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Photosynthesis, chlorophyll fluorescence, and yield of snap bean (*Phaseolus vulgaris* L.) genotypes differing in sensitivity to ozone

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Abstract

Understanding the impact of pollutant ozone (O_3) is a concern for agricultural production. This work was undertaken as the first comparative study of the effects of O_3 on the photosynthetic processes and yield of three snap bean (*Phaseolus vulgaris* L.) genotypes with known differences in sensitivity to O_3 (S156, R123 and R331). Previous information showed R123 and R331 to be tolerant and S156 sensitive. The purpose was to identify physiological subsystems that may mediate those differences in sensitivity. Plants were grown in environmentally controlled field chambers with four levels of O_3 (0, 15, 30 and 60 nmol mol^{-1}). Net assimilation (A) and fluorescence were measured throughout the growing season and yield data were collected at physiological maturity. All genotypes were tolerant of low O_3 (<30 nmol mol^{-1}) but the highest O_3 significantly reduced the yield in all three, with R331 and S156 being equally sensitive on a unit exposure basis. Yield reductions were correlated with A, especially during pod filling. No genotype showed any significant response of stomatal conductance (g_s) indicating equal O_3 fluxes into the leaves in all genotypes. Mesophyll conductance (g_m) was affected in S156 only, where it was reduced by 55% at 60 nmol mol^{-1} O_3. There was an upward trend in F_0, and a downward trend in the variable fluorescence ratio (F_v/F_m) with increasing O_3 for S156 but not for the other genotypes. S156 was the only genotype to show significant decreases in photochemical quenching (q_p) and R123 the only one to show significant decreases in non-photochemical quenching (q_n). The sequence of loss of Rubisco content and/or activity and changes in g_m, F_0, and F_v/F_m could not be resolved in time and may all have been the result of generalized tissue destruction rather than sequential attack on individual subsystems. S156 had the highest photosynthetic rate in clean air but appeared to have no significant capacity to protect Rubisco from attack or to up-regulate Rubisco activity at high O_3, thus there was no reserve capacity, while R123 was able to maintain both Rubisco activity and A within narrow ranges. These data suggest that S156 has comparatively little anti-oxidant capacity and/or is deficient in its ability to regulate Rubisco activity. For future studies the best contrasts for resolving questions of physiological sensitivity to O_3 would be obtained from R123 and S156.
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Keywords: Snap bean; Ozone sensitivity; Photosynthesis; Hormesis; Air pollution

1. Introduction

Widespread areas of crop production are currently at risk for damage from ambient atmospheric O_3 concentrations (Fowler et al., 1999). The International Panel on Climate Change (IPCC, 2001) predicts that atmospheric O_3 concentrations will continue to increase in the future. Therefore, it is important to understand the effects of chronic O_3 exposure on plant growth, development, and yield.

Ozone primarily enters plants through the stomata where it can dissolve in the apoplastic water. Ozone can directly react with the plasmalemma through ozonolysis or it can be converted into reactive oxygen species (ROS) such as hydroperoxide ($^{\bullet}O_2H$), superoxide ($^{\bullet}O_2^-$), and hydrogen peroxide (H_2O_2), that react with the plasmalemma and susceptible amino acids in membrane proteins or apoplastic enzymes as well as a variety of organic metabolites localized in the cell wall. These reactions alter cellular components and can lead to cell death, accelerated senescence, and the up or down regulation of genes (Long and Naidu, 2002; Fiscus et al., 2005). Visible symptoms of toxic O_3

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exposure include chlorosis or necrotic lesions on plant leaves (Miller et al., 1994; Sandermann, 1996; Guidi et al., 2000; Long and Naidu, 2002). Chronic O_3 exposure can also lead to reductions in leaf area, biomass, and yield (Miller et al., 1995; Fiscus et al., 1997, 2005; Long and Naidu, 2002; Morgan et al., 2003).

Much research on the cause of reduced growth and yield has focused on the decrease in photosynthetic capacity often found in plants exposed to O_3. Reductions in stomatal conductance (g_s), net photosynthetic CO_2 assimilation (A), and carboxylation efficiency have all been associated with O_3 exposure (Pell et al., 1992; Fiscus et al., 1997; Guidi et al., 2002; Morgan et al., 2003). Long and Naidu (2002) attributed these reductions to a loss of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), and decreased Rubisco activity. Chronic O_3 exposure also causes an acceleration of senescence that may be in part responsible for the reductions (Reid et al., 1998).

A major finding of the studies on O_3 effects on photosynthesis is that O_3 reduces g_s. In large part this reduction is the result of damage to the photosynthetic machinery leading to reduced fixation, increased internal CO_2 concentration (C_i), and finally to reduced g_s (Fiscus et al., 1997; McKee et al., 1995). However, most of these studies did not examine mesophyll conductance (g_m). Since O_3 and its converted ROS are known to react with cellular components, chronic O_3 exposure may also affect g_m at the very least through localized tissue destruction (necrotic lesions). Ozone has also been shown to alter photosynthetic electron transport in some plants via reduction in the efficiency of excitation capture in plants (Calatayud and Barreno, 2001; Castagna et al., 2001; Guidi et al., 2002). This type of photoinhibitory process may be related to membrane damage, a reduced number of intact or open photosystem II reaction centers, and an increase in dissipation of energy through alternative means such as heat (Castagna et al., 2001; Guidi et al., 2002; Rosenqvist and van Kooten, 2003). While these studies have examined the effects of O_3 on both carbon fixation and electron transport, the mechanisms or physiological differences that increase and/or decrease O_3 sensitivity in plants are still not known.

Disparities in O_3 sensitivity have been identified among genotypes of snap bean (*Phaseolus vulgaris* L.). Genotypes R123 and R331 are considered O_3 tolerant while genotype S156 is known to be highly sensitive (Burkey et al., 2005; Burkey and Eason, 2002). Open-top chamber studies have shown that pod yield and biomass production are suppressed in S156 while effects on R123 are minimal under moderate ozone stress (Burkey and Eason, 2002; Heagle et al., 2002; Burkey et al., 2005). There are no published reports of ozone effects on growth and yield for R331. However, the open-top chamber system has limited capacity to distinguish differences in ozone response at relatively low ozone concentrations (e.g. 15 nmol mol^{-1} versus 30 nmol mol^{-1}) and does not control the interactive effects of O_3 exposure with ambient temperature and vapor pressure. The exposure systems employed here are not subject to these limitations and thus provide a more complete characterization of the ozone response in these genotypes.

Photosynthesis data for S156 currently available in the literature are limited to mid-season measurements of net carbon exchange and leaf conductance taken midday under ambient conditions (Heagle et al., 2002). No published data for photosynthesis or leaf conductance are available for R331 and R123. Therefore, studies were undertaken to determine the effects of a range of chronic O_3 exposures on these three genotypes of snap bean.

There were three main objectives. First, to determine if the effect of O_3 on yield is correlated with its effects on gas exchange and/or electron transport parameters. Second, to gain insight into possible photosynthesis- and electron transport-related mechanisms that lead to differing sensitivity to O_3 in these three snap bean genotypes. Third, to determine specifically whether O_3 damage could be detected as effects on g_m.

2. Materials and methods

2.1. Experimental site and growth chambers

Studies were conducted during 2003 and 2004 at the USDA-ARS Plant Science Unit field site 5 km south of Raleigh, NC, U.S.A. Elevation was 110 m above sea level.

Plants were grown in closed, 2.44 m × 1.52 m, recirculating outdoor plant environment chambers (OPECs), admitting 90% of full sunlight, which were as previously described (Fiscus et al., 1999) except that the center height of the chamber lids was increased to 1.31 m resulting in a growth volume of approximately 3.7 m^3 in each chamber. Air flow was driven at the inlet ducts by dual centrifugal blowers with a no-load rating of 0.26 m^3 s^{-1} providing a real turnover of about 3 chamber volumes per minute. Temperature was controlled by 5.3 kW cooling units (Dayton Electric Mfg. Co., Niles, IL) with fine control imposed by 1.55 kW finned heaters (Vulcan Electric Co., Porter, ME) placed in the blower box and controlled with West ES6100 series process controllers (Danaher Industrial Controls (West Brand Products), Elizabethtown, NC) driven by signals from thermocouples in the exit ducts.

Because the heat exchange coils of the cooling units precipitate large quantities of water vapor the chambers were also equipped with pressurized misting nozzles within the growth volume controlled by Vaisala model HMW71Y humidity and temperature transmitters (Vaisala Inc., Woburn, MA) in the exit ducts providing control signals to West ES6100 series process controllers. The system allows control of vapor pressure throughout the entire growth cycle.

Each chamber was equipped with a charcoal filter between the cooling coil and the blowers that allowed reduction of O_3 concentration to very low levels because of the recirculating nature of the systems. Controlled amounts of O_3 were then dispensed between the blowers and the inlet duct bringing the chamber air to the desired O_3 concentration.

2.2. Plant culture

Three snap bean genotypes with known differences in sensitivity to O_3 were used: R123 and R331 (tolerant) and S156 (sensitive). Four plants of each genotype were placed randomly in each chamber. Seeds were planted 4 cm apart in 15 L pots containing Metro-Mix 220 (Scotts-Sierra Horticultural Products,

Marysville, OH). Osmocote Plus (Scotts-Sierra Horticultural Products, Marysville, OH.) slow release fertilizer (15–9–12: N–P–K) was thoroughly mixed in the planting media 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 daily. Plants were relocated randomly within chambers weekly during measurement periods to reduce possible position effects. The OPECs were set at day/night temperatures of 28/23 °C and a vapor pressure deficit (vpd) of 1.5 kPa.

2.3. Ozone exposures

Ozone was produced from dry oxygen by electrostatic discharge (Griffin Technics Corporation, Lodi, NJ) and monitored using a UV photometric analyzer (Thermo Environmental Instruments, Franklin, MA). Chamber O_3 was dispensed according to a diurnal O_3 concentration curve obtained by averaging 10 years of historical ambient concentration data recorded at this site. This ambient diurnal curve was scaled to dispense target 12-h average O_3 concentrations of 0, 15, 30, and 60 nmol mol^{-1} (Fig. 1). These targets resulted in AOT40 and SUM06 values of 0 for all exposures except for the 60 nmol mol^{-1} treatment which had values of 19,160 and 45,041 nmol mol^{-1} h, respectively. Target O_3 concentrations were achieved using mass flow controllers (Aalborg Instruments and Controls Inc., Orangeburg, NY) in a computerized feedback system. Ozone treatments were started 1 week after planting during emergence at concentrations of one-third the final target and increased in two additional steps of one-third during the second week. Throughout, time is given as weeks after planting.

2.4. Photosynthesis measurements

2.4.1. Gas exchange

Gas exchange characteristics were measured weekly from 2 to 8 weeks after planting (hereinafter referred to as "weeks")

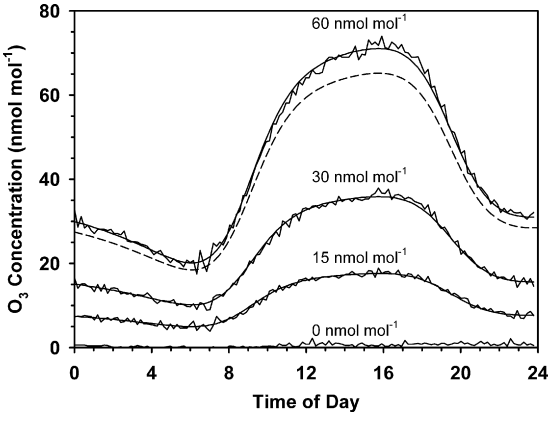

Fig. 1. Ten-year average diurnal ambient O_3 concentration standard curve (dashed line) and multiples calculated to achieve the specified target levels. Solid smooth lines are target levels for the treatments and the ragged lines are typical O_3 concentrations measured in the chambers over a single 24 h period. The standard curve multiplier was selected so that the 12-h mean O_3 concentration calculated between 0800 and 2000 h would match the targets specified on the figure.

for each genotype in each chamber using an open gas exchange system (LI-6400 photosynthesis system; LICOR, Lincoln, NE). Measurements were taken on the uppermost fully expanded leaf of randomly selected plants between 1000 and 1500 h EST. Net assimilation (A) was recorded at 10 concentrations of CO_2 supplied in the gas exchange cuvette (C_a), ranging from 300 to 1200 μmol mol^{-1}. Constant temperature (~27 °C), photon flux density (1500 μmol m^{-2} s^{-1}) and vpd of 1.5 kPa were maintained in the cuvette throughout the measurements. Following the procedure outlined by Long and Bernacchi (2003), estimates of the maximum rate of carboxylation of ribulose-1,5-bisphosphate (RuBP) via Rubisco ($V_{c,max}$), and the mitochondrial respiration rate (R_d) were obtained from the A versus intercellular CO_2 concentration (C_i) data. The estimates of $V_{c,max}$ and R_d were calculated by fitting the following equation (TableCurve 2D v5.01, SYSTAT Software Inc., Point Richmond, CA) (Farquhar et al., 1980):

$$A = \frac{C_i - \Gamma^*}{C_i + K_c(1 + O/K_0)} V_{c,max} - R_d \quad (1)$$

where A is the net rate of CO_2 uptake per unit leaf area (μmol m^{-2} s^{-1}), C_i the intercellular CO_2 concentration (μmol mol^{-1}), and O is the concentration of oxygen in air (mmol mol^{-1}).

K_c, K_0, and Γ^* were calculated and adjusted for leaf temperature using the equations of Bernacchi et al. (2001). For each A/C_i curve the stomatal limitation (S_l) was also assessed using the procedure described in Farquhar and Sharkey (1982). Also obtained from the gas exchange measurements were estimates of A and g_s at growth CO_2 concentration.

2.4.2. Fluorescence

Fluorescence measurements were taken on the uppermost fully expanded leaf of randomly selected plants over a range of C_i using the LI-6400 photosynthesis system fitted with an integral fluorescence chamber. Prior to measurements each leaf was dark adapted for 1 h. Dark adapted measurements of minimal fluorescence (F_0), maximal fluorescence (F_m), and variable fluorescence (F_v) as well as the F_v/F_m ratio were obtained. Leaves were then allowed to equilibrate for 30 min at a photon flux density of 1500 μmol m^{-2} s^{-1}. Measurements of the minimal fluorescence of a light adapted leaf (F_0'), maximal fluorescence during a saturating pulse (F_m'), and steady-state fluorescence (F_s) were obtained. From these measurements both photochemical (q_p) and non-photochemical (q_n) quenching components were calculated. Similar to measurements of gas exchange characteristics, a constant temperature (~27 °C), and vpd (1.9 kPa) were maintained throughout the measurements.

The quenching coefficients q_p and q_n were then used to determine the range of CO_2 concentrations in which the rate of electron transport (J) was constant. This range of CO_2 concentrations was then used to estimate g_m using the constant J method (Harley et al., 1991), where the variance of J was estimated over the range of CO_2 concentrations and the value of g_m that minimized the variance of J was considered the best estimate of g_m.

Table 1
Chamber environmental measurements averaged (±S.E.) across all replications

O_3 concentration target (nmol mol^{-1})	0	15	30	60
Measured O_3 concentration (nmol mol^{-1})	0.93	14.79	30.05	59.33
	0.07	0.04	0.11	0.22
Daytime chamber temperatures (C)	26.75	26.80	26.30	26.96
	0.07	0.05	0.12	0.12
Vapor pressure deficit (kPa)	1.40	1.41	1.39	1.47
	0.03	0.03	0.03	0.02
Daily ambient PAR integral (mol m^{-2}) (applies to all treatments)	24.712			
	1.213			

O_3 concentrations are 12 h means from 08:00 to 20:00.

2.5. Seed yield

Following senescence of the foliage and discoloration of the pods at the end of 8 weeks, irrigation was discontinued, and plants allowed to dry *in situ*. Pods were harvested after 12 weeks, weighed, and threshed manually to determine seed weight.

2.6. Experimental design and statistical treatment

The experiment was run over two 12-week periods in the winter of 2003 and spring of 2004 (21 November 2003 to 12 February 2004 and 20 February 2004 to 13 May 2004). The experimental design was a split plot in a randomized complete block design. Chamber was the main plot with genotype as the subplot, the two runs as blocking factor and two chambers per level of O_3 in each run. The treatment design was a 4×3 factorial with four O_3 treatments and three genotypes. Preliminary analysis showed no significant interactions between run and genotype or O_3, so for clarity of presentation, data for the autumn and spring runs were re-analyzed as four replications.

Data for A, g_s, $V_{c,max}$, g_m and S_l, were analyzed with repeated measures analysis (Littell et al., 2006), using a first order autoregressive covariance structure as implemented in the MIXED procedure of the SAS System for Windows version 9.1.3. Time was first treated as a discrete variable, with weeks as a classification factor, and means separation tests conducted using the LSD on least square means.

Data for assimilation (A and $V_{c,max}$) and fluorescence responses (F_0, F_v/F_m, q_p, and q_n) were further analyzed taking into account continuous effects of both time and O_3.

3. Results

3.1. Chamber environment

Mean OPEC O_3 concentrations, temperatures, vpds and daily PAR integrals are summarized in Table 1.

3.2. Seed yield

Seed yield per plant was significantly different among genotypes and among O_3 treatments (Fig. 2). There were significant differences among genotypes in seed yield per plant, averaged over all levels of O_3 ($p = 0.03$), and both the slopes and intercepts of the three genotypes for O_3 were significantly different ($p = 0.02$ and 0.04, respectively). R331 had significantly greater seed yield than either R123 or S156 across all levels of O_3 ($p < 0.05$). The highest seed yields per plant in all genotypes were measured in the 0 and 15 nmol mol^{-1} levels and were not statistically different within any genotype ($p = 0.49$, 0.27, and 0.41, for R123, S156, and R331, respectively). The highest level of O_3 (60 nmol mol^{-1}) significantly reduced seed yield compared with all other levels for all three genotypes ($p < 0.05$). Compared to 0 nmol mol^{-1} O_3, 60 nmol mol^{-1} reduced seed yield by 19%, 77% and 35% for R123, S156, and R331, respectively. Compared with 15 nmol mol^{-1} O_3, the corresponding reductions at 60 nmol mol^{-1} were 28%, 81%, and 39%. Sensitivity per unit of O_3 concentration, expressed as the slope of the decline in yield, was −0.089, −0.344, and −0.309 g seed (nmol mol^{-1})$^{-1}$, for R123, S156, and R331, respectively, when O_3-free air was taken into account. Corresponding sensitivities for the 15–60 nmol mol^{-1} range of O_3 concentrations were −0.187, −0.534, and −0.427 g seed (nmol mol^{-1})$^{-1}$. Appropriate contrasts showed that for either range of O_3 concentrations, the sensitivity slope of R123 was different from both of the others ($p = 0.035$) while there was no difference between S156 and R331 ($p = 0.489$).

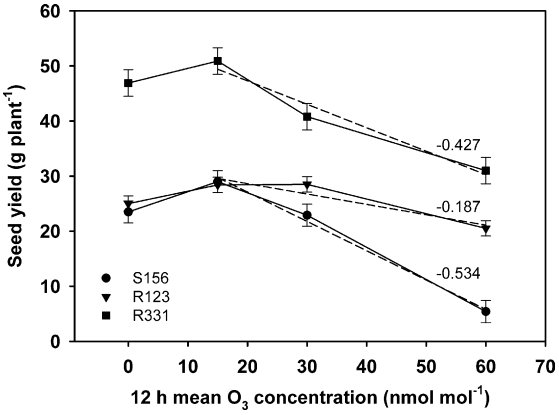

Fig. 2. Mean seed yields (±1S.E.) as a function of 12 h mean O_3 concentrations. Dashed lines are regressions over the three highest O_3 concentrations. Sensitivity slopes are given on the graph and for S156, R331 and R123 the constants were 37.8, 55.8 and 32.4, respectively, and r^2s were 0.993, 0.961 and 0.886, respectively.

Table 2
Assimilation (A) and the maximum RuBP saturated rate of carboxylation ($V_{c,max}$) least squares means computed from the repeated measures analysis for the entire growing season

Mean O_3 concentration (nmol mol^{-1})	A (μmol m^{-2} s^{-1})			$V_{c,max}$ (μmol m^{-2} s^{-1})		
	S156	R123	R331	S156	R123	R331
0	27.0 a A	21.8 a B	23.2 a B	159.7 a A	129.5 a B	124.8 a B
15	26.6 a A	23.4 a A	23.1 a A	148.2 ab A	140.2 a A	128.8 a A
30	23.5 a A	20.7 a A	22.0 a A	125.9 b A	121.4 a A	110.6 a A
60	16.6 b B	22.2 a A	19.7 a AB	90.4 c B	128.8 a A	112.5 a B

Means followed by the same letter are not statistically different at the 0.05 level according to LSD. Lower case letters separate means by ozone concentration within each genotype. Upper case letters separate means by genotype within a given ozone concentration.

3.3. Photosynthesis measurements

3.3.1. Net assimilation rate

Comparisons among discrete levels of O_3, averaged over the entire season, revealed that only S156 showed any significant decrease in A due to O_3 (Table 2). The 60 nmol mol^{-1} concentration reduced A by 38% compared with the 0 nmol mol^{-1} concentration. Compared to the other genotypes, A in S156 was both significantly higher at 0 nmol mol^{-1} and lower at 60 nmol mol^{-1}. Large variability in A for all genotypes throughout the growing season (Fig. 3) made it difficult to ascertain treatment effects, but consideration of the continuous effects of time and ozone (Table 3) alleviated the problem, showing that among all effects in the full model, comprising linear and quadratic effects of time and ozone and all interactions, the linear and quadratic trends of time, were significant for all three genotypes. Among effects involving ozone, the only significant one was the interaction of time and the quadratic effect of ozone in S156. In other words, photosynthesis changed with time, with a maximum between 2 and 8 weeks, but only in S156 was this change affected by ozone. In that genotype, increasing O_3 significantly accelerated the decline in A following flowering.

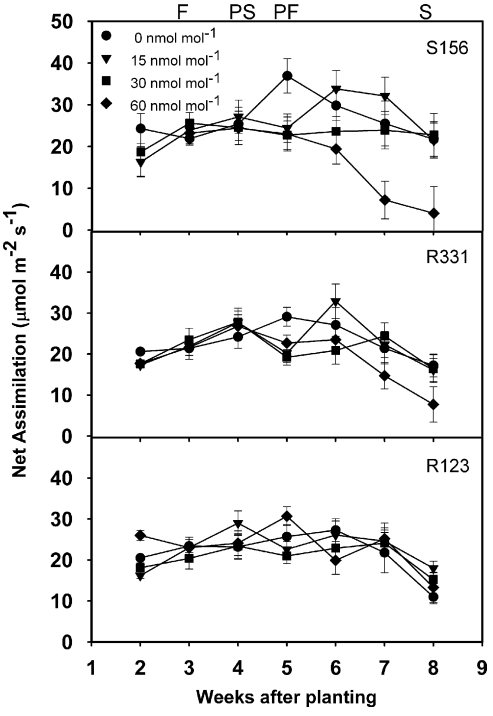

Fig. 3. Weekly mean assimilation (A) rates for each O_3 treatment throughout the growing season (±1S.E.). Approximate midpoints for beginning of each growth stage are indicated across the top of the graph. F: flowering; PS: pod set; PF: pod fill; S: senescence.

3.3.2. $V_{c,max}$

Averaged over the season, $V_{c,max}$ exhibited no difference between genotypes at 15 or 30 nmol mol^{-1} O_3 (Table 2). However, it was significantly higher in S156 than in either of the other genotypes at 0 nmol mol^{-1}, while at 60 nmol mol^{-1} $V_{c,max}$ was lower in S156 than in R123 with R331 intermediate but only significantly lower than R123.

Consideration of the continuous effects of time and ozone on $V_{c,max}$ (Table 3) showed that like A, $V_{c,max}$ changed with time, but that its course over time was only affected by ozone in S156, and in the same fashion as A.

3.3.3. Stomatal and mesophyll conductances and stomatal limitation

Only a few significant differences among genotypes existed for g_s, g_m, and S_l (Table 4). In the 15 nmol mol^{-1} treatment, g_s was higher in both S156 and R123 compared with R331. In the 60 nmol mol^{-1} treatment, g_m was higher in R123 compared with S156. At 30 nmol mol^{-1} O_3, S_l was higher in R123 compared with R331.

There were no significant season-long differences in mean g_s due to O_3 within any genotype (Table 4). However, there was considerable temporal variability in the measurements, as with A and $V_{c,max}$, with occasional significant differences developing throughout the season (data not shown) with the 60 nmol mol^{-1} treatment significantly lower than the 15 nmol mol^{-1} treatment at 4, 6 and 7 weeks in S156.

Significant differences existed in g_m among O_3 treatments only within S156 (Table 4). For example, g_m was 52.6% lower in S156 in the 60 nmol mol^{-1} treatment compared to the 0 nmol mol^{-1} treatment. Similar to g_s, there were individual time periods in which a snap bean genotype had significant differences in g_m among O_3 treatments. At 7 weeks S156 had significantly higher g_m for the 0 and 15 nmol mol^{-1} treatments compared to the 60 nmol mol^{-1} treatment, while R331 also had a significantly higher g_m at 15 nmol mol^{-1} compared to 60 nmol mol^{-1} (data not shown). In contrast, R123 never showed a significant decrease in g_m due to increasing O_3.

Table 3
Repeated measures analysis for assimilation (A) and the maximum RuBP saturated rate of carboxylation ($V_{c,max}$), using continuous effects of time and ozone (p values)

	Assimilation			$V_{c,max}$		
	S156	R123	R331	S156	R123	R331
$O_3 \times O_3$	0.1211	0.2468	0.2818	0.0893	0.9187	0.6076
Time	0.002	<0.0001	0.0004	<0.0001	0.0025	0.0036
Time × time	0.0024	<0.0001	0.0002	0.0002	0.0051	0.0016
$O_3 \times O_3 \times$ time	0.0036	0.206	0.0831	0.0007	0.86	0.7908

Time is weeks. S156, R123, R331 denote the genotype.

Table 4
Stomatal conductance (g_s), mesophyll conductance (g_m), and stomatal limitation (S_l) least squares means computed from the repeated measures analysis for the entire growing season

12-h mean O_3 concentration (nmol mol^{-1})	g_s (mol H_2O m^{-2} s^{-1})			g_m (mol m^{-2} s^{-1})			S_l		
	S156	R123	R331	S156	R123	R331	S156	R123	R331
0	0.48 a A	0.46 a A	0.48 a A	0.19 a A	0.21 a A	0.22 a A	0.27 a A	0.33 a A	0.27 ab A
15	0.51 a A	0.48 a A	0.38 a B	0.21 a A	0.24 a A	0.22 a A	0.26 a A	0.30 a A	0.29 a A
30	0.51 a A	0.43 a A	0.44 a A	0.14 ab A	0.18 a A	0.20 a A	0.24 a AB	0.30 a A	0.20 b B
60	0.41 a A	0.41 a A	0.40 a A	0.09 b B	0.18 a A	0.17 a AB	0.24 a A	0.31 a A	0.25 ab A

Means followed by the same letter are not statistically different at the 0.05 level according to LSD. Lower case letters separate means by O_3 concentration within each genotype. Upper case letters separate means by genotype within a given O_3 concentration.

Table 5
Minimal fluorescence (F_0) and the ratio of variable fluorescence to maximal fluorescence (F_v/F_m) least square means computed from the repeated measure analysis for the entire growing season

12-h mean O_3 concentration (nmol mol^{-1})	F_0			F_v/F_m		
	S156	R123	R331	S156	R123	R331
0	137.7 a A	136.4 a A	150.3 a A	0.75 ab A	0.73 a A	0.73 a A
15	149.9 a A	142.6 a A	153.3 a A	0.77 a A	0.77 a A	0.76 a A
30	161.5 a A	142.5 a A	154.0 a A	0.72 bc A	0.76 a A	0.75 a A
60	172.4 a A	144.0 a A	160.7 a A	0.68 c B	0.76 a A	0.74 a A

Means followed by the same letter are not statistically different at the 0.05 level according to LSD. Lower case letters separate means by O_3 concentration within each genotype. Upper case letters separate means by genotype within a given O_3 concentration.

The only differences in S_l within a genotype occurred in R331 between 15 and 30 nmol mol^{-1}, while at 30 nmol mol^{-1} S_l for R331 was only significantly smaller than in R123.

3.3.4. Fluorescence

Values of F_0, F_v/F_m, q_p, and q_n for each genotype and O_3 treatment averaged over the season are reported in Tables 5 and 6. LSD means separation tests conducted on these discrete values showed few differences among them. F_v/F_m was reduced at 60 nmol mol^{-1} in S156 and that value was also reduced compared to R123 and R331. Due to the paucity of differences in fluorescence variables over time, data were analyzed taking into account repetition of the measurements over time, but not the effects of time on responses. Both the slopes and intercepts of the effect of increasing O_3 were different among genotypes for all fluorescence variables (Table 7). Appropriate contrasts were

Table 6
Photochemical (q_p) and non-photochemical (q_n) quenching least square means computed from the repeated measures analysis for the entire growing season

12-h mean O_3 concentration (nmol mol^{-1})	q_p			q_n		
	S156	R123	R331	S156	R123	R331
0	0.44 ab A	0.44 a A	0.44 a A	0.84 a B	0.89 a A	0.86 a AB
15	0.49 a A	0.47 a A	0.44 a A	0.85 a A	0.87 ab A	0.87 a A
30	0.44 ab A	0.48 a A	0.44 a A	0.87 a A	0.88 ab A	0.87 a A
60	0.39 b B	0.52 a A	0.46 a A	0.87 a A	0.84 b A	0.85 a A

Means followed by the same letter are not statistically different at the 0.05 level according to LSD. Lower case letters separate means by O_3 concentration within each genotype. Upper case letters separate means by genotype within a given O_3 concentration.

Table 7
Intercept and slopes of the linear effects of varying O_3 concentration on fluorescence variables F_0, F_v/F_m, q_p, and q_n

Genotype	F_0		F_v/F_m		q_p		q_n	
	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope
S156	139.6915	0.597426	0.772733	−0.001548	0.471098	−0.001521	0.843524	0.000699
R123	138.5707	0.081110	0.761177	0.000121	0.433274	0.001360	0.888264	−0.000649
R331	150.3760	0.117706	0.742946	0.000144	0.428330	0.000394	0.869077	−0.000111
p	0.0007	0.0083	<0.0001	0.0124	0.0016	0.0127	<0.0001	0.0249

p-Values result from the repeated measures analysis of the effects of varying O_3 on fluorescence variables, testing equality of the intercepts and slopes of the linear effect.

therefore constructed to clarify those differences (Table 8). The change in every fluorescence variable with increasing O_3 concentration was significantly different between S156 and the other two genotypes, considered either alone or together. They were not different, however, between R123 and R331.

4. Discussion

Previously Heagle et al. (2002) found that S156 had a 90.1% reduction in final pod weight when comparing elevated O_3 concentrations (72 nmol mol^{-1}) to a control (23 nmol mol^{-1} O_3). They also reported a 15.4% reduction in final pod weight for an O_3 tolerant snap bean cultivar (Tenderette) over the same O_3 concentrations. In addition, the S156/R123 pod weight ratios from the current study were consistent with previous attempts to develop this pair of genotypes as an O_3 bioindicator system. Work by Burkey et al. (2005) indicated a mean ratio over 2 years of 0.46 in ambient air (AA) with a mean O_3 concentration of 48 nmol mol^{-1}. Interpolation of the pod weight data from this study (data not shown) yielded a ratio of 0.48, within 4% of theirs. We compared only their AA data to the present study since they suggested that the open-top chambers used in that study underestimated the impact of the O_3. Therefore, the yield reductions found were quantitatively consistent with previous studies conducted in an uncontrolled environment.

It was clear that S156 was very susceptible to O_3 injury and yield reduction. However, R331, that previously had been characterized as more tolerant to O_3 was found to be as sensitive as S156 when considered on the basis of mass of yield lost per unit of exposure. The sensitivity slopes of the yield curves between 15 and 60 nmol mol^{-1} (Fig. 2) showed that S156 and R331 are 2.8 and 2.2 times, respectively, as sensitive as R123 within the range of O_3 exposures used here. Even though R331 was nearly as sensitive as S156, owing to its substantially larger stature and yield in clean air it suffered a much smaller fractional yield loss in high O_3 concentrations. The current study also contained some suggestions about the effects of low concentrations of O_3 on snap bean yield. Though the differences were not significant in any genotype, the yield data (Fig. 2) suggest the possibility that all three genotypes may have benefited from low concentrations of O_3 (15 nmol mol^{-1} compared to 0 nmol mol^{-1}). This putative hormetic response might have been due to stimulation of anti-oxidant defenses but lacking further information it was not possible to say. In none of the three genotypes did the 30 nmol mol^{-1} O_3 treatment significantly reduce seed yield compared to 0 nmol mol^{-1} O_3. The reduction at 30 nmol mol^{-1} compared to the 15 nmol mol^{-1} exposure was significant in both S156 and R331 but not in R123 suggesting that the latter genotype may have a greater anti-oxidant capacity than the others or in some other way was able to protect its photosynthetic machinery and maintain A. This suggestion was strengthened by the lack of differences in g_s between genotypes at 60 nmol mol^{-1} O_3 implying that O_3 fluxes into the leaves were equal in all three genotypes. However when O_3 concentrations exceeded 15 nmol mol^{-1} in S156 and R331, their defenses were no longer able to cope and yield reductions ensued. In the case of R123 that threshold appeared to be in excess of 30 nmol mol^{-1} and even at the highest exposure the yield losses were far less per unit exposure. However, from a production perspective, R331 at 60 nmol mol^{-1} produced more seed than the other genotypes at their maxima.

Coinciding with our yield results there were varying differences in the effect of O_3 on the photosynthetic parameters of the three genotypes (Table 2). Some further insight into the relative relationships between yield and photosynthetic rates might be gained from Figs. 4 and 5. In Fig. 4 seed yield was plotted as a function of the mean seasonal midday assimilation rate (A). It is interesting that S156 had the highest mean A at 0 and 15 nmol mol^{-1} but suffered the largest decrease between 30 and 60 nmol mol^{-1}. In most respects the pattern for R331 resembled that for S156 except that it was displaced due to its larger stature and foreshortened due to its somewhat lower O_3 sensitivity. The most interesting pattern belonged to R123 which showed no significant changes in A and a yield effect only at the highest O_3 concentration. Thus, it might have been that the ability to maintain yield in R123 was related to its ability to maintain A, through regulatory processes, within a fairly narrow range over the O_3 concentrations used in this study.

Table 8
Repeated measures analysis of the effect of varying O_3 on fluorescence variables, testing differences between the slopes of the effects of ozone for three genotypes of snap bean ($p < 0.05$ indicates significant differences)

Contrast	F_0	F_v/F_m	q_p	q_n
156 vs. 123	0.0046	0.0084	0.0045	0.0087
156 vs. 331	0.0131	0.0099	0.0281	0.0498
123 vs. 331	0.5398	0.8276	0.3352	0.2354
156 vs. others	0.0022	0.0039	0.0044	0.0106

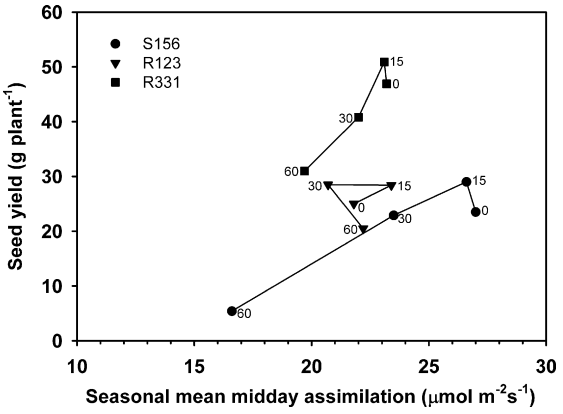

Fig. 4. Seed yield as a function of weekly mean midday assimilation rate (A). Numbers on the graph indicate 12 h mean O_3 concentrations. Significant differences among A ($p < 0.05$) are found in Table 2.

Fig. 5, a parametric plot of midday assimilation and $V_{c,max}$ provides a further illustration of the photosynthetic differences between S156 and R123. S156 followed a well defined trend where A was highly correlated with $V_{c,max}$ and both declined in response to increased O_3 concentration. The decreasing trend in $V_{c,max}$ between 0 and 15 nmol mol^{-1} suggested the possibility that the putative hormetic seed yield increase may have been due to stimulation of antioxidant action or effects on some process remote from actual carbon fixation, such as translocation. Also, the regular decline in A and $V_{c,max}$ strongly suggested that Rubisco was under attack over the entire range of O_3 concentrations used. S156 appeared to have little ability to up-regulate Rubisco activity, perhaps since it was operating near 100% of capacity even at the lowest O_3 concentrations. A similar pattern was observed for R331 except over a much more narrow range of both A and $V_{c,max}$.

The relational pattern for R123 is both more complex and instructive with A and $V_{c,max}$ operating over fairly narrow ranges with no significant differences in either. This seemed to suggest that R123 had a greater ability to compensate for O_3 damage by up-regulating Rubisco activity either through increasing the activation state or de novo synthesis using remobilized N from previously O_3-damaged tissue.

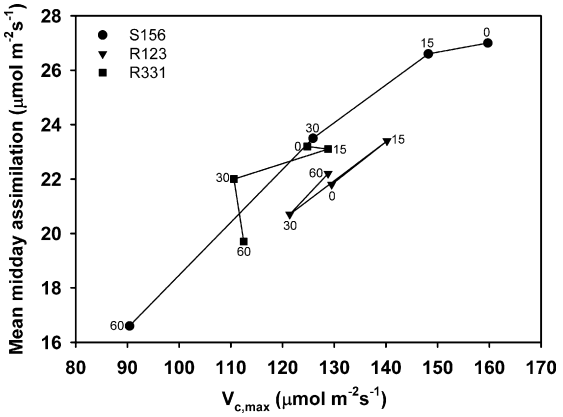

Fig. 5. Parametric plot of A and $V_{c,max}$. Numbers on the graph indicate 12 h mean O_3 concentrations. Significant differences among A and $V_{c,max}$ ($p < 0.05$) are found in Table 2.

F_v/F_m was significantly reduced and F_0 significantly increased in S156 indicating that electron transport was compromised by O_3. O_3 reduced the ability to capture light energy in S156 through the loss of intact and/or open photosystem II reaction centers. Light energy that was captured in O_3 exposed S156 plants was dissipated less through photosynthetic processes and at a greater rate through alternative means such as fluorescence, heat or the xanthophyll cycle.

In contrast neither R123 nor R331 showed any significant O_3 effects in the fluorescence data (Table 5). This indicated that even when there were significant reductions in photosynthetic parameters such as A, $V_{c,max}$, g_s, and g_m these genotypes were still able to efficiently capture and use light energy.

This study showed that plants sensitive to O_3 suffered decreased photosynthetic capacity through reductions in carbon fixation (A), and Rubisco activity ($V_{c,max}$), and may also have experienced a disruption of electron transport as indicted by reduced F_v/F_m and increased F_0. These results confirm those of previous studies such as Fiscus et al. (1997), Guidi et al. (2002), Long and Naidu (2002), Morgan et al. (2003) that report that O_3 decreased the photosynthetic capacity and the efficiency of excitation capture. Also confirming previous studies, our data indicated that there was no effect of O_3 on S_l and that the reduction in g_s was the result of decreased photosynthetic capacity. Reductions in g_m might also be the result of decreased photosynthetic capacity in the sense of down-regulating intact tissues but might also reflect the accumulation of tissue damage and cell death that would change the diffusive characteristics of the tissue system. The same question arises concerning any diminution of photosynthetic activity in systems facing oxidative stress, especially when expressed on a leaf area basis, as to whether we are measuring a smaller number of fully active photosynthetic units or a larger number of units that are operating in a compromised state.

Perhaps our most interesting results were found for R123. R123 did not show a significant reduction in photosynthetic capacity or electron transport due to exposure to O_3 but did show a yield reduction, perhaps due to increased diversion of photosynthate to maintenance and repair processes (Amthor, 1988). This supports previous research that reported that O_3 reduced Rubisco and/or Rubisco activity (Long and Naidu, 2002). Reductions in Rubisco and/or Rubisco activity would lead to reductions in g_s and g_m that were also found in this study. If this down regulation was prolonged it may eventually also lead to reduced electron transport as seen in S156. Reductions in electron transport may be due to the down regulation and/or loss of chlorophyll most likely from photoinhibition.

5. Conclusions

The objective of this study was to determine the effects of O_3 on three genotypes of snap bean with known sensitivities to O_3. We found that the effects of O_3 on the yield and photosynthetic parameters varied greatly depending on genotype and O_3 concentration. The highest O_3 concentration was found to significantly reduce yield for all three genotypes. However, all three genotypes were tolerant of low doses of O_3 and both R331

and S156 may in fact have benefited from a minimal O_3 exposure. One genotype (R331) previously thought to be tolerant of O_3 was found to be nearly as sensitive as our most sensitive snap bean genotype (S156) on a unit exposure basis. Given these data and the similarity of stature, the best physiological contrasts for future studies should be found between S156 and R123.

The yield reductions for S156 were clearly related to reductions in photosynthetic capacity due to O_3 and it is probable that any changes in g_s, g_m or fluorescence parameters followed or were coincidental with losses of Rubisco activity although the temporal resolution of the present data is not sufficient to resolve this question. The relationship between A and $V_{c,max}$ clearly shows that S156 has a higher inherent photosynthetic capacity in clean air than either R123 or R331 (Fig. 5) but was unable to translate that extra capacity into seed yield and began rapidly to lose capacity at O_3 concentration >15 nmol mol^{-1}. By contrast, R123 had a lower photosynthetic capacity in clean air but was able to stabilize Rubisco activity above 30 nmol mol^{-1} which may partially account for its lower proportional losses of productivity. Direct measurements of Rubisco content and activation states in S156 and R123 could clarify these relationships and help unravel the causes of the sensitivity differential between these two genotypes.

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