THE PENNSYLVANIA STATE UNIVERSITY
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ENVIRONMENTAL RESOURCE MANAGEMENT PROGRAM

# EVALUATING THE IMPACT OF NIGHTTIME OZONE EXPOSURE ON RESISTANT AND SENSITIVE *PHASEOLUS VULGARIS* L. GENOTYPES

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## ABSTRACT

Tropospheric ozone, a common air pollutant, proves harmful to humans and the environment, adversely affecting both plants and animals. Produced through natural and anthropogenic means, ozone enters a plant though the plant's stomata, resulting in visible foliar injury to sensitive species and triggering the formation of toxins that hasten aging, decrease photosynthesis, and promote necrosis. Consequently, exposure to ozone can reduce crop yield, prompting concern within the agricultural and food production industries. In order to detect damage from ambient ozone, the snap bean (*Phaseolus vulgaris* L.) genotype pair S156/R123 can be used as a bioindicator of ozone damage, displaying upper leaf stipple, stunted growth, and altered yield after ozone exposure (Burkey et al., 2005; Feng and Kobayashi, 2009; Ladd et al., 2011; Salvatori et al., 2013). Although many studies have presented a correlation between daytime ozone exposure and reductions in yield, limited research has been conducted pertaining to the effects of nighttime ozone exposure (Burkey et al., 2005; Feng and Kobayashi, 2009; Musselman and Minnick, 2000; Salvatori et al., 2013). Ozone production follows diurnal cycles with peak production in the afternoon and diminishment at night. However, ozone levels can remain high during nighttime, potentially inflicting plant injury. This greenhouse study investigated the impact of eleven target ozone concentrations of 0, 50, 75, 100, 125, 150, 175, 200, 225, 250, and 275 ppb upon sensitive and resistant genotypes of snap beans over a three-week, fifteen-day treatment period. Evaluation of the plants for foliar injury occurred throughout the exposure period, and mature pods were dried, counted, and weighed to evaluate yield. Nighttime ozone had a statistically significant effect on pod mass and pod number across the two genotypes. Across the range of nightly ozone exposures, the mean number of injured leaves between genotypes was also statistically significantly different. Moreover, the mean pod mass

between genotypes was statistically significantly different relative to the number of injured leaves.

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# Chapter 1

# Introduction

## *Ozone Regulatory History*

The ozone ($O_3$) layer resides naturally in the stratosphere, protecting the earth from ultraviolet radiation. Section VI of the Clean Air Act exists to protect and repair the ozone layer by banning the use of chlorofluorocarbons (CFCs), chemical compounds that destroy stratospheric ozone (US EPA, 2016). However, tropospheric ozone, concentrated within 10-16 kilometers of the earth's surface, harms the health of both humans and the environment (Ladd et al., 2011). In fact, ozone has been termed the most toxic, damaging air pollutant to plants (Agathokleous et al., 2016). Booker et al. (2009) estimated yield losses for ozone-sensitive crops such as snap beans, wheat, soybeans, barley, tomatoes, and potatoes in the range of 5-15%. The Environmental Protection Agency (EPA) regulates ozone concentrations in the United States as part of the National Ambient Air Quality Standards (NAAQS) for six criteria pollutants (CO, Pb, $O_3$, $NO_2$, $SO_2$, and particulate matter) under the amended 1990 Clean Air Act (US EPA, 2016). Ozone levels, as calculated using the fourth-high metric, must be under 70 parts per billion (ppb) according to the revised NAAQS, effective December 2015 (US EPA, 2016). Between the years of 2003 and 2004, the EPA introduced the $NO_x$ State Implementation Plan (SIP) Call Rule in attempt to reduce power plant and combustion emissions (US EPA, 2016). Although ground level ozone concentrations in the United States have decreased due to SIPs, ozone continues to

act as a costly air pollutant, adversely affecting agriculture in both the United States and in rapidly developing, highly populated areas of the world.

Current ozone regulations to protect human and environmental health are based on the fourth-high metric. The fourth-high metric is the fourth-highest ozone concentration averaged over an eight-hour period across three consecutive years. The revised 2015 NAAQS lowered the eight-hour ozone standard from 75 ppb to 70 ppb based on correlations between the 70 ppb level and the W126 cumulative exposure index, which is utilized to predict ozone impacts on vegetation. The W126 index value is the sum of weighted hourly ozone concentrations measured within three consecutive months during 12 hours of daylight (8 a.m. to 8 p.m.). In more simplistic terms, the W126 index value is derived by computing a concentration-weighted daily index value, then the monthly index value, followed by the consecutive 3-month value, and finally, the 3-year average. Higher ozone concentrations are weighted greater than lower values, and the three highest monthly sums from each year are used to calculate the 3-year average (US EPA, 2016).

Notably, the acceptable W126 index value of 17 ppm-hours fails to account for nighttime ozone concentrations despite findings of studies that suggest nighttime exposure contributes to plant injury (Musselman and Minnick, 2000; Goknur and Tibbitts, 2001; Günthardt-Goerg, 1996). Musselman and Minnick (2000) list the snap bean as a plant that displays nocturnal stomatal conductance, while Goknur and Tibbitts (2001) recorded gradual stomatal openings during dark periods in the Irish potato, and Günthardt-Goerg (1996) report that deciduous birch, poplar, and alder trees display foliar damage due to nighttime exposure. Thus, the correlation between the 12-hour W126 index value and the fourth-high metric suggests potential shortcomings; the W126 does not account for night ozone exposure, nor does the 70 ppb fourth-

high metric since the maximum ozone levels rarely occur at night (but theoretically could) (US EPA, 2016). However, evidence for nighttime ozone injury is difficult to compare across studies due to a lack of consistency, unrealistic ozone concentrations, unnatural study conditions, and varied plant growth stages (Lloyd, 2015). Consequently, the EPA lacks sufficient, consistent evidence that nighttime ozone exposures may contribute to plant damage and cannot justify extending the W126 index to measure ozone concentrations over a 24-hour period (US EPA, 2016).

### *Tropospheric Ozone Formation and Impact*

As a secondary air pollutant, tropospheric ozone forms in the atmosphere through photochemical reactions involving volatile organic compounds (VOCs) and nitrogen oxides ($NO_x$) (US EPA, 2016). Largely, intense heat in combustion chambers produces the gases necessary for ozone formation; power plants and vehicles release VOCs and $NO_x$. However, other sources, such as agriculture and industry also contribute to the formation of ozone (US EPA, 2016). To create ozone, ultraviolet light splits $NO_2$ into its constituent parts (NO + O). The singular reactive oxygen combines with ambient $O_2$ to produce ozone ($O_3$). The reverse reaction of ozone formation occurs simultaneously ($O_3 + NO \rightarrow NO_2 + O_2$), resulting in no net ozone production. However, when VOCs are present, $NO_2$ can regenerate without splitting ozone molecules, allowing ozone levels to increase (Allen, 2002).

The peak rates of ozone production and transport in the United States occur from April through September, when solar radiation is maximized. Typically, ozone production rates are highest in temperatures of 90 degrees Fahrenheit or above (US EPA, 2016). Ozone production

reaches its apex in the afternoon, slowing drastically and stopping once the sun sets. In areas where NO pollution is present, however, ozone is destroyed at night, and it cannot regenerate without the presence of sunlight. Typically, this explains why ozone levels decrease after sunset. However, despite ozone production decreasing after mid-afternoon, ozone levels may remain constant throughout the day and night (Ladd et al., 2011; US EPA, 2016).

Ozone and its precursors are transported via wind, easily moved hundreds of miles from urban to rural areas. Consequently, the impact of ozone often reaches geographically far beyond its point of formation (Ladd et al., 2011). Daytime ozone levels are commonly elevated in large cities in California, southeastern Texas, and in portions of the southeastern and northeastern United States during hot, dry periods (US EPA, 2016). However, mountainous regions, such as those in southern California and Colorado, tend to trap moving ozone and have high nighttime ozone levels (US EPA, 2013; Ladd et al., 2011). This mountainous concentration of ozone is part of a radiational or thermal inversion, where rising warm air masses from valleys reaches cold air near the mountaintop and becomes a stagnant air mass trapped with ozone (Ladd et al., 2011). In more pristine areas with clean air, ozone levels tend to remain high, as there are few pollutants to facilitate the back reaction of ozone formation.

Ozone enters a plant through its stomata, where it can dissolve in the water surrounding the plasma membrane. From there, the ozone may react directly with the water and solutes, forming reactive oxygen species (ROS), including hydroperoxide, superoxide, and hydrogen peroxide. Direct reactions of ozone and ROS with cellular components injure the plant's cells and membranes, reduce its ability to photosynthesize, weaken its reproductive and defensive abilities, and increase senescence (Flowers et al., 2007; Long and Naidu, 2002). Visible injury includes an interveinal upper leaf surface stipple, usually purple, blue, or black in color, which

forms due to the production and accumulation of anthocyanins (Ladd et al., 2011). Anthocyanin production is the plant's response to oxidative stress. Stipple generally progresses into chlorosis, or leaf yellowing, that forms due to a lack of chlorophyll pigmentation. Leaves that are wilted, dry, or that are gray or brown in color indicate necrosis, or cell death (Ladd et al., 2011). Necrosis may be followed by premature leaf drop.

Ozone sensitivity differs between plant genotypes and species. Plants that have a high stomatal conductance naturally take in more ozone, and plants that have low antioxidant levels have weaker defense mechanisms to fight ROS (Musselman and Minnick, 2000). During the nighttime, plants lose their capacity to metabolize ozone quickly due to decreased light, antioxidant production, and photosynthesizing ability. Consequently, nightly ozone exposure may damage the plant more than daytime ozone exposure, as its defensive mechanisms cannot be utilized as consistently as they are in the day (Musselman and Minnick, 2000). With a lower stomatal conductance at night, however, plants may not necessarily take in as much ozone as they would during the day. Decreased nightly stomatal conductance contrives a popular opinion that daytime ozone exposure inflicts far more injury upon the plant and that nighttime ozone is a negligible threat for ozone studies, contrary to studies that suggest otherwise (Günthardt-Goerg, 1996; Musselman and Minnick, 2000; Goknur and Tibbitts, 2001).

***Objectives:***

Due to food security concerns, yield losses from ozone pollution exceeding 5% have been deemed unacceptable by the EPA's Clean Air Scientific Advisory Commission (Frey, 2014). According to a meta-analysis study conducted at 406 different locations, the snap bean is an

ozone sensitive crop, losing around 19% of its potential yield in ambient ozone concentrations of 31 to 50 ppb (Burkey et al., 2005; Ladd et al., 2011; Feng and Kobayashi, 2009). Bioindicators, native or introduced organisms that display a typical response when exposed to stress, are used to evaluate air quality and environmental changes (Ladd et al., 2011). Snap beans are a popular air pollution bioindicator; they are grown in widespread areas, display consistent visual injury symptoms, and are genetically stable (Ladd et al., 2011). However, most snap bean studies have evaluated the impact of daytime ozone exposure with little regard to the impact of nighttime exposure. The effect of nighttime ozone exposure on agricultural species needs to be better understood to minimize yield loss and to revise the NAAQS for ozone, if necessary.

This study tested the effects of nighttime (8:00 p.m. to 7:00 a.m.) ozone exposure at target concentrations ranging from 0 to 275 ppb on ozone-sensitive (S156) and ozone-resistant (R123) snap bean genotypes. Foliar injury and yield were quantified. Thus, the experiment tested the response of foliar injury and yield against night ozone concentration for S156 and R123 snap beans.

# Chapter 2

# Materials and Methods

## *Experimental Location and Equipment*

This experiment was conducted in a university greenhouse on University Drive in State College, Pennsylvania. The greenhouse, situated next to the Forest Resources Lab, was equipped with 16 cylindrical, continuously stirred tank reactors (CSTR), each with a diameter of 1.5 meters and height of 1.5 meters (Figure 1). Air from within the greenhouse was pulled into the chambers with a blower system that completely exchanged the air within each chamber once per minute. The air from within the chambers was then released outside of the greenhouse. Each chamber was lined with a clear, plastic film that wrapped around its steel frame and had a 1000-watt Lumalux lamp on the ceiling. Ozone was produced via a model Z-08 ozone generator (Ozone Solutions, Hull, IA), and was distributed to each chamber through Teflon tubing. The generator used an electric current to generate ozone from dry air. Ozone monitors read each chamber's ozone level every 10 minutes. REAL Controls Inc. software recorded the ozone levels, continuous measurements of the temperature and humidity from Omega model HX93BC sensors (Omega Engineering, Inc., Stamford, Connecticut) located in each chamber and photosynthetically-active radiation (PAR) from LI-190R quantum sensors (LI-COR, Inc., Lincoln, NE), positioned at canopy height in the five daytime treatment chambers. The ambient air in the greenhouse was also continuously monitored for ozone levels, temperature, and relative humidity (Appendix A).

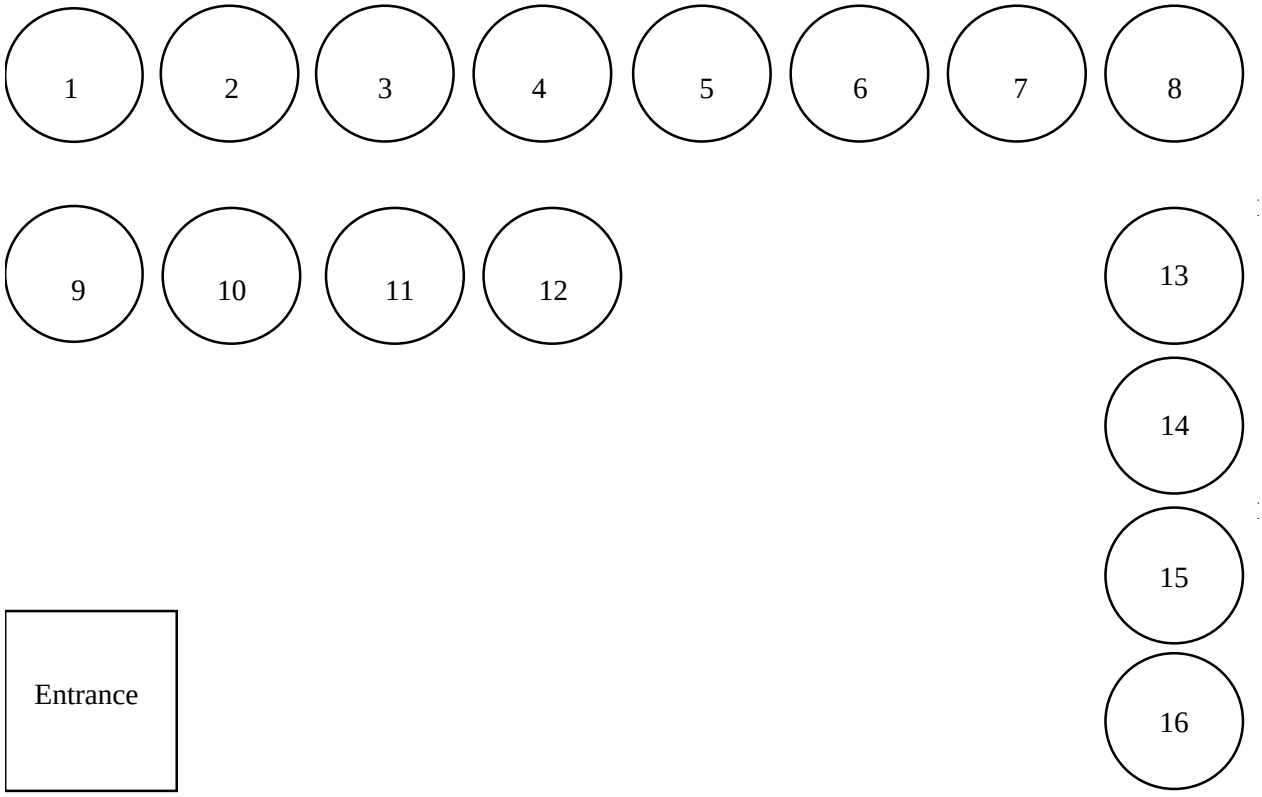


**Figure 1. Experimental set-up in the Forest Research Laboratory greenhouse. Each circle represents a fumigation chamber.**

***Plant Material***

Kent Burkey of the USDA-ARS in Raleigh, North Carolina provided snap bean (*Phaseolus vulgaris* L.) seeds of the ozone sensitive genotype (S156) and resistant genotype (R123). Seeds were planted on June 27, 2016 in 3.8-liter pots that had been sterilized overnight in a dilute bleach solution. The pots were filled with Sunshine Mix #4 and 15 grams of Osmocote Plus 15:9:12 from Sun Gro Horticulture in Agawam, Massachusetts and The Scotts Company LLC, respectively. Sunshine Mix #4 consisted of 63-73% peat moss, perlite, and dolomitic

limestone. Three seeds were planted in each pot and were thinned to one plant per pot by July 12, 2016, after the first trifoliate leaf had fully expanded (Figure 2). The plants were loosely staked to a bamboo support.


**Figure 2: Bean plants prior to ozone exposure.**

***Ozone treatments***

Fifty snap bean plants were used in this experiment: 44 treated plants (22 sensitive genotypes and 22 resistant genotypes) and 8 control plants. During the day (8:00 a.m. to 6:00 or 7:00 p.m.), the 44 treated bean plants were divided between five CSTRs and exposed to an ozone concentration of 50 ppb (Table 1). The plants were randomly rotated between chambers each day of exposure. The nighttime (8:00 p.m. to 7:00 a.m.) ozone treatments were applied in eleven treatment chambers. Two sensitive and two resistant plants were placed in each chamber. Total run time for the daytime exposures totaled 7030 minutes, and total run time for nighttime exposures totaled 10345 minutes (Table 1). Target ozone concentrations were as follows: 0, 50, 75, 100, 125, 150, 175, 200, 225, 250, and 275 ppb. Eight plants, four of each genotype, were placed in a control chamber with a day/night target ozone level of 0 ppb. Plants were exposed to ozone from July 18, 2016 (22 days after seeding) through August 5, 2016, for five days per week (Monday through Friday). On cloudy days, the greenhouse's shade cloth was removed during daytime exposures. The plants were randomized in their treatment chambers in order to minimize chamber effects. The plants were watered with distilled water twice per day between ozone exposures to keep the soil moisture near field capacity. After the end of the exposure periods, plants were moved from the chambers to the ambient greenhouse space and were watered as needed until the bean pods were fully developed.

| Date | Day Start | Day End | Run Time | Night Start | Night End | Run Time |
|---|---|---|---|---|---|---|
| July 18 | 8:30 | 19:10 | **10:40** | 20:00 | 7:45 | **11:45** |
| July 19 | 9:00 | 18:00 | **9:00** | 20:00 | 7:30 | **11:30** |
| July 20 | 9:25 | 18:40 | **9:15** | 20:00 | 7:30 | **11:30** |
| July 21 | 9:10 | 17:50 | **8:40** | 20:00 | 7:30 | **11:30** |
| July 22 | 12:20 | 17:50 | **5:30** | 20:00 | 7:30 | **11:30** |
| July 23 | No run | | | | | |
| July 24 | No run | | | | | |
| July 25 | 9:35 | 14:35 | **5:00** | 20:00 | 7:30 | **11:30** |
| July 26 | 9:50 | 18:05 | **8:15** | 20:00 | 7:30 | **11:30** |
| July 27 | 9:20 | 18:20 | **9:00** | 20:00 | 7:30 | **11:30** |
| July 28 | 11:50 | 17:50 | **6:00** | 20:00 | 7:30 | **11:30** |
| July 29 | 9:50 | 17:50 | **8:00** | 20:00 | 7:30 | **11:30** |
| July 30 | No run | | | | | |
| July 31 | No run | | | | | |
| August 1 | 12:45 | 18:15 | **5:30** | 20:00 | 7:30 | **11:30** |
| August 2 | 9:20 | 17:45 | **8:25** | 20:00 | 7:20 | **11:20** |
| August 3 | 9:55 | 18:20 | **8:25** | 20:00 | 7:30 | **11:30** |
| August 4 | 9:45 | 17:55 | **8:10** | 20:00 | 7:30 | **11:30** |
| August 5 | 10:20 | 17:40 | **7:20** | 20:00 | 7:20 | **11:20** |

**Table 1: Experiment run times for day and night exposures**

### *Ozone Injury Evaluation*

Between 6:00 p.m. and 7:45 p.m. during the exposure period (Table 1), trifoliate leaves on each plant were marked sequentially for development of visible injury each day. Photographs were taken of each group trifoliate leaves as the plants flowered and as injury developed. The date in which each plant started flowering was recorded. Leaf drop dates were also recorded.

### *Yield Measurement*

Pods remained on the bean plants to dry. Only bean pods with one or more seeds were considered mature. The mature pods were collected from each plant and placed in paper bags on August 23, 2016. The pods were dried in a $65.6^0$ C ($150^0$ F) oven before being weighed on August 30, 2016. The number of mature pods from each plant was also recorded.

### *Data Analyses*

Values reported for each night ozone treatment and genotype combination represent the mean of the two subsamples (plants) within each chamber. Values reported for the daytime controls represent the mean of four plants per genotype per chamber. Least squares regression was used to analyze the relationship between the dependent variables, foliar injury and pod yield, and nighttime ozone concentration. For each genotype, the null hypothesis was that the slope of the fitted line was equal to 0. Indicator-variable regression with an interaction term was used to test the null hypotheses that the slopes and intercepts of the two genotypes were equal. Statistical analyses were conducted using JMP Pro 12 software (SAS Institute Inc.).

# Chapter 3

## Results

Across the CSTRs, air temperatures typically reflected the ambient air temperature in the greenhouse. Maximum temperatures were reached on days with high solar radiation influxes. The mean daytime air temperature in the chambers was 29 ºC, with a minimum of 22 ºC and a maximum of 36 ºC, while the mean nighttime chamber temperature was 22 ºC, with a minimum temperature of 17 ºC and a maximum of 29 ºC. The mean relative humidity (RH) across the chambers during the day was 64%, ranging from 41% to 82%, and the mean nightly RH was 80%, ranging from 51% to 96%. The average photosynthetically active radiation (PAR) in the daytime was 376 µmol $m^{-2}$ $s^{-1}$, ranging from 309 µmol $m^{-2}$ $s^{-1}$ to 444 µmol $m^{-2}$ $s^{-1}$. Daytime ozone means were 9 ppb and 44 ppb in the control (n=1) and treatment (n=5) chambers, respectively. Actual mean ozone concentrations for the nighttime treatments were as follows: 8, 47, 79, 106, 125, 155, 172, 204, 223, 244, and 265 ppb (Appendix A).

**Table 2: Day of treatment when plant flowering began for S156 and R123 in relation to actual nightly ozone exposures (ppb).**

| Night Ozone (ppb) | Day of treatment when flowering started (R123) | Day of treatment when flowering started (S156) |
|---|---|---|
| 8 | 6 | 6 |
| 47 | 8 | 6 |
| 79 | 6 | 6 |
| 106 | 6 | 6 |
| 125 | 7 | 6 |
| 155 | 7 | 6 |
| 172 | 6 | 6 |
| 204 | 6 | 5 |
| 223 | 6 | 6 |
| 244 | 6 | 6 |
| 265 | 6 | 6 |

All bean plants flowered between the sixth and eighth day of the ozone treatment, as documented in Table 2. There were no significant relationships between ozone and flowering response for either genotype: $P = 0.5091$ for S156 and $P = 0.2372$ for R123.

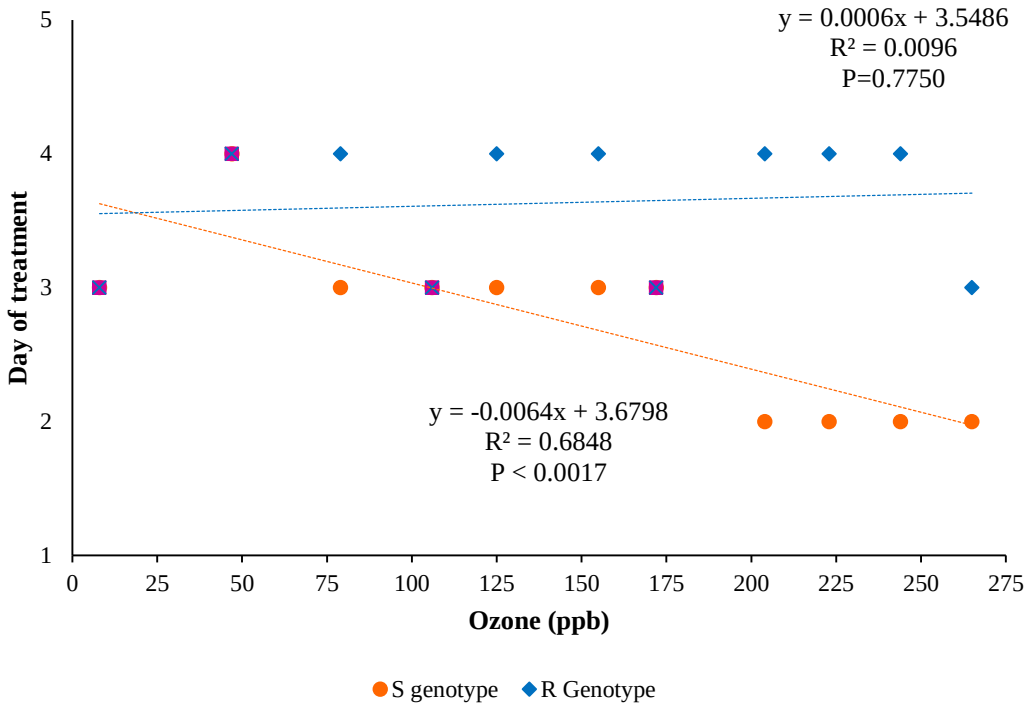


**Figure 3: Linear regression of the day of treatment when injury first appeared on S156 and R123 trifoliate leaves in relation to night ozone exposures. Purple square points represent data where S156 and R123 overlap.**

For the night ozone treatments above 200 ppb, foliar injury appeared one to two days earlier on S156 than R123 (Figure 3). Night ozone concentration was also significantly related to the onset of visible injury in S156 ($P = 0.0017$) but not R123. All plants showed visible injury by the fourth day of treatment.

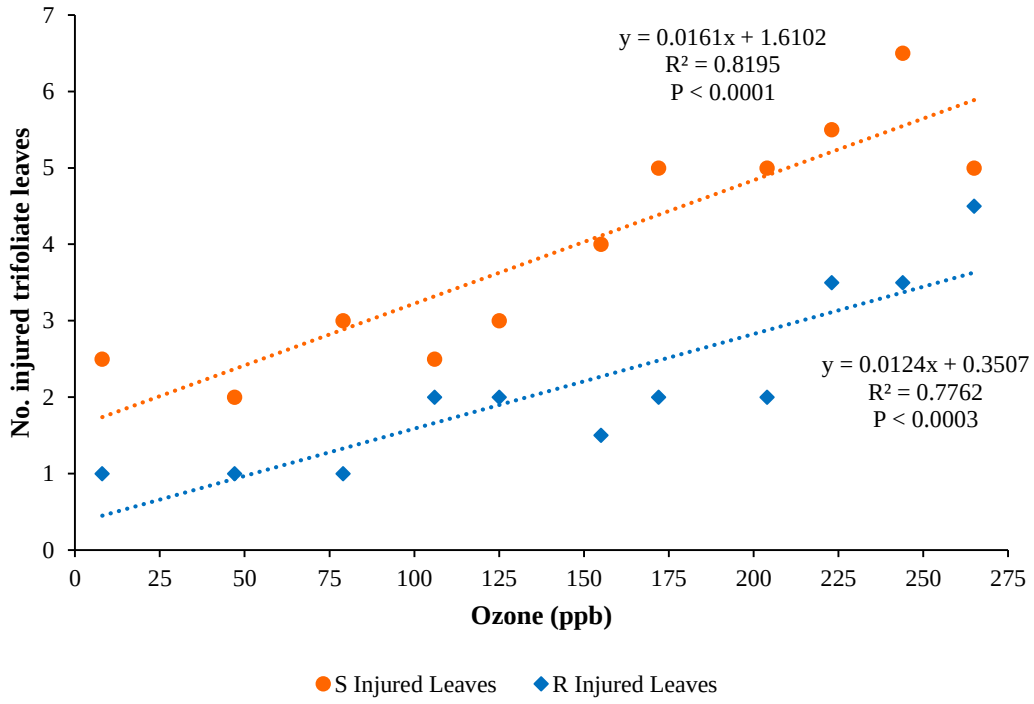


**Figure 4: Linear regression of the number of injured sensitive (S156) and resistant (R123) trifoliate leaves at the end of the treatment period in relation to ozone exposures.**

Figure 4 shows a strong linear relationship between the degree of injury and intensity of ozone exposure for both the sensitive and resistant genotypes. Nonetheless, the sensitive plants, in every case, displayed more injured leaves than the resistant plants for a given nighttime ozone treatment (ppb). Although the number of injured leaves showed a significant response to ozone in both genotypes (Figure 4), the slopes of the two regression lines are not significantly different ($P = 0.2760$; Table 3), indicating that the genotypes responded similarly to increasing ozone treatment. However, the intercepts are significantly different ($P = 0.0385$), reflecting the difference in magnitude of injury between genotypes (Table 3). In contrast, the daytime controls did not show symptoms of ozone injury.

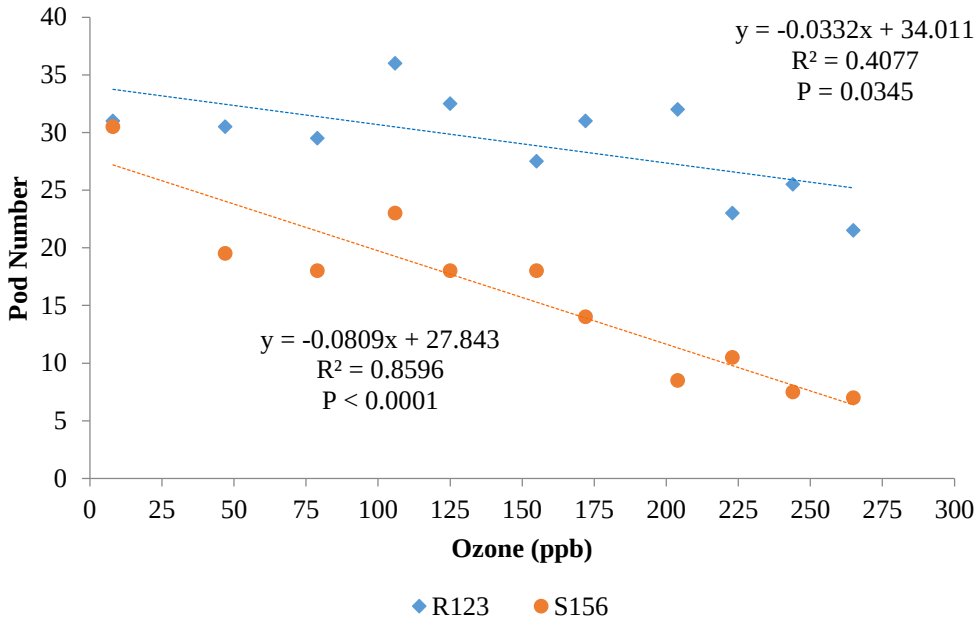


**Figure 5: Linear regression of sensitive (S156) and resistant (R123) genotype pod number in relation to night ozone exposures. Values for the daytime controls were 29.5 and 37 pods per plant for R123 and S156, respectively.**

Figure 5 shows that R123 produced a greater number of pods than S156 at every ozone exposure concentration above 8 ppb. There is a stronger linear relationship and effect of ozone displayed in the sensitive genotype ($R^2$= 0.8596; P < 0.0001) than the resistant genotype ($R^2$= 0.4077; P = 0.0345), and S156 had a more negative slope, about 2.4 times larger than R123, indicating that increasing night ozone had a greater negative effect on the number of pods produced per plant in S156. In addition, the slopes (P = 0.0127) and intercepts (P < 0.0001) of the genotypes were significantly different (Table 3), indicating a difference in both the rate and magnitude of ozone effects on pod number. Values for the daytime controls were 29.5 and 37 pods per plant for R123 and S156, respectively (data not shown).

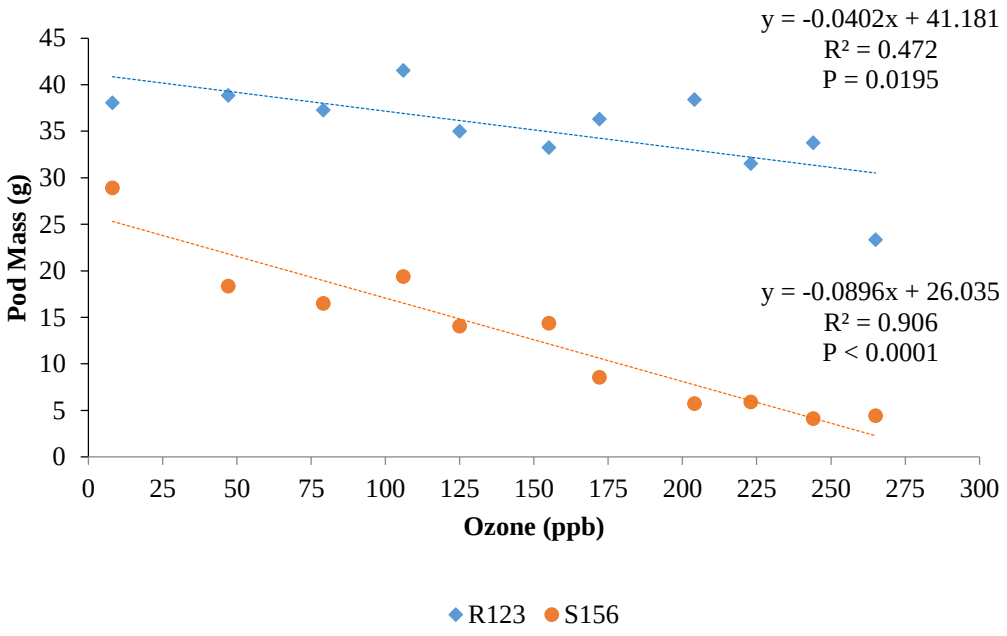


**Figure 6: Linear regression of sensitive (S156) and resistant (R123) genotype pod mass in relation to night ozone exposures. Values for the daytime controls were 37.76 and 38.22 g for R123 and S156, respectively.**

Figure 6 shows that R123 had greater pod mass than S156 at every ozone exposure concentration. The largest decrease in pod mass for the resistant genotype occurred between 244 and 265 ppb, with an overall yield reduction of 39% at 265 ppb relative to the 8 ppb night control. In contrast, S156 yield was reduced by 50% at 125 ppb and by 85% at 265 ppb, relative to the 8 ppb night control. The response of S156 pod mass to nighttime ozone treatments was better explained by a linear fit than for R123 (i.e., $R^2$ values of 0.906 vs. 0.472, respectively), and the relationship was also more significant for the sensitive ($P < 0.0001$) than the resistant ($P = 0.0195$) genotype. In addition, the slopes ($P = 0.0099$) and intercepts ($P < 0.0001$) of the genotypes were significantly different (Table 3), indicating a difference in both the rate and magnitude of ozone effects on pod mass. The slope coefficient for S156 was approximately 2.2

times larger than R123, indicating that S156 pod mass decreased at over twice the rate of R123 for each unit increase in nighttime ozone exposure. Pod mass in the daytime control was similar for R123 and S156 with 37.76 g and 38.22 g, respectively (data not shown).

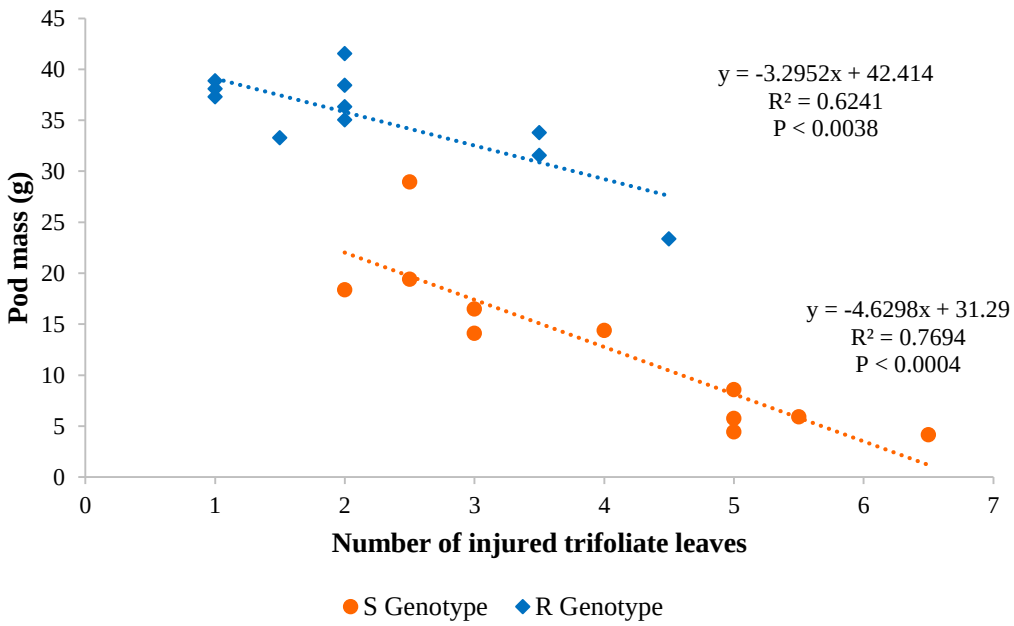


**Figure 7: Linear regression of S156 and R123 genotype pod mass in relation to the number of injured trifoliate leaves after treatment.**

Figure 7 shows a linear relationship between the number of injured leaves and pod mass for both genotypes; pod yield decreases as plant injury increases. The sensitive genotype displays a stronger linear fit, however ($R^2$=0.7694). The majority of observations for R123 are for 1 to 2 injured leaves. Thus, the best-fit line is leveraged by three points for the treatments with the greatest injury, and the lines for the two genotypes overlap only in the middle range of leaf injury. Although ozone had a significant effect on the response of both genotypes, the slopes of

the regression lines are not significantly different (Table 4), indicating a similar response in the rate of yield reduction with increasing foliar injury. However, the intercepts are significantly different (P = 0.0126; Table 4), with S156 producing lower pod mass than R123 at a given level of foliar injury.

**Table 3: Effects tests for pod mass, pod number, and injured leaf number vs. ozone treatment with genotype as an indicator variable. The interaction of ozone*genotype tests the null hypothesis that the slopes of the best-fit lines for S156 and R123 are equal.**

| | DF | Pod Mass | | Pod Number | | Injured Leaf Number | |
|---|---|---|---|---|---|---|---|
| | | **F-value** | **p-value** | **F-value** | **p-value** | **F-value** | **p-value** |
| Genotype (mean) | 1 | 272. | <0.0001 | 93.6 | <0.0001 | 46.5 | <0.0001 |
| Genotype (intercept) | 1 | 23.3 | <0.0001 | 21.3 | <0.0001 | 4.99 | 0.0385 |
| Ozone | 1 | 57.4 | <0.0001 | 43.8 | <0.0001 | 72.1 | <0.0001 |
| Ozone*Genotype | 1 | 84.3 | 0.0099 | 7.65 | 0.0127 | 1.26 | 0.2760 |

Across the range of nightly ozone exposures, the mean number of injured leaves between genotypes was significantly different ($F=46.59$, $p < 0.0001$). In addition, daytime treatment at 44 ppb is expected to produce a significant difference in the number of injured leaves between genotypes, in the absence of nighttime ozone exposure, given the significance of the intercept test ($F= 4.99$, $P = 0.0385$). Similar to the results presented for the individual genotypes in Figure 4, night ozone exposure also had a significant effect on the injured leaf number across the two genotypes ($F= 72.01$, $p < 0.0001$), but the interaction between the ozone level and the genotype was not significant. Thus, the null hypothesis (the slopes of the regression lines are equal) cannot be rejected.

As Table 3 shows, nighttime ozone and genotype had similar effects on pod mass and number. The mean pod mass and pod number were significantly different, on average, for the two genotypes (F= 272, P < 0.0001; F= 93.627, p < 0.0001 respectively). As expected, given the significance of the relationships between yield and night ozone for each genotype (Figure 5; Figure 6), ozone had a significant effect on pod mass and number across the two genotypes (F= 57.415, p < 0.0001; F= 43.859, p < 0.0001, respectively). As noted earlier, the interaction for ozone*genotype was also significant, indicating that the slopes of the regression lines are not equal.

**Table 4: Effects tests for pod mass vs. injured trifoliate leaf number with genotype as an indicator variable. The interaction of injured leaf number*genotype tests the null hypothesis that the slopes of the best-fit lines for S156 and R123 are equal.**

| | DF | Pod Mass | |
|---|---|---|---|
| | | **F-value** | **p-value** |
| Genotype (mean) | 1 | 64.8363 | <0.0001 |
| Genotype (intercept) | 1 | 7.6739 | 0.0126 |
| Injured Leaf Number | 1 | 41.2897 | <0.0001 |
| Injured Leaf Number*Genotype | 1 | 1.1723 | 0.2932 |

Table 4 shows that the mean pod mass between the sensitive and resistant genotypes was significantly different relative to the number of injured leaves (F= 64.8363, p < 0.0001). The injured leaf number also had a significant effect on pod mass across the two genotypes (F= 41.2897, p < 0.0001), with probabilities for the individual genotypes given in Figure 7. However, the interaction between injured leaf number and pod mass was not significant (p= 0.2932). Therefore, there is not enough evidence to reject the null hypothesis that the slopes of the regression lines are equal, meaning that pod mass decreases at a similar rate with

increasing leaf injury for both genotypes. However, since the intercepts are significantly different, R123 maintains a higher pod mass across the range of ozone treatments than S156.

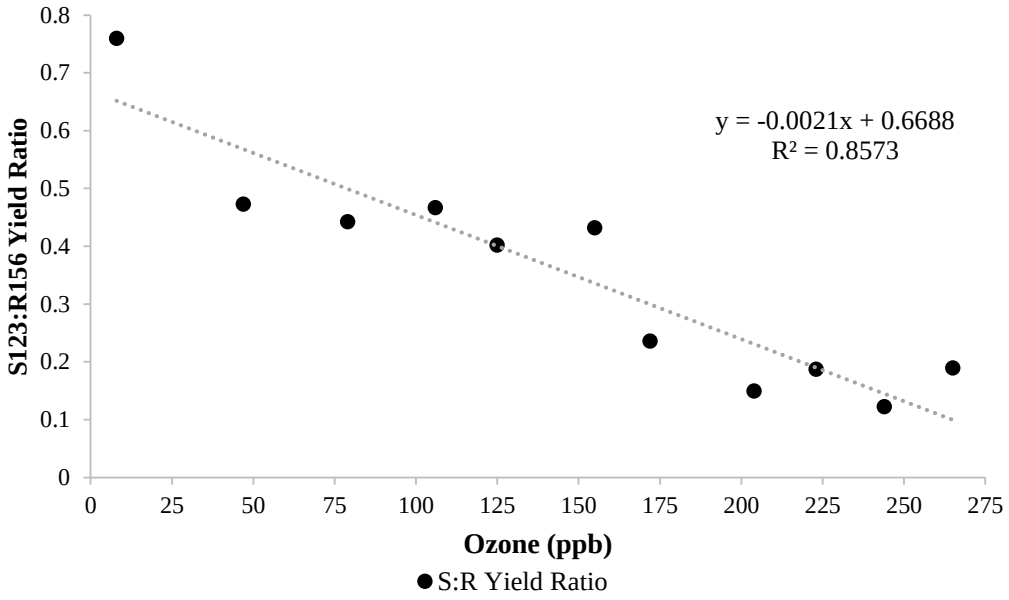


**Figure 8: S123:R156 pod mass ratio in relation to actual ozone treatment (ppb).**

Figure 8 shows that the pod mass ratio of the sensitive to resistant genotype decreased with increasing night ozone treatment, ranging from 0.19 to 0.76. The yield ratio displays a strong linear relationship with nightly ozone treatment ($R^2$=0.8573).

# Chapter 4

## Discussion

This experiment tested the effects of nighttime ozone exposure on phenology, foliar injury, pod number, and pod mass of S156 and R123 snap bean genotypes. Plants were exposed to 44 ppb ozone during the day followed by nighttime ozone treatments ranging from 8 to 265 ppb for 15 days over a three-week period. The night control plants were those exposed at 44 ppb during the day and 8 ppb at night. The experiment also included a control exposed to 9 ppb ozone during the day and 8 ppb at night (i.e., ambient + 0 ppb).

Changes in a plant's phenology can indicate a stress response. The control plants (9 ppb day/8 ppb night) flowered between 6-7 DAT, while the night controls (44 ppb day/8 ppb night) flowered between 6-8 DAT. Thus, day-only exposure did not have a significant effect on flowering. The treated bean plants all flowered between the $5^{th}$ and $8^{th}$ day of treatment (Table 2). Results from the nighttime treatments relative to results from the controls suggest that ozone did not have a significant effect on phenology. Interestingly, ozone stress has been shown to accelerate plant development (Booker et al., 2009; Salvatori et al., 2013). Ozone treatments began 15 days after seeding (DAS), prior to the full development of the second trifoliate leaf. It is plausible to assume that a significant effect on flowering time could have been observed if ozone treatments began a week or more prior to flowering. In a study conducted by Sanz et al. (2011), ozone hastened the early stages of flowering and ear stem growth of *Briza maxima* plants relative to controls. Moreover, ozone stress can shorten plant lifespans due to accelerated growth and increased senescence (Booker et al., 2009; Sanz et al., 2011). Consequently, heightened

senescence during the plant's pod formation, maturation, flowering, and growth stages limit plant production and yield (Booker et al., 2009; Salvatori et al., 2013).

Although flowering pattern showed no significant response to ozone, obvious symptoms of plant injury occurred early into the experiment and progressed during treatment. Night control S156 and R123 plants (44/8 ppb) began to display purple and white stipple 3 DAT (Figure 3), while control plants (9/8 ppb) never developed injury. Symptoms of plant injury appeared on S156 plants exposed at night ozone concentrations of 200 ppb and above just 2 DAT (Figure 3). All plants displayed stippling by 4 DAT, but the onset of injury was significantly related to night ozone exposure only for S156 (Figure 3). Since injury appeared sooner for the sensitive genotype, R123 may be better able to acclimate to, and defend against, some injury during the day (Matyssek et al., 1995). According to Salvatori et al. (2013), the resistant snap bean genotype has an overall higher detoxification and repairing capability due to antioxidant production relative to the sensitive genotype. However, antioxidant production occurs during photosynthesis, leading to the question of how R123 produces antioxidants and repairs itself under the stress of night ozone treatments when photosynthesis does not occur. Salvatori et al. (2013) suggest that R123 has a higher increase in maintenance respiration at night relative to S156.

The degree of injury progressed over time for all treatments, with a corresponding increasing degree of injury with increased ozone exposure. The nighttime treatment control plants (44/8 ppb) displayed minor to moderate injury by the end of the treatment period. On the S156 night controls, dark stippling and minor lesions on a mean of 2.5 trifoliate leaves developed, while minor purple and white upper leaf stippling appeared on only 1 trifoliate leaf of R123. However, the R123 controls (9/8 ppb) did not display any injury by the end of the

experiment. All treated plants displayed injury. Although both bean genotypes responded similarly in rate to increasing night ozone treatment, the sensitive genotype, predictably, suffered a greater magnitude of injury (Figure 4). For example, premature primary leaf drop occurred just 4 DAT on a S156 plant exposed at 265 ppb. Beginning the second week of treatment, necrotic, dry yellowed leaves affected by chlorosis hung onto many of the S156 plants, while the R123 plants displayed symptoms of earlier stages of ozone injury: dark purple or white interveinal stippling (Appendix B; Figure 10). For example, on July 20$^{th}$ (3 DAT), bifacial lesions appeared on the first trifoliate leaves of S156 plants exposed at 125 ppb and above, while the R123 plants exposed at 125 and above just started developing stipple. Just two days later (5 DAT), leaf yellowing and seed leaf drop was observed in S156 plants exposed at 223 ppb and above. As early as 5 DAT, the sensitive plants exposed at 204 ppb and above appeared noticeably smaller and their leaves were not as broad as the R123 plants', although the difference was most observable during the last week of treatment (Appendix B; Figure 9). Stark differences in injured trifoliate leaf number and plant growth from ozone injury did not solely occur at high ozone concentrations, however. On July 27 (10 DAT), the S156 plants exposed at 79 ppb displayed 3 injured trifoliate leaves. The first and second S156 trifoliate leaves were yellowed and wilted with bifacial lesions and necrotic patches, while the third trifoliate leaf displayed interveinal purple stipple (Appendix B; Figure 11). In contrast, the R123 plants exposed at 79 ppb had only one stippled trifoliate leaf that, despite injury, maintained its broad-leaf shape (Appendix B; Figure 11). These observations support the hypothesis that lower ozone exposure at night may inflict the same amount of foliar injury as a higher daytime exposure while also reducing plant biomass (Matyssek et al., 1995). Even though nighttime stomatal conductance was measured as half of the maximum daytime conductance in a study using *Betula pendula* cuttings (Maatyssek

et al., 1995), injury was evident from night exposure. Moreover, while research with Irish potatoes has described that plant stomata begin to open only near the end of the 12-hour nightly dark period, three hours of nightly exposure at the end of the dark period significantly contributed to higher ozone sensitivity and more severe injury observations in field environments (Goknur and Tibbitts, 2001). However, in contrast to the study conducted by Maatyssek et al. (1995), it took three times the ozone concentration at night to inflict the same amount of night injury as daytime injury in Goknur and Tibbitts' study (2001). Since plant injury from night exposures was identifiable, it is plausible that the bean plants responded in congruence with the aforementioned findings. The bean plants may not have experienced complete nightly stomatal closure, contributing to greater nightly conductance and increased ozone sensitivity.

Yield for the controls (9/8 ppb) averaged 29.5 pods per plant for R123 and 37 pods per plant for S156. Pod yield for the nighttime treatment control plants (44/8 ppb) averaged 31 and 30.5 for R123 and S156, respectively. R123 tends to produce fewer, larger bean pods that contain more seeds per pod, while S156 typically produces a greater number of smaller pods with fewer seeds per pod (Burkey et al., 2005; Salvatori et al. 2013). Flowers et al. (2007) found that R123 maintained pod yield that did not differ significantly across ozone concentrations of 0-60 nmol/mol. In this experiment, R123 plants produced more mature pods per plant than S156 at nighttime ozone concentrations of 47 ppb and above (Figure 5). Increasing night ozone had a stronger, more negative effect on pod production in S156 than R123, and there was a difference in the rate and magnitude of ozone effects on pod number between the two genotypes.

Pod mass for the control plants (9/8 ppb) was very similar between S156 and R123 (38.22 g and 37.76 g, respectively), generating a pod mass ratio of approximately 1. Pod mass for the nighttime treatment controls (44/8 ppb) was 28.95 g and 38.09 g for S156 and R123,

respectively, resulting in a pod mass ratio of 0.76. Although R123 has been known to produce fewer pods than S156, pod mass is generally similar across genotypes in ambient ozone concentrations of less than 30 ppb due to the larger size of R123 pods and more abundant S156 pods (Burkey et al., 2005; Salvatori et al., 2013). For the treated bean plants, the resistant genotype produced a greater mean pod mass (g) than the sensitive genotype for each level of ozone treatment (Figure 6). For every one increase in ppb, S156 pod mass decreased at a rate of -0.0896 grams and R123 decreased at a rate of -0.0402 grams. Thus, S156 pod mass decreased at more than two times the rate of R123 per unit increase in nighttime ozone exposure (Figure 6). Both the rate and magnitude of ozone effects on pod mass were different across genotypes due to different intercepts and slopes. The regression lines of pod mass relative to night ozone concentrations displayed a stronger relationship than pod number relative to night ozone across genotypes (Figure 5; Figure 6). Thus, pod mass is a better indicator of ozone effects for the sensitive and resistant genotypes because, under ambient ozone levels (less than 30 ppb), pod number differs, but the pod mass ratio is close to 1 (Booker et al., 2009; Burkey et al., 2005; Salvatori et al., 2013). Disparities in yield observations and results across studies may arise due to differing environmental conditions, plant culturing practices, and methods of yield evaluation (dried vs. fresh pods) (Booker et al., 2009; Salvatori et al., 2013).

Across the range of increasing night ozone exposures, S156:R123 yield ratios declined, ranging from 0.19 to 0.76, with a strong linear relationship between variables (Figure 8). For the controls, the yield ratio was approximately 1 (data not shown). Thus, it appears as though the day treatment alone reduced the S156:R123 ratio. In similar studies using charcoal filtered air and field chambers, yield ratios of approximately 1 were also observed (Burkey et al., 2005; Flowers et al., 2007). However, repeated trials are necessary to determine whether yield ratios vary with

experimental conditions, as beans are sensitive to changes in methodical and experimental conditions (temperature, relative humidity, light exposure, fertilizer, etc.; Booker et al., 2009; Salvatori et al., 2013).

The relationship between yield (g) and foliar injury was strong, displaying similar rates of decreasing yield with increasing foliar injury across genotypes (Figure 7). However, Agathokleous et al. (2016) discouraged the evaluation of ozone impact by using yield, and recommended the evaluation of visible injury for increased consistency. They justified these terms by stating that pod yield varied significantly between trials conducted in 2012 and 2013 due to high temperatures (93° F) that inhibited pollination, while the visible injury index was more consistent across the trials since leaf injury is unaffected by pollination. However, visual injury may not be consistent at all. Because visual injury measurements can be subjective, pod yield is often utilized as a consistent, strictly quantitative method for injury evaluation among scientific studies (Booker et al., 2009; Feng and Kobayashi, 2009). In fact, Booker et al. (2009) stated that ozone related yield losses have been reported without any visible foliar injury on the plant. In another study, the opposite was found to be true; five tomato cultivars displayed foliar injury, but did not experience a significant reduction in yield (Booker et al., 2009). By evaluating how pod mass and visible injury are related, the results in this experiment run contrary to the suggestions of Agathokleous et al. (2016) and found that the relationship between injured leaves and pod mass was significant (Table 4). Further, as previously discussed, S156 pod mass showed a better correlation than foliar injury with nighttime ozone concentration (Figures 4 and 6, respectively), again conflicting Agathokleous et al. (2016). In this study, graphical interpretations of data suggest that injury is a strong predictor of yield (Figure 7).

In summation, there is a linear relationship between nighttime ozone exposure and both injury and yield for the S156 and R123 snap bean genotypes between 8 and 265 ppb. However, ozone concentrations as high as those used in this experiment would not be observed in natural conditions. Natural, ambient surface ozone concentrations typically range from 31 to 50 ppb, with polluted areas and high temperatures pushing the higher end of that range (Feng and Kobayashi, 2009). It is projected that, by 2050, typical ambient ozone levels could reach 51 to 75 ppb (Feng and Kobayashi, 2009). Conclusions drawn from this study may be used in conjunction with future and existing air quality studies to better understand agricultural plant yield and plant pollution stress response. Subsequent studies should research higher day ozone levels that simulate pollution episodes in combination with nighttime treatments to determine the additive effects of nighttime ozone exposure.

# Appendix A

## Supplemental Material

Daytime summary of actual ozone levels, relative humidity, temperature, and PAR.

| | CHAMBER | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Ambient |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **OZONE** | **target (ppb)** | 200 | 150 | 100 | 175 | 225 | 125 | ZERO | 75 | 50 | 50 | 50 | 50 | ZERO | 50 | 250 | 275* | |
| | **mean** | | | | | | | | | 44 | 43 | 45 | 46 | 9 | 44 | | | 11 |
| | **sd** | | | | | | | | | 8 | 8 | 8 | 9 | 4 | 9 | | | 4 |
| | **max** | | | | | | | | | 63 | 59 | 61 | 65 | 27 | 71 | | | 35 |
| **RELATIVE** | **mean** | no data | 46 | 44 | 46 | 49 | 48 | 49 | 52 | 64 | 61 | 63 | 62 | 68 | no data | 60 | 58 | 52 |
| **HUMIDITY (%)** | **sd** | | 11 | 11 | 11 | 11 | 11 | 11 | 10 | 9 | 8 | 9 | 9 | 9 | | 9 | 9 | 8 |
| | **max** | | 82 | 80 | 82 | 83 | 84 | 85 | 85 | 88 | 86 | 87 | 88 | 92 | | 89 | 89 | 77 |
| | **min** | | 28 | 26 | 29 | 29 | 29 | 30 | 34 | 44 | 42 | 43 | 41 | 45 | | 40 | 38 | 35 |
| **TEMP (F)** | **mean** | 90 | 91 | 90 | 89 | 88 | 89 | 89 | 87 | 84 | 85 | 83 | 84 | 83 | no data | 84 | 84 | 86 |
| | **sd** | 9 | 6 | 6 | 6 | 6 | 6 | 5 | 5 | 3 | 4 | 4 | 4 | 4 | | 4 | 4 | 4 |
| | **max** | 103 | 104 | 104 | 101 | 101 | 102 | 100 | 99 | 95 | 97 | 95 | 97 | 95 | | 97 | 97 | 97 |
| | **min** | 0 | 73 | 74 | 73 | 73 | 73 | 73 | 72 | 73 | 73 | 72 | 72 | 71 | | 72 | 72 | 75 |
| **PAR** | **mean** | | | | | | | | | 328 | 444 | 393 | 409 | 309 | | | | |
| | **sd** | | | | | | | | | 225 | 314 | 260 | 334 | 204 | | | | |
| | **max** | | | | | | | | | 1410 | 1590 | 1481 | 1672 | 1259 | | | | |
| | **min** | | | | | | | | | 12 | 53 | 32 | 28 | 24 | | | | |

Nighttime summary of actual ozone levels, temperature, relative humidity, and PAR.

| | CHAMBER | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Ambient |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **OZONE** | **target (ppb)** | 200 | 150 | 100 | 175 | 225 | 125 | ZERO | 75 | 50 | 50 | 50 | 50 | ZERO | 50 | 250 | 275* | |
| | **mean** | 204 | 155 | 106 | 172 | 223 | 125 | 10 | 79 | | | | | 8 | 47 | 244 | 265 | 8 |
| | **sd** | 13 | 10 | 8 | 10 | 13 | 8 | 5 | 8 | | | | | 4 | 5 | 17 | 17 | 4 |
| | **max** | 236 | 176 | 147 | 198 | 299 | 145 | 24 | 105 | | | | | 23 | 60 | 305 | 295 | 29 |
| **RELATIVE** | **mean** | no data | 78 | 77 | 78 | 80 | 80 | 80 | 80 | 79 | 78 | 80 | 80 | 83 | no data | 85 | 85 | 75 |
| **HUMIDITY (%)** | **sd** | | 8 | 9 | 9 | 9 | 9 | 9 | 8 | 7 | 7 | 7 | 8 | 7 | | 7 | 7 | 7 |
| | **max** | | 91 | 91 | 92 | 93 | 94 | 94 | 93 | 90 | 90 | 91 | 91 | 95 | | 95 | 96 | 87 |
| | **min** | | 51 | 52 | 52 | 53 | 54 | 54 | 57 | 57 | 58 | 59 | 58 | 63 | | 67 | 65 | 53 |
| **TEMP (F)** | **mean** | 73 | 72 | 72 | 71 | 71 | 71 | 71 | 71 | 72 | 72 | 71 | 71 | 71 | no data | 71 | 71 | 73 |
| | **sd** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 3 | | 2 | 2 | 3 |
| | **max** | 84 | 83 | 83 | 82 | 81 | 81 | 81 | 80 | 82 | 82 | 80 | 80 | 80 | | 79 | 79 | 82 |
| | **min** | 64 | 63 | 63 | 63 | 63 | 63 | 63 | 63 | 64 | 64 | 64 | 64 | 64 | | 64 | 64 | 65 |
| **PAR** | **mean** | | | 6 | | | | 7 | | 4 | 6 | 6 | 5 | 4 | | 3 | | |
| | **sd** | | | 16 | | | | 18 | | 13 | 19 | 20 | 18 | 12 | | 11 | | |
| | **max** | | | 166 | | | | 175 | | 122 | 220 | 203 | 219 | 136 | | 136 | | |
| | **min** | | | 0 | | | | 1 | | 0 | 0 | 0 | 0 | 0 | | 0 | | |

# Appendix B

## Photographs

**Figure 9: Photograph taken at the end of treatment of a S156 plant exposed at 244 ppb (left) and a R123 plant exposed at 244 ppb (right).**


**Figure 10: Injury photographs taken on July 29, 2016 (17 DAT) of S156 (left) and R123 (right) plants treated with 100 ppb night ozone.**

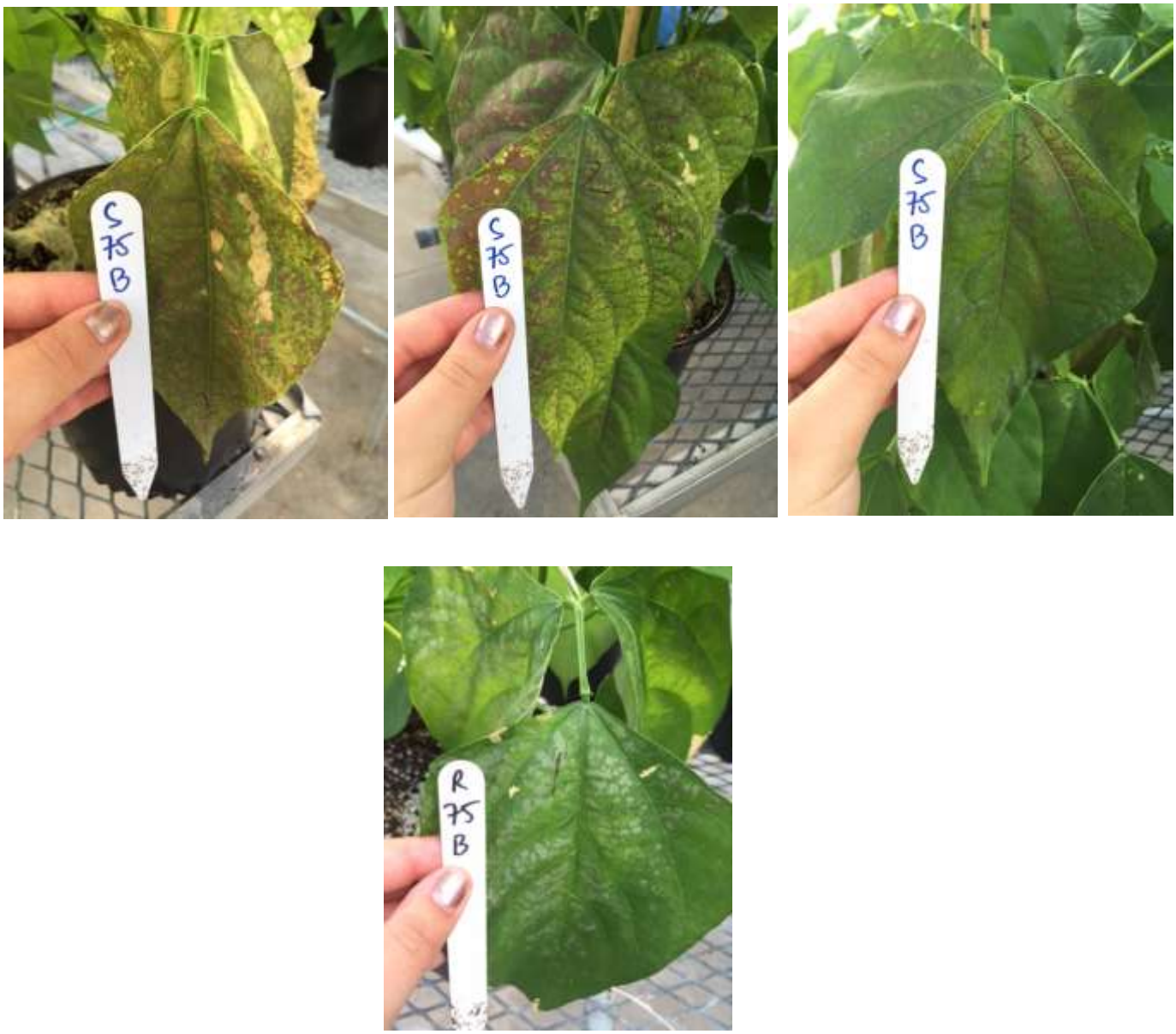


**Figure 11: Photographs taken on July 27, 2016 (10 DAT) of the magnitude of injury on S156 trifoliate leaves (above) vs. R123 trifoliate leaf (below) with a nightly exposure of 79 ppb.**

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# ACADEMIC VITA

# EMILY G. ISAACS

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**OBJECTIVE**

To learn more skills in order to educate new generations about the importance of agriculture and sustainability, utilizing my communication skills and environmental science background.

**EDUCATION**

- Pennsylvania State University, Schreyer Honors College — **State College, PA**
  B.S. in Environmental Resource Management — May 2017
- Maymester Trip — **Dublin, Ireland**
  *Walking in the Footsteps of the Irish during the Irish Potato Famine: Examinations of New World Crops in Old World Societies* — May 2016
- Baldwin High School — **Pittsburgh, PA**
  2009-2013

**EMPLOYMENT, INTERNSHIPS, RESEARCH AND TEACHING EXPERIENCE**

**Academic Programs Intern, The Sustainability Institute** — **State College, PA**
*Program coordinator, Research, Teaching* — March 2017-present

**Undergraduate Teaching Assistant, SOILS 102 (Introductory Soil Science Lab),** — **State College, PA**
*Lab Preparation, Teaching, Grading* — Fall 2016

**USDA-ARS, Pasture Systems and Watershed Management Research Unit,** — **State College, PA**
*Lab Technician/Water and Soil Sampling Processing* — May 2016-March 2017

**Schreyer Honors College Thesis Project,** — **State College, PA**
*Evaluating the Impact of Nighttime Ozone Fumigation on Resistant and Sensitive Phaseolus vulgaris L. genotypes.* — May 2016-Present

**Air Quality Center,** — **State College, PA**
*Intern/Undergraduate Research Assistant* — May-July, 2015

- Created QR code signage for plant identification and ozone symptoms
- Assisted with teaching Governor's School and Weather Camp students
- Facility management and upkeep

**Penn State Arboretum,** — **State College, PA**
*Intern for Public Garden Management* — May-August, 2014

- Learned about and worked with various plant fauna
- Facility management and upkeep
- Obtained basic mechanical skills (bobcat and mini-payloader)

**HONORS AND AWARDS**

| | |
|---|---|
| Dean's List | 2013-2017 |
| *Pittsburgh Tribune-Review* Outstanding Young Citizen | 2011-2013 |
| Walter McGough Memorial Award, Ligoneer Valley Writers' Poetry contest winner | 2013 |
| Susquehanna University Poetry contest winner | 2013 |
| Schreyer Honors College Academic Excellence Scholarship | 2013-2017 |
| Penn State Provost's Award | 2013-2015 |
| Dreibelbis Endowment for Excellence in Agriculture | 2015-2017 |
| J Adam Scholarship for Excellence in Agriculture | 2016-2017 |
| Highest Honors Baldwin High School | 2009-2013 |

**ACTIVITIES AND SERVICE**

| | |
|---|---|
| THON Hospitality Committee member | 2014-2017 |
| Secretary, Schreyer Honors College Student Council | 2014-2016 |
| THON dancer for Schreyer Honors College Student Council | 2015 |
| Co-Founder, Vice-President, and Public Relations Chair, Anti-Hunger Games | 2013-2016 |
| STOP HUNGER NOW organizer and volunteer | 2016 |
| SHO TIME (Schreyer Honors Orientation) mentor | August 2014, 2015, 2016 |
| Relay for Life participant | 2012-2015 |
| Senior care home visitor with Music Service Club | 2014 |
| Piano | |

**SKILLS**

Proficient in Microsoft programs, such as Word, Excel, and PowerPoint

*References available upon request*