

THE PENNSYLVANIA STATE UNIVERSITY
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DEPARTMENT OF ENVIRONMENTAL RESOURCE MANAGEMENT

EFFECTS OF NIGHTTIME OZONE EXPOSURE ON SENSITIVE (S156) AND RESISTANT
(R123) SNAP BEAN (*PHASEOLUS VULGARIS L.*) GENOTYPES

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ABSTRACT

Two genotypes of snap bean (*Phaseolus vulgaris* L.), ozone-sensitive S156 and ozone-resistant R123, were tested in order to determine damage from ambient and elevated nighttime ozone exposure. This experiment targeted 0 ppb and 150 ppb exposure levels over the course of 27 nights. Ozone damage was measured by three parameters: foliar injury using visual injury estimates and SPAD values, stomatal conductance rates, and yield measurements using pod number and mass. Mean visual injury estimates were higher for S156 than R123 at elevated ozone. SPAD values did not prove themselves a reliable tool for measuring injury. Stomatal conductance rates were significantly impacted by age at all timeframes, and younger leaves had higher conductance rates. Neither ozone, genotype, nor the interaction between ozone and genotype were significant factors for mean pod number, but all three parameters were significant factors for mean pod mass.

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Chapter 1

Introduction

Ozone Background

Ozone is a harmful pollutant that induces damage in humans and in plants ("Ground-level Ozone Basics", 2018). Ozone exposure can reduce a plant's capacity to perform photosynthesis, slow its growth, and increase its risk of insect damage, disease, weather damage, and damage from other pollutants (US EPA, 2017). Ozone is regulated by the EPA through National Ambient Air Quality Standards, or NAAQS (US EPA, 2013). While studies have been conducted regarding daytime effects of ozone on plants, less information is known on exposure-response relationships for nighttime exposure (US EPA, 2013). Since many species of plants exhibit nocturnal stomatal conductance, plants have the potential to absorb ozone during the nighttime hours (Caird et al., 2007).

Ozone (O_3) is a secondary pollutant formed at ground-level through photochemical reactions involving primary pollutants such as oxides of nitrogen (NO_x) and volatile organic compounds (VOCs). These primary pollutants are largely emitted by chemical solvents, industrial facilities, electric utilities, and gasoline vapors ("Ground-level Ozone Basics", 2018). The precursors to ozone are often associated with urban environments, where ozone levels are generally the highest. Wind can facilitate transportation of ozone, leading pollutants into rural areas ("Ground-level Ozone Basics," 2018).

Ozone is formed when sunlight breaks NO_2 into $\text{NO} + \text{O}$. The single oxygen molecule reacts with ambient O_2 to form ozone, O_3 . VOCs allow NO_2 to regenerate without breaking O_3 back into O and O_2 , propagating the elevation of ozone levels (Przyborski, 2003). NO scavenging, the reverse process of ozone formation, reduces ozone concentrations. However, in rural areas, nitrogen oxides are in smaller supply due to reduced industry and vehicular traffic, limiting ozone destruction overnight (US EPA, 2013).

The extent of injury observed from nighttime ozone exposure is relevant to policy makers and agriculturalists. High ozone levels cause significant reductions in crop yields (Ainsworth, 2017). According to Allen (1989), combined annual yield of corn, soybean, cotton, and winter wheat decreases by 5%, 10%, or 16% where ozone concentrations are respectively 40, 50, and 60 ppb as a seasonal 7-hour per day average. Moreover, it has been shown that plants can incur greater ozone injury at night than during the day, due to reduced root/shoot ratio from altered carbon allocation (Matyssek et al., 1995). Despite this, US NAAQS do not consider ozone flux into a plant or its biological defense mechanisms, and European legislation does not consider nighttime ozone exposure (Musselman and Minnick, 2000). Official EPA correspondence via the Clean Air Scientific Advisory Commission (CASAC) recommended that ozone be studied by the EPA to produce data to be reviewed for NAAQS standards (Frey, 2014). Beyond the loss of food quantity, CASAC stated that the visible injury on crops due to ozone damage decreases the economic value of these crops (Frey, 2014). Establishing a better-defined link between nocturnal ozone exposure and plant injury is necessary fully to understand the effects of ozone on plant health and to inform policy.

A common method for measuring the effects of environmental pollutants such as ozone is the use of bioindicators. Plant stress response can be measured using a pair of genotypes of a

given plant: both must exhibit similar growth under low ozone exposure, but one must show greater inhibition at a high level of exposure (Burkey et al., 2012). The first implementation of this system was reported in 1957, using tobacco (*Nicotiana tabacum* L.); (Heggestad, 1991). A more recent common system was the use of two clover genotypes (*Trifolium repens* L.) (ozone-sensitive NC-S and ozone resistant NC-R). While this system has been shown as a good measure of response to ozone exposure during growth periods, it presents practicality issues as it relies on vegetative propagation rather than seed propagation (Burkey et al., 2012).

Burkey et al. (2005) developed a newer pairing of bioindicators using snap bean (*Phaseolus vulgaris* L.) genotypes. In an experiment that ran from 2000 to 2001, they compared the ozone-sensitive Oregon-91 line of snap beans with its even more sensitive offspring, S156, and an ozone-resistant line, R123 (Burkey et al., 2005). The results of this experiment suggested that the pairing of the S156 and R123 genotypes made a viable system for detecting ambient ozone (Burkey et al., 2005).

Objective

The purpose of this experiment was to study the effect of nighttime ozone treatments on two genotypes of snap bean plants (*Phaseolus vulgaris* L.): S156 (ozone-sensitive) and R123 (ozone-resistant). The effects were measured by three parameters. Foliar injury was examined through visual injury estimates and SPAD values for chlorophyll density. Stomal conductance was measured with a porometer. Yield was measured through pod mass and number.

Chapter 2

Methods and Materials

Location and Equipment

This experiment was conducted in the greenhouse attached to the Forest Resources Lab at Penn State University in State College, Pennsylvania. The plants were housed in sixteen continuously stirred tank reactors (CSTR), built on a steel frame covered in a wrap of 76.2- μm transparent polytetrafluoroethylene (Teflon; Chemours, Wilmington, DE) film (Lloyd et al., 2018). Each cylindrical reactor had a height and diameter of 1.5 meters. A blower system pulled air into the chamber and then released it outside the greenhouse, at a rate that completely exchanged air in each chamber about once per minute (Lloyd et al., 2018).

Each CSTR was equipped with a 1000-watt Lumalux lamp, mounted over the top. Ozone was distributed to each CSTR through Telfon tubing and produced by an Ozone Solutions Z-08 ozone generator (Hull, IA), using electric current to drive the reaction. Software from REAL Controls Inc. recorded ozone levels every ten minutes, along with measurements for humidity and temperature via Omega Engineering HX93BC sensors (Stamford, CA) placed in each CSTR. This software used LI-COR LI-190R sensors (Lincoln, NE) at canopy height to record photosynthetically active radiation (PAR). Data were collected continuously in the ambient greenhouse air for environmental conditions including ozone, relative humidity, and temperature.

Plant Material

Three seeds of each snap bean genotype were planted on March 27, 2018, in sanitized 3.8-liter pots, containing 15 grams of Osmocote Plus 15:9:12 (Scotts Company LLC, Oxford, PA), added to Sunshine Mix #4 (Sun Gro Horticulture, Agawam, Massachusetts). The composition of Sunshine Mix #4 included 63-73% perlite, peat moss, and dolomitic limestone. Each set of three seeds was thinned to one plant after expansion of the first trifoliolate leaf expanded. Plants were tied loosely to a bamboo rod embedded in the soil for support and watered as needed with distilled water. Two days prior to the onset of ozone treatments, one plant of each genotype was placed in each CSTR.

Ozone Treatments

Ozone treatments began 27 days after seeding (DAS), on April 23rd. Half of the sixteen CSTRs were exposed to ambient greenhouse ozone levels, and the other half were treated with a target concentration of 150 ppb ozone. The plants flowered 41 DAS, on May 7th, and ozone treatments ended 59 DAS, on May 25th. Ozone exposure totaled 27 nights from 8 pm to 7 am, amounting to 297 hours of exposure.

Injury Measurements

On June 1st, 66 DAS, two methods were used to measure foliar injury on six trifoliolate leaves per plant. Each plant had more than six total leaves, but the oldest to youngest fully expanded leaves were studied and labeled: TF1, TF2, TF3, TF4, TF5, TF6, respectively. The

first method was a visual estimation of injury as percentage of affected leaf area across the trifoliolate leaf surface. The second method was to use a SPAD 502 Plus Chlorophyll Meter to measure leaf transmittance. SPAD measurements approximate chlorophyll density (Eguchi et al., 2006). Reported values represent the average of six measurements per trifoliolate leaf, with two measurements per leaflet.

Conductance Measurements

Stomatal conductance (gs) measurements were taken using an SC-1 Leaf Porometer. Values were recorded for abaxial and adaxial measurements on the center leaflet and then totaled for each leaf. Date, time, and leaves tested are recorded below (Table 1).

Table 1: Date, DAS, time, and leaves measured for conductance.

Date	DAS	Time	Leaf Measured
5/1	35	6:20pm-8:00pm	Second trifoliolate leaf
5/10	44	10:03pm-11:15pm	Youngest fully expanded leaf, A
5/22	56	5:21am-6:34am	Control plants only. Two leaves: A and B
5/22	56	7:40pm-10:50pm	Control plants only. Two leaves: A and B
5/23	57	11:32am-12:45pm	Control plants only. Two leaves: A and B
5/24	58	10:02pm-10:50pm	Control plants only. Two leaves: A and B

Yield Measurements

Pods were harvested on June 15th, 80 DAS. This included the collection of any pod with one or more seeds in it, which were placed in paper bags and then dried in an oven at 65.6°C.

The dry pods were then counted and weighed.

Data Analysis

Genotype and ozone treatments were applied using a split-plot, factorial structure and randomized complete block design, with eight blocks. Ozone was the whole plot factor with genotype as the split-plot. JMP Pro software (SAS Institute, Inc.) was used to run a mixed model analysis to determine the effects of genotype, ozone, and the interaction of genotype and ozone on response variables. Results were considered significant at $p = 0.05$.

Chapter 3

Results

Greenhouse Conditions

The mean daytime temperature was 24.4°C, with a minimum of 15°C and a max of 33.9°C. The mean nighttime temperature was 18.3°C, with a minimum of 13.9°C and a maximum of 25°C. Mean daytime relative humidity was 55%, ranging from 21% to 91%. Mean nighttime relative humidity was 67%, ranging from 35% to 92%. Mean daytime photosynthetically active radiation (PAR) was 409 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, ranging from 0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to 2096 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Mean nighttime PAR was 2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, ranging from 0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to 122 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. PAR were available for half of the chambers (N = 8).

Ozone Exposure Concentrations

Mean daytime ambient ozone concentration in the control chambers was 10 ppb, with a max of 32 ppb. Mean nighttime ambient ozone concentration in control chambers was 13 ppb, with a max of 40 ppb. Mean nighttime ozone concentration in treatment chambers was 145 ppb, with a max of 181 ppb. On the first day of ozone treatments, sunrise occurred at 6:20 am, and sunset occurred at 7:59 pm (Time and Date AS April, 2019). On the last day of ozone treatments, sunrise occurred at 5:46 am and sunset occurred at 8:31 pm (Time and Date AS May, 2019).

Foliar Injury

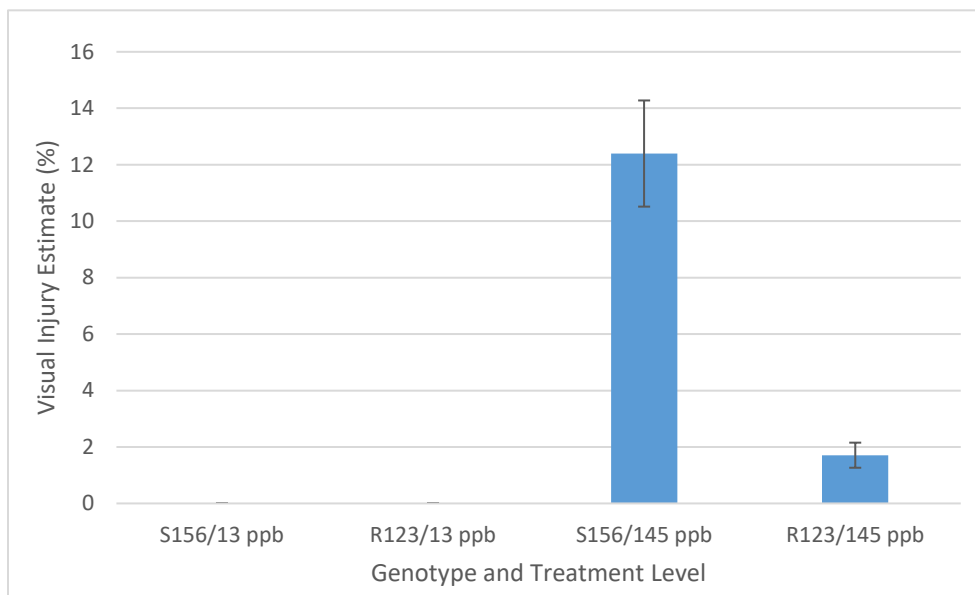


Figure 1: Mean visual injury estimates for sensitive (S156) and resistant (R123) genotypes at exposure to ambient and elevated ozone levels, averaged across six leaves. Error bars represent standard error.

Visually estimated as percent leaf area, all leaves on both genotypes of the control treatment exhibited 0% injury. At elevated ozone treatment, S156 showed higher injury levels, with a mean of 12%, ranging from 0% to 55%. R123 showed injury values with a mean of 2%, ranging from 0% to 15% (Figure 1).

Table 2: p-values for visual injury estimates for each trifoliolate leaf, including effects of ozone, genotype, and their interaction. Significant values are highlighted.

	Dfden	TF1	TF2	TF3	TF4	TF5	TF6
O3	7	0.051	0.075	0.040	0.016	< 0.001	0.002
Gn	14	0.050	0.058	0.033	0.010	< 0.001	0.002
O3 x Gn	14	0.050	0.058	0.033	0.010	< 0.001	0.002

Ozone had a significant effect on injured leaf area for leaves TF3, TF4, TF5, and TF6. Genotype and the interaction between ozone and genotype were significant for leaves TF1, TF3, TF4, TF5, and TF6 (Table 2).

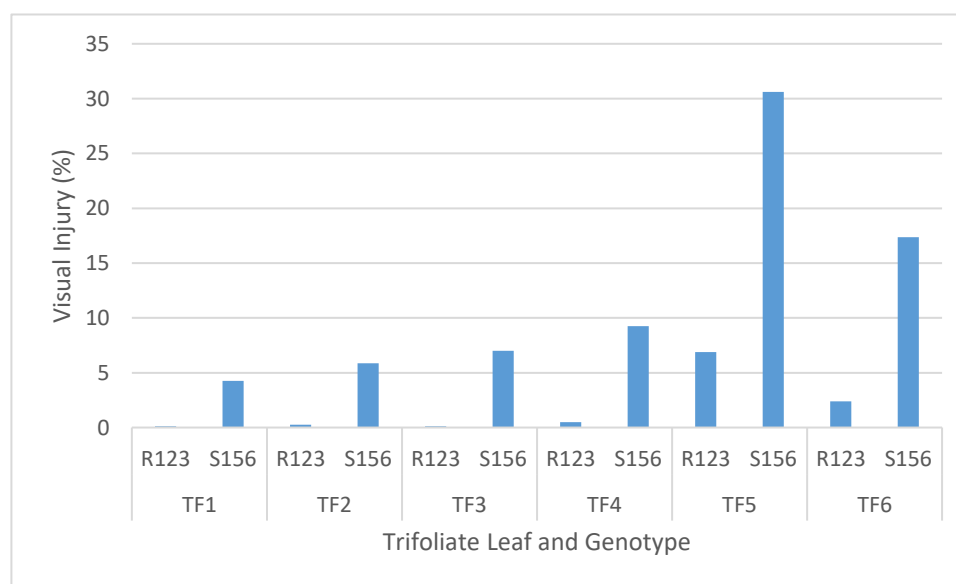


Figure 2: Mean visual injury percentage for each trifoliolate leaf, at elevated ozone treatment.

At elevated ozone treatment, mean visual injury percentage was higher for S156 in every trifoliolate leaf measured (Figure 2).

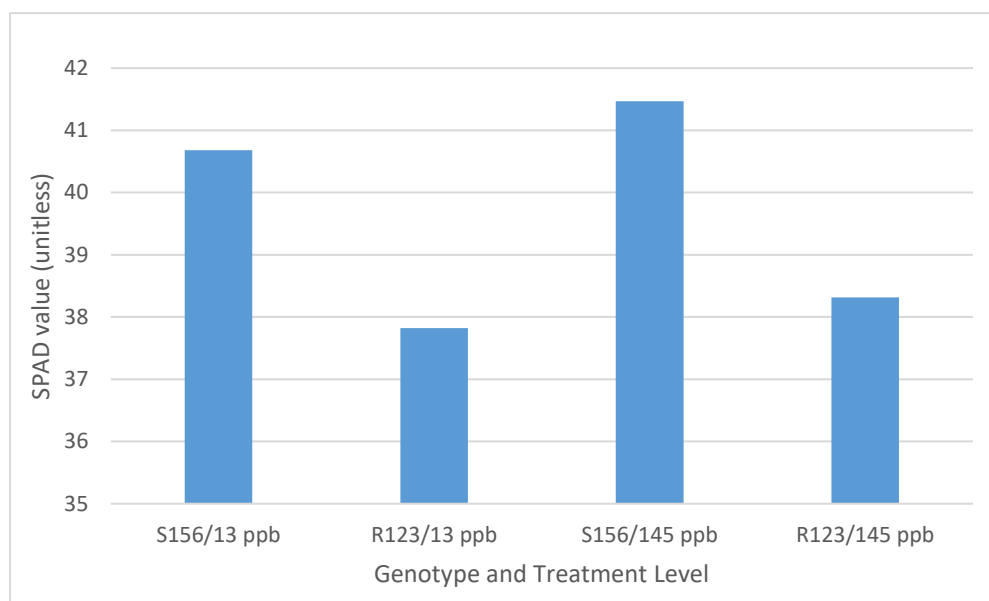


Figure 3: Mean SPAD values for sensitive (S156) and resistant (R123) genotypes at ambient and elevated ozone levels, averaged across six leaves.

Mean SPAD value for S156 under ambient ozone was 40.7, and 41.5 under elevated ozone. Mean value for R123 under ambient ozone was 37.8, and 38.3 under elevated ozone (Figure 3).

Table 3: p-values for SPAD data for each trifoliolate leaf. Significant values are highlighted.

	Dfden	TF1	TF2	TF3	TF4	TF5	TF6
O3	7	0.974	0.120	0.919	0.331	0.605	0.747
Gn	14	0.006	0.036	0.773	0.101	0.019	0.010
O3 x Gn	14	0.524	0.410	0.661	0.665	0.691	0.837

SPAD values among the six leaves were not significantly affected by ozone. There was a significant effect for genotype on the two oldest and two youngest leaves. However, their interaction was not significant (Table 3).

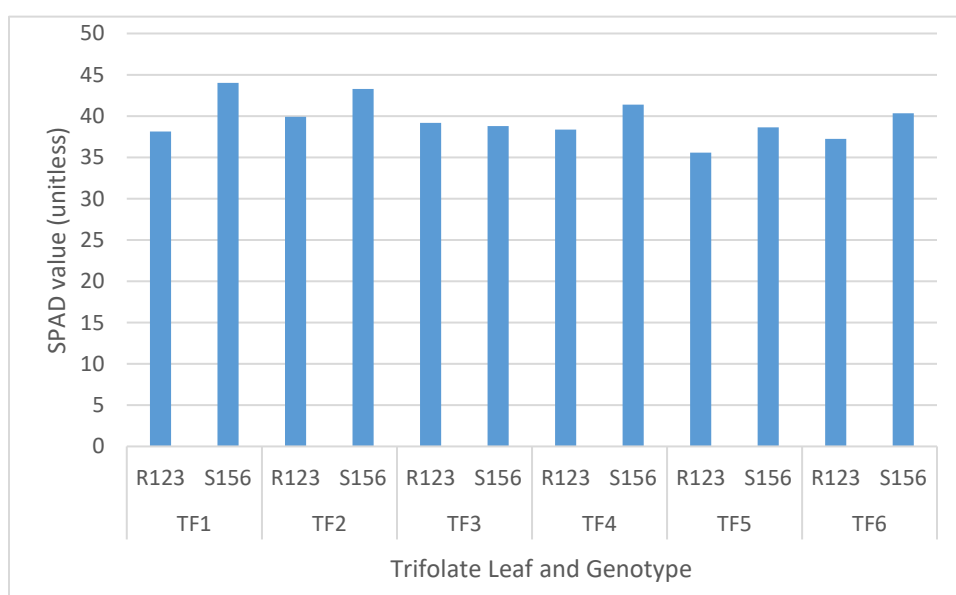


Figure 4: Mean SPAD value for each trifoliolate leaf, averaged across ozone levels for each genotype.

Mean SPAD value was significantly higher for S156 in leaves: TF1, TF2, TF5, and TF6, with no significant difference for TF3 and TF4 (Figure 4).

Table 4: Mean SPAD value for each trifoliolate leaf, averaged across ozone levels for each genotype.

TF1	R123	38.1
	S156	44.0
TF2	R123	39.9
	S156	43.3
TF3	R123	39.2
	S156	38.8
TF4	R123	38.4
	S156	41.4
TF5	R123	35.6
	S156	38.6
TF6	R123	37.3
	S156	40.3

Averaged across ozone treatments, the highest mean SPAD value for R123 was in leaf TF2, at 39.9. The lowest mean SPAD value for R123 was in leaf TF5, at 35.5. The highest mean SPAD value for S156 was in leaf TF1, at 44.0. The lowest mean SPAD value for S156 was in leaf TF5, at 38.6 (Table 4).

Conductance Levels

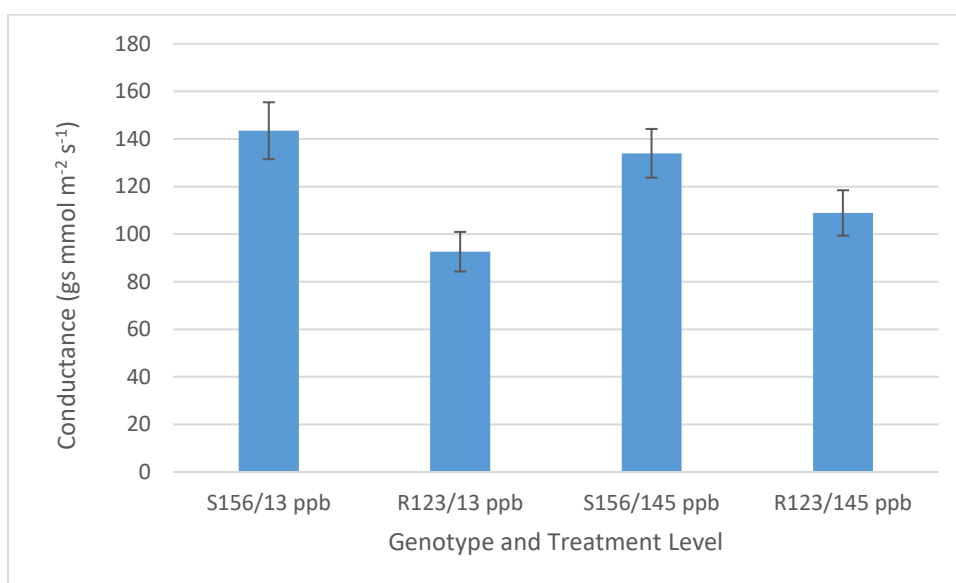


Figure 5: Mean nighttime conductance levels for each genotype and treatment level, averaged for values taken on 05/01/18 (18:20-20:00) and 05/10/18 (22:03-23:15). Error bars represent standard error.

The mean nighttime stomatal conductance value for S156 at ambient ozone was 143.5 mmol m⁻² s⁻¹, and at elevated ozone treatment it was 134.0 mmol m⁻² s⁻¹. At ambient ozone treatment, mean conductance value for R123 was 92.6 mmol m⁻² s⁻¹, and at elevated ozone treatment it was 108.9 mmol m⁻² s⁻¹ (Figure 5).

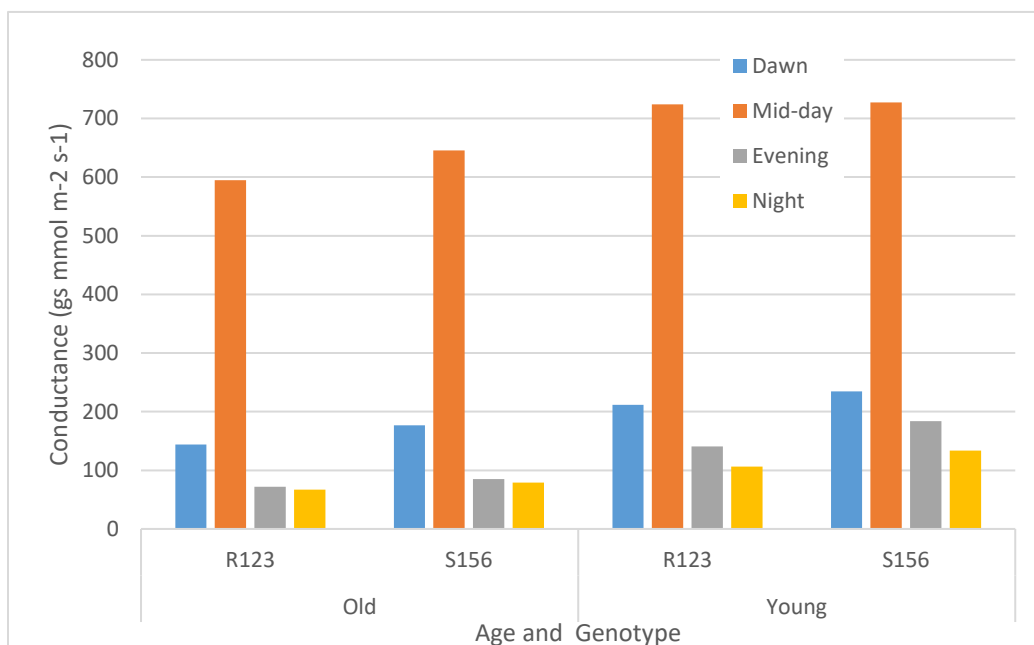


Figure 6: Mean conductance levels at dawn, mid-day, evening, and night for old and young trifoliolate leaf for resistant (R123) and sensitive (S156) genotypes at ambient ozone.

Figure 6 shows how average conductance changes throughout the day as given for an old and a young trifoliolate leaf on each plant. Dawn measurements were taken from 5:21 to 6:34 am., mid-day measurements were taken from 11:32 am to 12:45 pm., evening measurements were taken from 7:40 to 8:50 pm., and night measurements were taken from 9:55 to 10:50 pm.

Figure 6 shows that the highest levels occurred mid-day, with a sharp drop in the evening and night. Conductance rates increased at dawn. The younger leaves of both genotypes had higher conductance levels than the old leaves at every timeframe.

Table 5: p-values for conductance rates, including effects of genotype, age, and interactions between the two. Significant values are highlighted.

	Dawn	Mid-day	Evening	Night
Gn	0.0028	0.2461	0.0313	0.1235
Age	0.0003	0.0042	0.0002	0.0001
Age x Gn	0.7214	0.4509	0.4006	0.356

Genotype had a significant effect on conductance rates at dawn and in the evening. The effect of leaf age was significant at all four measurement times. The interaction between leaf age and genotype was not significant (Table 5).

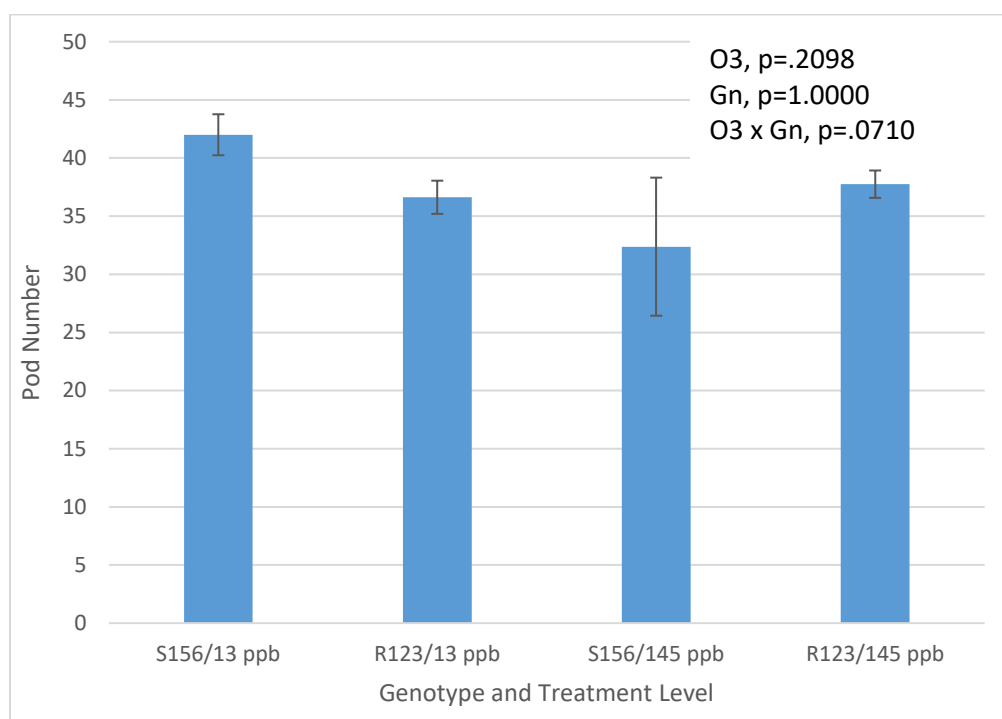
Yield

Figure 7: Mean number of pods harvested from sensitive (S156) and resistant (R123) genotypes at ambient and elevated ozone levels. Error bars represent standard error.

For S156, mean pod number was 42.0 pods under ambient conditions and 32.4 pods under ozone treatment. R123 mean pod number was 32.6 under ambient conditions and 37.8 under ozone treatment. Neither genotype, ozone, nor their interaction was statistically significant (Figure 7).

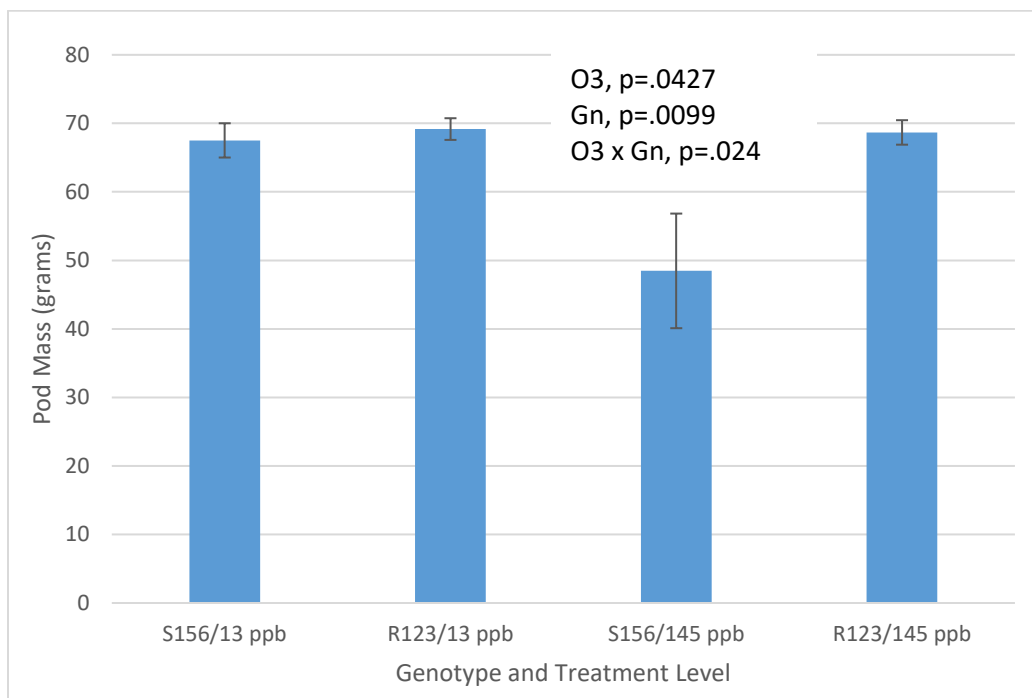


Figure 8: Mean mass of pods harvested from sensitive (S156) and resistant (R123) genotypes at exposure to 0 ppb ozone or 150 ppb ozone. Error bars represent standard error.

For S156, mean pod mass decreased from 67.51 grams under ambient conditions to 48.47 grams under ozone treatment. R123 mean pod mass decreased from 69.17 grams under ambient conditions and 68.68 grams under ozone treatment. Genotype, ozone, and their interaction were statistically significant (Figure 8).

Chapter 4

Discussion

Mean SPAD values in this experiment were shown to be higher in the S156 genotype than R123 (Figure 3). Agathokleous et al. (2016) also reported the SPAD value for S156 leaves (38.97 ± 0.56) as higher than R123 (31.30 ± 0.65). However, a separate study stated that SPAD values for foliar injury have compromised reliability for plants exhibiting moderate to severe ozone injury (Neufeld et al., 2006). It is not fully understood why SPAD readings are unreliable in the presence of ozone stippling (Neufeld et al., 2006). This suggests that SPAD data are not adequately sensitive to describe ozone-induced foliar injury. In this experiment, mean estimated visible injury was rated higher for S156 than R123 for every leaf measured at elevated ozone, whereas mean visible injury for both genotypes was zero at ambient ozone levels (Figure 2; Figure 1). These data may signify that visible injury more accurately depicts the differing sensitivities between R123 and S156; however, estimates are subjective and may be influenced by human bias.

Nighttime conductance rates for this experiment showed higher mean values for S156 than R123 at ambient and elevated ozone levels (Figure 5). This agrees with data from Salvatori et al. (2013), which present the same conclusion. However, in this experiment, mean nighttime conductance for S156 was lower at elevated ozone than at ambient ozone, and mean conductance for R123 was higher at elevated ozone than at ambient ozone (Figure 5). This contradicts the findings of Salvatori et al. (2013), whose results indicated higher conductance levels for both genotypes at elevated ozone levels.

Table 5 shows that age was a significant factor for conductance rates at all times, and Figure 6 shows that the younger leaves had higher conductance rates at all times of day. This may signify that the rate of ozone assimilation had an effect on visible injury observed; faster ozone assimilation from higher conductance rates in younger leaves may generate acute injury as opposed to chronic injury. Ainsworth (2017) likewise stated that crops can generate acute exposure injury at ozone levels above 100 ppb. Figure 6 shows a rise in conductance levels for S156/R123 at dawn, which may be an active time for injury to occur. Table 5 shows that genotype was statistically significant at dawn, and Figure 6 shows that young S156 leaves had higher mean conductance than R123 at this time. This may help explain why S156 had overall higher mean visible injury (Figure 1).

Final mean pod number (Figure 7) was not significantly affected by genotype, ozone, or their interaction. Final mean pod mass (Figure 8), however, was significantly affected by all three parameters. Figure 8 shows that the interaction for mean pod mass between ozone and genotype was statistically significant with a p-value of 0.024. This agrees with the results from a study by Burkey et al. (2012), which also found statistical significance in the interaction between ozone and genotype for the yield of S156 and R123 snap beans. Burkey et al. (2012) noted how this relationship demonstrates the differential responses to ozone treatment between S156 and R123. Figure 8 shows how the mean pod mass for S156 decreased at elevated ozone.

Nighttime ozone treatment had a statistically significant impact on average pod mass (Figure 8). Ozone exposure incites great losses in crop yields (Ainsworth, 2017). Mills et al. (2018) reported that, among global values, the United States experiences the highest levels of maize and soybean yield loss due to ozone damage, with 6.1% and 12.4% losses respectively on an annual basis. Worldwide, these and other crops like wheat and rice are also impacted (Mills

et al., 2018). Genotypic variation can have pronounced effect on the degree of yield reduction: especially in soybeans and rice (Mills et al., 2018). It has been estimated elsewhere that annual losses for maize and soybean in the United States are 10% and 5% respectively, amounting to \$9 billion in losses every year (McGrath et al., 2015). This information highlights how critical ozone regulation is. McGrath et al. (2015) state that United States air quality regulations have helped to minimize ozone-related crop losses, and that more stringent regulations would garner even greater benefit. It would also be beneficial to plant ozone-resistant varieties of crops to minimize yield losses. However, more research needs to be done to make ozone-resistant crops widely available (Ainsworth, 2017).

In conclusion, mean visual injury estimates were higher for S156 than R123 at elevated ozone (Figure 1). SPAD values did not prove themselves a reliable tool for measuring injury (Table 3). Stomatal conductance rates were significantly impacted by age at all timeframes (Figure 1), and younger leaves had higher conductance rates (Figure 6). Neither ozone, genotype, nor their interaction were significant factors for mean pod number (Figure 7), but all three parameters were significant factors for mean pod mass (Figure 8).

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ACADEMIC VITA

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Education

Pennsylvania State University

- Graduation Spring 2019
- Bachelor of Science in Environmental Resource Management
- Schreyer Honors Scholar
- Schuylkill Honors Scholar

Schuylkill Haven Area High School

- Graduation Spring 2015
- Salutatorian

Honors and Awards

Schreyer Honors College Academic Excellence Scholarship	2015-2019
College of Agricultural Sciences Undergraduate Research Award	2018
Dean's List	2015-2018
Provost's Award	2016
Chancellor's Award	2016
President's Freshman Award	2015
Eagle Scout, Boy Scouts of America	2013

Employment

Student Waste Handler, PSU Environmental Health and Safety March 2018-present

- Collect and transport regulated medical waste
- Test chemical fume hoods, eyewashes, and safety showers
- Record and arrange data
- Operate independently and on a team
- Account for effective time management of various projects

Office Supplies/Tech Sales and Services, Staples August 2015-February 2018

- Address customer service concerns
- Solve technical problems on an array of computers
- Manage inventory
- Make recommendations on products and services