

RESEARCH PAPER

Influence of atmospheric vapour pressure deficit on ozone responses of snap bean (*Phaseolus vulgaris* L.) genotypes

Edwin L. Fiscus¹, Fitzgerald L. Booker^{1,*}, Walid Sadok² and Kent O. Burkey¹

¹ US Department of Agriculture, Agricultural Research Service, Plant Science Research Unit, and Department of Crop Science, North Carolina State University, 3127 Ligon Street, Raleigh, NC 27607, USA

² University of Florida, Agronomy Physiology Laboratory, Gainesville, FL 32611, USA

* To whom correspondence should be addressed. E-mail: fitz.booker@ars.usda.gov

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Abstract

Environmental conditions influence plant responses to ozone (O₃), but few studies have evaluated individual factors directly. In this study, the effect of O₃ at high and low atmospheric vapour pressure deficit (VPD) was evaluated in two genotypes of snap bean (*Phaseolus vulgaris* L.) (R123 and S156) used as O₃ bioindicator plants. Plants were grown in outdoor controlled-environment chambers in charcoal-filtered air containing 0 or 60 nl l⁻¹ O₃ (12 h average) at two VPDs (1.26 and 1.96 kPa) and sampled for biomass, leaf area, daily water loss, and seed yield. VPD clearly influenced O₃ effects. At low VPD, O₃ reduced biomass, leaf area, and seed yield substantially in both genotypes, while at high VPD, O₃ had no significant effect on these components. In clean air, high VPD reduced biomass and yield by similar fractions in both genotypes compared with low VPD. Data suggest that a stomatal response to VPD *per se* may be lacking in both genotypes and it is hypothesized that the high VPD resulted in unsustainable transpiration and water deficits that resulted in reduced growth and yield. High VPD- and water-stress-induced stomatal responses may have reduced the O₃ flux into the leaves, which contributed to a higher yield compared to the low VPD treatment in both genotypes. At low VPD, transpiration increased in the O₃ treatment relative to the clean air treatment, suggesting that whole-plant conductance was increased by O₃ exposure. Ozone-related biomass reductions at low VPD were proportionally higher in S156 than in R123, indicating that differential O₃ sensitivity of these bioindicator plants remained evident when environmental conditions were conducive for O₃ effects. Assessments of potential O₃ impacts on vegetation should incorporate interacting factors such as VPD.

Key words: Air pollutants, bioindicator, ozone, snap bean, transpiration, vapour pressure deficit.

Introduction

McLaughlin and Taylor (1981) demonstrated that foliar uptake of ozone (O₃) and sulphur dioxide was enhanced at high humidity in kidney beans (*Phaseolus vulgaris* L.). Conditions that minimize water stress during exposures, such as adequate soil moisture and high humidity, generally increase foliar injury from O₃ (McLaughlin and Taylor, 1981; Mills, 2002). Their influence on O₃ uptake via effects on stomatal conductance and the physiological sensitivity of the plant appear to partly mediate O₃ damage (Taylor *et al.*, 1988). However, previous research on the effects of humidity and atmospheric vapour pressure deficit (VPD) on plant responses to O₃ has been done in short-term

controlled-environment or greenhouse experiments, typically at subambient photosynthetically active radiation (PAR) levels (Otto and Daines, 1969; Dunning and Heck, 1973; McLaughlin and Taylor, 1981; Mortensen, 1992; Balls *et al.*, 1996). Empirical modelling has been employed to address some of these limitations and extend applicability of the findings (Ball *et al.*, 1998; Bungener *et al.*, 1999; Benton *et al.*, 2000; Emberson *et al.*, 2000; Pleijel *et al.*, 2007). Although lower VPD (higher humidity) led to greater O₃ injury in most, but not all (Bungener *et al.*, 1999), conditions and plant species tested and modelled, improved assessment of O₃ interactions with VPD should include

a wide range of plant-response variables evaluated under realistic light levels in otherwise controlled-environment conditions.

It is important to understand how environmental variables such as temperature, VPD, soil moisture and fertility, and light affect plant responses to O_3 . For example, understanding the differential effects of environmental factors on O_3 responses may ultimately contribute to refinement of O_3 -concentration-based (McLaughlin and Taylor, 1981; Fiscus *et al.*, 2005) and O_3 -flux-based risk assessment methods (Emberson *et al.*, 2000; Pleijel *et al.*, 2007). Also, improved understanding of the basic physiological processes that influence plant responses to O_3 could aid in genotype selection strategies for maintaining future food production under changing climate conditions. The intention of this research was to establish the degree to which sustained differences in atmospheric VPD might affect plant responses to O_3 . To achieve this objective, this study used the outdoor plant environment chambers (OPECs) previously described by Flowers *et al.* (2007), in which temperature, humidity, $[CO_2]$, and $[O_3]$ can be controlled and which admit 90% of daily ambient sunlight.

Materials and methods

Field site and plant material

The experiments were conducted during the summer of 2008 at the USDA-ARS Plant Science Research Unit field site 5 km south of Raleigh NC, USA. Elevation was 110 m above sea level.

Two snap bean genotypes with known differences in sensitivity to O_3 , S156 and R123 (Burkey *et al.*, 2005), were grown in eight OPECs. Seeds were planted 4 cm apart in 15-l pots containing Metro-Mix potting medium (Sun Gro Horticulture, Bellevue, WA, USA). Osmocote Plus (Scotts-Sierra Horticultural Products, Marysville, OH, USA) slow-release fertilizer (N/P/K 15:9:12) was thoroughly mixed in the planting medium at the time of planting. Seedlings were thinned to one plant per pot upon full expansion of the first trifoliate leaf and irrigated to the drip point nightly with a drip emitter in each pot. At 11 days after planting the soil was treated with Marathon (imidocloprid; [(6-chloro-3-pyridinyl)-methyl]-*N*-nitro-2-imidazolidinimine; Olympic Horticultural Products, Mainland, PA, USA) to control thrips and Ridomil Gold; ((*R*)-2-[(2,6-dimethylphenyl)-methoxyacetyl-amino]-propionic acid methyl ester; Novartis Crop Protection, Greensboro, NC, USA) to control *Phytophthora* and *Pythium*.

Ozone was produced from dry oxygen by electrostatic discharge (Griffin Technics Corporation, Lodi, NJ, USA) and monitored using a UV photometric analyser (Thermo Scientific, Franklin, MA, USA). Chamber O_3 was dispensed following a 10-year summertime daily average ambient $[O_3]$ curve determined for this site (Flowers *et al.*, 2007). Multiples of the standard curve were calculated to describe target 12-h average $[O_3]$ of 0 and 60 nl l⁻¹. Ozone exposures started at 18 days after planting (DAP) at one-third of the final target and were increased an additional one-third for the next 2 d with the full exposure levels reached at 21 DAP. Target $[O_3]$ was achieved using mass flow controllers (Aalborg Instruments and Controls, Orangeburg, NY, USA) in a computerized measurement and feedback control system. The 60 nl l⁻¹ 12-h mean target resulted in daily AOT40, SUM06, and W126 values of 245, 534, and 295 nl l⁻¹ h, respectively (Fiscus *et al.*, 2005). Over the duration of the experiment (62 d) the resultant total exposures were 15.2, 33.1, and 18.3 μ l l⁻¹ h. Chamber $[CO_2]$ was also

controlled at about 390 μ mol mol⁻¹ using a separate feedback control system drawing from a 12.7 metric ton liquid CO_2 receiver.

Experiments

Eight O_3 -tolerant (R123) and eight O_3 -sensitive (S156) plants were placed in a randomized pattern in each of the eight chambers. The treatment design consisted of two O_3 treatments (12 h mean $[O_3]$ of 0 and 60 nl l⁻¹) and two atmospheric VPD levels (1.26 and 1.96 kPa) at a constant temperature of 26 °C with two replications.

Measurements

Starting at 32 DAP, one pot of each genotype from each chamber, which had been well watered the previous evening, was enclosed in a white plastic bag tied around the base of the stem to prevent soil evaporation and weighed. For each of the next three mornings, these pots were again weighed to determine water loss during the previous day and a mass of water added to the pot equal to the water loss. After a 3-d water loss period, the water loss per day was averaged and the plants destructively sampled for biomass and leaf area measurements (Model 3100C leaf area meter; LI-COR, Lincoln, NE, USA). Rooting medium was washed from the roots. Plant components were dried in a drying barn and then weighed. Water use per unit leaf area was calculated for each plant. This procedure was repeated with an additional set of plants from each chamber for a total of 4 weeks. After the fourth sampling week, three of the remaining plants of each genotype per chamber were grown to physiological maturity, and the mature brown pods were harvested, weighed, and shelled. Seeds were counted and weighed and the mean individual seed mass was calculated. Data from the final growth period before yield harvest were not included in the analysis as there was virtually no leaf area remaining in the high O_3 treatments in either genotype.

Transpiration responses of the two snap bean genotypes to VPD were also examined in a glasshouse in Gainesville, FL, USA. Experiments were performed as described by Fletcher *et al.* (2007) and Sadok and Sinclair (2009) to characterize the transpiration response to atmospheric VPD for each genotype. Transpiration rates of four plants for each genotype were measured in a set of eight 0.3-m diameter \times 0.3-m tall transparent chambers over a range of VPDs from \sim 0.6 to 3.5 kPa. For each experiment the eight chambers were run simultaneously and the VPD in each was adjusted to two or three levels and maintained for approximately 1 h at each level. The transpiration at each VPD was determined by the mass change during the 1-h exposure. At the end of each experiment, the leaf areas were destructively measured and the transpiration expressed on a unit leaf area basis. The experiment was repeated twice on plants of similar age (38 DAP \pm 2 d) and the results combined for a total sample of eight plants per genotype. During the two experiments, mean temperature was 29 °C and PAR averaged 357 \pm 35 μ mol m⁻² s⁻¹.

Data analysis

The experiment was a completely randomized three-way (2 \times 2 \times 2) factorial split-plot with O_3 and VPD treatments as the main plots and genotype as the subplot. Results were analysed by analysis of variance using a mixed model (Littell *et al.*, 1996) (PC SAS for Windows, version 9.2). Repeated measures with a compound symmetry covariance structure was used to analyse data obtained over time (harvests) (Littell *et al.*, 1996). Shoot and root biomass data were natural log transformed to stabilize the variance. Plant water use data from the first four sampling periods were combined for calculation of seasonal means. Reported values are least squares means and associated standard error. Measurements were made on samples obtained from two replicate chambers. Differences were considered statistically significant if $P \leq 0.05$ and marginally statistically significant if $P \leq 0.1$.

For the glasshouse transpiration-VPD measurements statistical analyses were performed using the R-package 'stats' (Team RDC, 2009). Transpiration data were fitted to a linear model using the 'lm' function which is an adaptation of the S-language function of the same name developed by Chambers (1992). Slopes and intercepts were compared by analysis of covariance and leaf areas were compared by a Welch two-sample t-test.

Results

Experimental conditions

The mean daily chamber environmental exposure data demonstrated good control and separation of the O₃ and VPD treatments as well as acceptable daily PAR levels ($39.8 \pm 1.3 \text{ mol m}^{-2} \text{ d}^{-1}$; Table 1).

Shoot and root biomass

The adverse effect of O₃ on above-ground biomass production during the growing season was greatest in the low VPD treatment (VPD \times O₃ interaction $P \leq 0.02$; Table 2, Fig. 1). Shoot biomass was 31% lower on average across the season in the 1.26/60 (low VPD/added O₃) treatment compared with the 1.96/60 (high VPD/added O₃) treatment. Overall, S156 was more sensitive (–30%) than R123 (–9%) to O₃, as indicated by shoot biomass production across harvests (genotype \times O₃ interaction $P \leq 0.06$; Table 2, Fig. 1). R123 shoot weight was on average slightly higher (13%) than S156.

In R123, high VPD caused an increase in root dry mass in both clean air and added O₃, but not in S156 (genotype \times VPD \times O₃ interaction $P \leq 0.03$; Table 2, Fig. 2). Overall, added O₃ reduced root mass per plant by 16%, while root mass was 11% higher in the high VPD compared with low VPD treatment (Table 2, Fig. 2).

Leaf and root weights reached their maximum values at 42 DAP and declined thereafter (harvest $P \leq 0.001$). Average stem weight among treatments was stable from 42 DAP onwards, and pod weight increased as DAP progressed (harvest $P \leq 0.001$). Harvest interactions with other

treatment factors were not statistically significant ($P > 0.1$; Table 2).

Plant biomass ratios of S156/R123 were calculated to assess the snap bean genotypes as an O₃ bioindicator system at different VPDs. Average biomass ratios for S156/R123 were not significantly different among the 1.96/0, 1.96/60, and 1.26/0 treatments (0.93 ± 0.06 , 0.87 ± 0.06 , and 1.05 ± 0.06 , respectively). However, the biomass ratio declined to 0.71 ± 0.06 in the 1.26/60 (low VPD/added O₃) treatment (VPD \times O₃ interaction $P \leq 0.03$). None of the harvest effects were statistically significant ($P \geq 0.3$).

Seed yield

Ozone showed its maximum effect at low VPD, reducing yields by 55–72% (Fig. 3) (VPD \times O₃ interaction $P \leq 0.003$; Table 3). At high VPD, the O₃ effect on seed yield was totally muted, showing no significant difference in either genotype compared to the clean air treatment. The decrease in seed yield with O₃ at low VPD was caused by fewer seeds per plant and lower mass per seed (Table 4). Added O₃ decreased the seed count and mass per seed by 32–56% at low VPD while neither genotype showed an O₃ effect at high VPD (VPD \times O₃ interaction $P \leq 0.02$; Tables 3 and 4).

In all VPD and O₃ treatments, seed yield and number of seeds per plant were 59% and 49% higher, respectively, in R123 than in S156 ($P \leq 0.001$; Tables 3 and 4). Mass per seed was not significantly different between genotypes. The VPD treatment effect and the genotype \times VPD \times O₃ interaction for seed yield, number of seeds per plant, and mass per seed were not statistically significant (Table 3). The S156/R123 ratios for seed weight were not significantly different among treatments ($P \geq 0.1$).

Leaf area and mean daily water use

At low VPD, O₃ reduced average seasonal leaf area by 21–39% (VPD \times O₃ interaction $P \leq 0.1$; Tables 3 and 5). At

Table 1. Outdoor plant environment chamber environmental data. Chamber temperatures, vapour pressure deficits (VPDs), and [CO₂] are shown as daytime means \pm SE, collected throughout the experiment: daytime was delimited by the time of morning and evening when photosynthetically active radiation (PAR) transitioned above and below $50 \mu\text{mol m}^{-2} \text{ s}^{-1}$, respectively. [O₃] values are 12-h means \pm SE (0800–2000h), starting at 21 DAP. The mean daily PAR integral was $39.8 \pm 1.3 \text{ mol m}^{-2} \text{ d}^{-1}$.

Treatment (VPD/[O ₃])	Temperature (°C)	VPD (kPa)	[O ₃] (nl l ⁻¹)	[CO ₂] ($\mu\text{mol mol}^{-1}$)
1.26/0	26.2 ± 0.6	1.25 ± 0.01	2.4 ± 0.3	393 ± 2
1.26/60	26.4 ± 0.4	1.26 ± 0.01	59.0 ± 0.1	393 ± 2
1.96/0	26.2 ± 0.6	2.00 ± 0.02	3.2 ± 0.1	392 ± 2
1.96/60	26.4 ± 1.1	2.06 ± 0.02	59.1 ± 0.1	392 ± 2

Table 2. Analysis of variance results (P values) for main treatment effects and interactions for natural log transformed shoot and root biomass per plant data. VPD, vapour pressure deficit.

Effect	Shoot weight plant ⁻¹	Root weight plant ⁻¹
Harvest	0.001	0.001
VPD	0.3	0.04
O ₃	0.002	0.01
Genotype	0.03	0.4
Harvest \times VPD	0.5	0.8
Harvest \times O ₃	0.5	0.9
Harvest \times genotype	0.8	0.7
Genotype \times VPD	0.7	0.04
Genotype \times O ₃	0.06	0.9
VPD \times O ₃	0.02	0.1
Genotype \times VPD \times O ₃	0.3	0.03
Harvest \times genotype \times VPD \times O ₃	0.8	0.6

Table 3. Analysis of variance results (*P* values) for main treatment effects and interactions for seed harvest (seed weight per plant, number of seeds per plant, and mass per seed), leaf area, and daily water use per unit leaf area VPD, vapour pressure deficit; –, not applicable.

Effect	Seed weight plant ^{−1}	Seeds plant ^{−1}	Mass seed ^{−1}	Leaf area	Daily water use (leaf area) ^{−1}
Harvest	–	–	–	0.01	0.01
VPD	0.5	0.3	0.3	0.07	0.3
O ₃	0.001	0.002	0.003	0.03	0.04
Genotype	0.001	0.001	0.1	0.03	0.02
Harvest × VPD	–	–	–	0.9	0.9
Harvest × O ₃	–	–	–	0.6	0.4
Harvest × genotype	–	–	–	0.6	0.2
Genotype × VPD	0.9	0.6	0.7	0.9	0.5
Genotype × O ₃	0.9	0.6	0.4	0.3	0.08
VPD × O ₃	0.003	0.02	0.01	0.1	0.03
Genotype × VPD × O ₃	0.7	0.6	0.4	0.9	0.7
Harvest × genotype × VPD × O ₃	–	–	–	0.9	0.9

Table 4. Final harvest number of seeds per plant and mass per seed Values are least squares means ± SE.

Treatment (VPD/[O ₃])	Seeds plant ^{−1}		Mass seed ^{−1} (mg)	
	R123	S156	R123	S156
1.26/0	391 ± 27	298 ± 27	245 ± 15	232 ± 15
1.26/60	265 ± 27	132 ± 27	165 ± 15	150 ± 15
1.96/0	310 ± 27	220 ± 27	217 ± 15	213 ± 15
1.96/60	280 ± 27	189 ± 27	227 ± 15	187 ± 15

high VPD, the O₃ effect was smaller in both genotypes. Mean seasonal leaf area was reduced by high VPD in clean air within R123 and S156 by 26–28%, while there was little VPD effect in the added O₃ treatment. Leaf area was 23% higher in R123 than in S156 (*P* ≤ 0.03; Tables 3 and 5). The genotype × VPD × O₃ interaction was not statistically significant (Table 3). Leaf area peaked at 42 DAP and declined thereafter (harvest *P* ≤ 0.01; data not shown). Harvest interactions with other variables were not statistically significant (Table 3).

The O₃ treatment at low VPD significantly increased daily water use by 23–38%, but not at high VPD (VPD × O₃ interaction *P* ≤ 0.03; Tables 3 and 5). Mean daily water use per unit leaf area for both R123 and S156 was increased by high VPD in clean air by 31% and 14%, respectively. Seasonal daily water loss was 12% higher in S156 than in R123 (*P* ≤ 0.03; Tables 3 and 5). The genotype × VPD × O₃ interaction was not statistically significant (Table 3). Daily water use per unit leaf area generally increased during the experiment (harvest *P* ≤ 0.01; data not shown). Harvest interactions with other variables were not statistically significant (Table 3).

Production water use efficiency (WUE; g seed plant^{−1} (kg d)^{−1}; Table 5) were computed from the seed yields and the seasonal integrals of the measured water use. In clean air, WUE was reduced 45% and 42% by high VPD in R123

and S156, and with added O₃, WUE was increased by 41% and 60% in R123 and S156, respectively. The O₃ effect at low VPD reduced WUE by 60% in R123 and by 78% in S156. At high VPD, the O₃ effect was essentially zero for R123 and −39% for S156.

Transpiration response to VPD

The results of the VPD–transpiration response experiments are shown in Table 6. Neither genotype expressed a break-point in the response curve and, although the leaf areas of S156 plants were significantly smaller than the R123 genotype, there were no significant differences in either the slopes or intercepts of the response curves.

Discussion

VPD clearly influenced plant responses to O₃, with detrimental effects on biomass production, seed yield, and leaf area most severe in the low VPD treatment (Figs. 1, 2, 3; Tables 4, 5). These results were consistent with previous greenhouse experiments and modelling studies in which low VPD (high humidity) increased the adverse effects of O₃, in part by increasing stomatal conductance and thereby O₃ uptake (Otto and Daines, 1969; McLaughlin and Taylor, 1981; Mills, 2002; Pleijel et al., 2007). In addition, McLaughlin and Taylor (1981) suggested that high transpiration rates at high VPD could reduce O₃ transport across the leaf cell wall, thus attenuating O₃ injury. In the current study, the higher daily water loss rate in the 1.96/0 (high VPD/clean air) treatment indicated that transpiration was increased in this treatment compared with the 1.26/0 (low VPD/clean air) treatment (Table 5). In the 1.96/60 (high VPD/added O₃) treatment, therefore, the higher transpiration rate possibly provided some protection against O₃ injury. Transpiration was also higher in the 1.26/60 (low VPD/added O₃) treatment than in the 1.26/0 (low VPD/clean air) treatment even though leaf area was decreased.

Table 5. Seasonal leaf areas, daily water use per unit leaf area (DWULA), and calculated production water use efficiencies (WUE) Values for leaf area and DWULA are least squares means \pm SE. WUE values were calculated from the final harvest seed mass and the integrated water use measured over the season.

Treatment (VPD/[O ₃])	Leaf area (m ² plant ⁻¹)		DWULA (kg m ⁻² d ⁻¹)		WUE (g seed plant ⁻¹ (kg d) ⁻¹ of water use)	
	R123	S156	R123	S156	R123	S156
1.26/0	0.953 \pm 0.101	0.864 \pm 0.101	1.187 \pm 0.114	1.341 \pm 0.114	2.83	1.81
1.26/60	0.752 \pm 0.101	0.525 \pm 0.101	1.464 \pm 0.114	1.852 \pm 0.114	1.13	0.40
1.96/0	0.709 \pm 0.101	0.621 \pm 0.101	1.559 \pm 0.114	1.532 \pm 0.140	1.55	1.05
1.96/60	0.733 \pm 0.101	0.504 \pm 0.101	1.369 \pm 0.114	1.724 \pm 0.128	1.59	0.64

Table 6. Transpiration responses of R123 and S156 snap bean genotypes to vapour pressure deficit as determined in chamber treatments in a greenhouse
Statistical significance: *, $P \leq 0.05$; ns, $P > 0.05$.

Parameter	R123	S156	P
Leaf area (m ²)	0.0855 \pm 0.0085	0.0595 \pm 0.0022	*
Leaf dry mass (g)	4.10 \pm 0.52	2.90 \pm 0.14	ns
Slope (mmol m ⁻² s ⁻¹ kPa ⁻¹)	1.478 \pm 0.112	1.586 \pm 0.114	ns
Intercept (mmol m ² s ⁻¹)	-0.720 \pm 0.028	-0.816 \pm 0.219	ns
n	29	31	
r ²	0.87	0.87	

These responses suggest that both genotypes lacked, or suffered reduced stomatal control, as a result of the O₃ exposures at low VPD.

This was unexpected, given that stomatal conductance typically declines or remains unchanged in many plants exposed to chronic O₃ (Fiscus *et al.*, 1997, 2005; Long and Naidu, 2002; Pleijel *et al.*, 2007). In a previous study with the same snap bean genotypes treated in the same chambers used herein at a VPD of 1.4 kPa, there was no significant season-long difference in mean conductance due to O₃ in either genotype (Flowers *et al.*, 2007). There was, however, considerable temporal variability in the measurements, as with net photosynthesis, with significant differences developing in the 60 nl l⁻¹ O₃ treatment in S156 at later points in the experiment. In a month-long experiment with S156 and another snap bean genotype (R331) treated with 22 or 70 nl l⁻¹ O₃ (8 h average) in open-top chambers, neither net photosynthesis nor stomatal conductance were significantly affected (Paoletti and Grulke, 2010). Average VPD in the experiment was calculated to be 2.2 kPa, which might have contributed to reduced sensitivity of the plants to O₃. Stomatal closure time in response to changes in light intensity, however, was longer in O₃-treated plants compared with the control (Paoletti and Grulke, 2010).

In hydroponically grown bush bean, no consistent effect of O₃ on cumulative transpiration was observed (Tingey *et al.*, 1994). Daily water use per unit leaf area was not significantly altered in soybean (*Glycine max* (L.) Merr.) treated from germination to maturity with 72 nl l⁻¹ (12 h

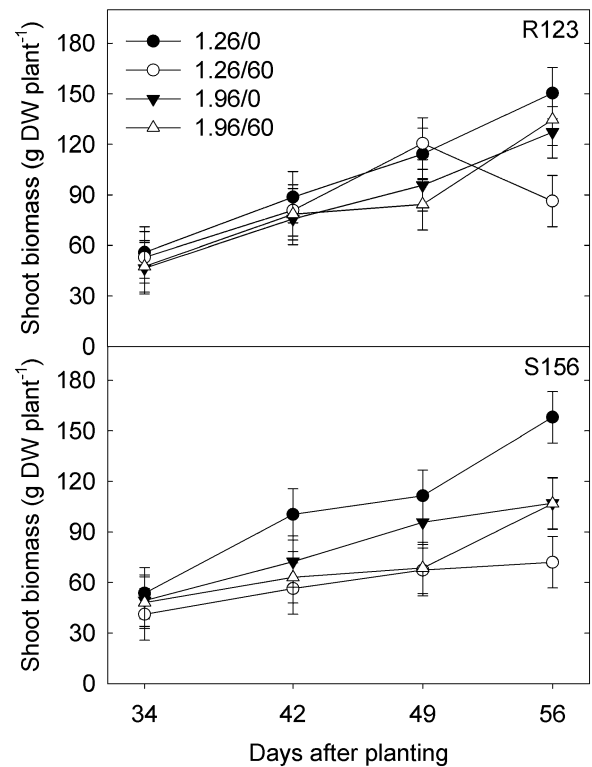


Fig. 1. Shoot (leaf, stem, and pod) dry mass (least squares means \pm SE) for periodic biomass harvests during the growing season. Genotypes are indicated in the upper right corner of each panel. Treatments: low VPD/clean air (1.26/0); low VPD/added O₃ (1.26/60); high VPD/clean air (1.96/0); and high VPD/added O₃ (1.96/60). DW, dry weight.

average) O₃ in open-top chambers (Booker *et al.*, 2004). A decrease in evapotranspiration in soybean exposed to elevated O₃ in a free-air system was suggested to be related to decreased stomatal conductance and leaf area (Bernacchi *et al.*, 2011).

An increase in stomatal conductance due to O₃ has been inferred from measurements of increased transpiration in O₃-treated plants (van den Driessche and Langebartels, 1994; McLaughlin *et al.*, 2007; Mills *et al.*, 2009). For example, water use by well-watered Norway spruce (*Picea abies* (L.) Karst.) trees treated with 400 nl l⁻¹ O₃ in solar

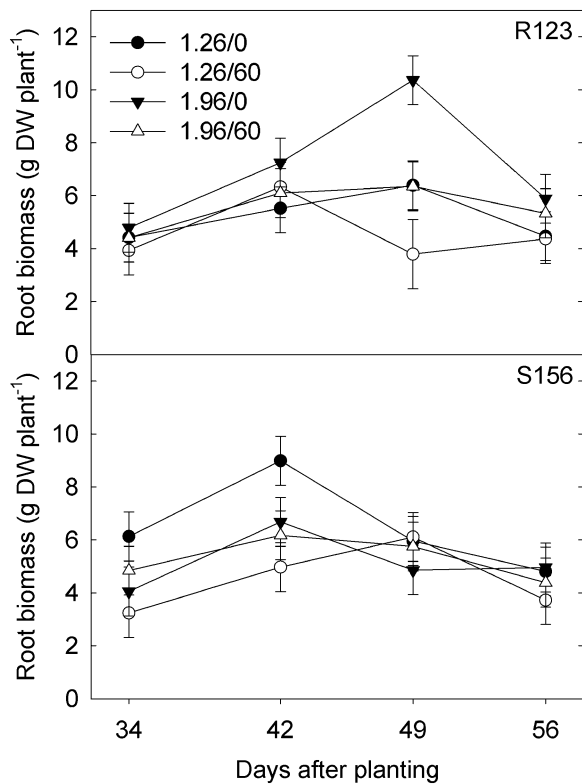


Fig. 2. Root dry mass (least squares means \pm SE) for periodic biomass harvests during the growing season. Genotypes are indicated in the upper right corner of each panel. Treatments: low VPD/clean air (1.26/0); low VPD/added O_3 (1.26/60); high VPD/clean air (1.96/0); and high VPD/added O_3 (1.96/60).

domes (dome-shaped greenhouses) was 19% higher than in control plants (van den Driessche and Langebartels, 1994). Increased transpiration of canopies of mature trees in a southern Appalachian forest was detected in response to increasing ambient O_3 levels (McLaughlin *et al.*, 2007). Reduced stomatal control of water loss following O_3 exposure was suggested to account for these responses (van den Driessche and Langebartels, 1994; McLaughlin *et al.*, 2007). Increased stomatal conductance in Norway spruce exposed to $80 \text{ nl l}^{-1} O_3$ has been reported (Barnes *et al.*, 1990). Stomatal conductance increased in oak saplings (*Quercus kelloggii* Newb. and *Q. douglasii* Hook. and Arn.) treated for 2 months with $70 \text{ nl l}^{-1} O_3$ in open-top chambers (Paoletti and Grulke, 2010). Similarly, stomatal conductance of undamaged inner canopy leaves of two grassland species, *Dactylis glomerata* L. (Poaceae) and *Leontodon hispidus* L. (Asteraceae), exposed to a range of O_3 concentrations ($21\text{--}102 \text{ nl l}^{-1}$, 24 h average) in solar domes for 140 days increased with increasing O_3 concentration (Mills *et al.*, 2009). In addition, water loss from excised leaves was greater with O_3 , suggesting an inability to close the stomata in response to stem detachment (Mills *et al.*, 2009). It has been suggested that O_3 can induce sluggish stomatal responses to environment changes, which can affect plant sensitivity to O_3 (Paoletti and Grulke, 2010). Taken together, these studies suggest that O_3 effects on stomatal conductance vary depending on interactions among the

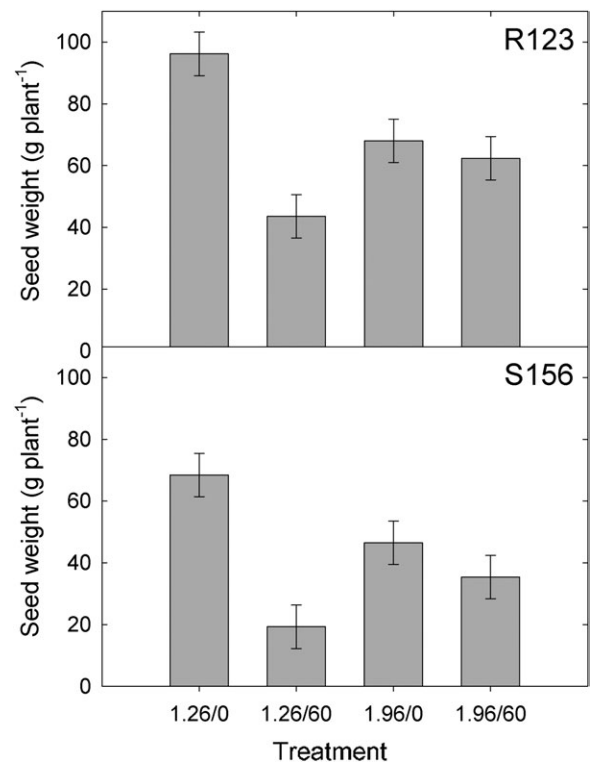


Fig. 3. Final harvest seed mass (least squares means \pm SE) from mature brown pods. Genotypes are indicated in the upper right corner of each panel. Treatments: low VPD/clean air (1.26/0); low VPD/added O_3 (1.26/60); high VPD/clean air (1.96/0); and high VPD/added O_3 (1.96/60).

pollutant, the environmental conditions, and the physiology of the plant.

It has been proposed that O_3 -induced ethylene production uncouples the control of abscisic acid (ABA) on stomatal closure (Mills *et al.*, 2009; Wilkinson and Davies, 2010). Accordingly, plants experiencing a stress that stimulates ABA production, such as high VPD, would, with O_3 exposure, be limited in their stomatal closure response (Wilkinson and Davies, 2010). In the current study, however, there was no significant effect of the 1.96/60 (high VPD/ O_3) treatment on shoot biomass, seed yield, or leaf area, and no indication that stomatal control was impaired at high VPD (Figs. 1, 3; Table 5). It was at low VPD that stomatal control appeared to be compromised by O_3 . Without thorough measurements of stomatal conductance and leaf temperature, it can only be speculated that O_3 caused an increase in whole-plant stomatal conductance or that increases in leaf temperature due to O_3 increased transpiration.

Confounding interpretation of causality is the lack of information concerning leaf temperatures, which can have substantial effects on the leaf-to-air vapour pressure gradient, which is a superior descriptor of the driving gradient for transpiration than air VPD alone. If transpiration reduced leaf temperature by 3°C below air temperature, a not uncommon observance in the field at this location, the leaf-to-air VPD in the current instance might be reduced

from 1.26 kPa to 0.7 kPa and from 1.96 kPa to 1.40 kPa. Alternatively, if O_3 induced an increase in leaf temperature due to stomatal closure, leaf-to-air VPD would increase, producing a somewhat compensatory increase in transpiration. Bernacchi *et al.* (2011) measured a 0.3–0.7 °C increase in canopy temperature in soybean treated with elevated O_3 , but evapotranspiration was still lower than in plants exposed to ambient O_3 . The water loss rates and leaf areas reported in the current study do not provide a suitable basis, relative or otherwise, for calculating stomatal conductances or O_3 uptake into the leaves, as would be possible with measured stomatal conductance that included leaf temperature measurements.

Ozone effects on shoot biomass, leaf area, and yield were accompanied by a decrease in root biomass. The reduction in root mass in response to O_3 is well known and thought to result from changes in carbohydrate partitioning, early senescence of lower leaves that preferentially supply photosynthate to roots, and phytohormone imbalances (Cooley and Manning, 1987; Andersen, 2003; Grantz *et al.*, 2006). High VPD counteracted this effect and offered a substantial degree of protection from O_3 , possibly due to development of a water stress and the resultant consequences of increased root growth in R123 and decreased stomatal conductance and O_3 uptake. The lack of increased root mass at high VPD in S156 suggests the possibility that this genotype may lack any means to respond to high evaporative demand other than by reducing leaf area. No VPD-associated changes in root mass occurred in S156 in either clean air or with added O_3 .

In regard to plant responses to VPD, these genotypes apparently lack any direct response to VPD *per se* so that transpiration rate early in development is a function of VPD only. In this sense, these plants would fall into the ‘- regulation’ or unregulated categories discussed by Sperry *et al.* (2002), in which plants transpire at rates determined solely by the environment until some supply or plant-transport parameter becomes limiting. Thus, increasing the VPD would cause transpiration to increase without constraint until it exceeds the uptake or transport capacity of the plant. As a result, a plant water deficit may develop, inducing a growth regulator response leading to a degree of stomatal regulation, increased root growth, reduced leaf area and photosynthetic capacity, and a modified plant-water balance. This scenario is consistent with the snap bean responses observed in this study in clean air. The observed increase in transpiration rates under these circumstances was qualitatively consistent with the increased evaporative demand and linear nature of the transpiration–VPD curves from the glasshouse study (Table 6). Taken together, these separate observations support the hypothesis that in clean air there may be little stomatal sensitivity to VPD or stomatal regulation of water loss in these genotypes. Continued unabated, a threshold may be reached above which water uptake and transport within the plant can no longer match the evaporative demand. Stomatal conductance may then decrease to stabilize transpiration at some maximal rate (Bunce, 1981; Turner *et al.*, 1984). In

many species, the threshold for this response occurs in the range of 2.0–2.5 kPa (Turner *et al.*, 1984; Fletcher *et al.*, 2007) or, rarely, as low as 1.0 kPa (Turner *et al.*, 1984). The reduced conductance might reduce production of new leaf area and, in the case of R123, stimulate root growth. What is clear is that high VPD in clean air decreased seed yield by similar proportions in both genotypes (–31%).

The O_3 -sensitivity difference between genotypes was evident for biomass production at low VPD. The usefulness of these genotypes as an O_3 bioindicator pair, as indicated by the S156/R123 ratio, was apparent for plant biomass production when VPD was favourable for O_3 effects. Interpretation of plant responses to O_3 should include consideration of differential effects of environmental factors such as VPD in the analysis.

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