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Ozone Gardens: Impacts of Air Quality on Native Plants in Chicago

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Ozone Gardens: Impacts of Air Quality on Native Plants in Chicago

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Abstract:

Air quality impacts are hard for the public to understand where air pollution is not visible. Visual indications of plant damage like stipples can help residents in cities with understanding how poor air quality impacts both plants and humans. This is particularly important since the effects of climate change and air pollution are difficult to observe at small temporal and spatial scales. Ozone gardens with ozone-sensitive plants are a space to visualize ozone damage on leaves of plants. They are a practical way to express these complicated scientific topics to the public, especially in heavily polluted areas like Chicago. Some plants are particularly susceptible to foliar leaf damage from ozone exposure known as stipples and that damage can be visually quantified, making them bioindicator species. Native plants that are bioindicators for ozone and snap beans with an increased sensitivity to ozone can be used as a tool for visualizing the damaging effects of ozone air pollution in ozone gardens. Three test sites were chosen to install ozone bioindicator gardens to test this phenomenon. Photosynthesis and conductance were measured at two sites while soil moisture was measured at all three sites to compare plant health throughout the experiment. Ozone concentrations and stipple counts were monitored in July and August of 2023. In ozone gardens installed in the Hilton Chicago O'Hare Airport, Peggy Notebaert Nature Museum (PNNM), and a residence in Highland Park, the hypothesis is that native and sensitive plant species will exhibit stipples while the resistant species will have significantly less to no stipples. Ozone concentrations and stipple percent coverage of leaves is expected to have a direct relationship. If the ozone garden is water stressed, the effect of ozone damage will be diminished, leading to both sensitive and resistant species having fewer stipples. Through generalized linear mixed models, Cutleaf coneflower (*Rudbeckia laciniata)* was found

to have significant stipple coverage which supported its use as a native bioindicator for ozone detection. Common milkweed (*Asclepias syriaca)* and Wild lupine (*Lupinus perennis)* were found to not have significant stipple coverage under increased ozone, supporting their use as ozone damage resistant species. The R123 and S156 snap bean (*Phaseolus vulgaris)* variants were found to have significant stipple coverage and supports their use in ozone gardens. The R123 and S156 snap bean were found to be significantly different from each other through Tukey's honestly significant difference test, supporting the use of R123 snap bean as a resistant species to S156 snap bean. Research is needed in more of the Chicagoland area and additional ozone gardens could help support the significance between ozone concentrations and stipple coverage.

Introduction:

I: Ozone formation and harm

Air pollution has been improving over the last five decades in the United States (Environmental Protection Agency Green Book, 2024). Previously, poor air quality was evident with primary pollutants from smokestacks degrading visibility. These polluted atmospheres were common in cities where visibility has subsequently improved like Los Angeles, New York, and Chicago (Ashok & Barrett, 2016). Despite the significant reduction of visible air pollution, some non-visible air pollutants have not decreased as much and remain a serious public health concern. Without the visual impact of polluted air, less public attention is focused on further improving air quality. Ozone gardens allow community members to visually experience the impact of continuing poor air quality.

Historically, major highly populated cities experienced a high amount of photochemical smog, which can appear as a colored gas in the atmosphere. This type of air pollution was described in Glasgow by the public health official at the time, Des Voeux, as ‘smog’ by combining the words smoke and fog due to the pollution’s distinct visible presence (Brimblecombe, 2005). Although smog has been reduced, most of the pollution in the air today is not visible to human beings. One of these gases is ozone, which is harmful to human and plant health and is a tropospheric or ground-level pollutant. Confusion arises in public understanding since ozone is also naturally occurring. Ozone forms a layer in the stratosphere or upper atmosphere that protects humans from UV radiation (Butler et al., 2020). Another source of confusion is that ozone is not a primary pollutant emitted from specific sources.

Volatile organic compounds (VOCs) and nitrous oxides (NOx) undergo a reaction to form ozone, a secondary pollutant that does not have a visible point of formation or is formed secondarily (Pinto et al., 2010). In the troposphere, ozone is formed by VOCs from natural as well as anthropogenic sources and NOx pollution from combustion of fossil fuels. Automobiles and airplanes contribute to VOCs and NOx through the use of fossil fuels (Trousdell et al., 2016). VOCs and NOx in the atmosphere react with sunlight to form ozone with oxygen after photolysis. Higher levels of ozone pollution are observed miles away from large cities since ozone is formed secondarily and can be carried by wind (Klumpp et al., 2006a & Klumpp et al., 2006b). For example, NO has a shorter lifespan than NO_2, which can be carried more easily away from cities with wind to form ozone with oxygen after photolysis in suburban and rural areas. Since ozone is not produced from a primary source, it can be present even if there are no visible sources of air pollution in the vicinity.

High tropospheric ozone concentrations are unhealthy for people and a high risk to human respiratory health (Cisneros et al., 2010). Ozone can negatively affect our respiratory system by causing airways to constrict and trap air when breathing in ozone polluted air which leads to wheezing and shortness of breath. Ozone inflames the airways which damages the lining in spots. Chronic exposure to tropospheric ozone can lead to asthma and is linked to an aggravation or worsening of respiratory issues or symptoms. There is also a link to premature mortality along with an increase in emergency room visits and hospital admissions (Loughner et al., 2020).

Like humans, plants are also impacted by tropospheric ozone. Similarly to how the lining of the airways of our respiratory systems can be inflamed and damaged in spots by breathing in air polluted with ozone, ozone uptake through the stomata of plants causes internal oxidative damage (Ronan et al., 2020). This damage slows and may stop the process of photosynthesis, making ozone the most damaging air pollutant for a wide range of plants.

II: Plant physiology process affected by ozone

Tropospheric ozone pollution affects the photosynthesis process after entering through the stomata which are major mechanisms of plants. Stomata are small openings typically on the bottom of the leaves of plants that range from ten to eighty micrometers in length. They regulate the gas exchange from the leaves of plants by opening and closing to prevent water loss and allow carbon dioxide to diffuse from the atmosphere for photosynthesis. Guard cells control the opening and closing of the stomata with their turgor or rigidity. Flexing of the guard cells increases stomatal conductance and allows for the diffusion of carbon dioxide gas to occur simultaneously with the release of water vapor (Haworth et al., 2021). Plants' stomatal responses vary with environmental conditions such as closing and opening when temperatures fluctuate. Stomatal closure is the earliest response to the environmental stressor drought. Droughts have increased in frequency due to climate change, which has become more prevalent (Flexas and Medrano, 2002). The physiology of plants is greatly affected by stressors, including tropospheric ozone uptake through the stomata of plants and drought decreasing stomatal conductance.

After ozone has entered through the stomata, it travels to the spongy mesophyll layer. Here, the ozone oxidizes and damages vascular bundles of xylem and phloem as it continues to diffuse as carbon dioxide does in the plant. The oxidation causes burn damage that leads to the death of cells. Ozone makes its way into the palisade layer, where photosynthesis occurs after traveling through the spongy layer. In the palisade layer above the spongy layer, ozone continues to oxidize and damage plant organs such as the spongy and palisade mesophyll until it reaches the upper epidermis (Kim et al., 2020 & Michaels et al., 2022). The remaining ozone then damages and oxidizes the upper epidermis, leaving a visible mark.

The visible marks that tropospheric ozone causes on the top of a leaf of a plant between the leaf veins are called stipples (Lombardozzi, 2021). A stipple is a spot on the leaf of a plant where ozone has burned or destroyed the chloroplasts or photosynthetic structures inside the leaf. After the ozone has traveled upwards through the plant organs, the top of the leaf is left with a brown burned spot of damage where the photosynthetic structure was once green.

These stipples can be categorized into damage data based on the percentage of leaf coverage by stipples. Experiments have shown that these stipple counts, or visible foliar injury data, are mainly attributable to ozone pollution and depict the damage caused to plants by ozone (Agathokleous et al., 2017). Stipples are a visible representation of the oxidant damage caused by tropospheric ozone from a biochemical and plant physiological perspective.

Damage by ozone is very harmful for crop production. Ozone effects on photosynthesis are being studied to mitigate the damage of ozone on crop production yield such as the resistance of ozone uptake (Salvatori et al., 2013). Water stress was found to be a factor that could change the typical stipple damage caused by ozone (Lombardozzi et al., 2012). Water stress or drought closes the stomata which lowers the uptake of ozone and hence the production of stipples. In tulip poplar saplings, ozone pollution coupled with drought was found to change the expected response from the plant organs and reduce uptake of ozone (Shang et al., 2019). Drought stress has been shown to protect plants from ozone uptake by inducing stomatal closure (Carminati & Javaux, 2020).

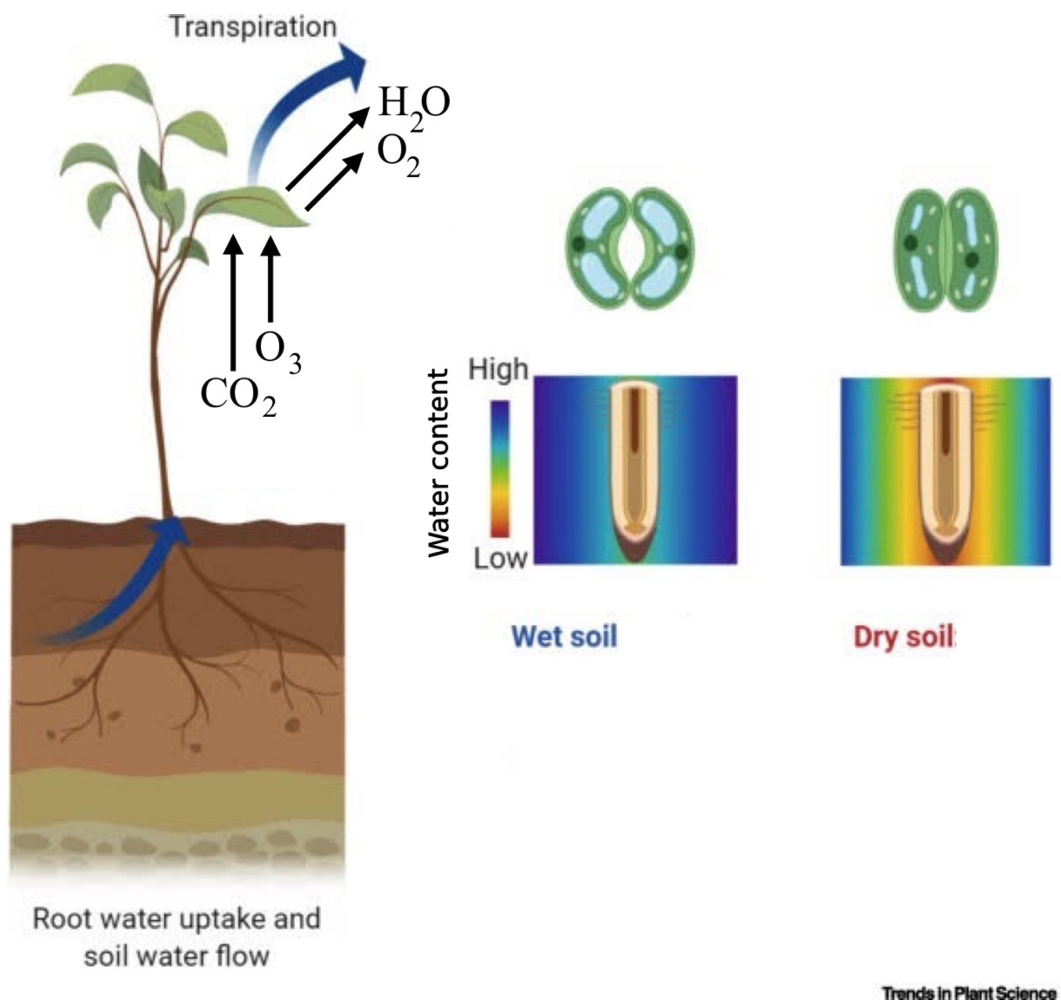


Figure 1. An adapted figure showing the uptake of ozone and stomata variability (Carminati & Javaux, 2020).

Figure 1 illustrates the gas exchange that occurs with ground-level ozone and natural processes during stomatal conductance. Soil with higher water content will cause the stomata to open as compared to closing in soil with lower water content, restricting gas exchange.

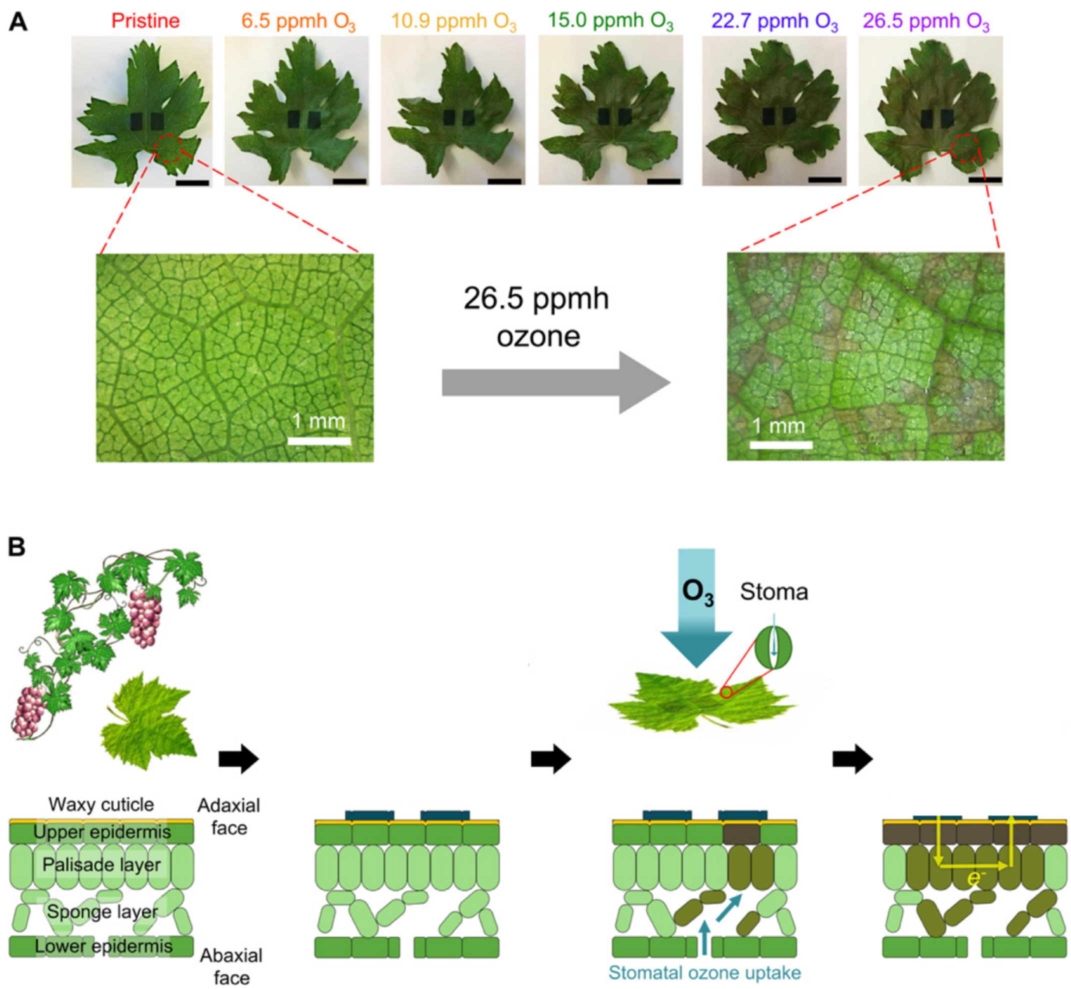


Figure 2. An adapted figure showing a view of the visible leaf damage present after varying degrees of ozone exposure (Kim et al., 2020).

The uptake of ozone from the stomata of a leaf can be viewed at the cell level as well as the damage left on the top of the leaf after exposed to ozone. Since this damage can be seen with the eye, it can be quantified to evaluate the leaf's health and environmental ozone pollution, making it a bioindicator.

III: Bioindicators

Indicator species or bioindicator species are animals or plants that can be used to infer the state or health of an ecosystem. For example, the presence of certain species of fish has historically been used to quantify the health of rivers and streams (de Castilhos Ghisi et al., 2020). The presence of certain species of fish would be bioindicators for a clean body of water because some fish may only survive with high oxygen content. The presence of the indicator species would signify the state of the environment, such as sufficient oxygen content.

Native plants have also been used as indicator species to monitor air quality. Some plants are particularly susceptible to stipple damage from ozone exposure and that damage can be quantified, making them bioindicator species. North American native pollinator host plant species like *Asclepias syriaca*, common milkweed, have been monitored for visible foliar injury from ozone due to their high sensitivity (Smith et al., 2003). This native species and *Rudbeckia laciniata*, Cutleaf coneflower, have been used in visible foliar monitoring ozone gardens in St. Louis, Missouri and other parts of the United States (Fishman, 2014). Ozone damage to these native plants has been widely researched and sensitivity to ozone was consistently found.

Wild lupine, *Lupinus perennis*, is a native species in the legume plant family that may also display ozone sensitivity due to ozone being the most damaging air pollutant to most plants (Ronan et al., 2020). This North American native species is also a host plant for the endangered Karner blue butterfly of Chicago and the Great Lakes region (Pascale & Thiet, 2016). Since this plant is a native pollinator species like *Asclepias syriaca*, and *Rudbeckia laciniata*, *Lupinus perennis* might show similar signs of ozone sensitivity.

Snap beans, in the legume family, are models for other annual crops which are widely consumed throughout the world (Morgan et al., 2003). *Phaseolus vulgaris*, or snap beans, have been bred by scientists at North Carolina State University to be a useful bioindicator to measure visible foliar injury data in the field (Burkey et al., 2005). The R123 snap bean variant is ozone-tolerant and has been tested to have a higher yield and lower visible foliar injury than its counterpart, the S156 ozone-sensitive snap bean variant (Burkey et al., 2012). Both variants have been widely utilized and tested in various research where it was consistently found that the R123 snap bean variant was resistant to ozone damage while the S156 snap bean variant was sensitive to ozone damage.

IV: Science outreach

Native plants and snap beans with an increased sensitivity to ozone can be used as a tool for visualizing the damaging effects of ozone pollution. Visual aids of stipple damage show the public the effects of ozone pollution. The stipples are a proxy for how ozone affects us and our lungs (Salvatori et al., 2013). Visual aids like stipples displaying degrees of damage help in understanding our environment and the damage that we are being left with by climate change and air pollution that are otherwise more complicated to understand because they are not easily visible in everyday life.

In this growing global age of information, science communication is widely necessary to keep the world rightly informed on harder to understand concepts such as invisible ozone (Rowland, 1993). There is an increase in populations affected by non-visible airborne pollution that might not understand the damage, even in this time with growing access to information.

Bioindicator species are a useful tool for helping affected populations understand the effects of ozone air pollution within their communities.

V: Community science and informal science education

Community science is scientific work done by members of the community or general public. It has also been referred to as citizen science and is done in collaboration with professional scientists and scientific institutions. Community science can be an effective way of scientific outreach to the public and spaces that they are in. Urban community gardens are ways to incorporate community science and boost community engagement with scientific material. Giving the public a place to plant and maintain vegetables or other crops with access to resources such as master gardeners to learn about the growing process allows community science to occur in urban community gardens (Brown-Fraser et al., 2015). Community gardens with the use of community scientists have been proven to positively impact environmental awareness, involvement in science, public health, and wellness.

Community scientists can contribute to scientific data collection effectively like scientists, even if they might not perform it at an expert level. For example, some community participants effectively identified species and contributed to a garden diversity study (Egerer et al., 2019). A simple questionnaire asking gardeners or the community scientists to report on the species in their gardens was well taken by participants and there was effective data taken on the presence of many species per garden plot. Species listed was less on average than that of the professionals who then measured the species present in each garden, but, many species were still recorded with wide community participation.

Active learning can also be achieved through community science. Active learning was facilitated through effective communication with community scientist data monitors during the data collection process in community science projects (Probert et al., 2022). Effective communication about the scientific learning process and outcomes of the project were key to minimizing uncertainties and increasing the active learning benefits of the community science project. Active learning encouraged through community science can be an effective form of informal education valuable for scientific outreach.

Informal education is possible through academic research institutions, botanical gardens, museums, and more. These spaces allow for learning to occur outside of traditional classroom settings which may allow for a different and deeper connection to scientific material (Krishnan et al., 2019). For example, a botanical garden displays plant-based exhibits meant to visually please visitors. These visually pleasing displays can help to draw in the observer to then read the signs denoting the species of plants used, informally educating them on a scientific topic.

VI: Ozone Gardens

Ozone gardens are useful and beneficial for measuring the visible foliar damage to multiple species in a communicable way to the general community. In the spring, an ozone garden can be planted to monitor the stipple damage on the leaves of sensitive plants. These gardens should be planted with plenty of space between plants to adequately record data throughout the growing season. The simpleness of the garden structure of ozone gardens allows for other tests such as for drought stress on the uptake of ozone from plants. Ozone gardens are helpful tools that are valuable for science communication and are an effective form of outreach on complex scientific material.

Scientific outreach is possible through ozone garden implementation. ozone gardens located in the St. Louis area were helpful tools to examine ozone visible foliar damage across species and communicate it to the public. *Asclepias syriaca* and *Rudbeckia* were used in visible foliar monitoring ozone gardens in St. Louis, Missouri and other parts of the United States due to their sensitivity to ozone and easy visibility of stipples due to ozone damage (Fishman, 2014). These constructed ozone gardens are a part of a monitoring program created to increase scientific outreach to a greater public across the country and beyond because of the easily communicable stipple damage. Students involved in Saint Louis Science Center's (SLSC) Youth Exploring Science (YES) program for disadvantaged high schoolers were involved in the ozone garden project and learned about air pollution damage as well as gained experience overseeing plant research.

Having studies conducted in ozone gardens allows for the use of community scientists such as the students from the YES science outreach program, which provide invaluable contributions to data collection and ozone pollution awareness (DeForest Hauser et al., 2015). These gardens consist of multiple species of ozone-sensitive plants in easily accessible plots located in community centers like museums and conservatories. Community scientists would be able to visit these institutions and periodically record individual leaf percent coverage by stipples. These periodical counts during the heightened months of ozone pollution in the summer should give the community scientists active learning.

Ozone gardens with community scientists are a form of informal education. Active learning and informal education can be achieved through stipple counts recorded by community scientists. The act of counting stipple coverage and assigning a level of damage to the plant is a process that assigns the quantity of pollution to a visible and tangible entity. This process is a nontraditional way of making invisible pollution like ozone into something more comprehendible. The nontraditional look into atmospheric pollution damage could be a more palpable way to get the greater public to want to understand complicated air pollution. Members of the public are also capable of passive and informal education or learning about ozone pollution even without these active stipple count recordings taken periodically throughout the summer. This informal education by the public is possible through marked signs in the ozone gardens in these cultural spaces and institutions (Groom et al., 2017). Marked signs about the process of stipple formation beside the ozone gardens allow informal education by anyone visually drawn into the garden who wants to read more about it.

VII: Hypothesis

This study aims to explore how ozone gardens are a feasible way to express complicated scientific topics to the public. This scientific outreach is needed, especially in heavily polluted areas like Chicago. In ozone gardens installed in O'Hare, Peggy Notebaert Nature Museum, and a residence in Highland Park, the hypothesis is that native and sensitive plant species will exhibit stipples while the resistant species will have significantly less to no stipples. If no high ozone is recorded by the ozone sensor, then no stipples are expected. If the ozone garden is water stressed, the effect of ozone damage will be diminished, leading to both sensitive and resistant species having fewer stipples.

Methods:

Three test sites were chosen to install ozone bioindicator gardens to measure the coverage of stipples to test the hypotheses. Photosynthesis and conductance were measured at two sites while soil moisture was measured at all three sites to compare plant health throughout the experiment. EPA ozone sensor data were used in the research project as well as ozone sensor data from a sensor installed by DePaul University at the Peggy Notebaert Nature Museum. Ozone gardens were established at each site using native species grown in the DePaul University Greenhouse and other species from seeds including snap bean sensitive and resistant cultivars provided by Dr. Kent Burkey of North Carolina State University in the beginning of June 2023. Stipple percentage coverage counts of individual leaves were taken weekly from each site in the months containing heightened levels of ozone in July and August of 2023.

I: Garden locations

To obtain a possible variation in ozone monitor readings between geographical areas in the greater Chicago area, specific locations were chosen for ozone bioindicator gardens. Locations were also chosen based on their proximity to active EPA ozone air quality sensors as well as one location for its potential for public community outreach and partnership with DePaul University.

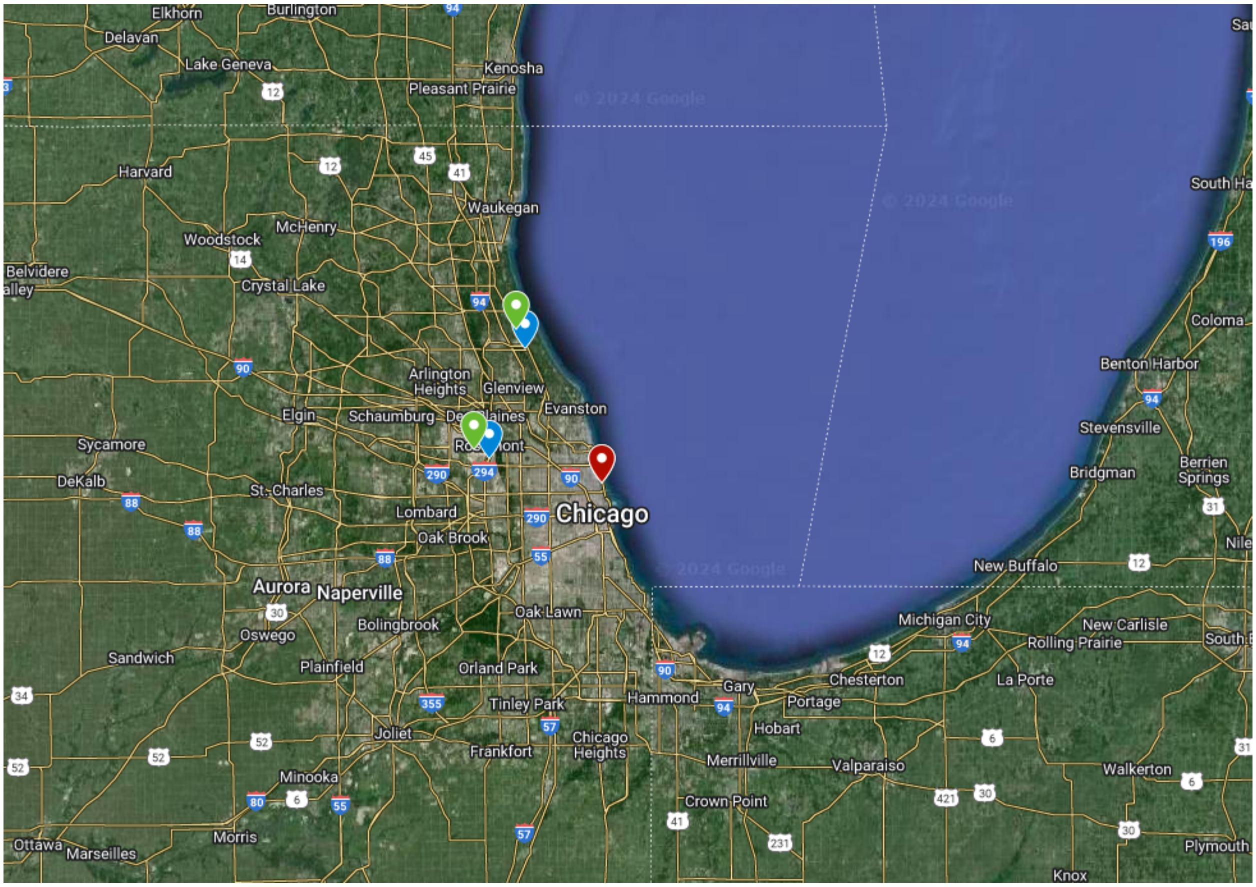


Figure 3. PNNM, ORD, and HP sites in the selected study area. Blue markers indicate locations of EPA ozone monitors. Green markers indicate locations of ozone gardens. The red marker indicates the location of the PNNM personal ozone monitor and ozone garden.

A location was chosen in Peggy Notebaert Nature Museum (PNNM) for its partnership with DePaul University to increase public community outreach on air quality. An ozone sensor was installed for comparison data; it is also about 100 meters from the garden location. Based on the criteria of proximity to active EPA ozone sensors, ozone bioindicator gardens were chosen to be installed at the Hilton Chicago O’Hare Airport (ORD) and a residence in Highland Park, Illinois (HP). ORD can be seen west of the PNNM site and HP can be seen furthest to the north.

II: Constructing gardens

PNNM and HP gardens were constructed to have the most similar conditions in separate areas of the greater northern Illinois/Chicagoland area. They were both planned to be 3 meters by 3 meters square gardens. In the plot, plants were placed with approximately 0.3 meter of space around each plant starting from the top left facing the garden, or from the south-west most point. A 0.6 meter path for measurement access was planned to be placed in the middle of the six plants in each row, with five rows total. The plant list for each garden can be seen in the Table 1.

Table 1. Plant list for each site with common and species name as well as plant type.

Common Name	Species Name	Plant Type
Cutleaf coneflower	*Rudbeckia laciniata*	Native ozone bioindicator
Common milkweed	*Asclepias syriaca*	Native ozone bioindicator
Wild lupine	*Lupinus perennis*	Native prospective ozone bioindicator
R123 Resistant snap bean	*Phaseolus vulgaris*	Non-native ozone-resistant cultivar
S156 Sensitive snap bean	*Phaseolus vulgaris*	Non-native ozone-sensitive cultivar

The garden structure for the PNNM and HP sites can be viewed approximately to scale in Figure 4.

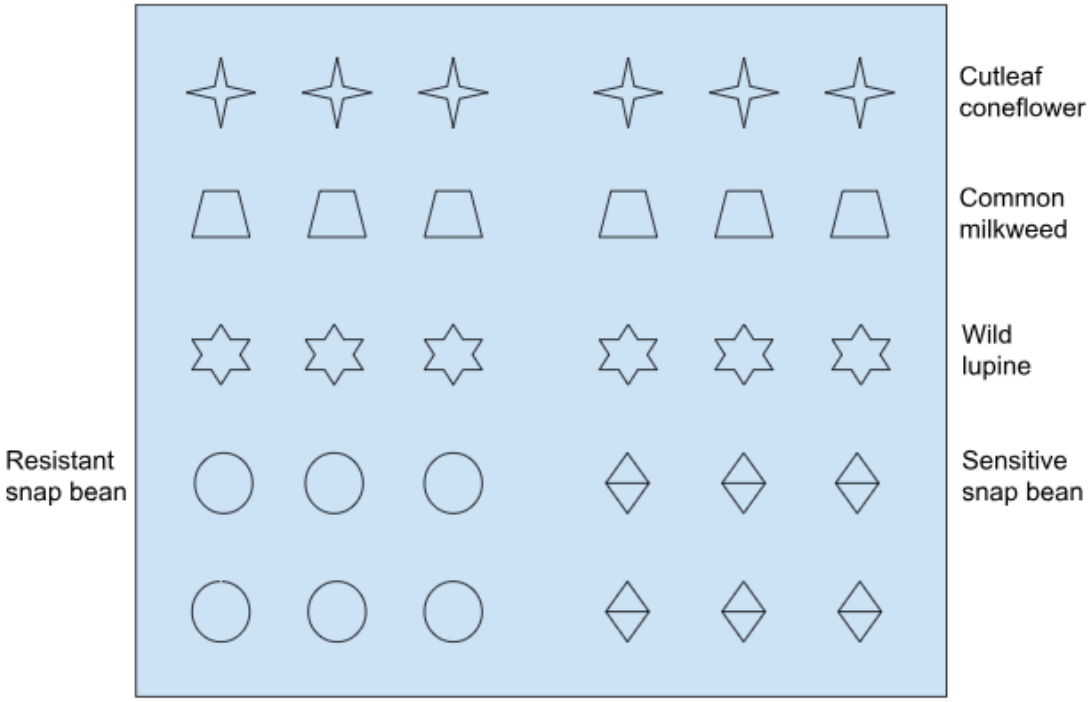


Figure 4. PNNM and HP garden structure with plant types correlating to shapes. There is an approximate 0.61 meter path splitting rows of native plants and groupings of resistant and sensitive snap bean down the middle of the garden. The dimensions of the garden are 3 meters by 3 meters with approximately 0.3 meters of space between each plant.

Rows of plants in the HP garden ran east-west, parallel to a fence. Rows of plants in the PNNM garden ran north-west, parallel to the museum building. The rows of plants were selected to range from tallest to shortest total plant height. Starting with the tallest plant height in the back row to the shortest plant height in the front row, with the back row closest to the fence in HP and the back row closest to the museum building in PNNM, the selected native plants that occupied each row were *Rudbeckia laciniata, Asclepias syriaca,* and *Lupinus perennis.* In the fourth and fifth row, the first three plants starting from the left facing the front of the garden were the R123 or ozone-tolerant variant of *Phaseolus vulgaris* and the last three plants that followed the path break were the S156 or ozone-sensitive variant of *Phaseolus vulgaris.*

The ORD garden was constructed to mimic similar conditions in the PNNM and HP gardens. Six large plastic pots usually used for decorative planting outside of the lobby of the O'Hare Hilton Hotel Airport were used to house plants. The garden structure for the ORD site can be viewed approximately to scale in Figure 5.

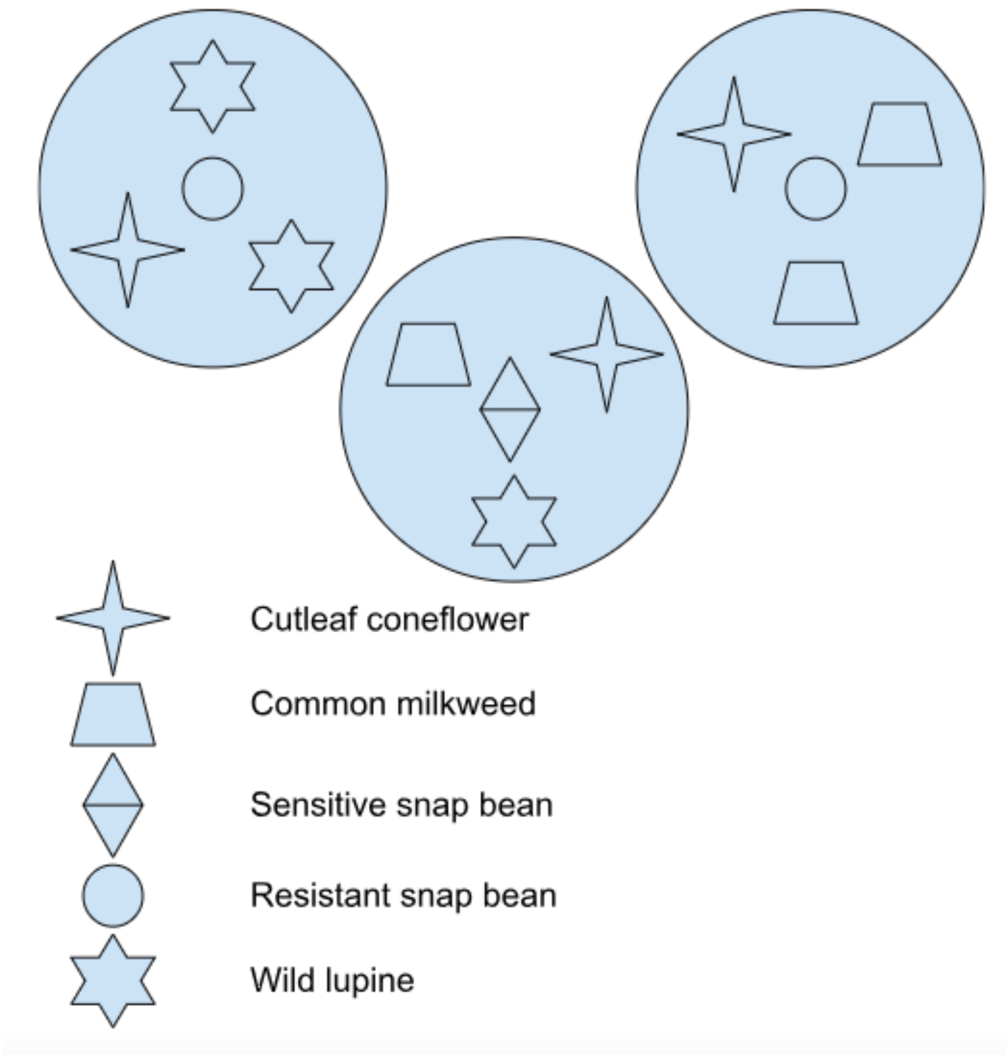


Figure 5. ORD site garden structure and key with plant types according to shape. Each landscaping pot was 0.91 m in diameter and 0.76 m in height.

In each pot, all three native plants were spaced with approximately 0.3 meter of space around each plant. In three of the pots, two R123 or the ozone tolerant variant of *Phaseolus vulgaris* were placed in the center with approximately 0.3 meter of space around each plant. In the other three pots, two S156 or the ozone sensitive variant of *Phaseolus vulgaris* were placed in the center with approximately 0.3 meter of space around each plant.

III: Installing gardens

The HP garden was planted on May 27th, the PNNM garden was planted on May 30th, and the ORD Garden was planted on June 1st. During installation, an extra native plant per species was added to each row of the PNNM and HP garden structure to account for possible individual plant mortality. An extra native plant per species was added to three pots at the ORD site to account for possible individual plant mortality.

A difference in the conditions of the gardens was that the PNNM garden was watered approximately half the number of times the HP garden was watered. The HP garden was watered most days of the week while the PNNM garden was watered at most three times a week. The ORD garden was watered the least, at most twice a week. The decorative pots in the ORD garden potentially created a hydrological regime that was different from the other two sites planted in situ.

IV: Measurements

Soil moisture was measured for each site using a HydroSense Soil Water Measurement System (Campbell Scientific, CD620, CS620). Volumetric water content was measured in percent at a rod length of 20 cm in ten randomly chosen areas in the PNNM, HP, and ORD sites. For the ORD site, four pots were randomly chosen to be measured twice while two pots were measured once. This random selection of pots chosen for measurement were changed for each visit throughout the experiment.

The LI-6400/XT Portable Photosynthesis System (LI-COR Environmental) (give manufacturer) was used to measure conductance and photosynthesis in the plants at the PNNM and HP sites. Settings in the LI-6400/XT Portable Photosynthesis System were at a CO_2R (reference) level of 400 ppm, a leaf temperature of 30 degrees Celsius, a flow of 500 μmol s^{-1}, the leaf fan on fast, and a PAR (photosynthetic active radiation) of 1000 μmol m^{-2} s^{-1}. These settings were the same for both sites as well as the data collection procedure. This procedure involved taking readings of one random leaf per plant for three plants of each type of species/variant. The leaf was placed into the system chamber, where the leaf area present in the select square leaf chamber would be closed off from the outside environment for five minutes to give ample time for the leaf in the chamber and readings to stabilize. Readings were then logged by the instrument and a subset recorded into a field journal to make fifteen measurements total each visit.

Stipple counts were measured by observed stipple coverage in percent of ten randomly selected leaves per plant per species/variant. Percent stipple coverage classifications per each individual leaf were zero percent damage, 1-6 percent damage, 7-25 percent damage, 26-50 percent damage, 51-75 percent damage, and 76-100 percent damage. Online examples of photographs of multiple species of plants in each classification were available to train the assessment of stipple coverage provided by the US National Center for Atmospheric Research (NCAR) (define abbreviation if this is the first time used) before data collection.

A 2B Technologies (Broomfield, CO) Model 205 Dual Beam Ozone Monitor was installed in an outdoor shed at the PNNM site. Attached to the monitor's air inlet was a filtered air tube funneled into the outside of the shed, pictured at the PNNM site in Figure 6. The filter was for particulate matter and should not have affected the readings. The data collection rate was set to collect every five minutes.

Figure 6. Filtered air tube installed in an outdoor shed at the PNNM site. The connected ozone monitor was located within the outdoor shed.

The Model 205 Dual Beam Ozone Monitor was installed and read ozone measurements for approximately one month prior to the start of stipple counting, photosynthesis, and soil moisture measurements. Data was downloaded each visit to the PNNM site from the Ozone Monitor as CSV files.

Data collection visits to each site occurred weekly starting at approximately 12 pm, from July 5th, 2023, to August 28th, 2023. Stipple percent coverage observations, photosynthesis, soil moisture measurements, and Ozone Monitor measurements were taken together at each visit to the PNNM site.

Stipple percent coverage observations, LI-6400/XT Portable Photosynthesis System measurements, and soil moisture measurements were all taken together at each visit to the HP and PNNM sites. Stipple percent coverage observations and soil moisture measurements were taken together at each visit to the ORD site. Separate site protocols can be seen in Table 2. Sites that contain an “X” for a certain measurement were taken for each visit for the duration of the experiment per site.

Table 2. PNNM, HP, and ORD field data collection.

Site	PNNM	HP	ORD
Stipple percent coverage	X	X	X
Soil moisture	X	X	X
LI-6400/XT Portable Photosynthesis System	X	X	
Model 205 Dual Beam Ozone Monitor	X		

Photosynthesis measurements were taken the next rain-free day following the planned visit while all other measurements were collected on the planned visit for PNNM and HP sites during rain events. The data collection dates for collecting stipple percent coverage and soil moisture along with model 205 Dual Beam Ozone Monitor measurements for the PNNM site can be viewed in Table 3. The date with an asterisk (*) signifies the start of soil moisture data collection for all sites thereafter.

Table 3. ORD, PNNM, and HP ozone and stipple coverage data collection dates.

	ORD	PNNM	HP
week 1	7/5/2023	7/6/2023	7/8/2023
week 2	7/14/2023	7/13/2023	7/16/2023
week 3	7/20/2023	7/21/2023 *	7/23/2023
week 4	7/27/2023	7/28/2023	7/31/2023
week 5	8/3/2023	8/4/2023	8/6/2023
week 6	8/11/2023	8/10/2023	8/13/2023
week 7	8/17/2023	8/18/2023	8/20/2023
week 8	8/24/2023	8/25/2023	8/28/2023

The data collection dates for collecting photosynthesis and conductance measurements can be viewed in Table 4. The date with an asterisk (*) again signifies the start of soil moisture data collection for all sites thereafter and the date with a double asterisk (**) signifies that only snap bean variant data was collected.

Table 4. PNNM and HP photosynthesis and conductance data collection dates.

	PNNM	HP
week 2	7/13/2023 **	7/16/2023
week 3	7/21/2023 *	7/23/2023
week 4	7/30/2023	7/31/2023
week 5	8/4/2023	8/8/2023
week 6	8/10/2023	8/16/2023
week 7	8/18/2023	8/20/2023
week 8	8/27/2023	8/28/2023

Ozone sensor data in Rosemont and Northbrook were downloaded from the EPA website for the summer of 2023 for the ORD and HP sites. The Rosemont ozone EPA monitor site name was Cook County Trailer and ID was 17-031-3103. The Northbrook EPA ozone monitor site name was Northbrook Water Plant and ID was 17-031-4201.

Version 4.3.0 of RStudio was used to construct plots and conduct statistical analysis. The a priori window of time used to calculate Accumulated dose of ozone Over a Threshold of 40 ppb (AOT40) hours measurements was four days or 96 hours. AOT40 measurements taken from EPA ozone sensors in Rosemont and Northbrook as well as measurements taken from the Ozone Monitor installed in Lincoln Park over the study time were multiplied by this window of time and averaged from the date of stipple collection to the previous four days or 96 hours in RStudio. AOT40 hours calculations, along with stipple coverage, photosynthesis, conductance, and volumetric water content were used as fixed effects with time as a random effect to create generalized linear mixed models. The libraries MASS, logspline, glmmTMB, and fitdistrplus were used to fit the distribution and create models for statistical analysis. Multiple generalized linear mixed models were tested using the glmmTMB function after transforming the distribution with a gamma fit function. The libraries emmeans and lsmeans were used to conduct a post hoc Tukey Honestly Significant Difference (HSD) test on the model that had the lowest Akaike Information Criterion (AIC) score that fit the data set the best.

Results:

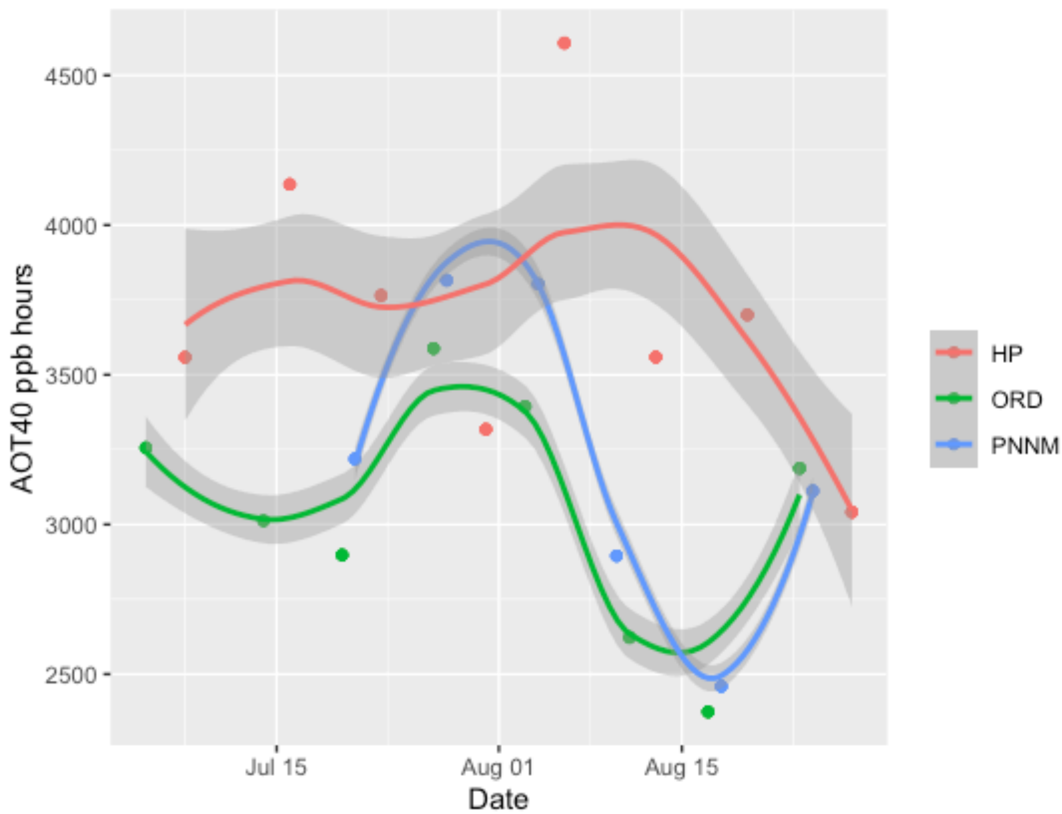


Figure 7. AOT40 or accumulated doses of ozone over a threshold of 40 ppb measurement calculations using the 96-hour window from the start of July to the end of August. The HP site can be seen in red, the ORD site in green, and the PNNM site in blue.

AOT40 calculated from the EPA sites (HP, ORD) and the monitor installed at the PNNM differed from each other but also demonstrated some similarities (Figure **7**). The HP site contained the highest AOT40 ppb hours measured throughout the study period while the ORD site contained the lowest values. The PNNM site had higher AOT40 than the ORD site on the last week of the eight-week study period but was otherwise lower. The HP site contained variability although all points measured higher than both the ORD and the PNNM site except for two out of the eight weeks of the study period, near the start of August and again near the end of August. The PNNM site, which was the only site to have a personally installed ozone monitor, contained the highest variability in AOT40 ppb hours measurements. The first two weeks of data at the PNNM site were unusable and missing from the figure due to a pump in the ozone monitor getting clogged from particulate matter from wildfire smoke.

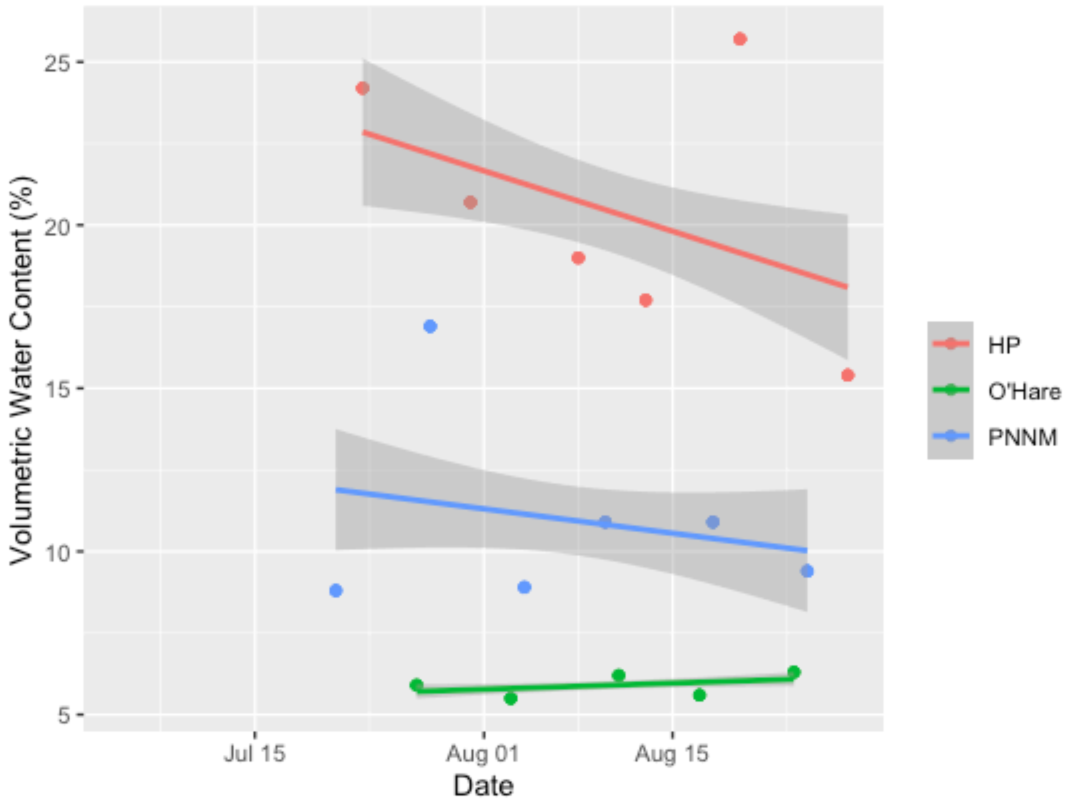


Figure 8. Volumetric water content from the start of July to the end of August for all three sites. The HP site can be seen in red, the ORD site in green, and the PNNM site in blue.

Figure 8 shows the volumetric water content at each site over time. Measurements for volumetric water content started during the third week of sampling for all sites, totaling six weeks of data. The HP site had the highest volumetric water content throughout the study period while the ORD site had the lowest. The PNNM site had no significant trend in volumetric water content throughout the study period. Both the PNNM and HP sites had the highest variability in volumetric water content, and the HP site had slightly higher variability than the PNNM site. The higher variability in the HP site can be seen by having the highest amount of spread in volumetric water content, with only two points falling within the gray spread surrounding the line of best fit while the PNNM site contains three points falling within the gray spread surrounding the line of best fit.

Both the HP and PNNM sites became drier throughout the study period, with slight variability towards the end of the study period at the HP site during the second to last week of measurement that trended upwards and got wetter. The ORD site, which was in large landscaping pots as compared to large garden plots at the HP and PNNM sites, was consistently dry throughout the study period and can be pictured in Figure 9.

Figure 9. Landscaping pots at the ORD site at the start of the study period. There were six pots total in the outdoor patio of the ORD site.

Volumetric water content averaged below seven percent throughout the whole study period at the ORD site. The pots were filled to the top of the brim with general gardening soil.

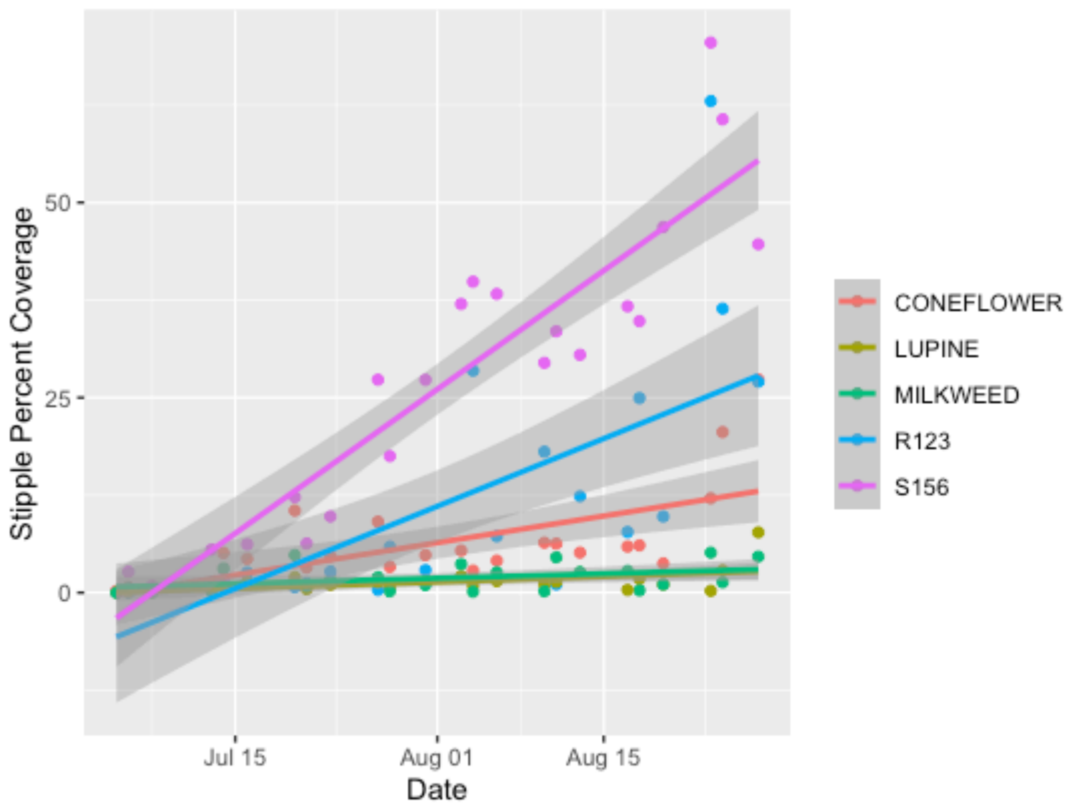


Figure 10. Stipple percent coverage measured from the start of July to the end of August for all sites. The S156 sensitive snap bean can be seen in purple, the R123 resistant snap bean in blue, Cutleaf coneflower in orange, Common milkweed in green, and Wild lupine in yellow.

Plant type differed in stipple coverage but had similar stipple build-up trends over the study (Figure 10). The S156 sensitive snap bean reached an average of over 50% stipple coverage by the end of the study period. This is followed by the R123 resistant snap bean and Cutleaf coneflower. The R123 resistant snap bean reached an average of over 25% stipple coverage of all plants by the end of the study period. Cutleaf coneflower reached an average of over 10% stipple coverage of all plants by the end of the study period. Common milkweed and

Wild lupine have the least and most similar stipple counts, both reaching an approximate average of 5% stipple coverage by the end of the study period.

As expected, the S156 sensitive snap bean showed the most amount of stipple coverage with the highest positive increase throughout the study period. The R123 resistant snap bean was used as a control for the coverage of stipples throughout the study period. It had the second highest stipple average coverage by the end of the study period, higher than all the native bioindicator species.

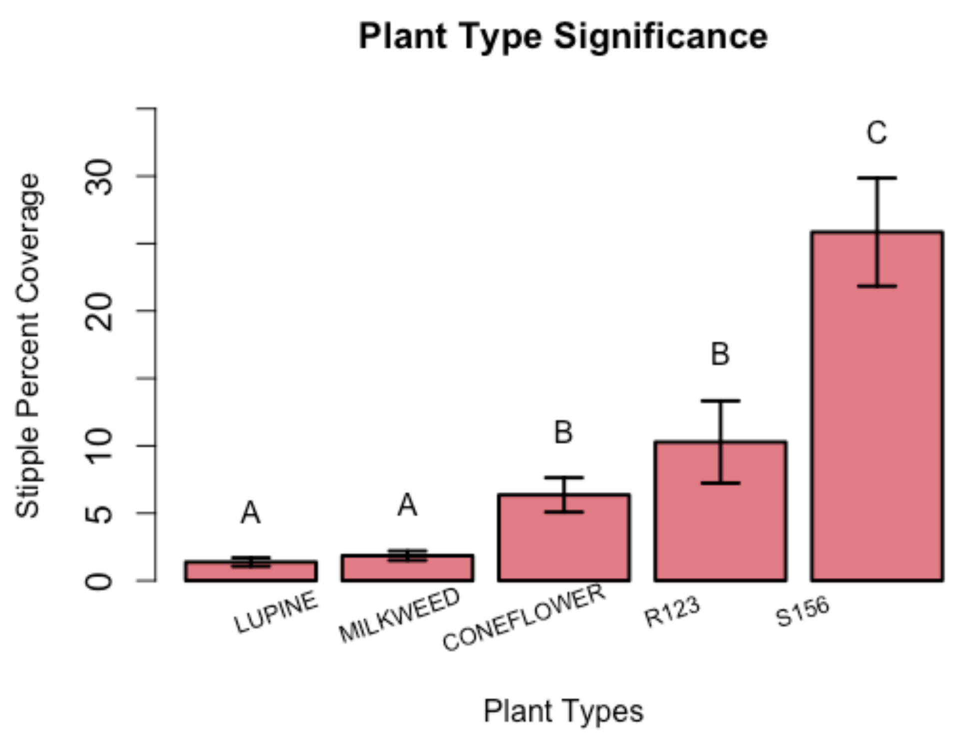


Figure 11. Plant types and their differences shown with each type's stipple percent coverage mean and standard error. "A" refers to Wild lupine and Common milkweed, "B" refers to Cutleaf coneflower and R123 resistant snap bean, and "C" refers to S156 sensitive snap bean.

Tukey's post hoc test was conducted for pairwise comparisons on the generalized linear mixed model of stipple coverage versus plant type. Cutleaf coneflower was found to be highly significant from Wild lupine, Common milkweed, and the S156 sensitive snap bean (P = < 0.0001). Cutleaf coneflower was not found to be highly significant from the R123 resistant snap bean (P = 0.2300). This relationship can be seen on Figure 11 due to the standard errors of the plant types overlapping. The S156 sensitive snap bean and the R123 resistant snap bean were significantly different from Wild lupine and Common milkweed (P = < 0.001). Wild lupine and Common milkweed were found to not be significantly different from each other (P = 0.8839). This relationship can be seen with Common milkweed and Wild lupine overlapping standard errors in Figure x. Finally, the S156 sensitive snap bean and the R123 resistant snap bean were found to be significantly different from each other (P = 0.0010).

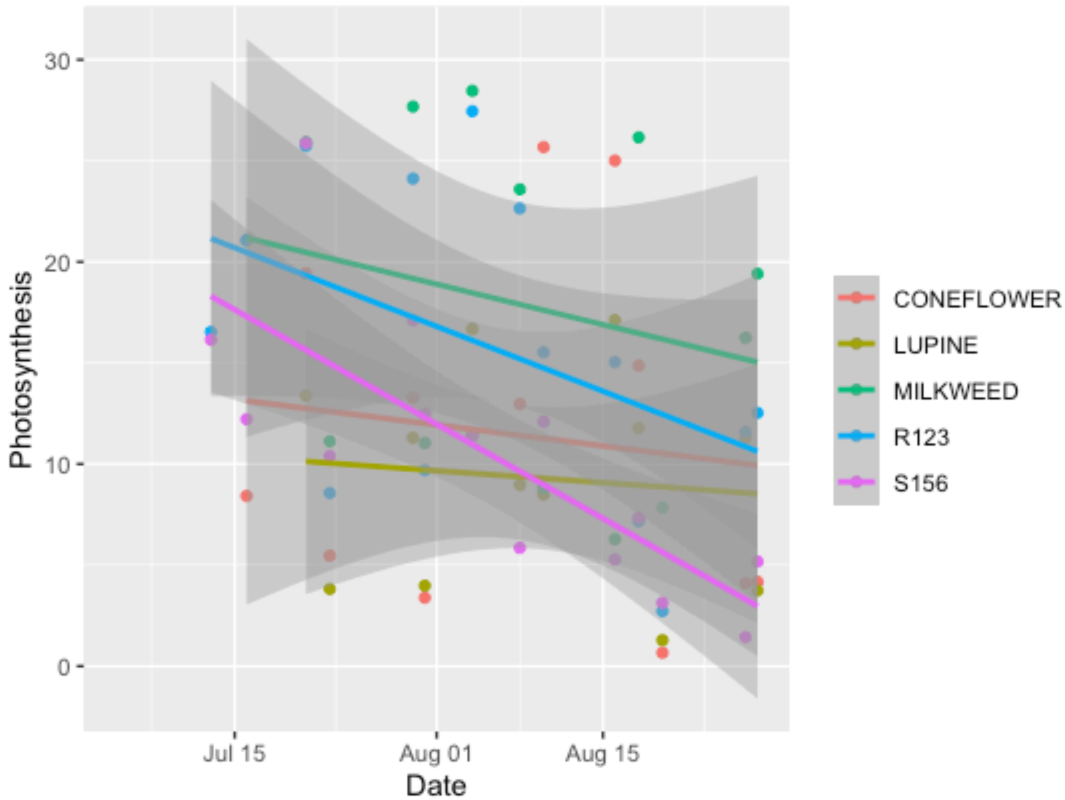


Figure 12. Photosynthesis measured from the start of July to the end of August for all sites. The S156 sensitive snap bean can be seen in purple, the R123 resistant snap bean in blue, Cutleaf coneflower in orange, Common milkweed in green, and Wild lupine in yellow.

Measurements for photosynthesis started during the third week of sampling for the HP and PNNM sites, totaling six weeks of data. The S156 sensitive snap bean had the highest decline in photosynthesis throughout the study period as expected. This trend is followed by the R123 resistant snap bean and Common milkweed. Wild lupine remained the most unchanged. Cutleaf coneflower experienced a similar, but slightly higher decline.

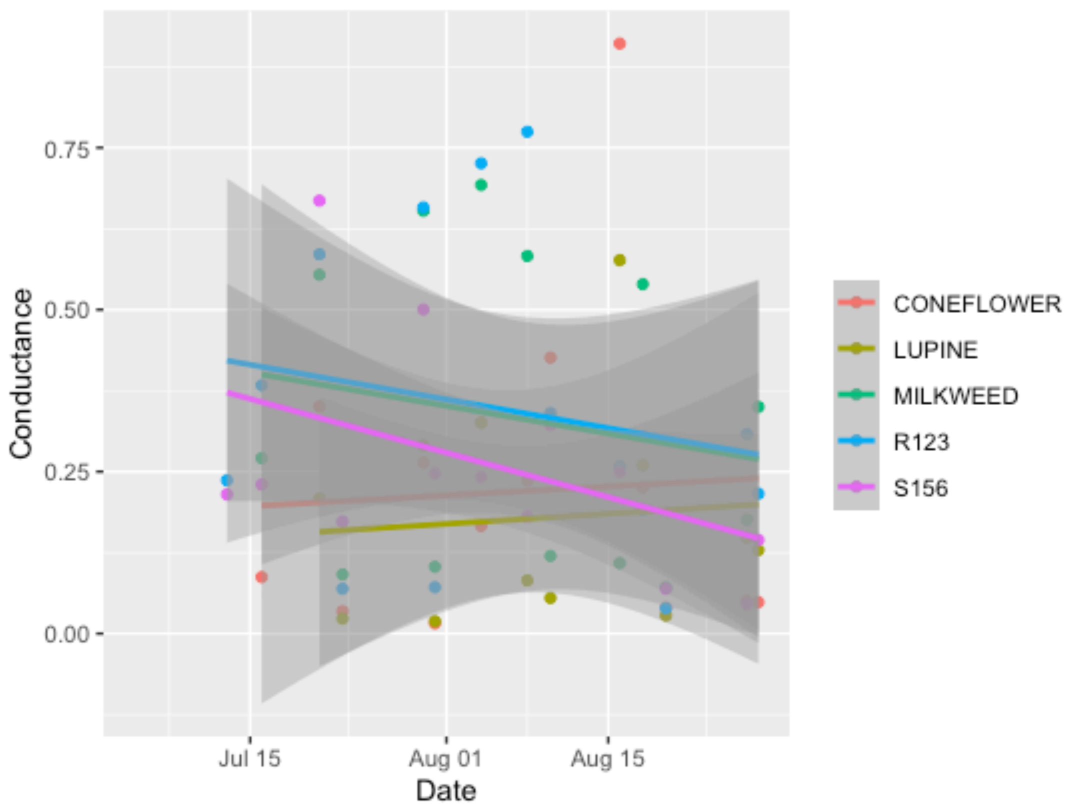


Figure 13. Stomatal conductance in m/s measured from the start of July to the end of August for all sites. The S156 sensitive snap bean can be seen in purple, the R123 resistant snap bean in blue, Cutleaf coneflower in orange, Common milkweed in green, and Wild lupine in yellow.

Measurements for conductance started during the third week of sampling for the HP and PNNM sites, totaling six weeks of data. The S156 sensitive snap bean had the highest decline in conductance throughout the study period. This trend is followed by the R123 resistant snap bean closely followed by Common milkweed. Wild lupine and Cutleaf coneflower had parallel slight increases in conductance throughout the study period.

Table 5 gives information for all generalized linear mixed models tested throughout the study period. All variables of interest: stipple percent coverage (Stipple), accumulated does of ozone over a threshold of 40 ppb using the 96-hour window (AOT40 ppb hours), volumetric water content (VWC), and plant species or variant (Plant), were used to make models for statistical analysis.

Table 5. AIC values found using statistical analysis in RStudio

Model	AIC	Delta AIC
Null (Stipple ~ 1)	548.8	-92.0
Stipple ~ Plant + AOT40 ppb hours	458.7	-1.9
Stipple ~ Plant + AOT40 ppb hours + VWC	460.2	-3.4
Stipple ~ Plant + VWC	458.3	-1.5
Stipple ~ Plant	456.8	0.0

All variables were tested in generalized linear mixed models. A null model was first created using no other additional variables and weeks as a random factor to make it a random effect. This is because all variables were consistently measured by week and week as a random effect minimizes the variability due to differences within a week. The random factor of weeks was used for every following generalized linear mixed model of stipple coverage explained by plant type.

The AIC values represent scores that can be used to compare multiple models to find out the best one. The best model would usually have the lowest AIC value, which incorporates the best fit of the variables and the model's parsimony. The Delta AIC values represent the change in

AIC values from the best model, the stipple coverage explained by plant type generalized liner mixed model with an AIC score of 456.8. The second-best model and the one used for further analysis was the stipple coverage explained by plant type and VWC model with an AIC score of 458.3. This model was used for further analysis due to stipple coverage being analyzed with plant type in a separate Tukey's post hoc test. This model was also the best scored model which contained two additional variables (Plant + VWC).

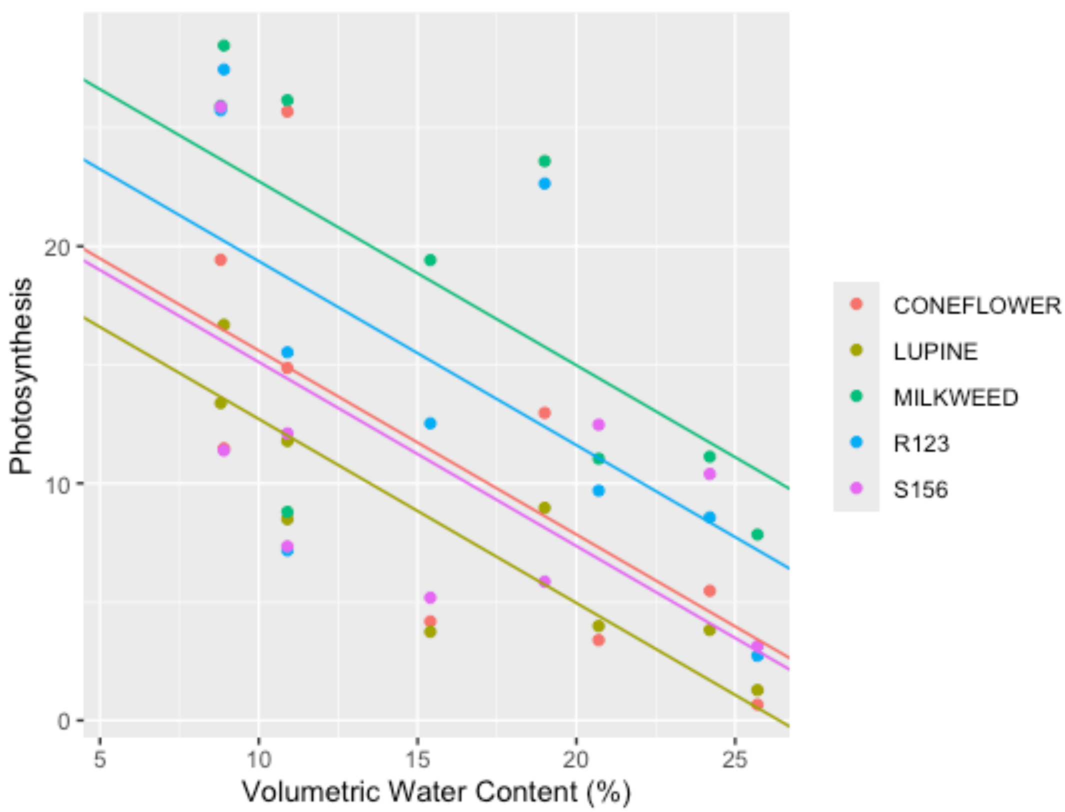


Figure 14. Photosynthesis and volumetric water content for all types of plant. The S156 sensitive snap bean can be seen in purple, the R123 resistant snap bean in blue, Cutleaf coneflower in orange, Common milkweed in green, and Wild lupine in yellow.

Lines correlating to each plant type were found in RStudio using the model constraining all slopes to be the same for each plant type. All plant types experienced a decline in photosynthesis with an increase in volumetric water content. Common milkweed had the highest photosynthesis while Wild lupine had the lowest. Cutleaf coneflower and the S156 sensitive snap bean had lower photosynthesis than the R123 resistant snap bean. When tested in RStudio, volumetric water content had a P-value of 4.11e-12, meaning that volumetric water content is significantly correlated to photosynthesis explained by plant type. This is a counterintuitive result because they are significantly negatively correlated when they should be positively correlated. The expected result was a significant positive correlation between photosynthesis and volumetric water content because with higher volumetric water content photosynthesis was supposed to be higher with less restraint due to drought stress. The opposite was found and a significant negative correlation was found with photosynthesis and volumetric water content.

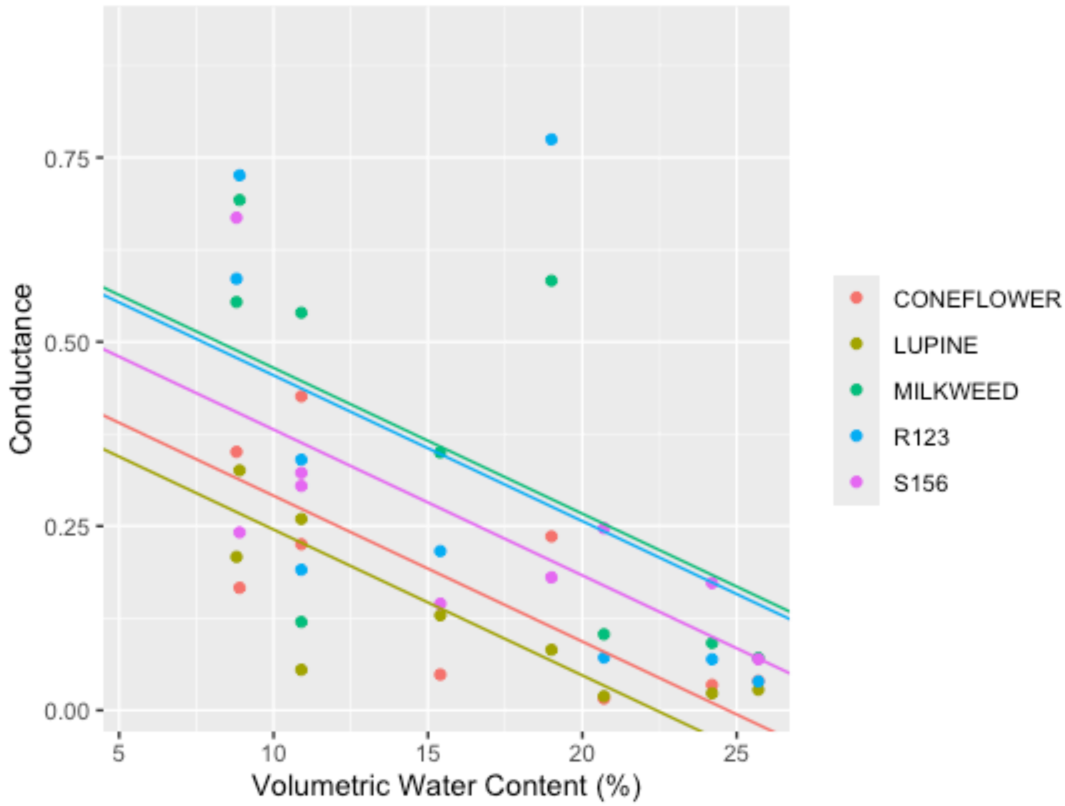


Figure 15. Stomatal conductance and volumetric water content for all types of plants. The S156 sensitive snap bean can be seen in purple, the R123 resistant snap bean in blue, Cutleaf coneflower in orange, Common milkweed in green, and Wild lupine in yellow.

Lines correlating to each plant type were found in RStudio using the model constraining all slopes to be the same for each plant type. All plant types experienced a decline in conductance with an increase in volumetric water content. Common milkweed and the R123 resistant snap bean had the highest conductance. Wild lupine had the lowest conductance. Cutleaf coneflower and the S156 sensitive snap bean had lower conductance than the R123 resistant snap bean and Common milkweed. When tested in RStudio, volumetric water content had a P-value of 2.56e-14, meaning that volumetric water content is significantly correlated to conductance explained by plant type. This would be a counterintuitive result because they are significantly negatively correlated when they should be positively correlated. The expected result was a significant positive correlation between conductance and volumetric water content because with higher volumetric water content conductance was supposed to be higher with less restraint due to drought stress. The opposite was found and a significant negative correlation was found with conductance and volumetric water content.

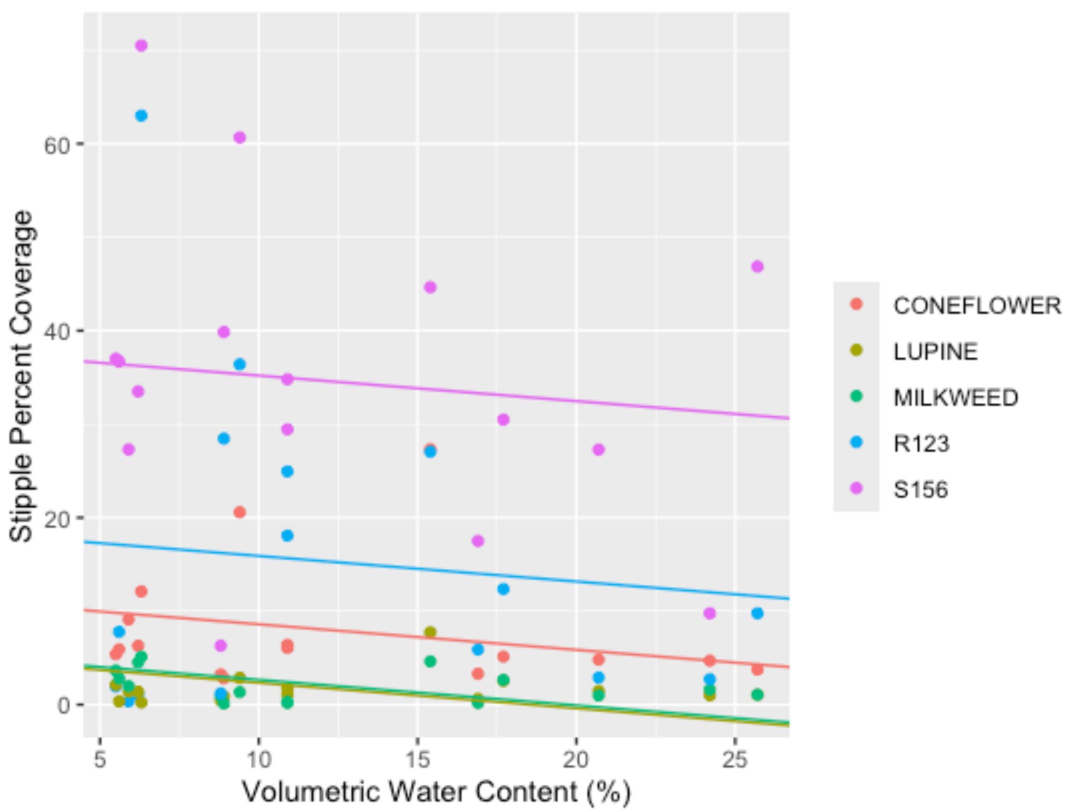


Figure 16. Stipple percent coverage and volumetric water content for all types of plant. The S156 sensitive snap bean can be seen in purple, the R123 resistant snap bean in blue, Cutleaf coneflower in orange, Common milkweed in green, and Wild lupine in yellow.

Lines correlating to each plant type were found in RStudio using the model constraining all slopes to be the same for each plant type. All plant types experienced a slight decline in stipple coverage with an increase in volumetric water content. When tested in RStudio, volumetric water content had a P-value of 0.4600, meaning that volumetric water content is not significantly correlated to stipple coverage explained by plant type.

Both the S156 sensitive snap and the R123 resistant snap were highly significant with stipple coverage due to plant type (P = < 2e-16) when tested in a generalized linear mixed model of stipple coverage versus plant type with a random effect of time. This finding shows that the variants had a significant effect on stipple coverage and stipple measurements for both variants have a significant difference from zero. It was also found that Cutleaf coneflower had a significant effect on stipple coverage (p = 1.08e-12). Common milkweed (P = 0.0123) and Wild lupine (P = 0.0966) had no significance with stipple coverage.

When tested using a generalized linear mixed model of photosynthesis measured by plant species, all plants had high significance with photosynthesis (P = < 2e-16). This shows that plant species and variants had a significant effect on photosynthesis.

When tested using a generalized linear mixed model of stomatal conductance measured by plant species, all plants had high significance with conductance. Wild lupine had the most significant relationship with conductance (P = 3.22e-13), followed by Cutleaf coneflower (P = 2.48e-11), and the S156 sensitive snap bean (P = 5.07e-10). The R123 resistant snap bean (P = 1.31e-06) and Common milkweed (P = 1.18e-06) were also found to be significantly related to conductance.

Discussion:

Our hypothesis that native and sensitive plant species will exhibit stipples while the resistant species will have significantly fewer stipples was partially supported through statistical analysis. The S156 sensitive snap bean and the R123 resistant snap bean both had a highly significant relationship with stipple coverage when tested for significance to stipple coverage due to plant type in a generalized linear mixed model. The only other plant type that had a significant relationship with stipple coverage when tested was from Cutleaf coneflower. Common milkweed and Wild lupine both did not have a significant relationship with stipple coverage.

When plant type was tested by stipple coverage using Tukey's post hoc HSD test, the S156 sensitive snap bean was significantly different from all other plant types. Cutleaf coneflower was significantly different from all other plant types except for the R123 resistant snap bean. The S156 sensitive and the R123 resistant snap bean were significantly different from Common milkweed and Wild lupine, showing that stipples significantly affect the S156 sensitive and the R123 resistant snap bean over Common milkweed and Wild lupine. The S156 sensitive snap bean had significantly more stipples than the R123 resistant snap bean, supporting their uses as sensitive and resistant controls for ozone indication. Common milkweed and Wild lupine were significantly different from all other plant types except each other for having the least amount of stipple coverage.

The significantly greater coverage of stipples of the S156 sensitive snap bean and the significant coverage of stipples of the R123 resistant snap bean supports our hypothesis that the sensitive plant species will exhibit stipples and our hypothesis that the resistant species will have significantly less stipples. The significant coverage of stipples of Cutleaf coneflower supports its use as a native ozone bioindicator species, but does not support our hypothesis that native bioindicator plant species will exhibit stipples while the resistant species will have significantly less stipples. This is because the resistant species and Cutleaf coneflower were found to not have significantly different stipple coverage. The coverage of stipples of Common milkweed does not support our hypothesis that native bioindicator plant species will exhibit stipples. Although this specific test was not stated in our original hypothesis, the coverage of stipples of Wild lupine does not support the use of this plant as an ozone bioindicator species. The significantly less coverage of stipples of Common milkweed and Wild lupine to Cutleaf coneflower, the R123 resistant snap bean, and the S156 sensitive snap bean supports their use as resistant species in ozone gardens.

Our second hypothesis that if no high ozone is recorded by the ozone sensor, then less to no stipples are expected was not supported by statistical analysis. Ozone or AOT40 ppb hours calculations and stipple percent coverage were found to not have a significant correlation. The possibility of the not significant correlation between ozone and stipple coverage may have been due to the low number of three sites that were tested for ozone air pollution and other variables through the ozone garden within the area. Another possibility may have been due to ozone not varying enough because it was always relatively high during the testing period. This may have been a possibility because the study period was from the start of July to the end of August, which are months when ozone is usually high.

Other studies have found a significant direct effect of high ozone on stipple production or coverage. These studies have included many sites, as well as years studying the effects of ozone on stipple production. One study utilized tobacco Bel-W3 (sensitive to ozone) to visualize and confirm the gradient of ambient ozone from northern to southern Europe in over 100 sites

(Klumpp et al., 2006b). More sites in the Chicagoland area could have helped to diminish the variability in data and obtained results like these studies.

Another reason for the not significant correlation between ozone and stipple coverage may be due to the wildfire smoke in the first two weeks out of the eight weeks of testing. The PM or particulate matter concentrations are increased during wildfires, which offsets the ozone increase (Zhang et al., 2022). This is because there is an increase in NO during a wildfire which binds with ozone to temporarily decrease ozone concentration, similar to the city/suburban effect. The first two weeks of ozone data that were affected by wildfire smoke could have also been a reason for the not significant relationship between stipple coverage and AOT40 ppb hours.

Our third and last hypothesis that if the ozone garden is water stressed, the effect of ozone damage will be diminished, leading to both sensitive and resistant species having less stipples was somewhat supported through statistical analysis. The ORD site was water stressed with a volumetric water content of less than 8% throughout the study period because plants with less than 30% are in extreme water shortage (Ladányi et al., 2021). Both the PNNM and HP sites got drier throughout the study period seen by the negative trend versus time in volumetric water content in Figure 8. They were also water stressed because they had volumetric water contents of less than 30% throughout the study period. This qualitative observation may help to explain why the association of ozone concentration to stipple percent coverage was not significant or that the effect of ozone damage was diminished, partially supporting our hypothesis. In some studies, researchers found that the uptake of ozone from the stomata of susceptible plants is diminished with the increase in drought, decreasing the effects of ozone (Lombardozzi et al., 2012). The plants might have been undergoing this phenomenon of water stress reducing the stippling effects of ozone.

Photosynthesis and conductance were both significantly correlated with volumetric water content at both sites (Figure 12 and Figure 13). This correlation was negative, meaning that as volumetric water content increased, photosynthesis and conductance decreased. This finding was counterintuitive to how photosynthesis and conductance are usually associated with volumetric water content. It would not further support our third hypothesis that both the HP and PNNM sites were water stressed during the study period or that the effect of ozone damage was diminished. This counterintuitive result could be due to the natural life cycle of the plants. Since the study period was during July and August, the plants could have been nearing the end of their life cycle and be experiencing normal declines in photosynthesis and conductance. The ozone damage could have also been negatively affecting the photosynthesis and conductance of the plants since the start of the study period. This is because the stipple coverage data collected showed stipple coverage as soon as the first day of testing.

The finding that there was the most ozone or AOT 40 ppb hours produced in the HP site over the PNNM and the ORD sites was consistent with past studies. The PNNM and ORD sites are both located within the city of Chicago, or in Cook County while the HP site is located just outside the area in Lake County. Higher ozone concentrations were found in the suburbs in this and past studies due to the reactions that form ozone (Fishman et al., 2014). Ozone can become consumed by the NO emitted into the atmosphere in cities from higher traffic and other fossil fuel combustion activities. The reaction can be seen below:

$$NO + O_3 \rightarrow NO_2 + O_2$$

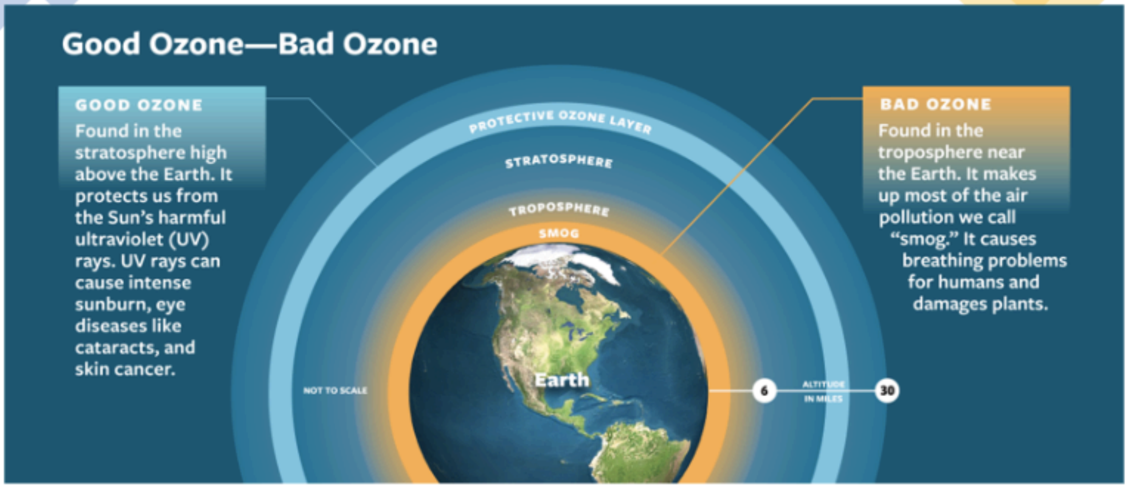
NO also has a shorter lifespan than NO_2, which can be transported by wind to rural or suburban areas. Here, NO_2 can create ozone with oxygen after photolysis, farther away from the city center (Pinto et al., 2010).

$$NO_2 + h\nu \rightarrow NO + O$$

$$O + O_2 \rightarrow O_3$$

The location of the ozone garden at the PNNM site in Lincoln Park was placed in a public-facing and highly foot-trafficked area near the entrance of the museum. There were two signs in visible areas. One indicated the purpose of the garden with visual stipple coverage of plant types. The other sign explained the pollutant ozone and how it differed from the ozone layer we wish to protect. These signs were read by informal learners passing by the garden and can be viewed in Figure 16.

What is Ozone?



Adapted from the National Center for Atmospheric Research
https://www2.cgd.ucar.edu/research/ozone-garden/

Collaboration between DePaul University and
Peggy Notebaert Nature Museum

Welcome to the

Ozone Bioindicator Garden

These gardens visualize how ground level ozone pollution damages plants with cultivars that are sensitive to ozone pollution by showing visible signs of damage.

This damage is in the form of stipples which are brown colored spots on the top sides of leaves.

Coneflower | Snap Bean | Milkweed

- No Damage
- Moderate Damage
- Severe Damage

Collaboration between DePaul University and
Peggy Notebaert Nature Museum

Adapted from the National Center for Atmospheric Research
https://www2.cgd.ucar.edu/research/ozone-garden/

Figure 16. The Peggy Notebaert Nature Museum (PNNM) site ozone garden, including signage behind the rows of plants.

While collecting data with the LI-6400/XT Portable Photosynthesis System, I was approached by the public with questions about the ozone garden at the PNNM site. These individuals' ages ranged from young children to older adults visiting the museum or walking in Lincoln Park. They engaged in active learning and asked questions about what I was measuring and how it affected them. Some would not engage me and instead went to the signs to engage in informal learning themselves. While there was no official experiment done on this aspect of the ozone garden, there was active informal learning happening. A future incorporation of a public survey of engagement would be a feasible way to measure effectiveness of the ozone garden.

Ozone gardens remain a feasible and effective way of communicating the damage of ozone to the community and greater population. Signage in gardens has the potential to be studied in environmental education as was seen in the PNNM site. The S156 sensitive snap bean, the R123 resistant snap bean, and Cutleaf coneflower, were shown to be significantly associated with stipple coverage throughout the study period. Their use in an ozone garden should be able to visualize the damage of ozone air pollution effectively throughout a given study period to test local ozone air pollution concentrations against air sensor readings. The effects of water stress on stipple coverage due to ozone air pollution can be studied further as there were relevant associations found with soil volumetric water content, photosynthesis, and conductance.

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