Effects of Nighttime Ozone Treatment at Ambient Concentrations on Sensitive and Resistant Snap Bean Genotypes

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ADDITIONAL INDEX WORDS. *Phaseolus vulgaris*, nocturnal stomatal conductance, R123, S156, biomonitor

ABSTRACT. The effect of nighttime ozone (O₃) exposure, alone and in combination with daytime O₃ treatment, was tested on yield of an O₃-resistant (R123) and an O₃-sensitive (S156) snap bean (*Phaseolus vulgaris* L.) genotype. Three trials, with exposure durations ranging in length from 14 to 21 days, were conducted in continuous stirred tank reactors located within a greenhouse. The effects of day-only (0800–1900 HR = 11 hours·day^{−1}) and day + night (0800–1900 HR + 2000–0700 HR = 22 hours·day^{−1}) exposure timings were compared. The Fall 2014 trial also tested the effect of nighttime-only (2000–0700 HR = 11 hours·day^{−1}) O₃ exposure. Nighttime O₃ exposure alone, at 62 ppb, did not cause foliar injury and had no effect on the yield of either genotype. In combination with daytime O₃ exposure, nighttime O₃ concentrations up to 78 ppb did not impact yields or show a consistent effect on nocturnal stomatal conductance (*g_{sn}*). When data were pooled across the day and day + night exposures times, mean daytime O₃ levels ≥62 ppb caused foliar injury and significant yield decreases in all three trials. Under control conditions, R123 and S156 produced similar pod masses in two of the three trials. In all three trials, R123 produced significantly greater yields by mass than S156 with elevated O₃. Nighttime conductance measurements suggested that S156 and R123 have inherently different *g_{sn}* rates and that cumulative O₃ exposure can increase *g_{sn}* in both genotypes.

Across the globe, tropospheric ozone harms human and environmental health (World Health Organization, 2006), while also reducing crop yields (Ashmore, 2005). In the United States, the U.S. Environmental Protection Agency (USEPA) regulates O₃ air pollution under the National Ambient Air Quality Standards (NAAQS). As a secondary air pollutant, O₃ forms through photochemical reactions involving nitrogen oxides (NO_x) and volatile organic compounds (VOCs), which are produced by anthropogenic and natural processes. Both O₃ and its precursors migrate from urban sources to rural and remote locations, resulting in widespread impacts (USEPA, 2013).

Ambient O₃ exposure contributes to yield losses for economically important plant species, including O₃-sensitive cultivars of soybean [*Glycine max* (L.) Merr.], wheat (*Triticum aestivum* L.), potato (*Solanum tuberosum* L.), bean, tomato (*Solanum lycopersicum* L.), watermelon [*Citrullus lanatus*

(Thunb.) Matsum and Nakai], onion (*Allium cepa* L.), turnip (*Brassica rapa* L.), and lettuce (*Lactuca sativa* L.) (Booker et al., 2009; USEPA, 2013). Ozone, an oxidant gas, diffuses into leaves through stomatal pores and can either dissolve in water or react directly with biochemical compounds. The resulting reactive oxygen species damage membranes and other cell components, reducing photosynthesis, accelerating aging, and negatively affecting growth and reproduction (Booker et al., 2009; USEPA, 2013). Ozone exposure can also reduce crop quality and nutritive value (USEPA, 2015).

Sensitivity to O₃ varies both within and among species. In general, plants that have higher stomatal conductance (*g_s*), which allows a greater flux of O₃ into the leaf (Reich and Amundson, 1985; Temple, 1991), and lower levels of ascorbic acid, an antioxidant and key plant defense signaling molecule (Baier et al., 2005; Conklin and Barth, 2004), are more susceptible to injury. Within a species, different genotypes may vary in their molecular response to O₃ (Baier et al., 2005; Gravano et al., 2004; Kangasjärvi et al., 2005; Langebartels et al., 2002; Pell et al., 1997; Schraudner et al., 1998; Wohlgemuth et al., 2002). In addition, environmental factors, such as light level and water availability, can interact with O₃ stress and alter injury severity (Orvar et al., 1997; Wilkinson and Davies, 2010).

Booker et al. (2009) reviewed studies of O₃ impacts on agronomic and horticultural crops, reporting yield losses

Received for publication 31 Aug. 2017. Accepted for publication 15 Nov. 2017. Funding provided by the Pennsylvania Department of Environmental Protection, Bureau of Air Quality; the Pennsylvania Agricultural Experiment Station; and the Department of Plant Science, The Pennsylvania State University.

We thank Jim Savage, Jon Ferdinand, and Scott DiLoreto for their technical expertise; Kent Burkey for providing seed and expertise; Rick Bates and Jim Sellmer for reviewing the manuscript; and Martha Schupp and Emily Isaacs for greenhouse assistance.

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ranging from $\approx 5\%$ to 15% for O_3 sensitive plants. Based on a meta-analysis of 81 publications, Feng and Kobayashi (2009) estimated that mean ambient O_3 concentrations of 31–50 ppb, relative to base concentrations (<26 ppb), reduced the yield of six major crops as follows: bean (19%), rice [*Oryza sativa* L. (17.5%)], wheat (9.7%), barley [*Hordeum vulgare* L. (8.9%)], soybean (7.7%), and potato (5.3%). Notably, plants may have been exposed to actual O_3 concentrations in excess of 50 ppb following the diurnal cycle of O_3 over the 7- to 12-h averaging period. The USEPA's Clean Air Scientific Advisory Commission suggested that O_3 -induced crop yield reductions exceeding a threshold of 5% relative loss are “unacceptably high,” causing adverse impacts on public welfare (USEPA, 2015). Therefore, ambient O_3 levels pose a definite concern to food production, and standards designed to protect vegetation require continual review.

In the latest revision of the NAAQS for O_3 , effective 28 Dec. 2015, the USEPA Administrator strengthened the secondary standard (i.e., protecting public welfare, including the yield and quality of agricultural crops) from 75 to 70 ppb. However, the legislation retains a “fourth-high metric” format and 8-h averaging time, identical to the primary standard used to protect public health (USEPA, 2015). In contrast to a cumulative exposure index, the fourth-high metric is based on the fourth-highest daily maximum O_3 concentration (i.e., averaged over an 8-h period within a 24-h window), averaged across three consecutive years.

The 70-ppb level of the fourth-high metric was correlated with the cumulative, concentration-weighted W126 index. The W126 takes into account all hourly O_3 concentrations within the 12-h window from 0800 to 2000 HR (Lefohn et al., 1988; Lefohn and Runeckles, 1987; USEPA, 2015). Despite evidence showing injury due to nighttime O_3 exposure (Goknur and Tibbitts, 2001; Lee and Hogsett, 1999; Matyssek et al., 1995; Winner et al., 1989), the 12-h W126 index does not include elevated O_3 episodes occurring between 2000 and 0800 HR (USEPA, 2015). Public comment on the proposed NAAQS for O_3 suggested that the W126 cumulative index, as the sole calibration for the hourly maximum fourth-high metric, should include concentrations for the entire 24-h period within 1 d (USEPA, 2015; also see A.S.L. & Associates, n.d.). Therefore, the 12-h W126, when calculated from 0800 to 2000 HR, may not adequately describe exposure–response relationships to protect sensitive vegetation where nighttime O_3 exposure is a factor (Musselman et al., 2006).

Nocturnal stomatal conductance has been reported for a wide range of species (Caird et al., 2007; Dawson et al., 2007; Musselman and Minnick, 2000), demonstrating the potential for O_3 uptake from ambient air at night. Furthermore, researchers observed injury resulting from nighttime or dark period O_3 exposure, using both indoor (Goknur and Tibbitts, 2001) and outdoor chambers (Günthardt-Goerg, 1996; Lee and Hogsett, 1999; Matyssek et al., 1995). However, no clear nighttime exposure–response relationships have been developed for O_3 to date, and evidence documenting the cumulative effects of nighttime O_3 exposure remains limited (USEPA, 2013).

Nighttime O_3 exposure occurs when O_3 concentrations remain elevated in the surface layer of the atmosphere because of limited destruction via dry deposition and NO scavenging. Based on available monitoring data, the USEPA (2013) identified mountainous areas of southern California, the front-range

of Colorado, and the Great Smoky Mountains as potential locations of concern for elevated nocturnal O_3 levels. However, nighttime O_3 exposure may cause injury in other locations. Typically, O_3 is produced photochemically during daylight hours, reaching a peak in midafternoon and decreasing rapidly, often to near 0, after sunset. Rural areas downwind of urban pollution sources can experience a delay in this cycle, leading to more uniform daily O_3 concentrations. This attenuation of the typical diurnal pattern results from additional time required to transport O_3 and precursors from urban sources, along with continued O_3 production during transport (USEPA, 2013).

In the residual layer of the atmosphere, which forms above a stable surface layer when vertical mixing is limited (Stull, 1988), O_3 concentrations can also remain relatively constant throughout the day and night (Emberson et al., 2000; Forlani et al., 2005; Musselman and Minnick, 2000; Orendovici-Best et al., 2010; USEPA, 2013; Winner et al., 1989). Therefore, vegetation growing at higher elevations may be exposed to greater nighttime O_3 levels. For example, at a mountainous site in rural central Pennsylvania, the mean O_3 concentration from 9 June to 9 Sept. 2003 was 45 ppb (Orendovici, 2005, see Table 9). However, nighttime O_3 levels often exceeded daytime concentrations, with maximum values around 100 ppb (Orendovici, 2005, see Fig. 9). Given the potential for nighttime O_3 exposure, particularly in rural areas and high-elevation sites (Emberson et al., 2000; Forlani et al., 2005; Musselman and Minnick, 2000; USEPA, 2013), it is important to consider effects on plants.

To determine if nighttime O_3 exposure causes injury and influences yield of O_3 -resistant (R123) and O_3 -sensitive (S156) snap bean genotypes (Burkey et al., 2005, 2012; Flowers et al., 2007; Reinert and Eason, 2000), the effects of day-only (0800–1900 HR = $11 \text{ h} \cdot \text{d}^{-1}$) and day + night (0800–1900 HR + 2000–0700 HR = $22 \text{ h} \cdot \text{d}^{-1}$) exposure times at control (3–15 ppb), low (25–49 ppb), and high concentrations (up to 78 ppb) were compared. Ozone treatments represented realistic nighttime levels based on observations from central Pennsylvania (Orendovici, 2005). The S156 and R123 genotypes were developed by the USDA-ARS Plant Science Research Unit in Raleigh, NC (Flowers et al., 2007; Reinert and Eason, 2000), and have been used to study flux-effect relationships by the International Cooperative Program on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation, 2012).

Materials and Methods

The experiments were conducted within a greenhouse on the University Park campus of The Pennsylvania State University (lat. 40.805640°N, long. 77.852356°W). Ozone treatments were administered in continuous stirred tank reactors [CSTR (Heck et al., 1978)]. The cylindrical CSTR chambers measured 1.5 m diameter by 1.5 m height and were enclosed with 76.2- μm transparent polytetrafluoroethylene (Teflon; Chemours, Wilmington, DE) film. Each chamber was equipped with an overhead 1000-W lamp having a spectral distribution of 350–700 nm, with peaks at 550 and 650 nm (Lumalux; GTE Products Corp., Sylvania Lighting Center, Danvers, MA). Outside air was passed through activated charcoal filters to reduce O_3 levels within the greenhouse. Mechanical blowers pulled the filtered greenhouse air into the CSTR chambers, with the full chamber volume exchanging about once per minute and exhaust released to the outside. An O_3 generator (Z-08; Ozone

Solutions, Hull, IA) was used to produce O₃ from dry air via electric current. Ozone was distributed to the CSTRs via manually adjusted valves and polytetrafluoroethylene tubing. Ozone levels in each chamber were monitored at 10-min intervals and adjusted using computerized feedback to the O₃ generator. Air samples were collected at a rate of $\approx 1 \text{ L} \cdot \text{min}^{-1}$ using a network of computer-controlled solenoid valves and measured with two photometric ozone analyzers (model 49; Thermo Environmental Corp., Franklin, MA). Ozone measurements were recorded via custom data acquisition software (REAL Controls, Salix, PA). The software also tracked air temperature and relative humidity (RH) data from sensors (HX93BC; Omega Engineering, Stamford, CT) installed in each chamber. Photosynthetically active radiation (*PAR*) was recorded during the 2015 trials from four quantum sensors (LI-190R; LI-COR, Lincoln, NE), which were positioned at canopy height in one chamber per experimental block.

PLANT MATERIAL. Seeds of the O₃-resistant (R123) and O₃-sensitive (S156) snap bean genotypes were provided by Dr. Kent Burkey of the USDA-ARS in Raleigh, NC. For the first two trials, Fall 2014 and Spring 2015, seeds were sown in 2.8-L pots. In the third trial, Fall 2015, 3.8-L pots were used. Pots were steam-sterilized at 82 °C for 20 min. Each pot was filled with commercial potting media containing 63% to 73% peatmoss, perlite, and dolomitic limestone (Sunshine Mix #4; Sun Gro Horticulture, Agawam, MA) that had been mixed with 15 g of 15N–3.93P–9.96K controlled-release fertilizer (Osmocote Plus; The Scotts Co., Marysville, OH). Three bean seeds were planted in each pot under ambient greenhouse conditions. Planting dates for the three trials are given in Table 1. When the first trifoliate leaves began to expand, plants were thinned to one per pot. Each seedling was tied to a 1-m bamboo stake for support.

OZONE EXPOSURES. All three trials included four O₃ treatments plus a control. The four O₃ treatments were a combination of O₃ concentration and treatment time as follows: 1) low O₃, day-only, 2) high O₃, day-only, 3) low O₃, day + night, and 4) high O₃, day + night (i.e., corresponding to day/night O₃ levels of low/ambient, high/ambient, low/low, and high/high, respectively). The Fall 2014 trial included a fifth, night-only treatment time at low and high O₃ concentrations (i.e., corresponding to day/night O₃ levels of ambient/low and ambient/high, respectively). The day-only (0800–1900 HR) and night-only (2000–0700 HR) treatments were $\approx 11 \text{ h}$ in duration, whereas the combined day + night treatments were $\approx 22 \text{ h}$. Because the O₃ supply to each chamber was fixed, plants in the day-only O₃ treatments were rotated into a spare control chamber (one per block), subjected to greenhouse ambient conditions + 0 ppb added O₃, at night. For the night-only treatment in Fall 2014, plants were located in the spare control chamber during the day

and moved into their respective O₃ treatment chamber at night. Randomized complete blocks of four chambers were calibrated by O₃ level as follows: control (ambient + 0 ppb), spare (ambient + 0 ppb), low, and high. The Fall 2014 trial included two blocks, with target O₃ concentrations of 30 and 60 ppb for the low and high chambers, respectively. For each genotype, eight plants per O₃ treatment and 14 plants for the control were divided between the two blocks. The Spring and Fall 2015 trials had four blocks, with target O₃ concentrations of 45 and 75 ppb for the low and high chambers, respectively. For each genotype, eight plants per treatment were divided between the four blocks.

Plants were acclimated to chamber conditions at least 48 h before O₃ treatment. Ozone concentrations followed a square-wave pattern, reaching target levels within 15 min from the start of each fumigation period. On cloudy days, solar radiation was supplemented with the 1000-W lamps located above each chamber. Water was supplied as needed to maintain soil moisture near pot capacity, usually twice per day, between treatment times. Plants were randomized daily within their respective treatment chambers to minimize positional effects. On days when O₃ treatments were not conducted, plants remained in the CSTR chambers with the doors open.

Ozone exposures commenced on 1 Oct. 2014 [15 d after seeding (DAS)], 23 Apr. 2015 (26 DAS), and 2 Oct. 2015 (28 DAS) for the three experiments. The number of exposure days ranged from 14 to 21 among trials (Table 1). At the conclusion of each trial, plants were removed from the CSTRs and randomized by block on greenhouse benches. Plants were grown under ambient light conditions and watered to pot capacity every 2–3 d until pods reached maturity. In Spring 2015, initial signs of chlorosis were observed on 3 May [36 DAS [10 d after treatment (DAT)]], and symptoms increased during the exposure period. During pod maturation, after plants had been removed from the CSTRs, most plants developed severe chlorosis as a result of nutrient deficiency from watering with high-calcium tap water. As a result, plants were watered with distilled water in Fall 2015.

NOCTURNAL STOMATAL CONDUCTANCE. A leaf porometer (SC-1; Meter Group, Pullman, WA) was used to measure abaxial g_{sn} during the time period from 2200 to 0100 HR. The terminal leaflet of a mature trifoliate leaf (i.e., leaf diameter ranging from 18 to 30 cm) was used for measurements. Leaves with visible injury symptoms exceeding 20% of the leaf area were excluded from the analysis. At the time of g_{sn} measurements, mature leaves of S156 were consistently smaller than R123 in the high O₃ treatments. Conductance was measured following daytime O₃ treatment on 30 Apr. and 7 May (33 and 40 DAS, respectively) in Spring 2015 and on 14 and 27 Oct. (40 and 53 DAS, respectively) during Fall 2015. Data reported for 14 Oct.

2015 represent the mean of two plants per replication. On the other three dates, one plant per replication was measured.

YIELD. Before harvesting, pods remained on the plants to dry. In a few cases, pods on S156 plants remained yellowish-green, whereas most of the R123 pods were brown. Any pod with one or more fully developed seeds was considered mature (*sensu* Burkey et al., 2005).

Table 1. Seeding dates for snap bean with the number of days until flowering, harvest, and the start and end of O₃ treatment for Fall 2014, Spring 2015, and Fall 2015 trials.

Trial	Seeding date	Time after seeding (d)				Total O ₃ exposure (d) ^z
		Start O ₃	Flower	End O ₃	Harvest	
Fall 2014	16 Sept.	15	29	38	92	18
Spring 2015	28 Mar.	26	33	45	102	14
Fall 2015	4 Sept.	28	34	53	79	21

^zCumulative number of O₃ treatment days; exposure times were $\approx 11 \text{ h} \cdot \text{d}^{-1}$ for the individual day and night treatments and $22 \text{ h} \cdot \text{d}^{-1}$ for the day + night treatments.

For each plant, the number of mature pods was recorded. In Fall 2014, each pod was opened and threshed by hand on 17 Dec., and the number of seeds per pod was counted. Seeds were dried at 21 °C for 24 d before weighing. In Spring and Fall 2015, pods harvested on 8 July and 23 Nov. were oven-dried at 66 °C for 48 h and weighed.

STATISTICAL ANALYSIS. The experimental design was a randomized complete block with four chambers per block and either two (Fall 2014) or four replications (Spring and Fall 2015). Chambers were the experimental units. The treatments followed an augmented factorial structure (Lentner and Bishop, 1993), with a factorial combination of two genotypes (i.e., S156, R123) and four day/night O₃ combinations (i.e., low/ambient, high/ambient, low/low, and high/high), along with a control (ambient/ambient). The Fall 2014 trial included two additional night-only O₃ treatments (i.e., ambient/low and ambient/high).

Data from the three trials were analyzed separately. Response variables recorded in Fall 2014 were pod number, seed number, seed mass, seeds per pod, and mean seed mass (grams per seed). Pod number, pod mass, and g_{sn} were recorded for Spring and Fall 2015. Values for multiple plants within an experimental unit were averaged before analysis. Block was considered a random effect. All data were subjected to analysis of variance in JMP Pro 12.1.0 (SAS Institute, Cary, NC) using the mixed model platform with restricted maximum likelihood methodology (REML) and Satterthwaite estimation for df. Single degree-of-freedom contrasts were used to test the significance of the main effects genotype, O₃ level, and time (i.e., day vs. day + night), as well selected interactions (Marini, 2003). Differences were considered significant at $P \leq 0.05$.

Results and Discussion

OZONE TREATMENTS. Table 2 provides a summary of mean O₃ concentrations, separated into day and night exposure periods, for each of the three trials. Mean O₃ levels in the control and spare chambers (greenhouse ambient + 0 ppb added O₃) ranged from 3 to 15 ppb over the duration of the three experiments. In Fall 2014, target O₃ concentrations for the low and high chambers were 30 and 60 ppb, respectively, and observed means were within 5 ppb (Table 2). In Spring and Fall 2015, target O₃ concentrations for the low and high treatments were 45 and 75 ppb, respectively. Mean O₃ concentrations

observed in the low and high chambers for Spring and Fall 2015 were within 1 and 4 ppb of target levels, respectively.

Because O₃ was generated via an electric current using dry air, instead of pure O₂, small amounts of N₂O₅ and N₂O were also likely produced (Harris et al., 1982). Using electric discharge, production of N₂O₅ from dry air has been estimated at 0.02–0.025 mmol N₂O₅ per mmol O₃ (Brown and Roberts, 1988; Mortensen and Jørgensen, 1996). However, in open-top chambers, actual levels of HNO₃, the hydrated form of N₂O₅, were lower in the added O₃ treatment than ambient air (Bytnerowicz et al., 1995). Although pure O₂ is the ideal source gas for high-voltage O₃ generation, cost and maintenance issues are prohibitive for long-term studies (Mortensen and Jørgensen, 1996; Olszyk et al., 1990). Given the low O₃ concentrations in this study, it is unlikely that incidental production of other trace oxidants impacted results.

GREENHOUSE ENVIRONMENT. Mean daytime air temperatures in the CSTRs ranged from 24 to 25 °C during the three trials, with nighttime means in the range of 20 to 22 °C (Table 3). Maximum daytime air temperatures did not exceed 37 °C, and a minimum temperature of 12 °C occurred once in Fall 2015. Mean RH in the CSTRs ranged from 49% to 57% during the day and 46% to 56% at night for the three trials (Table 3). RH values near 50% represent realistic conditions for O₃ exposure, given that O₃ production is generally greatest during hot, dry weather conditions associated with stagnant high pressure systems (Ryan et al., 2000; USEPA, 2013).

PHENOLOGY AND ONSET OF INJURY. In Fall 2014, O₃ treatments began at 15 DAS, before full expansion of the second trifoliate leaf (19 DAS for all plants). Plants bloomed by 29 DAS and began pod fill at 35 DAS. At 37 DAS, the second to last day of O₃ treatment, the largest pod was ≈5 cm in length. Plants grew more slowly in Spring 2015, with the second trifoliate leaf expanded at 24 DAS. Ozone treatments also began later, at 26 DAS, and all plants flowered by 33 DAS. The largest pods reached ≈5 cm at 37 DAS. In Fall 2015, plants had at least three to four expanded leaves when O₃ exposures began at 28 DAS. All plants flowered by 34 DAS, and pods were 12–15 cm in length at 44 DAS.

No injury was observed for the night-only O₃ treatments in Fall 2014. By contrast, daytime O₃ exposure produced stippling, bleaching, and mesophyll collapse on foliage of both genotypes, with curling on the leaf margins of S156 exposed to high O₃ levels. In Fall 2014, foliar injury symptoms appeared 5 DAT in the high O₃ treatments. Injury progressed in both

genotypes with cumulative exposure to high O₃, but no symptoms were apparent at low O₃ (mean = 25 ppb). At the conclusion of the trial, S156 plants exposed to 62 ppb mean O₃ during the day appeared stunted, with smaller leaves relative to R123 plants, and developed greater foliar injury. Injury symptoms appeared on plants treated with high O₃ (mean = 74 ppb) on 2 DAT in Spring 2015. At 3 DAT, stippling was also evident on plants in the 45-ppb treatments. By 17 DAT, early leaf abscission occurred in the 75-ppb treatments. In Fall 2015, O₃ injury appeared in the high and low O₃

Table 2. Mean, SD, and maximum (max) concentrations for ambient, low, and high O₃ treatments, reported for day (0800–1900 HR) and night (2000–0700 HR) exposure times, over the duration of Fall 2014, Spring 2015, and Fall 2015 exposures.

Time		O ₃ concn (ppb) ^z								
		Fall 2014			Spring 2015			Fall 2015		
		Amb ^y	Low	High	Amb ^y	Low	High	Amb ^y	Low	High
Day	$\bar{X}$	3	25	62	9	44	74	9	44	75
	SD	2	4	8	3	5	11	5	9	9
	Max	14	38	91	22	61	426	49	71	117
Night	$\bar{X}$	3	27	62	8	46	75	15	49	78
	SD	3	4	9	4	4	6	5	6	8
	Max	12	41	91	20	61	94	52	71	117

^zOzone levels represent the mean across chambers for each treatment.

^yAmb = ambient (no added O₃) includes means for the control and spare chambers.

treatments 1 and 5 DAT, respectively. At 16 DAT, premature leaf abscission occurred on plants of both genotypes exposed to 75 ppb O₃. Across the three trials, mean daytime O₃ exposures greater than 45 ppb caused foliar injury to both genotypes. However, only the high O₃ treatment (mean daytime O₃ ≥ 60 ppb) caused early leaf abscission.

Table 3. Mean, SD, minimum (min), and maximum (max) values across all treatment chambers for air temperature, relative humidity (RH), and photosynthetically active radiation (PAR), reported for day (0800–1900 HR) and night (2000–0700 HR) exposure times, over the duration of Fall 2014, Spring 2015, and Fall 2015 exposure periods.

		Air temp (°C)			RH (%)			PAR (μmol·m ⁻² ·s ⁻¹) ^z	
		Fall 14	Spring 15	Fall 15	Fall 14	Spring 15	Fall 15	Spring 15	Fall 15
Day	$\bar{X}$	24	25	24	57	49	49	507	537
	SD	3	4	3	10	15	8	408	303
	Min	19	14	12	32	16	24	7	1
	Max	37	36	36	81	84	79	2,313	1,763
Night	$\bar{X}$	21	22	20	56	46	53	7	1
	SD	1	2	1	11	16	8	23	3
	Min	18	17	15	34	17	35	0	0
	Max	22	26	24	90	83	77	300	71

^zPAR values were recorded from one chamber per block ($n = 4$) in Spring and Fall 2015.

FALL 2014: YIELD. Given the absence of visual injury in the Fall 2014 night-only (2000–0700 HR) O₃ exposures, it was not surprising that night O₃ treatment (high O₃ mean = 62 ppb) did not significantly affect any yield parameter (Table 4). Among the control and night-only O₃ treatments, seed mass for R123 and S156 varied by only 1.6% and 5.7%, respectively. Other studies have also failed to demonstrate significant injury from night-only O₃ exposure. Günthardt-Goerg (1996) found that night-only O₃ treatment at 75 ppb did not produce foliar injury on a sensitive tobacco (*Nicotiana tabacum* L.) cultivar (BelW3). For turnip plants grown in open-top chambers, there was no significant difference in total plant biomass for plants exposed to ambient O₃ during either the day or night in three separate trials (Winner et al., 1989, see Table 1 therein). Using Plexiglas chambers, Goknur and Tibbitts (2001) found that a dark period O₃ concentration of ≈300 ppb

Table 4. Yield parameters of O₃ resistant (R123) and O₃ sensitive (S156) snap bean genotypes and single degree-of-freedom contrasts comparing the effects of day (0800–1900 HR) and night (2000–0700 HR) O₃ treatment combinations in Fall 2014 ($n = 2$).

Treatment	Genotype	Ozone ^z		Pods (no.)	Seeds		Seed mass	
		Day	Night		(no.)	(no./pod)	(g)	(g/seed)
1 (Control)	R123	AMB	AMB	12.9	57.9	4.55	16.32	0.290
2	R123	LOW	AMB	11.5	55.5	4.82	16.45	0.301
3	R123	AMB	LOW	12.5	56.4	4.55	16.51	0.304
4	R123	LOW	LOW	13.9	62.0	4.46	17.33	0.289
5	R123	HIGH	AMB	11.4	51.3	4.51	15.00	0.303
6	R123	AMB	HIGH	13.1	61.3	4.70	16.58	0.273
7	R123	HIGH	HIGH	11.8	51.4	4.35	14.25	0.284
8 (Control)	S156	AMB	AMB	17.1	59.2	3.52	16.77	0.295
9	S156	LOW	AMB	17.3	60.5	3.52	17.93	0.313
10	S156	AMB	LOW	15.8	56.3	3.58	17.03	0.314
11	S156	LOW	LOW	16.3	60.4	3.74	16.91	0.290
12	S156	HIGH	AMB	8.4	22.9	2.75	7.28	0.346
13	S156	AMB	HIGH	16.9	61.5	3.66	17.78	0.305
14	S156	HIGH	HIGH	7.5	21.9	2.92	6.43	0.299
Contrast ($P > F$) ^y								
Control: Gn (1 vs. 8)				0.013	0.874	0.001	0.778	0.935
Night-only O ₃ treatments								
C vs. L (1 + 8 vs. 3 + 10)				0.430	0.614	0.799	0.842	0.713
C vs. H (1 + 8 vs. 6 + 13)				0.973	0.513	0.223	0.578	0.943
L vs. H (3 + 10 vs. 6 + 13)				0.411	0.256	0.326	0.719	0.661
Day O ₃ treatments ^x								
Gn (2 + 4 + 5 + 7 vs. 9 + 11 + 12 + 14)				0.769	0.001	0.001	0.001	0.572
C vs. L (1 + 8 vs. 2 + 4 + 9 + 11)				0.787	0.779	0.346	0.536	0.880
C vs. H (1 + 8 vs. 5 + 7 + 12 + 14)				0.001	0.001	0.002	0.001	0.682
L vs. H (2 + 4 + 9 + 11 vs. 5 + 7 + 12 + 14)				0.001	0.001	0.001	0.001	0.750
Time (2 + 5 + 9 + 12 vs. 4 + 7 + 11 + 14)				0.769	0.693	0.728	0.588	0.429
Interaction								
O ₃ × Gn (5 + 7 + 9 + 11 vs. 2 + 4 + 12 + 14) ^w				0.001	0.001	0.004	0.001	0.728

^zMean O₃ levels were AMB (no added O₃) = 3 ppb day and night; LOW = 25 ppb day, 27 ppb night; HIGH = 62 ppb day and night.

^yEach contrast compares individual treatments or combinations of treatments as noted by treatment numbers. C = control; L = low O₃; H = high O₃; Gn = genotype; Time = day vs. day + night.

^xLow and high O₃ levels are pooled across day and day + night treatment times.

^wCompares response of genotypes to low and high O₃, pooled across day and day + night treatment times.

O₃ was required to induce similar foliar injury to potato as exposure to 100 ppb in the light, over 3-h time periods (see Fig. 5 therein).

Under control conditions, S156 produced significantly more mature pods than R123 in Fall 2014 [$P = 0.031$ (Table 4)]. However, R123 had significantly more seeds per pod than S156 ($P < 0.001$), with means of 4.55 and 3.52, respectively. Therefore, although S156 tended to produce more pods than R123, R123 had a greater number of seeds in each pod, leading to similar yields by mass (S156:R123 = 1.03). Total seed number and mean seed mass (grams per seed) were not significantly different for the two genotypes. Similarly, Burkey et al. (2005) observed greater pod numbers with fewer seeds per pod in S156 than R123, leading to similar yields by mass, when plants were grown in open-top chambers with charcoal-filtered air. Therefore, mass-based yield can be compared more directly than pod number for the two genotypes.

High O₃ treatment during the day (mean = 62 ppb) significantly reduced all yield parameters ($P < 0.001$), except mean seed mass, relative to both the control and low O₃ (mean = 25 ppb) treatments (Table 4). However, yields under low O₃ were not significantly different from the control. Flowers et al. (2007) also found that both R123 and S156 tolerated O₃ concentrations less than 30 ppb. Notably, the interaction of genotype and O₃ (low vs. high) was significant for all yield parameters except mean seed mass. Pooled across the day and day + night timings, the increase in mean daytime O₃ from 25 to 62 ppb decreased total seed mass by 39% for S156 but only 9% for R123. Similarly, total seed number declined by 63% (S156) and 13% (R123), with decreases of 22% (S156) and 4.5% (R123) in the number of seeds per pod (Table 4). Therefore, exposure to 62 ppb O₃ had a greater impact on both pod initiation and maturation in S156 than R123. Mass yield ratios (S156:R123) for the low and high O₃ treatments were 1.03 and 0.47, respectively, compared with 1.03 for the control.

SPRING AND FALL 2015: YIELD. In contrast to Fall 2014 (Table 4) and Spring 2015, R123 produced significantly greater pod mass than S156 under control conditions in Fall 2015 [$P < 0.001$ (Table 5)]. In Fall 2015, plants were relatively larger when transferred into the CSTRs, with three to four expanded trifoliate leaves. In addition, higher *PAR* levels and lower air temperature likely enhanced growth in Fall 2015 compared with Spring 2015 (Table 3). Ratios of S156:R123 pod mass in the controls for Spring and Fall 2015 were 0.91 and 0.77, respectively. For plants grown outdoors in charcoal-filtered air, over

two subsequent years (seasonal mean O₃ = 25–31 ppb), the S156:R123 yield ratio was about 1.0 (Burkey et al., 2005). Similarly, Flowers et al. (2007) noted a seed yield ratio of ≈ 1 for S156 and R123 plants grown in environmentally controlled field chambers (mean O₃ = 0 ppb). However, plants grown in the same facility, exposed to 0 ppb O₃ in combination with low and high vapor pressure deficit treatments, had seed mass ratios of about 0.72 and 0.70, respectively (Fiscus et al., 2012). Agathokleous et al. (2017) showed that small increases in air temperature, particularly in the range of 31 to 34 °C, can substantially decrease pod number and affect the mass of pods and seeds for S156 and R123. Therefore, S156:R123 yield ratios may vary with environmental factors (e.g., temperature, RH, *PAR*, nutrient availability) and cultural practices (e.g., container volume, media composition) and should be interpreted with caution (Burkey et al., 2012).

Data for the day and day + night treatments were pooled to compare the effects of the low and high O₃ treatments relative to the control and each other. Across genotypes, the low O₃ treatments (mean = 44 ppb) significantly decreased pod number ($P < 0.001$) and mass ($P < 0.001$) in Fall 2015 only (Table 5). In Fall 2015, plants were 2-d older at the beginning of treatments, and the exposures lasted 7-d longer than in Spring (Table 1). High O₃ (mean = 74 and 75 ppb for Spring 2015 and Fall 2015, respectively) significantly reduced pod number and mass

Table 5. Yield parameters of O₃ resistant (R123) and O₃ sensitive (S156) snap bean genotypes and single degree-of-freedom contrasts comparing the effects of day (0800–1900 HR) and day + night (0800–1900 HR + 2000–0700 HR) O₃ treatments in Spring 2015 and Fall 2015 ($n = 4$).

Treatment	Genotype	Ozone ^z		Pod no.		Pod mass (g)	
		Day	Night	Spring	Fall	Spring	Fall
1 (Control)	R123	AMB	AMB	22.5	24.9	31.47	37.16
2	R123	LOW	AMB	21.4	20.9	28.01	31.14
3	R123	LOW	LOW	22.3	21.0	30.03	32.13
4	R123	HIGH	AMB	19.3	16.9	27.26	21.23
5	R123	HIGH	HIGH	18.6	17.1	24.55	21.44
6 (Control)	S156	AMB	AMB	28.1	26.8	28.62	28.73
7	S156	LOW	AMB	26.6	22.5	29.28	23.54
8	S156	LOW	LOW	24.9	21.0	27.01	22.95
9	S156	HIGH	AMB	18.9	14.5	19.51	13.45
10	S156	HIGH	HIGH	18.9	14.8	18.19	11.75
Contrast ($P > F$) ^y							
Control: Gn (1 vs. 6)				0.004	0.197	0.265	0.001
O ₃ treatments ^x							
Gn (2 + 3 + 4 + 5 vs. 7 + 8 + 9 + 10)				0.041	0.280	0.004	0.001
C vs. L (1 + 6 vs. 2 + 3 + 7 + 8)				0.177	0.001	0.347	0.001
C vs. H (1 + 6 vs. 4 + 5 + 9 + 10)				0.001	0.001	0.001	0.001
L vs. H (2 + 3 + 7 + 8 vs. 4 + 5 + 9 + 10)				0.001	0.001	0.001	0.001
Time (2 + 4 + 7 + 9 vs. 3 + 5 + 8 + 10)				0.681	0.760	0.400	0.740
Interactions							
O ₃ × Gn (4 + 5 + 7 + 8 vs. 2 + 3 + 9 + 10) ^w				0.035	0.033	0.020	0.833
Time × Gn: L (2 + 8 vs. 3 + 7)				0.312	0.425	0.237	0.500
Time × Gn: H (4 + 10 vs. 5 + 9)				0.808	1.000	0.696	0.416

^zMean O₃ levels were AMB (no added O₃) = 9 ppb day, 8 (spring) and 15 (fall) ppb night; LOW = 44 ppb day, 46 (spring) and 49 (fall) ppb night; HIGH = 74 (spring) and 75 (fall) ppb day, 75 (spring) and 78 (fall) ppb night.

^yEach contrast compares individual treatments or combinations of treatments as noted by treatment numbers. C = control; L = low O₃; H = high O₃; Gn = genotype; Time = day vs. day + night.

^xLow and high O₃ levels are pooled across day and day + night treatment times.

^wCompares response of genotypes to low and high O₃, pooled across day and day + night treatment times.

relative to both the control and low O₃ treatments [all $P < 0.001$ (Table 5)]. However, the interaction of genotype and O₃ (low vs. high) had a significant effect on pod number in Spring ($P = 0.035$) and Fall 2015 ($P = 0.033$). The decreases in pod number at high O₃ during Spring and Fall were greater for S156 (27% and 33%, respectively) than R123 (13% and 11%, respectively). For pod mass, the interaction was significant in Spring ($P = 0.020$) but not Fall 2015. In Spring 2015, R123 pod mass was 11% lower under high O₃ than low O₃, whereas S156 pod mass was 33% less. By contrast, R123 pod mass decreased by 33% in Fall 2015, with a 46% reduction for S156. Therefore, the high O₃ treatment had greater effects on yield in Fall 2015, when exposures were 7-d longer in duration (Table 1). Flowers et al. (2007) also observed a significant decrease in the seed yield, by mass, of both genotypes at 60 ppb O₃. Compared with the control, they reported 19% and 77% reductions for R123 and S156, respectively. In contrast to the present study, O₃ treatments began 1 week after planting, starting at one-third of the target O₃ level and increasing in two steps during the second week of treatment. Their approach provided more time for the plants to acclimate to O₃ stress, as well as the opportunity to respond at a younger age than in the present study, which appeared to minimize R123 yield losses relative to S156. Fiscus et al. (2012), using environmentally controlled field chambers, noted $\approx 55\%$ and 72% reductions in seed yield for R123 and S156, respectively, at low vapor-pressure deficit (1.26 kPa) and 60 ppb O₃. Using open-top field chambers, Heagle et al. (2002) observed a striking decline of 90% pod mass for S156 when exposed to 73 ppb relative to 23 ppb, but the study did not include R123.

In contrast to the present results, mature pod (>4 cm with seeds) mass decreases of only 28% and 30% for R123 and S156, respectively, were reported for plants exposed to a mean of 78 ppb O₃ for 20 d in enclosed chambers (Salvatori et al., 2013). The relatively minor impact of O₃ on yield was accompanied by overall low yields. For example, the researchers reported 13.8 and 19.2 pods per plant for R123 and S156, respectively, under control conditions. In comparison, the number of pods per plant for R123 and S156 in the present study ranged from 12.9 to 24.9 and 17.1 to 28.1, respectively (Tables 4 and 5). The minimum values were recorded in Fall 2014, when twice as many plants were grown in each CSTR, leading to space constraints and competition for light that likely reduced yields. In the study by Salvatori et al. (2013), plants were potted in a combination of field soil and sand, which may have inhibited aeration and drainage. In addition, light was provided entirely by metal halide lamps, resulting in low *PAR* levels at leaf height (i.e., 350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Exposure to high light levels (>1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) can increase the negative impacts of O₃ on photosynthesis (Guidi et al., 2000). Therefore, suboptimal growth conditions may have confounded the effects of O₃ treatment observed by Salvatori et al. (2013).

Daytime O₃ treatment (mean = 44–75 ppb) resulted in significantly lower pod mass for S156 relative to R123 in both Spring and Fall 2015 (Table 5). Pooled across treatment times, pod mass yield ratios of S156:R123 at low O₃ were 0.97 and 0.73 in Spring and Fall 2015, respectively. At high O₃, those ratios declined to 0.73 (Spring) and 0.59 (Fall).

Similar to Fall 2014, the timing of O₃ exposure (i.e., day vs. day + night) did not significantly affect yield responses in Spring or Fall 2015 (Table 5). For tobacco, there was no difference in the onset of foliar symptoms between day-only

and continuous (i.e., day + night) O₃ exposures, but biomass was not reported (Günthardt-Goerg, 1996). Winner et al. (1989) obtained inconsistent results for turnip grown in open-top chambers in which charcoal filters were used to reduce ambient O₃ from between 50 and 100 ppb to ≤ 25 ppb. Plants exposed continuously to ambient O₃ had significantly lower biomass than plants exposed during the day only (i.e., charcoal-filtered air at night) in one of three trials, whereas treatments were not significantly different in the other two trials. Therefore, more evidence is required to support the hypothesis that nighttime O₃ exposure, at realistic concentrations, exacerbates daytime O₃ injury.

NOCTURNAL STOMATAL CONDUCTANCE. Nighttime conductance measurements in Spring and Fall 2015 showed significant differences between genotypes in the control (ambient + 0 ppb) on two of four observation dates (Table 6). On each date, S156 maintained higher g_{sn} , ranging from 73.8 to 94.1 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, relative to R123, with g_{sn} in the range of 48.0 to 61.9 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. These results support the observation that S156 has higher g_{sn} relative to R123 (Salvatori et al., 2013), potentially leading to higher nighttime O₃ fluxes per unit leaf area. However, compared with the present study, Salvatori et al. (2013) reported lower g_{sn} values for both genotypes under control conditions (mean O₃ = 4 ppb) when measured from 39 to 42 DAS, with means of 28 and 12 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for S156 and R123, respectively. In the current experiment, plants were watered 2–3 h before measurement, which likely resulted in maximal g_{sn} . In addition, daytime *PAR* values were higher, with means of 507–537 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in (Table 3), compared with the artificial lighting used by Salvatori et al. (2013), which supplied a constant 350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Greater light availability in the CSTRs likely supported higher rates of photosynthesis and transpiration, as well as increased g_{sn} , relative to enclosed growth chambers (Easlon and Richards, 2009).

At elevated O₃, g_{sn} varied significantly between genotypes on all four dates, with higher g_{sn} for S156 in each O₃ treatment (Table 6). Rates of g_{sn} for S156 ranged from 66.0 to 185.5 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and values for R123 were between 33.8 and 69.7 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Salvatori et al. (2013) reported g_{sn} for O₃-treated S156 and R123 plants of ≈ 88 and 44 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively, in agreement with the present study.

Across genotypes and exposure times, O₃ had a significant effect on g_{sn} only on 27 Oct. (Table 6). There was no difference between the control and low O₃ treatment, but g_{sn} rates in the high O₃ treatment were significantly greater than values from the control ($P = 0.009$) and low O₃ treatment ($P < 0.001$). The interaction of O₃ (low vs. high) and genotype was also significant on 27 Oct. ($P = 0.002$), with a much greater increase in g_{sn} for S156 (90%) in the high treatments than R123 (29%). In addition, the interaction was significant between the control and high O₃ treatment on 14 Oct. ($P = 0.032$) because of a decrease (–23%) in g_{sn} for R123 and increase for S156 (7%) with elevated O₃. The effects of O₃ treatment may have been more advanced on 27 Oct. (25 DAT) relative to the three earlier sampling times (7–14 DAT), leading to greater g_{sn} at high O₃ in both genotypes.

Similarly, Salvatori et al. (2013) reported that exposure to 75 ppb O₃ (7 h·d^{–1} for 20 d) caused a significant increase in g_{sn} and dark respiration (R_d) compared with control conditions, when measured from 17 to 20 DAT, for both genotypes. However, the relative increases were greater for R123 ($g_{\text{sn}} = 271\%$ and $R_d = 29\%$) than S156 ($g_{\text{sn}} = 209\%$, $R_d = 9\%$). They hypothesized that

Table 6. Nocturnal stomatal conductance (g_{sn}) for O₃ resistant (R123) and O₃ sensitive (S156) snap bean genotypes and single degree-of-freedom contrasts comparing the effects of day (0800–1900 HR) and day + night (0800–1900 HR + 2000–0700 HR) O₃ treatments for Spring (April, May) and Fall 2015 (October) trials ($n = 4$).

Treatment	Genotype	Ozone ^z		g_{sn} (mmol·m ⁻² ·s ⁻¹)			
		Day	Night	30 Apr.	7 May	14 Oct. ^y	27 Oct.
1 (Control)	R123	AMB	AMB	57.0	61.9	54.4	48.0
2	R123	LOW	AMB	42.8	56.6	—	33.8
3	R123	LOW	LOW	69.7	65.5	—	38.5
4	R123	HIGH	AMB	62.0	56.9	39.0	50.1
5	R123	HIGH	HIGH	55.7	46.2	45.2	43.1
6 (Control)	S156	AMB	AMB	94.1	73.8	88.3	88.9
7	S156	LOW	AMB	72.2	95.8	—	89.2
8	S156	LOW	LOW	73.6	66.0	—	67.5
9	S156	HIGH	AMB	71.4	67.9	107.0	185.5
10	S156	HIGH	HIGH	73.6	129.8	81.5	112.5
Contrast ($P > F$) ^x							
Control: Gn (1 vs. 6)				0.004	0.403	0.067	0.025
O ₃ treatments ^x							
Gn (2 + 3 + 4 + 5 vs. 7 + 8 + 9 + 10)				0.014	0.001	0.001	0.001
C vs. L (1 + 6 vs. 2 + 3 + 7 + 8)				0.134	0.723	—	0.295
C vs. H (1 + 6 vs. 4 + 5 + 9 + 10)				0.176	0.403	0.769	0.010
L vs. H (2 + 3 + 7 + 8 vs. 4 + 5 + 9 + 10)				0.849	0.552	—	0.001
Time (2 + 4 + 7 + 9 vs. 3 + 5 + 8 + 10)				0.305	0.289	0.437 ^w	0.009
Interactions							
O ₃ × Gn (4 + 5 + 7 + 8 vs. 2 + 3 + 9 + 10) ^y				0.800	0.061	0.032 ^u	0.002
Time × Gn: L (2 + 8 vs. 3 + 7)				0.133	0.062	—	0.288
Time × Gn: H (4 + 10 vs. 5 + 9)				0.612	0.001	—	0.011

^zMean O₃ levels were AMB (no added O₃) = 9 ppb day, 8 (spring) and 15 (fall) ppb night; LOW = 44 ppb day, 46 (spring) and 49 (fall) ppb night; HIGH = 74 (spring) and 75 (fall) ppb day, 75 (spring) and 78 (fall) ppb night.

^yOnly the control and high O₃ treatments were sampled. The number of contrasts was restricted to five to control familywise error rate.

^xEach contrast compares individual treatments or combinations of treatments as noted by treatment numbers. C = control; L = low O₃; H = high O₃; Gn = genotype; Time = day vs. day + night.

^wValue represents effect of time at high O₃ (4 + 9 vs. 5 + 10).

^yCompares response of genotypes to low and high O₃, pooled across day and day + night treatment times.

^uValue represents interaction for C vs. H (1 + 9 + 10) vs. (4 + 5 + 6).

R123 responds to O₃ injury by increasing R_d , which leads to higher g_{sn} , and therefore, is better able to repair O₃-induced injury than S156. However, our data do not support their hypothesis and show a greater relative increase in g_{sn} for S156 than R123 in response to O₃ exposure. Hoshika et al. (2013) also reported increased g_s measured after 10 h of darkness, relative to day g_s , for S156 exposed to 150 ppb O₃ for 1 h. However, absolute g_s values did not actually increase relative to the control treatment and ranged from ≈ 21 to 29 mmol·m⁻²·s⁻¹ among the O₃ treatments for “well-watered” plants (see Figs. 3 and 5 therein). Therefore, their conclusions should be interpreted with caution. Other researchers also reported increased g_{sn} for holly oak (*Quercus ilex* L.) following O₃ exposure (Vitale et al., 2008). Measured at the whole-plant level, O₃ exposure increased daily water use per unit leaf area for both R123 and S156, suggesting increased day and/or night g_s (Fiscus et al., 2012). Ozone impairs the ability of guard cells to close stomata (Caird et al., 2007; Paoletti and Grulke, 2010), providing another mechanism to explain elevated g_{sn} , in addition to enhanced maintenance respiration (Salvatori et al., 2013).

The time of O₃ exposure had a significant effect on g_{sn} only on 27 Oct. ($P = 0.009$), when rates were generally higher for the day than the day + night treatments (Table 6). However, the interaction of time × genotype was also significant ($P = 0.011$) for the high O₃ treatment, reflecting a larger difference in g_{sn} between the day (185.5 mmol·m⁻²·s⁻¹) and day + night (112.5 mmol·m⁻²·s⁻¹) exposure times for S156 than R123 (50.1 vs. 43.1 mmol·m⁻²·s⁻¹). The opposite trend occurred for the significant time × genotype interaction on 7 May ($P = 0.001$), with greater g_{sn} for S156 receiving the day + night timing (129.8 mmol·m⁻²·s⁻¹) than the day-only timing (67.9 mmol·m⁻²·s⁻¹) at high O₃. For R123, differences on 7 May in day and day + night rates at high O₃ were small (i.e., 56.9 vs. 46.2 mmol·m⁻²·s⁻¹, respectively). Therefore, the impact of exposure time on g_{sn} was significant but inconsistent for S156.

To date, few studies have investigated the effects of nighttime O₃ exposure on g_{sn} . Skärby et al. (1987) reported increased g_{sn} in a single shoot ($n = 1$) of scots pine (*Pinus sylvestris* L.) exposed to O₃ at night, relative to a shoot that was not exposed to O₃. The inconclusive effects observed here may have been a sampling artifact because of variability in leaf injury levels and/or differences in leaf maturity. Although data on g_{sn} across leaf age

are lacking, anatomically based estimates of conductance within mesophyll intercellular air spaces suggest that young S156 leaves allow greater gas exchange rates than adult leaves of both S156 and R123 (Villányi et al., 2013). In addition, epidermal cells in younger leaves of S156, those that developed after O₃ exposure, stopped dividing earlier than cells from mature leaves. Consequently, cells expanded for a longer period of time, producing larger guard cells and stomatal apertures as a result of prior O₃ exposure (Villányi et al., 2013). Therefore, conductance should ideally be measured on leaves of similar ages because O₃ exposure affects anatomical development.

Leaf injury and repair also likely affect g_{sn} (Salvatori et al., 2013). Therefore, it would be informative to measure leaves of different age and injury classes to determine whether the impact of O₃ exposure on g_{sn} is consistent. Other studies have focused on the first or second “fully expanded” trifoliate leaf from the top of the plant, shifting to younger leaves as the plant produces new growth over time (Elagöz et al., 2006; Flowers et al., 2007; Hoshika et al., 2013; Salvatori et al., 2013; Villányi et al., 2014). However, the definition of “fully expanded” does not appear uniform among studies. Importantly, leaf maturity has

been shown to influence sensitivity to O₃. Among different developmental stages, mature trifoliate leaves that had expanded to about 70% to 95% of full size were the most sensitive to O₃ (i.e., in terms of visual foliar injury), relative to expanding leaves at 50% to 70% of full size, which had intermediate injury levels, and young leaves, which were the least sensitive (Lee and Bennett, 1982). Therefore, sampling the youngest “fully expanded” trifoliate leaf may not be an objective measure of how leaf gas exchange responds to O₃ exposure.

Conclusion

Under control conditions, S156 and R123 produced similar yields by mass in Fall 2014 and Spring 2015 but not Fall 2015 (Tables 4 and 5). Yield mass ratios of S156:R123 ranged from 1.03 (Fall 2014) to 0.77 (Fall 2015) and were likely affected by environmental factors (Agathokleous et al., 2017). R123 produced significantly greater pod mass than S156 in the Fall 2015 control, and in all three trials, R123 had significantly greater mass yields than S156 under elevated daytime O₃.

Nighttime O₃ exposure alone, at 62 ppb, did not cause foliar injury and had no effect on the yields of S156 and R123. In combination with daytime O₃ exposure, nighttime O₃ treatment at up to 78 ppb did not impact yield or show a consistent effect on g_{sn} . Because plants generally have a lower capacity to detoxify O₃ at night, without a constant supply of metabolites from photosynthesis, an equivalent O₃ dose may cause greater injury at night than during the day (Musselman and Minnick, 2000). Therefore, the “effective flux,” which accounts for plant defense mechanisms and therefore overall sensitivity to O₃, has the greatest potential to accurately predict O₃ injury (Heath et al., 2009; Musselman et al., 2006). Recent work has demonstrated diurnal variation in plant sensitivity to O₃ (Grantz, 2014; Grantz et al., 2013), but more research is required to establish the effects of nighttime O₃ exposure.

Nighttime conductance measurements suggested that S156 and R123 have inherently different g_{sn} rates and that cumulative O₃ exposure can increase g_{sn} in both genotypes [i.e., results for 27 Oct. reflected the longest cumulative exposure (Table 6)]. However, exposure time (day vs. day + night) did not have a consistent effect on g_{sn} . High O₃ treatment caused a greater relative increase in g_{sn} for S156 than R123, contrary to the results reported by Salvatori et al. (2013). Notably, elevated g_{sn} may create a positive feedback when nighttime O₃ levels are high, increasing O₃ flux into the leaf (Salvatori et al., 2013). However, different sampling strategies and potential variation of g_{sn} with leaf maturity and injury confound results to date. Future experiments should test a larger range of night O₃ concentrations, in combination with daytime exposure, on the physiology and yield of S156 and R123. Improved understanding of how g_{sn} varies with leaf characteristics, as well as temporally through the night, will help predict the effects of elevated nighttime O₃.

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