Visualizing ozone pollution in the Denver Metro Area using a bioindicator garden

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Abstract

Since the industrial revolution, widespread fossil fuel combustion has significantly increased atmospheric pollution. The combustion of fossil fuels releases nitrogen oxides (NOx) and volatile organic compounds (VOCs), which form tropospheric (ground-level) ozone when exposed to sunlight. Ozone directly harms plants by disrupting their ability to absorb carbon dioxide, release oxygen, and carry out photosynthesis. Bioindicator species are plants that have visible responses to environmental conditions, such as ozone, and can act as "canaries in the coal mine," indicating the health of their environment. In this study, we used a garden of ozone bioindicator species (ozone garden) as a cost-effective tool for monitoring and visualizing the effects of anthropogenic ozone pollution. Our goal was to understand the damage tropospheric ozone has on plants in Denver using two varieties of beans: a common ozone-sensitive variety and a modified ozone-tolerant variety as a control. For a full growing season, we monitored the temperature, ozone concentration, and leaves for ozone damage. **We found that the ozone-sensitive cultivar exhibited significantly more foliar injury compared to the tolerant cultivar ($p<0.05$) in response to tropospheric ozone.** We also found that ozone-sensitive snap beans experience a latency period in their response to foliar injury. All foliar injury data were added to a nationwide database of ozone gardens to contribute to understanding the relationship between ozone concentration and damage severity across different locations.

Keywords: Ozone garden; ozone pollution; bioindicator species; ozone garden; snap beans

1 INTRODUCTION

Since the industrial revolution, widespread fossil fuel combustion has significantly increased atmospheric pollution[1]. Emissions from these combustions include nitrogen oxides (NOx) and volatile organic compounds (VOCs), which form tropospheric (ground-level) ozone when exposed to sunlight[2]. Although stratospheric and tropospheric ozone are chemically identical, stratospheric ozone shields earth from harmful UV rays while tropospheric ozone is a dangerous air pollutant (hereafter, ozone pollution refers to tropospheric ozone). High ozone concentrations are prevalent in urban areas due to vehicle emissions and industrial activity[3].Even moderate ozone levels can have consequences on animals, plants and ecosystems, that include worsening respiratory conditions, inhibited plant growth, and reduced biodiversity and carbon sequestration[4].

Ozone directly harms plants by disrupting the plant's ability to absorb carbon dioxide, release oxygen, and carry out photosynthesis[2]. These processes are essential for plant growth and the presence of ozone greatly reduces agricultural productivity[5]. The U.S. loses $500 million in crop production annually due to ozone pollution[6]. Ozone enters plants through tiny pores called stomata, which are crucial for photosynthesis as they control the rate of intaking carbon dioxide and releasing oxygen. Once inside the plant, ozone damages the leaf cells that produce the sugars necessary for plant growth, leading to slowed growth, and weakened defense mechanisms, increasing plants' susceptibility to disease, bug damage, and damage from extreme weather[7]. Due to the cumulative impact of ozone damage on the cellular and structural components necessary for photosynthesis, the damage is often irreversible under prolonged exposure[4]. Given the direct impact on plant health, active measures are needed in high-risk areas such as the Denver metro area, which frequently surpasses ozone pollution safety thresholds.

While the Environmental Protection Agency's (EPA) 70 PPB ozone standard protects human health, under the National Ambient Air Quality Standards (NAAQS), plants are vulnerable at levels as low as 40 PPB[4]. Days above 70 PPB trigger Ozone Action Alert Days, where open burning is prohibited and sensitive groups are advised to limit outdoor activity. The EPA monitors state's ozone concentrations to determine their attainment status with the NAAQS as either "attainment" (below standard) or "nonattainment" (exceeds standard) which is organized by severity as marginal, moderate, serious, severe, and extreme[8]. Denver's sunny conditions and high traffic volume make it particularly susceptible to elevated ozone levels, and as of 2022, the city was reclassified as a "severe" nonattainment area[9]. In the 2023 summer growing season (June to October), the Denver metro Area issued 35 Ozone Action Alert Days and nearly 95% of the season had ozone concentrations above 40 ppb. This reveals the necessity to monitor ozone concentrations, raise awareness about the damaging effects of ozone in the Front Range, and increase public education on how to avoid contributing to the problem.

Ozone gardens are a useful and cost-effective tool for monitoring and visualizing the effects of anthropogenic pollution using bioindicator species (plants that have visible responses to environmental conditions). Plants differ in their sensitivity to ozone pollution, as their susceptibility to damage is influenced by stomatal uptake and antioxidant defenses[2]. As a result, certain species consistently exhibit ozone damage and are used as bioindicators. Bioindicator species act as "canaries in the coal mine" as they can indicate the health of their environment[10]. The USDA's Agricultural Research Service developed snap bean (Phaseolus vulgaris) bioindicators for the purpose of detecting ozone pollution[11]. Through many controlled fumigation trials, sensitive (S-156) and resistant (R-331) varieties were produced. Other ozone bioindicators include common milkweed (Asclepias syriaca), cutleaf coneflower (Rudbeckia laciniata), and le chipper potato (Solanum tuberosum). The effects of ozone pollution are clear on the sensitive snap beans in the forms of chlorosis (yellowing of leaves and stems), necrosis (cell death), and stippling. Stippling is solely a symptom of high ozone exposure and manifests as dark spotting on the leaves, caused by the plant's defensive production of anthocyanin pigments[2]. Ultimately, using bioindicator species to monitor high-risk areas is crucial for understanding how geography affects plants' response to ozone stress.

In this study, we test the hypothesis that elevated tropospheric ozone levels in the Denver metro area cause significantly greater foliar damage in ozone-sensitive snap beans compared to ozone tolerant cultivars, demonstrating the value of ozone gardens as tools for environmental monitoring and public education. While the ozone garden is located at the University of Denver, the data was used to extrapolate to the broader Denver metro area. This was due to the distance of the temperature and ozone concentration monitoring stations, and the lack of other established ozone gardens with available data around Denver at the time of the study. In collaboration with faculty from the Departments of Geography and the Environment and Biology, we developed an ozone monitoring garden for the University of Denver campus. We established perennial species for long-term monitoring of ozone and we investigated the impacts of ozone pollution on snap beans by comparing a sensitive (S156) and a tolerant cultivar (R123). We added the plant monitoring data to the National Ozone Garden Network to contribute to the broader understanding of how ozone pollution varies across geographic regions. Here, we present the results of leaf damage and productivity for a single growing season, combined with monitoring of daily temperature and ozone. We found heightened damage to the sensitive cultivar when compared to the tolerant cultivar. We discuss how visible foliar damage helps contextualize the impact of human activities and provides an accessible method for both measuring ozone levels and raising public awareness. The establishment of the ozone garden has allowed for the creation of a yearly Ozone Pollution Lab, where Environmental Science students at the University assess damage to the species. Through studying these plants, we gain important insights into the pervasive threat of ozone pollution, which can help spread awareness about the issue.

2 METHODS

2.1 Creation of the DU Ozone Garden

The ozone garden has two goals: annual monitoring of snap beans and long term monitoring of bioindicator perennials such as coneflower and milkweed. In spring 2023, we obtained tolerant (R123) and sensitive (S156) cultivars of snap beans (Phaseolus vulgaris) from the Ozone Garden Network, based at the University Corporation for Atmospheric Research in Boulder, Colorado. We started the season with 42 seedlings in total, using 20 tolerant cultivars as the control group and 22 sensitive cultivars as the experimental group. We planted the seeds the first week of May in the

University of Denver's (DU) green room, located on the top floor of Olin Hall. We began hardening the plants in the last two weeks of May to prepare them for transplanting outside. This process involved taking the seedlings to the ozone garden (located behind DU's community garden as seen in Figure 1.) daily for increasing periods of time. We started with one hour every other day, then increased the duration by 30 minutes on alternating days. After the two-week hardening period, we transplanted the beans into the ozone garden and labeled each plant with its cultivar and plant number. We hung a shade cloth over the beans intermittently throughout June to ease their transition into full sunlight. At the end of June, we marked a leaf from each plant's bottom, middle, and top layers to ensure consistent monitoring throughout the season. To promote optimal growth, we watered the garden daily for the first month, then reduced watering to every other day, and weeded weekly. Proper watering and care of the snap beans ensured reliable ozone damage data.

In the 2023 season, we also prepared milkweed seeds in the greenhouse, but they did not survive being transplanted into the garden. In the 2024 growing season, we transplanted 13 milkweed sprouts that we had started in the greenhouse and hardened to adjust to full sun. Additionally, we transplanted two established milkweed plants (both about 1 year old) from the University of Colorado Denver Ozone Garden. Using perennial milkweeds allows the ozone garden to be a long-term project. This is advantageous because the cumulative nature of ozone damage means that its effects on these plants will become more evident with each passing year[4]. We analyze only the 2023 season snap-bean data in the following sections.

2.2 Environmental Monitoring 2023

In order to provide a thorough environmental context for the ozone garden experiment, we collected daily data on temperature (°F), and ozone concentration (PPB). We placed a standard rain gauge on the fence behind the ozone garden and checked and emptied it daily to ensure the measurements were accurate. We used the National Weather Service's online data to obtain Denver's daily high temperatures, which are measured by the Denver Water Department located at 1600 W 12th Ave, Denver, CO 80204 (approximately 4.7 miles from the ozone garden). We acquired the daily high ozone concentration from the Colorado Department of Public Health and Environment's (CDPHE) Air Quality Summaries. We used the Highlands Monitoring site (located at 8100 S University Blvd, Centennial CO 80122, approximately 7.5 miles from the

Figure 1. Ozone Garden Location. This image (courtesy of Google Earth) shows the ozone gardens location on S Vine St between the DU Bridge Community Garden and DU's Boettcher West. The ozone garden is highlighted in a red box.

ozone garden) because it is in a mixed use area similar to the environment around DU. Additionally, we noted all ozone action alert days. This environmental data allowed us to monitor how variations in temperature impact ozone concentrations in the summer months.

2.3 Garden Monitoring 2023

Consistent and thorough data collection was essential for determining the effects of ozone damage, and was achieved through a structured monitoring process that included visual assessment and yield measurement. Before transplanting the seeds, we completed NCAR's ozone damage training to ensure that we could discern between ozone damage and other forms of plant damage. We randomly chose one leaf from the bottom, middle, and top of each plant to tag with a pipe cleaner to ensure consistent monitoring. From July 13th, 2023 until October 24th 2023, we monitored the garden every other day for ozone damage using NCAR's 1-6 leaf damage rating scale (Table 1.).

On each monitoring day, we observed two plants from each cultivar. To account for minor environmental variations, we selected the first and last plants of each cultivar's set for assessment (e.g., plants 1 and 20 on the first day, 2 and 19 on the second, and so on). We thoroughly observed the pipe cleaner-marked leaves from each layer for dark stippling, and then ranked their ozone damage from 1 (0% damage) to 6 (76-100% damage).

Each monitoring day, we entered this data into the Ozone Garden Network's "data entry" portal,

Table 1 Ozone Damage Classification. Classification of ozone damage on leaves based on the percentage of affected area. Damage severity is ranked from 1 (least damage) to 6 (most severe). The accompanying images were sourced from the NCAR Ozone Garden Data Entry portal.

Class	1	2	3	4	5	6
Damage %	0%	1-6%	7-25%	26-50%	51-75%	76-100%
Picture						

Figure 2. Ozone Damage on Sensitive Plant. Example of ozone damage on a bioindicator sensitive snap bean cultivar. This image was taken on October 8, 2023 of sensitive plant #8, and would be classified as level 6 damage. Characteristic symptoms include chlorosis (yellowing), particularly visible on the upper leaves, necrosis (drying) of the bottom leaves, and stippling (dark spots avoiding leaf veins) across all leaves.

contributing to their nationwide study of ozone pollution effects. Additionally, we counted all bean pods 1 inch and longer on the plants being monitored. We recorded the leaf damage and number of bean pods into a spreadsheet. After the bean pods reached maturity on the plant and dried, we harvested and stored them in separate paper bags for each plant. We collected bean pods from the plants until they died in late October. Then, we removed all beans (seeds) from their dry pods and placed them on paper towels to dry for two weeks, keeping them separated by plant. After this two-week period, we counted and weighed every plant's dried beans and calculated the average number produced and average weights for both varieties. This data provides insight into the physiological reactions of sensitive and tolerant snap bean cultivars under high ozone conditions in Denver, CO.

2.4 Data Analysis

We completed all statistical analysis in R v4.2.3[12] and RStudio v2024.12.1+563[12], and used the readxl (v#1.4.5) package to format our data. To test for a relationship between daily temperature and ozone concentrations, we used the function cor.test() to conduct a Pearson's correlation between the daily temperature and ozone concentrations. We used the function t.test to conduct a Welch Two Sample t-test (unequal variance t-test) to test for differences between sensitive and resistant bean cultivars in average ozone damage and reproductive output estimated by number of beans produced and bean mass.

3 RESULTS

As ozone concentrations peak on hot, sunny, and stagnant days, it was crucial to record the temperature daily (Figure 1). The data reveal a general trend of increasing temperatures from June to July, followed by a gradual decline through November. The substantial interquartile range in October highlights a period of significant temperature fluctuation.

Obtaining the daily ozone concentrations was also necessary to see how it varies depending on the temperature from June 2023 to October 2023. We confirmed that temperature and ozone concentration are highly correlated (Pearson's correlation $r = 0.6$, $p < 2.2e-16$). From June to October, ozone varied from

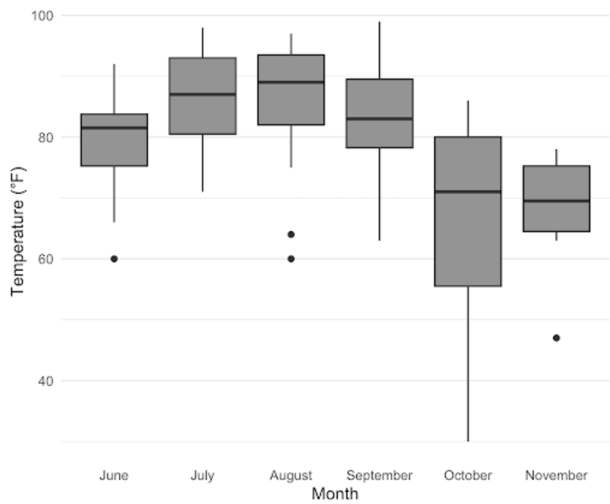

Figure 3. Time series of temperature (orange bars) in Denver, 2023. This data shows the distribution of daily temperatures from June to November.

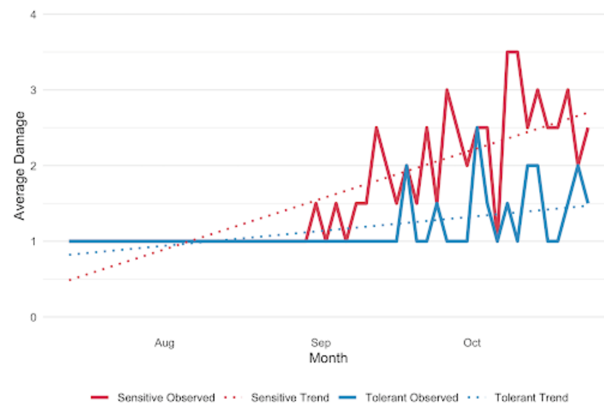

Figure 5. Average leaf damage over time in Denver (2023) for sensitive (red line) and tolerant (blue line) snap bean cultivars exposed to ozone. The dotted lines show the linear regression model for both treatments. The x-axis shows months starting in August due to no recorded damage prior to this, even though the data collection began on July 13th 2023. The y-axis represents the average damage rating, ranging from 1 (lowest) to 3.5 (highest observed).

10 PPB to 83 PPB (Figure 4). Within this time frame, 94.6% of days were above 40 PPB (when physiological damage begins), 11.8% of days were above 70 PPB (EPA standard), and there were 35 ozone action alert days issued by the Colorado Department of Public Health and Environment.

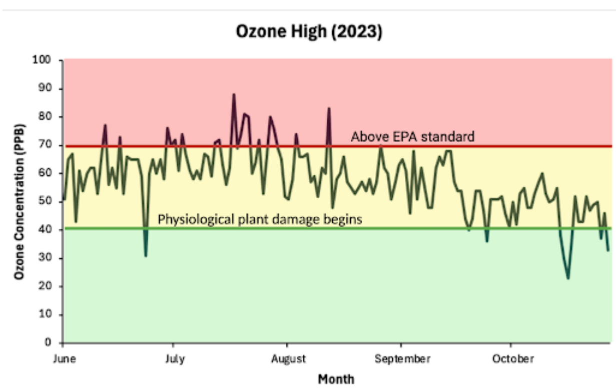

Figure 4. Time series of ozone high concentrations in Denver, 2023 growing season (June to October). The green horizontal line at 40 PPB indicates the threshold at which physiological plant damage typically begins. The red horizontal line at 70 PPB represents the National Ambient Air Quality Standards (NAAQS) set by the EPA for ground-level ozone; concentrations above this line exceed the standard.

We plotted both cultivars' average damage throughout the season (Figure 5), revealing that the sensitive cultivar reached higher levels of damage by the end of the season.

We plotted the overall range of damage for both cultivars to visualize how much more damage the sensitive cultivar experienced (Figure 6). We found a statistically significant difference in the two cultivars' response to tropospheric ozone [t.test, $p = 2.5e-6$]. The tolerant group had an average damage rating of 1.173 while the sensitive groups had an average damage rating of 1.678, revealing that the sensitive group experienced higher levels of ozone damage, with a mean difference of about 0.5 units. On average, the sensitive group produced about 12.5 beans per plant, and the tolerant group produced about 13.6 beans per plant and there was not a significant difference in bean yield ($p =0.69$). At the end of the season the sensitive group had an average bean weight of 10.8 g per plant, and the tolerant group had an average bean weight of 14.4 grams per plant, but there was not a significant difference ($p = 0.2606$).

4 DISCUSSION

Overall, this analysis confirmed that bioindicator snap beans (sensitive cultivar) experience significant physiological damage from ozone pollution in the Denver Metro Area. The highest ozone concentrations aligned with the hottest months of July and August. Interestingly, the most physiological damage was measured in the cooler months of September and October. Similar results were observed in Pringle's study of bioindicator coneflower in Philadelphia, Pennsylvania[4]. Despite year-round production, tropospheric ozone reaches the highest concentrations in the warmest months, resulting in cumulative damage seen most significantly in older leaves[4]. Research on bioindicator coneflowers has found a "latency period" characterized by a slow onset of foliar damage

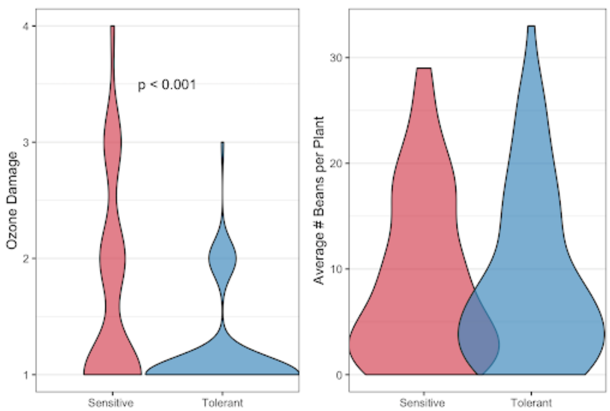

Figure 6. Distribution of average ozone damage ratings between cultivars and average number of beans per cultivar. Violin plots of the distribution of ozone damage ratings in the sensitive snap bean cultivar (red) and the tolerant cultivar (blue). Sensitive plants exhibited a wider range of damage, with more instances at higher damage levels, whereas the majority of damage in the tolerant group remained at the lowest level. A two-sided Welch's t-test indicated a significant difference in means ($t = 4.98$, $df = 129.8$, $p = 2.597 \times 10^{-6}$). There was not a significant difference in the average number of beans per plant between the two cultivars.

that rapidly intensifies later in the season, regardless of decreasing ozone concentrations[4]. Our study paralleled Pringle's observation that foliar damage begins slowly in the early summer months, and quickly accelerates around August[4]. While Pringles' study used a different bioindicator species in a different environment, our results support the idea of a latency period in the snap beans foliar injury response to ozone.

Although we obtained interesting results that add to the understanding of how ozone concentrations and plant responses vary with geographic region, there are two caveats to acknowledge. The first limitation on this study is that the temperature and ozone data used were obtained from monitoring stations located 5-7 miles from the University of Denver. These were the closest monitoring stations available that consistently uploaded verified data, but ideally, they would have been much closer to the garden to provide a more accurate representation of the plants' environments. Secondly, in September 2023, a second person took over monitoring. While we both took the same training, there is inevitably some subjectivity in defining the level of damage on the leaves.

This project supports nationwide research on tropospheric ozone, and has also become an outdoor classroom for DU students and community members. A core class sequence for environmental science majors "Environmental Systems" has begun using the garden in a lab to give students the experience of identifying damage and understanding the physical impacts of ozone pollution. This connects DU students to real-world nationwide datasets, as the garden is a part of the Ozone Garden Network operated by the National Center for Atmospheric Research (NCAR). The university corporation for atmospheric research obtained grant funding from NASA to support the expansion of ozone gardens across the US to better understand how geography and climate is affecting plant damage. NCAR's dataset from nationwide ozone gardens is being used to understand precise levels of when damage appears, how severe the damage gets, and how this damage varies across regions and climates.

Although our research successfully demonstrated ozone concentration effects on snap bean bioindicators in Denver, refinements would yield more accurate results. We would specifically recommend randomizing cultivar placement for a double-blind design, expanding the sample size, and implementing weekly monitoring of all plants. Additionally, focusing more on the aesthetic of the garden may help to engage community members. Recognizing the physical impacts of ozone pollution is a critical step to empower local communities to engage in discussions about environmental health and protection.

In the 2024 growing season, we focused more on the aesthetic appeal and educational draw of the garden. We installed three signs from the Ozone Garden Bioindicator Garden Network to explain why it is important to care about ozone, how the bioindicator garden works, and steps individuals can take to reduce their contribution to ozone pollution. We also planted 15 milkweed plants with the goal of long-term perennial monitoring, as they return every growing season. This ensures that even without planting new seeds, the garden will remain useful for visualizing ozone pollution. In preparation of the 2025 growing season, we have acquired seed potatoes and snap bean, milkweed, and coneflower seeds. It is our hope that a vibrant garden accompanied by informative signs will encourage people to think about how their actions are affecting the natural world around them.

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6 EDITOR'S NOTES

This article was peer-reviewed.

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