exposures, O₃ increases cell membrane permeability to K⁺, resulting in stomatal closure (Heath and Taylor 1997). Ponderosa pine seedlings exposed to elevated O₃ concentration have shown both decreased (Weber et al. 1993) and increased g_s (Beyers et al. 1992) relative to seedlings grown in charcoal-filtered air. However, both responses can be explained by the effect of substomatal CO₂ concentration (C_i) on stomatal aperture (Cowan 1977). In the first study, exposure to elevated O₃ concentration reduced photosynthetic rate, increasing C_i, and resulting in a proportional closure of stomatal aper-

ture. In the second study (Beyers et al. 1992), exposure to both elevated O₃ concentration and drought stress reduced the leaf area of seedlings, and translocated N elevated both foliar N and photosynthetic rate of the remaining foliage with a proportional increase in stomatal aperture. However, exposure to elevated O₃ concentration may also decouple the relationship between assimilation and g_s (Matyssek et al. 1995, Tjoelker et al. 1995, Grulke 1999), lowering water-use efficiency.

Ozone exposure alone has been shown to increase nighttime g_s in other conifer species (Keller and Häslér 1984, Skärby et al. 1987, Wieser and Havranek 1993). Physical damage of epidermal cells and abnormal maintenance of open stomata in birch resulted from the combined effects of sulfur oxides and nitrogen oxides (*Betula* spp., Neighbour et al. 1988). The combined effects of SO₂ and O₃ compromised cell wall integrity of guard cells and reduced stomatal control in Norway spruce (Maier-Maercker and Koch 1995). The concentrations of NO₂ and HNO₃ alone at the western sites were not expected to elicit aberrations in stomatal behavior directly (Wellburn 1990, Bytnerowicz et al. 1998). However, the background O₃ exposure combined with NO₂ and HNO₃ may have contributed to the responses observed.

Drought stress throughout the summer at the eastern site, and drought stress in late summer at the western sites appeared to promote nighttime stomatal closure. Higher drought stress at SP relative to CR could explain the increasingly sluggish stomatal response in late summer (Figure 7): more light would be required to overcome higher concentrations of foliar abscisic acid (ABA) (Christmann et al. 1995). During the day, drought stress in combination with elevated O₃ concentration had the opposite effect on beech (*Fagus sylvatica* L.; Davidson et al. 1992, Pearson and Mansfield 1993) and Norway spruce (Wallin and Skärby 1992, Karlsson et al. 1997).

Calculated O₃ uptake at night accounted for 9% (CR), 5% (SP) and 3% (BF) of the 24-h total O₃ uptake in early summer, but was negligible in late summer at all sites. Calculations of O₃ uptake were more sensitive to day length and daytime g_s than to a 10 ppb difference in O₃ concentration. Although it represents only a small portion of the total daily O₃ uptake at these sites, nighttime O₃ uptake could still be physiologically stressful. Concentrations of foliar antioxidants, and their regeneration capacity are lower at night (Schupp and Rennenberg 1988, Faria et al. 1996, Tausz et al. 2001). Both ascorbate and glutathione require NADPH regenerated by photosynthetic electron transport to stay reduced and scavenge oxidants (Foyer et al. 1994). Other species exposed to O₃ during the night have shown similar or higher O₃-induced injury than plants growing in elevated O₃ concentrations during the daytime, despite lower nighttime g_s (Matyssek et al. 1995, Günthardt-Georg et al. 1997). In this study, mature trees had lower nighttime g_s than pole-sized trees, and foliage from mature trees is known to have greater antioxidant protection on a leaf surface area basis (Tegischer et al. 2002, Wieser et al. 2002). Daytime g_s was similar between pole-sized and mature ponderosa pine (Grulke and Retzlaff 2001). Pollutant uptake combined with the absence of light-derived energy to scavenge oxidants might have contributed to the poor canopy health of pole-sized trees at SP.

Stomata of ponderosa pine were partially open at night, at 5 to 20% of daytime values. Stomata of both pole-sized and mature trees were open, but pole-sized trees generally had higher nighttime conductance. Nighttime conductance declined through the growing season at all sites despite accumulating exposure to O₃, nitrous oxides and nitric acid exposure. The decline in g_s and the increase in sluggish response to low irradiances through the growing season were most likely related to an increase in drought stress and accompanying hormonal increase in foliar ABA concentration (Christmann et al. 1995). There was no evidence for cuticular degradation or stomatal occlusions that would result in the observed pattern of responses. Early summer nighttime uptake of O₃ was low (3 to 9%), and probably should not be accumulated for the purposes of determining tree response to cumulative O₃ exposure in natural stands. However, nighttime uptake of oxidants may have deleterious physiological effects because oxidants absorbed by foliage at night are not detoxified as well as during the day. Coupled with relatively high daytime uptake, high physiological activity required for growth, nighttime uptake of oxidants in early summer could contribute to the overall declining health of forest trees, especially of pole-sized trees.

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