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EFFECTIVENESS OF *AILANTHUS ALTISSIMA* AS A BIOINDICATOR OF OZONE POLLUTION

A Thesis in

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Abstract

Ground-level ozone is one of the most significant air pollutants in the US; it is harmful to human health, vegetation, and ecosystems. Concurrently, *Ailanthus altissima* (tree-of-heaven, Ailanthus) is one of the most invasive plant species in the US. A series of exposure experiments and field surveys were conducted during 2010 and 2011 to evaluate the effectiveness of Ailanthus as a bioindicator of ozone pollution. To explore the uniformity of Ailanthus' reaction to ozone, Ailanthus seedlings from 6 locations (Reno, NV; Corvallis, OR; Bloomington, IN; Mineral, VA; State College, PA; and Far Rockaway, NY) were exposed to ozone using continuously stirred tank reactor chambers within a greenhouse. Seedlings from the Corvallis, OR seed source were planted in the field and monitored for ambient ozone-induced foliar injury along with staghorn sumac, black cherry, common milkweed, and dogbane to evaluate Ailanthus' performance compared to other ozone bioindicators. Potted Ailanthus seedlings were exposed in a greenhouse to 5 different ozone concentrations to determine if a relationship existed between concentration of ozone exposure and foliar injury. Plants from Corvallis, OR were significantly more susceptible to ozone exposure than were other seed sources ($P < 0.0152$). Ambient ozone levels in Pennsylvania during the growing season were sufficient to induce foliar symptoms on all surveyed flora species, including Ailanthus, in the field, and Ailanthus preformed comparably to the other bioindicators. Also, a positive linear relationship existed between ozone exposure concentration and foliar injury on Ailanthus plants ($R^2 = 0.8543$). We conclude that, aside from the variability in the germplasm, Ailanthus is an effective bioindicator of ground-level ozone pollution.

Table of Contents

List of Figures

List of Tables

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Introduction

Tropospheric ozone is harmful to human health, vegetation, and ecosystems (US EPA 2003). When vegetation is exposed to ozone, various plant physiological processes are impaired, including photosynthesis, water use efficiency, rate of senescence, dry matter production, flowering, pollen tube extension, and yield (Krupa et al. 2001, Krupa 1997). Ozone is responsible for $500 million in reduced crop production in the United States each year (US EPA 2003). Therefore, it is important to monitor levels of ozone in the environment and its effect on ecosystems.

A biological indicator species, or bioindicator, is an organism whose function, population, or status can be used to help determine the health of its ecosystem. A useful bioindicator is common, sensitive to the disturbance or stress, provides an easily recognizable and measurable response, responds in proportion to the degree of stress, and exhibits genetic stability. Bioindicators are important for environmental monitoring since it is not always possible to use electronic environmental-testing equipment in the field.

Several species of plants are useful bioindicators of ozone pollution, such as *Prunus serotina* Ehrh. (black cherry) and *Asclepias syriaca* L. (common milkweed) (US DOI 2003). One example of an organization that uses these plants is the Forest Inventory and Analysis (FIA) program of the United States (US) Forest Service. The FIA program monitors ozone-induced foliar injury data on a nationwide grid of ozone biomonitoring plots. Since the discovery that leaf stipple on grape was caused by ozone (Richards et al. 1958), adaxial stipple has been the classic symptom used to evaluate ozone injury on broadleaved bioindicators in the field (Skelly 2000, Skelly et al. 1987).

Ailanthus altissima (Mill.) Swingle (tree-of-heaven, Ailanthus) is a deciduous tree in the Quassia Family (Simaroubaceae). Ailanthus is considered an invasive species in the US, Australia, New Zealand, and several countries in southern and eastern Europe because of the tree's ability to rapidly colonize disturbed areas and suppress competition with allelopathic chemicals. Ailanthus is considered to be a bioindicator of ozone pollution by the US Department of Interior (US DOI 2003). However, a detailed study on the effectiveness of Ailanthus as a bioindicator and the species' consistency of bioindicator characteristics across the country has not been undertaken. Furthermore, the FIA has yet to include Ailanthus in its surveys of ozone-induced foliar injury (USDA 2011). The objective of this thesis is to evaluate Ailanthus for its efficacy as a bioindicator of ozone pollution.

Two different experimental approaches were used to fulfill this objective. Ailanthus seedlings were exposed to ozone under controlled conditions in a greenhouse to test for variability in species' reaction to ozone, species' sensitivity to ozone, and a relationship between ozone exposure concentration and foliar damage. Additionally, Ailanthus seedlings were planted in a field site in order to observe Ailanthus' reaction to ambient levels of ozone in Pennsylvania and to compare Ailanthus' response to other bioindicators of ozone pollution.

Methods

Facilities

A greenhouse equipped with a charcoal filtration air supply system (resulting in indoor ambient ozone levels of <8 ppb) on the Pennsylvania State University campus in University Park, Pennsylvania, USA was used in this research. Within the greenhouse, 16 continuously stirred tank reactor (CSTR) chambers were utilized. CSTR chambers were connected to an ozone generator and a computer monitoring system capable of measuring and controlling the concentration of ozone in each chamber. Temperature was monitored in the chambers in 2011 using thermocouples and data were recorded on data loggers.

The field survey was conducted at the Russell E. Larson Agricultural Research Center at Rock Springs in Centre County, Pennsylvania. The Ailanthus included in our survey were planted in prepared plots in a mowed field. The plots were subject to invasion by weeds, the most dominant being *Medicago falcate* L. (yellow-flowered alfalfa). The survey also included fragmented northeastern temperate forest habitat and abandoned pasture habitat. Nearby vegetation in the forest habitat was dominated by *Elaeagnus umbellate* Thunb. (autumn olive) and *Prunus serotina* Ehrh. (black cherry), and the abandoned pasture habitat was dominated by *Dactylis glomerata* L. (orchardgrass) and *Phleum pretense* L. (timothy). The dominant soil type in the area was Hagerstown silt loam (fine, mixed, semiactive, mesic, Typic Hapludalf) (Braker 1981). The average temperature during the growing season (May 1 - September 30) was 19.8 ºC and the average precipitation was 0.7 cm per day in this region. Ambient ozone concentrations for the field survey were measured at EPA AIRS Site #42-027-0100, located approximately 16.8 km northeast of Rock Springs.

2010 Experimental Procedures

Ailanthus seed was acquired from 6 locations across the US, and within each location, the seed set was sampled from one mother plant (Fig. 1). The seed sources were: Reno, Nevada (NV); Corvallis, Oregon (OR); Bloomington, Indiana (IN); Mineral, Virginia (VA); State College, Pennsylvania (PA); and Far Rockaway, New York (NY).

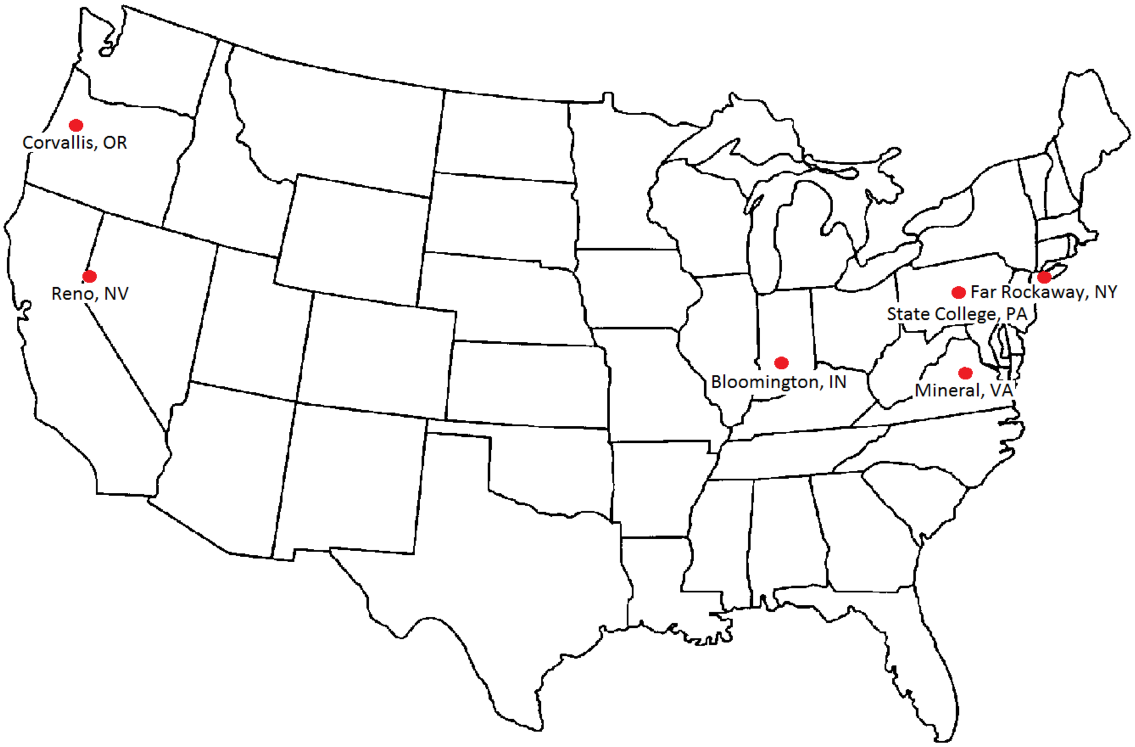

Figure 1. Spatial distribution of *Ailanthus altissima* seed sources used in the 2010 exposure experiment. Each dot designates the location of a seed source.

In preparation for planting, seeds from each seed source were placed in the laboratory refrigerator at approximately 2 °C for 2 weeks to simulate over-wintering. Seeds were germinated on flats in the greenhouse, and 64 seedlings from each seed source were planted into individual 1.95 L (0.5 gal) pots containing SUN GRO Metro-Mix 200 series growing medium (Sun Gro Horticulture Canada Ltd, Bellevue, WA) and fertilized with approximately 5.0 g (<1 tsp) of Osmocote Plus (15N-9P-12K) fertilizer (The Scotts Company LLC, Marysville, OH). Seedlings were arranged randomly on benches within the greenhouse and were watered as needed throughout the experiment. Prior to ozone exposure, 4 seedlings from each seed source were randomly assigned to 1 of the 16 CSTR chambers and to a location within that chamber. These locations were maintained throughout the experiment.

Controlled exposures were initiated on August 9 when the seedlings were approximately 17 cm tall. Although seedlings were similar in age, height growth varied among seed sources. Mean initial height of each seed source in cm was 22.2 (NY), 18.3 (NV), 17.9 (VA), 17.6 (PA), 13.9 (IN), and 12.1 (OR). Seedlings nearest to 17 cm tall were selected for the experiment. During exposure to ozone, 7 of the 16

CSTR chambers were set at 75 ppb ozone, 7 at 120 ppb, and 2 chambers were set at 0 ppb ozone (the control treatment). The 75 ppb ozone concentration was chosen since it was near the threshold of the US Environmental Protection Agency (EPA) National Ambient Air Quality Standards (NAAQS) for ozone. The 120 ppb ozone concentration was selected to represent the ozone concentration in a highly polluted city. The ozone generator was turned on (thereby raising the ozone level in each CSTR chamber to its assigned concentration) for 8 hr/day, 5 days/week (M-F) for 3 weeks.

Plants were visually evaluated for ozone injury at the end of each fumigation week (Sunday) using a modified Horsfall-Barratt classification scale (Horsfall and Barratt 1945). Each plant was rated from 0-5 for amount (AMT) and severity (SEV) of foliar injury. The rating system corresponds to the following percentages: 0 = 0%; 1 = 1-6%, 2 = 7-25%, 3 = 26-50%, 4 = 51-75%, and 5 = >75%. The AMT of injury describes the percentage of leaves on the plant that were injured, and the SEV of foliar injury describes the percentage of surface area that was injured on the affected leaves. Observations were also made on the foliar injury, including its color.

The AMT and SEV ratings for each plant were converted to percentage values representing the midpoint of each injury class as follows: 0 = 0%, 1 = 3.5%, 2 = 16%, 3 = 38%, 4 = 63%, and 5 = 88%. The AMT percentage was multiplied by the SEV percentage for each plant to represent whole plant injury, INJ_P (i.e., AMT* SEV = INJ_P). The mean of whole plant injury values for each seed source, denoted INJ_{SS}, was also calculated. A $\log_{10}$ data transformation was used to attain equal variances. A two-way Analysis of Variance (ANOVA) was used to test for significant differences ($\alpha = 0.05$) of final (week 3) injury values among seed sources and treatments, and a Tukey HSD was used to identify significant differences ($\alpha = 0.05$) among final injury values per seed source. Data were analyzed using the Statistical Analysis System (SAS) statistical software (SAS Institute Inc. 2011).

2011 Experimental Procedures

Field component. Ailanthus seed from the OR seed source were germinated and grown in the greenhouse using the same procedures as in 2010. Thirty-six seedlings (mean height 24 cm) were planted on June 13 at Rock Springs. Plants were supplemented with water during dry weather. Ozone-induced foliar injury was visually evaluated weekly for 9 weeks (July 18 – September 13) using the same methodology as in 2010.

Four native species of suspected or known ozone bioindicators (US DOI 2003) in the Rock Springs area were surveyed for ozone-induced injury in addition to Ailanthus. Surveyed plants consisted of 10 individuals each of *Rhus typhina* L. (staghorn sumac), *Prunus serotina* Ehrh. (black cherry), *Asclepias syriaca* L. (common milkweed), and *Apocynum cannabinum* L. (dogbane). These species were assessed for ozone-induced foliar injury during the same time period that Ailanthus was rated, using the same techniques.

Ambient ozone concentrations for the survey time period were downloaded from EPA AIRS Site #42-027-0100. The hourly ozone averages of 60 ppb or greater were added cumulatively over the growing season to calculate the SUM60 ozone index. The SUM60 ozone index was selected because it has been correlated with ozone-induced foliar symptoms within eastern US forests (Hildebrand et al. 1996).

Greenhouse component. Ailanthus seed from the OR seed source were germinated and grown in the greenhouse using the same procedures as in 2010. In 2010, it was observed that the ambient temperature within chambers varied and that plants in the hotter chambers developed less ozone-induced injury. Therefore, temperature in all chambers was monitored for 1 week prior to start of exposure in 2011 to establish a baseline. The overall mean temperature for each chamber was calculated, and data were used to classify the chambers into low (mean 24.6 ºC), medium (mean 25.2 ºC), and high (mean 25.8 ºC) temperature blocks (3 chambers per block).

Ozone exposures were initiated on July 11 when the seedlings were approximately 23 cm tall. Target ozone concentrations were 40 ppb, 60 ppb, 80 ppb, 100 ppb, and 120 ppb, which represented the broad range of ozone concentrations found in the US. Three CSTR chambers were programmed for each target ozone concentration, and 1 chamber was programmed to 0 ppb as a control. Each repetition of ozone concentration was randomly assigned to a chamber within each temperature block. Plant heights were measured at exposure initiation. Daily exposures were conducted 8 hr/day, 5 days/week (M-F) for 4 weeks, and plants were evaluated for ozone-induced foliar injury each Sunday using the same methodology as in 2010.

Ozone-induced foliar injury ratings were converted into percentages, and the AMT percentage was multiplied by the SEV percentage for each plant to represent whole plant injury, INJ_P, as in 2010. The mean final (week 4) plant injury values per chamber were calculated. A multiple regression analysis was developed using the mean final INJ_P values, mean ozone concentration, mean initial plant height (cm), and mean temperature (ºC) for each chamber. Two interaction terms were also used in the model: the interaction between temperature and ozone (teoz), and the interaction between height and ozone (heoz). Data were analyzed using the Statistical Analysis System (SAS) statistical software (SAS Institute Inc. 2011).

Results

2010

The amount of injury on Ailanthus leaves was influenced by the concentration and duration of ozone exposure and by seed source (Fig. 2). Foliar injury was greater in the 120 ppb treatment than the 75 ppb treatment, and injury increased over the duration of the experiment in both treatments. No foliar injury was observed on any plants in the control chambers (0 ppb). Plants from all seed sources in the 75 ppb treatment exhibited foliar injury after 1 week of ozone exposure except the plants from the NY seed source. Plants from the OR source exhibited the greatest amount of injury during the first week of exposure to 75 ppb ozone and exhibited the greatest amount of injury for the duration of the exposure. The plants from PA, NV, IN, and VA sources exhibited similar amounts of foliar injury symptoms throughout the 75 ppb exposure; plants from the NY source displayed the least amount of injury. Plants from all seed sources exhibited foliar injury after 1 week of exposure in the 120 ppb treatment. The plants from OR source exhibited the greatest amount of injury after the first week of exposure to 120 ppb ozone, and they continued to exhibit the greatest amount of injury for the duration of the exposure. Remaining plants displayed similar amounts of injury at the start and conclusion of the 120 ppb exposure. Overall, the plants from the OR seed source displayed the greatest amount of foliar injury and the plants from the NY source displayed the least amount of foliar injury in both the 75 ppb and 120 ppb treatments. The plants from the NY source were relatively insensitive to the low ozone treatment but showed symptoms at the greater ozone concentration.

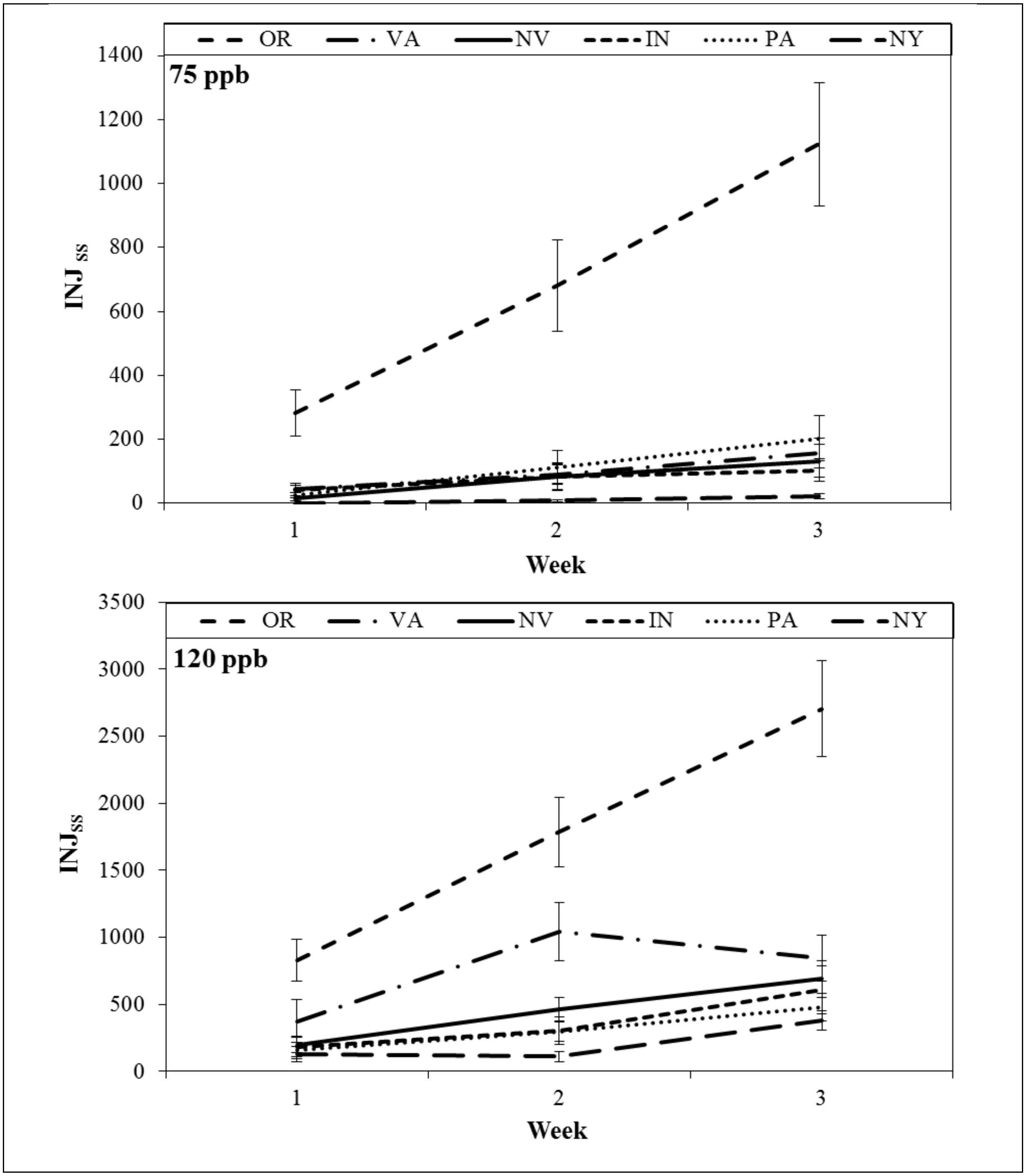

Figure 2. Mean foliar injury among *Ailanthus altissima* seedlings from each seed source (INJ_{SS}) per week for 75 ppb and 120 ppb ozone treatments in the 2010 exposure experiment. Error bars represent ± SE.

The two-way ANOVA indicated that ozone concentration and seed source had significant effects on foliar damage (P = <.0001). Tukey's test indicated that seedlings from the OR seed source were significantly different from seedlings from the other seed sources (Table 1). Furthermore, seedlings from the Mineral, VA seed source were significantly different from seedlings from the Far Rockaway, NY seed source. Remaining seed sources were statistically similar.

Table 1. Number of exposed Ailanthus plants and mean final (week 3) ozone-induced foliar injury among seedlings from 6 seed sources and all (0 ppb, 75 ppb, and 120 ppb) treatments ± SE.

Collection location	No. Exposed Plants	Mean[A] Final INJ_{SS}[B]	
Corvallis, OR	64	1674.68 ± 215.61	c
Mineral, VA	64	437.92 ± 88.44	b
Reno, NV	64	359.15 ± 73.20	ab
Bloomington, IN	64	311.08 ± 84.32	ab
State College, PA	63	298.51 ± 58.44	ab
Far Rockaway, NY	61	128.80 ± 35.78	a

[A] Denotes mean of the plant injury values for each seed source and all treatments ± SE.

[B] Means followed by the same letter are not significantly different ($\alpha = 0.05$) according to Tukey's test.

2011

Field component. The Ailanthus seedlings planted at Rock Springs displayed ozone-induced foliar injury symptoms when exposed to ambient ozone concentrations (Fig. 3). Ailanthus first exhibited injury on August 8. The level of injury increased in a non-linear fashion for the duration of the survey, except during August 15-22 when the injury decreased. The final mean whole plant injury value, INJ_P, for Ailanthus was 177.5. All Ailanthus plants displayed black stipple in the beginning of the monitoring period (August 8-22). The black stipple had transitioned completely to tan stipple on 31 of 36 plants (86%) by the conclusion of the survey.

All non-Ailanthus ozone bioindicators displayed ozone-induced foliar injury symptoms by the end of the monitoring period. Sumac plants exhibited the greatest foliar injury over the course of the experiment. Sumac plants were already exhibiting ozone-induced foliar injury symptoms at the initiation of the surveying period on July 18, and symptom severity increased rapidly over the duration of the survey. The mean INJ_P exhibited by sumac plants at the conclusion of the survey was 1842.0, which was more than 20 times greater than that of the most ozone-resistant species surveyed, dogbane. The rest of the surveyed species, including Ailanthus, displayed similar trends in mean injury per plant over the course of the experiment. Foliar-injury symptoms were first observed on common milkweed on August 1, and the average final INJ_P for milkweed was 396.4. Foliar-injury symptoms were first observed on black cherry leaves on August 15, and the average final INJ_P for black cherry was 475.2. Ozone-induced foliar injury symptoms were observed on dogbane plants on August 15 with a mean INJ_P of 84.4 at the conclusion of the survey.

The ambient levels of ozone at Rock Springs, as measured at the EPA AIRS Site #42-027-0100, first increased over 60 ppb ozone on May 6. The SUM60 of ozone continued to increase via a series of high ozone events over the course of the experiment. The highest ozone events occurred on June 8 and July 21-22. There were also periods of low ozone from August 2-17 and August 21 - September 12 when the SUM60 index did not increase. However, ozone-induced foliar injury increased even during periods of low ambient ozone concentration. Ozone-induced foliar injury was not observed on the majority of surveyed plants until the SUM60 had reached approximately 11,600 ppb.hrs (around August 8). The SUM60 at the conclusion of the survey was approximately 13,000 ppb.hrs.

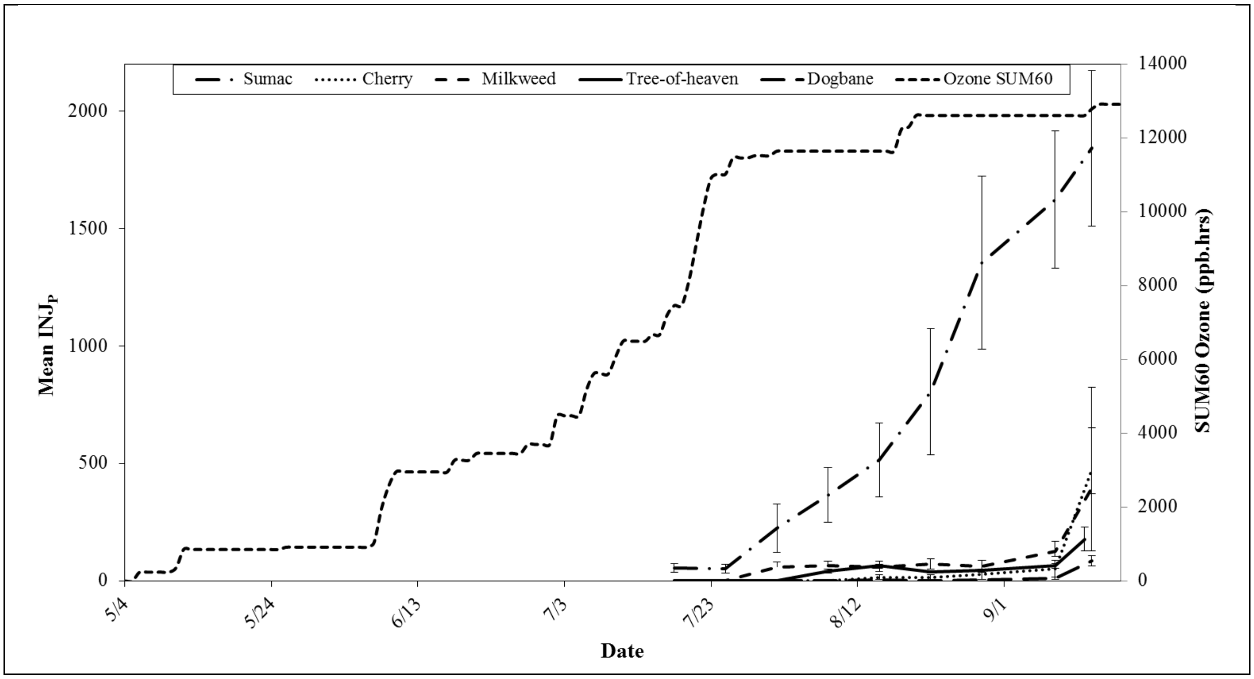

Figure 3. Cumulative ozone concentrations of 60 ppb or greater for the 2011 growing season (SUM60) and mean plant injury values (INJ$_P$) per species and sampling date in survey. Error bars represent ± SE.

Greenhouse component. The level of foliar injury on Ailanthus was influenced by the concentration and duration of ozone exposure (Fig. 4). Ailanthus did not exhibit injury when exposed to the control or 40 ppb ozone treatments. Ailanthus plants exposed to 60 ppb ozone did not exhibit any foliar injury symptoms during the first 2 weeks of the experiment, but 1 of 12 plants (8.3%) exhibited foliar injury during weeks 3 and 4 of exposure. Plants in the 80 ppb ozone treatment exhibited a small amount of injury symptoms during the first 3 weeks of exposure, which increased during week 4 of exposure. Plants in the 100 ppb ozone treatment exhibited levels of foliar injury symptoms similar to the 80 ppb treatment during the first week of exposure, and symptoms increased steadily over the course of the experiment until week 3 of exposure. Plants in the 120 ppb treatment exhibited an amount of foliar injury symptoms similar to the 100 ppb treatment during the week 1 of exposure, and the level of foliar injury symptoms increased steadily over the course of the experiment.

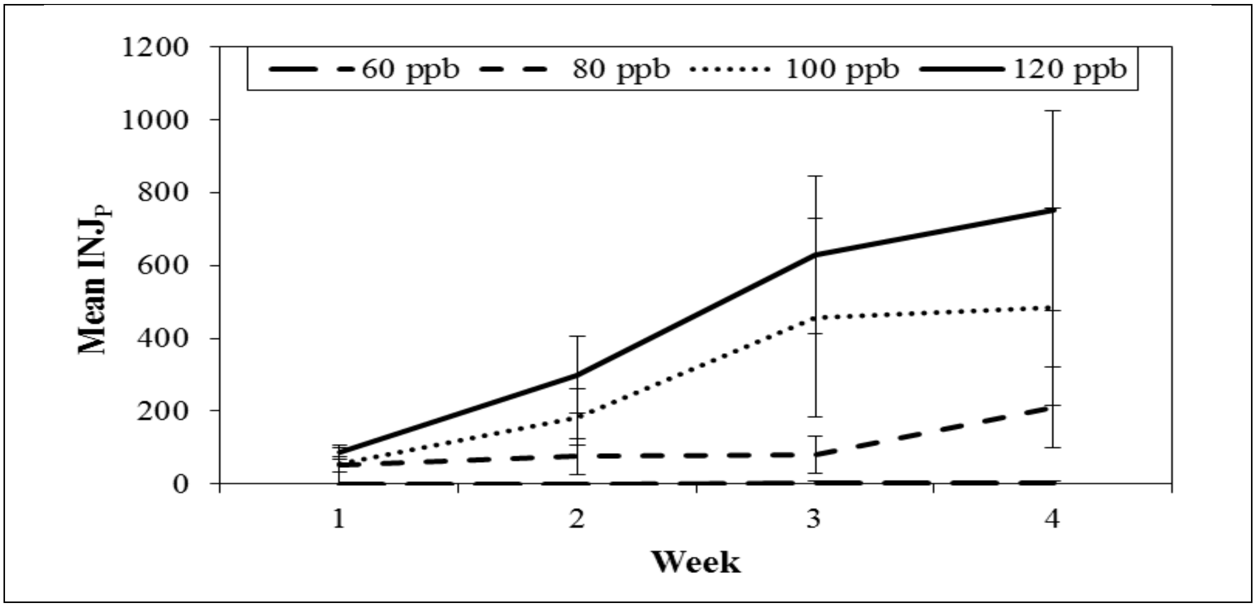

Figure 4. Mean plant injury values (INJ$_P$) observed on *Ailanthus altissima* per treatment and week of 2011 exposure experiment. No injury was observed on the control (0 ppb) or 40 ppb treatments. Error bars represent ± SE.

Multiple regression analysis indicated a significant relationship ($P = 0.0006$, $R^2 = 0.8543$) between variables in the model (ozone concentration, initial height, temperature, teoz, and heoz) and final INJ$_P$ values. The influence of all variables was significant, and ozone concentration had the greatest influence on INJ$_P$, followed by teoz, heoz, temperature, and height, respectively. Using the model output, the following model equation was constructed:

$INJ_P = -34992 + 424 * temp + 705 * ozone + 76 * height - 8 * teoz - 2 * heoz$

This regression model predicts that INJ$_P$ will increase with an increase in ozone concentration and will decrease with an increase in temperature and initial plant height.

Discussion

It was determined that seedlings from the OR seed source were significantly more susceptible to injury from ozone exposure than seedlings from the other seed sources. Although all seedlings used in the 2010 experiment were similar in age, the seedlings from OR were slower-growing and smallest in initial height. The smaller plants, containing younger leaves and less biomass, may have been more susceptible to ozone injury. However, others have reported that younger trees (<4 year) are affected less by ozone than older trees (Wittig et al. 2007) and younger leaves are affected less by ozone than older leaves (Zhang et al. 2010). Since tree age and height are correlated, these results imply that taller trees should be more susceptible to ozone injury, which is not supported by our findings. Alternatively, it may be that the seedlings in our experiment all exhibited a similar number of damaged leaves, and since the smaller seedlings had less total leaves, their AMT percentage ratings were higher, indicating a higher susceptibility.

The high susceptibility to ozone of seedlings from the OR seed source may also be related to genetics of the seed source. When Ailanthus was introduced to North America, it was first imported to Pennsylvania in 1784 and then to New York in 1820. These introductions were both from English stock imported from China. The final introduction of Ailanthus to the US was in California during the mid-1800’s gold rush when immigrating Chinese brought Ailanthus directly from China to California. Thus, seeds from OR may have differing genetics from other Ailanthus seed sources because they descended from a different introduction into the US and/or originated from a different part of China. Alternatively, different seed sources may have evolved tolerance to ozone based on the historical amount of ambient ozone stress present in each location. However, we determined that historical ambient ozone concentrations in Corvallis, OR were likely not significantly different from the other locations studied (unpublished data). Therefore, it is unlikely that adaptation to ozone stress occurred. Regardless of explanation, these results suggest a lack of genetic stability of Ailanthus, which is not a favorable trait of an effective bioindicator. Since seedlings from the OR seed source were the most susceptible to ozone-induced foliar injury, they may be the best choice for bioindicator of all seed sources tested.

Ailanthus planted at Rock Springs exhibited foliar injury symptoms from exposure to ambient levels of ozone. Ailanthus plants displayed black stipple in the beginning of the monitoring period, which gradually changed to tan stipple. In contrast, Ailanthus exposed to ozone in CSTR chambers under controlled conditions only displayed tan stipple. These 2 differing patterns of Ailanthus response to ambient ozone have also been observed in CSTR chambers and in the field by others (Davis and Coppolino 1974, Davis and Orendovici 2006). This discrepancy may be related to differences in ozone concentrations and environment of exposure between CSTR chambers and field conditions. For example, ozone concentrations in the CSTR chambers may not have been low enough, spaced out enough, or coupled with the right temperature, light, or humidity conditions to induce black stipple. Additionally, there could be some other parameter in the field environment, such as another air pollutant, which was inducing a reaction in the Ailanthus leaves to produce black stipple instead of tan.

All species surveyed at Rock Springs exhibited ozone-induced foliar injury symptoms by the end of the survey period. The EPA's secondary standard for ozone pollution, which was developed to protect crops and vegetation against injury, is that the 3-year mean of the fourth-highest daily maximum 8-hour mean ozone concentrations measured at each monitor within an area over each year must not exceed 75 ppb. The ambient concentration of ozone at Rock Springs, as measured by a nearby federal monitoring station, was not in violation of the EPA's secondary standard for ozone pollution ("8-Hour Ozone…" 2012). These results suggest that the current NAAQS for ozone may not be sufficient to protect sensitive vegetation from ozone exposure-induced injury.

Ozone-induced foliar injury was not observed on most plants until SUM60 had reached approximately 11,600 ppb.hrs. This implies that 11,600 ppb.hrs ozone may be the threshold concentration to which these plant species start to exhibit symptoms under the environmental conditions of central PA. These thresholds are similar to what others have estimated. For example, the threshold value of SUM60 ozone needed to cause foliar symptoms on other species of ozone-sensitive plants has been estimated to be approximately 9000 ppb-hrs or < 10,000 ppb.hrs in Missouri and South Carolina, respectively (Davis 2011, Davis 2009). However, we also observed that ozone-induced foliar injury increased even in periods of low ambient exposure. Therefore, there may be lag time between the increase in SUM60 ozone and an increase in the foliar injury symptoms exhibited by plants, as moderated by environmental conditions such as soil moisture.

Alternatively, ozone inducing more severe symptoms later in the growing season may be unrelated to SUM60. Our results support others who have noted that exposure induced more severe symptoms later in the growing season (Davis and Coppolino 1974). One explanation may be that older leaves are more affected by ozone than younger leaves (Zhang et al. 2010); thus, the sensitivity to ozone of the plants in our survey increased over the course of the growing season as their leaves aged. An alternative explanation is that later in the growing season is closer to the plants' fall senescence. As autumn approaches, deciduous trees begin to shut down and shed their leaves according to photoperiod, and this process of shutting down physiological functions may make the leaves more vulnerable to ozone injury. Others have shown that exposure to elevated levels of ozone increases rate of senescence in deciduous trees (Dermody et al. 2005).

Ailanthus exposed to ozone in the greenhouse chambers displayed greater foliar injury with increasing ozone concentration, except in the control and 40 ppb treatments. Ailanthus did develop symptoms when exposed to the 60 ppb and 75 ppb ozone treatments, which suggests that it is sensitive to relatively low concentrations of ozone. Overall, greater ozone-induced foliar injury and defoliation were observed in 2010 than 2011. Specifically, the seedlings from OR had a mean INJ_P for the 120 ppb treatment of 2705 ± 360 in 2010 and 627 ± 216 in 2011 after 3weeks. This may be due to the 2010 exposure occurring later in the growing season when plants were closer to their fall senescence or due to differences in starting height between these 2 experiments, as previously discussed. In 2010, the Ailanthus seedlings had a mean height of 12.1 cm at the start of exposure; in 2011, the Ailanthus had a mean initial height of 23.4 cm. Other possible reasons for this discrepancy include differences in temperature, humidity, light, or other growth factors between the 2 years. In addition, genetic differences in ozone-sensitivity as related to the seed sources (e.g. multiple paternal contributions) may have played a role.

Our regression model indicates a positive linear relationship between foliar injury and ozone concentration, as well as a negative linear relationship between foliar injury and temperature or initial height of the plant. The positive linear relationship between injury and ozone concentration implies that Ailanthus responds in proportion to the degree of stress, which is an important attribute of an effective bioindicator. The negative linear relationship between injury and temperature may be explained if the plants experienced moderate stress in the CSTR chambers that were warmer than ambient temperatures. It has been reported that plants respond to moderate stress by closing stomata (Reynolds-Henne, 2010), and it has also been reported that ozone injures leaves by entering through the stomata and reacting with water inside the apoplast (US EPA 2006). If the plants in the warmer chambers were experiencing stress induced by ozone exposure or increased temperature, they might close their stomata and intake less ozone, resulting in less foliar injury. Alternatively, the warmer chambers may have led to dryer soil, and lack of soil moisture generally decreases stomatal conductance of plants, which reduces the amount of ozone entering the leaf that can cause injury (Grulke et al. 2003, Matyssek et al. 2006, Panek, 2004). These regression results also suggest that shorter plants and leaves are more susceptible to injury induced by ozone exposure, which may be explained by a variety of factors, as previously discussed.

Conclusions

Ailanthus seedlings from all seed sources tested exhibited visible injury when exposed to ozone in CSTR chambers, though susceptibility to ozone differed among seed sources. Seedlings from the OR seed source were more vulnerable to foliar injury from ozone exposure than other seed sources. In the field, Ailanthus plants exhibited a level of visible injury comparable to commonly accepted ozone bioindicators. Foliar injury on Ailanthus seedlings can be induced at relatively low ozone concentrations, and a positive linear relationship between the concentration of exposure and the amount and severity of foliar injury symptoms was determined. We conclude that, aside from the variability in the germplasm, Ailanthus is an effective bioindicator of ground-level, ambient ozone pollution.

Appendices

Appendix A: Literature Review

A. Air Quality and Society

Air quality is a term used to describe the relative condition of ambient air. When air quality is high or good, it means that the ambient air has a low concentration of pollutants and is safe to breathe, and when air quality is low or bad, it means that the air may be polluted and harmful to sensitive populations, such as persons with asthma or emphysema. By definition, an air pollutant is any substance in the air that could, in high concentration, harm humans, animals, vegetation, or other materials. Air pollutants include ozone, sulfur oxides, nitrogen oxides, carbon monoxide, carbon dioxide, volatile organic compounds, particulate matter, and ammonia (US EPA 2012). Air pollutants are produced by combustible engines; burning coal, oil, and other fossil fuels; and manufacturing chemicals (US EPA 2012). When humans are exposed to these compounds, they can irritate the eyes, throat, and lungs (US EPA 2012). Some symptoms of air pollution exposure include burning eyes, cough, tightness of the chest, and in extreme cases, death (US EPA 2012).

Throughout early history, humans were mostly unaware of the concept of air quality and their influence upon it. As cities and industries grew over time, their negative impact on air quality also increased. During the industrial revolution, large machines and factories came into operation, powered by the burning of fossil fuels, such as coal and oil. By the 1950's people were becoming aware of the consequences of their unchecked air pollution, especially after the fatal air pollution incidences of Donora, Pennsylvania in 1948 and the London killer smog in 1952. In 1956, Great Britain passed a Clean Air Act, requiring the use of cleaner fuels, better furnaces, and electric heat to reduce the amount of air pollution being released into the environment. In 1963, the United States' Congress passed a Clean Air Act, which requires the Environmental Protection Agency (EPA) to develop and enforce regulations to protect the public and our environment from exposure to airborne contaminants that are considered to be hazardous. The regulations include the establishment of National Ambient Air Quality Standards (NAAQS) for criteria air pollutants, which are ozone, particulate matter, carbon monoxide, nitrogen oxides, sulfur dioxide, and lead (US EPA 2012). In part, the US Clean Air Act was amended in 1970 and 1990 due to a growing public concern over the health effects associated with air pollution.

The regulation and enforcement mandated by the Clean Air Act has had a positive influence on our air quality in the United States. Since 1990, national annual air pollutant emissions have declined significantly (US EPA 2012). Direct $PM_{2.5}$ emissions (particulate matter in the air, including dust, dirt, soot, smoke, and liquid droplets, that are less than 2.5 micrometers in diameter) have declined by more than half; PM_{10} and SO_2 emissions have declined by more than 60 percent, and NO_x and VOC emissions have declined by more than 40 percent (US EPA 2012). The combined emissions of the 6 common pollutants and their precursors ($PM_{2.5}$ and PM_{10}, SO_2, NO_x, VOCs, CO, and lead) declined 59 percent on average from 1990 to 2010 (US EPA 2012). The associated air quality benefits of this decline have likely lead to improved health, longevity, and quality of life for all Americans.

B. Ozone

Ozone is a molecule that consists of 3 oxygen atoms (molecular formula: O_3) and occurs as a gas in the Earth's upper atmosphere and at ground level. Ozone in the stratosphere is mostly produced naturally from ultraviolet rays reacting with oxygen. Tropospheric (or ground-level) ozone is created by chemical reactions between oxides of nitrogen (NO_X) and volatile organic compounds (VOC) in the presence of sunlight (US EPA 2003). Sources of NO_X include motor vehicles, utilities, and combustion; and sources of VOCs include industrial/commercial processes, motor vehicles, and consumer solvents (US EPA 2003). Therefore, an increase in these activities leads to an increase in tropospheric ozone concentration. Ozone is also the main component of urban smog (US EPA 2003).

In the stratosphere, ozone filters out UV rays from the sun that would be harmful to plants and animals on Earth (US EPA 2003). This ozone is part of the "ozone layer," which helps to allow life on Earth. In the troposphere, breathing ozone can trigger a variety of health problems including chest pain, coughing, throat irritation, and congestion (US EPA 2003). It can worsen bronchitis, emphysema, and asthma (US EPA 2003). Tropospheric ozone also can reduce lung function and inflame the lining of the lungs, and repeated exposure may permanently scar lung tissue (US EPA 2003).

C. Plant Response to Ozone and Symptoms

In addition to human health, tropospheric ozone is harmful to vegetation and ecosystems (US EPA 2003). Ozone causes negative effects on a number of plant processes, including photosynthesis, water use efficiency, rate of senescence, dry matter production, flowering, pollen tube extension, and yield (Krupa et al. 2001, Krupa 1997). It leads to foliar injury, reduced agricultural crop and commercial forest yields, reduced growth and survivability of tree seedlings, and increased susceptibility to diseases, pests and other stresses (US EPA 2003). In the United States alone, tropospheric ozone is responsible for an estimated $500 million in reduced crop production each year (US EPA 2003).

Ozone enters plants through stomata in the leaves (US EPA 2006). Once inside the substomatal cavity, ozone is thought to rapidly react with water in the apoplast and form reactive oxygen species, such as hydrogen peroxide, superoxide, and hydroxyl radicals (US EPA 2006). These chemicals injure the inside of plant leaves and impair physiological functions (US EPA 2006). For example, when injury is done to the plasma membranes of cells, fluidity, permeability, potassium exchange capacity and calcium exclusion abilities are all affected, which prevent the cell from functioning normally (US EPA 2006). This injury to the leaves is visible to the naked eye as a dark or tan adaxial stipple, chlorosis, and necrosis (US EPA 2006). The plant must expend energy to repair the injury done and defend itself against further harm. Therefore, cellular injury from ozone exposure can change rates of leaf gas exchange, growth, and reproduction at the individual plant level (US EPA 2006). These ozone-induced effects at the individual plant level can greatly impact the ecosystem and cause changes in ecosystem services, such as carbon storage, water production, nutrient cycling, and community composition (US EPA 2006).

D. Importance and Use of Bioindicators

A biological indicator species, or bioindicator, is an organism whose function, population, or status can be used to determine something about the health of its ecosystem. Many bioindicators are species that are sensitive to a particular pollutant, and when in the presence of that pollutant, exhibit specific symptoms that can be interpreted by a knowledgeable observer. A classic example of a bioindicator is the common canary. Before the invention of air quality testing equipment, coal miners in the UK and US would bring canaries into coal mines as an early-warning sign for toxic gases, including carbon monoxide and methane. The birds, which are more sensitive to toxic gases than humans, would become sick before the miners, thus giving the miners time to escape or take protective measures. Numerous species of insects in the order Ephemeroptera are also used as bioindicators. The order Ephemeroptera includes several species of mayflies in North America, which spend the first part of their lives living in streams as mayfly nymphs. Many species of mayfly nymphs are sensitive to chemical pollution and low levels of dissolved oxygen in the water, and thus, the presence or absence of these animals can indicate the pollution status of a body of water.

Bioindicators are important for environmental monitoring since it is not always feasible or practical to bring environmental-testing equipment into the field for analysis. When this is the case, bioindicators may be used to estimate the presence, absence, or amount of a particular pollutant. The most useful bioindicators are those that are common, are sensitive to the disturbance or stress, provide an easily recognizable and measurable response, respond in proportion to the degree of stress, and exhibit genetic stability.

The Forest Inventory and Analysis (FIA) program of the United States' Forest Service is an example of a federal program that uses bioindicators routinely. The FIA runs a continuous census of America's forests in which surveyors gather a myriad of information on the nation's forests, including forest health. The "Ozone Indicator" is one of the forest health variables measured by the FIA program. In this program, ozone-induced foliar injury data are collected from field sites on a nation-wide grid of ozone biomonitoring plots. These foliar data consist of ratings of the frequency and severity of ozone-induced injury determined on the leaves of bioindicator plant species, such as blackberry, black cherry, milkweed, and yellow poplar. The FIA program monitors ozone bioindicators because they offer information on forest condition and health by providing a visible link between ozone exposure and the effects of that exposure on productivity, biodiversity, and aesthetics.

E. Ailanthus altissima

Ailanthus altissima (tree-of-heaven, Ailanthus) is a deciduous tree in the Quassia Family (Simaroubaceae). It is a fast-growing, medium-sized tree that reaches heights between 17 and 27 meters (56 and 90 ft) with a diameter at breast height of about 1 meter. The bark is smooth and light grey and often develops light tan fissures and becomes rougher as the tree ages. The leaves are large, odd or even pinnately compound and contain 10–41 leaflets organized in pairs. Ailanthus reproduces both sexually, through seeds, and asexually by vegetative sprouts. The flowers are small, yellowish green to reddish in color and appear in large panicles up to 50 cm in length at the end of new shoots. The flowers of the male tree have an unpleasant odor likened to peanuts or cashews. The fruit is a papery, somewhat twisted, winged samara that occurs in large clusters from September to October. Ailanthus has been shown to produce a chemical (ailanthone), which inhibits the growth of many other plant species (Heisey and Heisey 2003).

Ailanthus is native to both northeast and central China and Taiwan, and it is not considered invasive in its native range. The tree was first brought from China to the United States in 1784. It was initially regarded as a beautiful garden specimen and valued for its tolerance of difficult growing conditions. However, gardeners soon lost interest in the tree after becoming familiar with its suckering, weedy habits and its foul odor. Despite this, Ailanthus was used extensively as a street tree during much of the 19th century. Currently, Ailanthus is considered an invasive species in the United States, Australia, New Zealand, and several countries in southern and Eastern Europe because of the tree's ability to quickly colonize disturbed areas and suppress competition with allelopathic chemicals. However, in some parts of the world, Ailanthus is still used as an ornamental plant for its wood, medicinal properties, and as a host plant to feed silkworms of the moth *Samia cynthia*. In the US, Ailanthus can be found in at least 44 of the 50 states (88%), including Hawaii (unpublished data).

F. Ailanthus Reactions to Ozone

Ailanthus exposure to ozone has been observed and documented several times in the literature. The first mention of Ailanthus's sensitivity to ozone was made by Davis and Coppolino (1974). In this experiment, more than 1000 plants representing 15 species and/or cultivars of woody ornamentals were exposed to 0.25 ppm ozone for 8 hours at bi-weekly intervals throughout the 1973 growing season (Davis and Coppolino 1974). Of 35 Ailanthus plants exposed in their trial, 26 of them were susceptible (74.3%) (Davis and Coppolino 1974). They noted that the first symptom observed on Ailanthus was a uniform, light tan stipple and most leaflets were affected on injured leaves (Davis and Coppolino 1974). Later in the growing season, exposure induced more severe symptoms, which appeared as large, scattered black stipples as well as chlorosis (Davis and Coppolino 1974). Leaves that became chlorotic ranged in color from light green to yellow (Davis and Coppolino 1974). Also, leaflet tips became necrotic, necrotic patches appeared between leaf veins, and occasionally, an entire leaflet became brown and desiccated (Davis and Coppolino 1974).

Gravano et al. published 2 papers documenting Ailanthus' exposure to ozone in 2 separate experiments in Italy (Gravano et al. 1999; Gravano et al. 2003). In the first experiment, Gravano et al. tried to reproduce symptoms they had seen in the field by exposing Ailanthus plants to 50 or 100 nl/l (*i.e.* 50 or 100 ppb) ozone for 5 hours a day for 15 days in fumigation chambers (Gravano et al. 1999). In the second experiment, Ailanthus plants were placed outside in 2 sites and exposed to a concentration of 31 ppm/h ozone at the high ozone site and 11 ppm/h at the low ozone site (Gravano et al. 2003). In the second experiment, Ailanthus plantlets started exhibiting symptoms when ozone values reached 5 ppm/h (Gravano et al. 2003). In both experiments, they noted that ozone symptoms consisted of whitish (ivory) stipples on the adaxial surface of the leaf that turned quickly into brown necrotic spots, and the older leaves and leaflets inserted in the basal portion of the compound leaves were the most affected organ (Gravano et al. 1999; Gravano et al. 2003). Gravano et al also noted that the injured leaflets were shed early and the growth of the whole plant was reduced due to the ozone exposure (Gravano et al. 1999; Gravano et al. 2003)

Davis and Orendovici evaluated several tree species in the Forsythe National Wildlife Refuge in New Jersey for the percentage of plants exhibiting ozone-induced foliar symptoms between 1993-1996 and 2001-2003 (Davis and Orendovici 2006). They observed that plants most sensitive to ozone included Ailanthus (12.8% individuals exhibiting stipple), which was the third most sensitive of the plants studied, behind common milkweed (26.4%) and wild grape (23.3%) (Davis and Orendovici 2006). Davis and Orendovici quantified ozone exposure as SUM60, which is defined as the cumulative ozone concentration of 60 ppb or greater for the growing season, and during the experiment, Ailanthus was exposed to a SUM60 ranging from 25,000 to 50,000 ppb-h each year (Davis and Orendovici 2006). In the study, they noted that classic, dark adaxial stipples was the most common foliar symptom; however, Ailanthus often exhibited a typical light-brown to tan stipples, which was not recorded as an ozone-induced symptom (Davis and Orendovici 2006).

Appendix B: Additional Tables and Figures

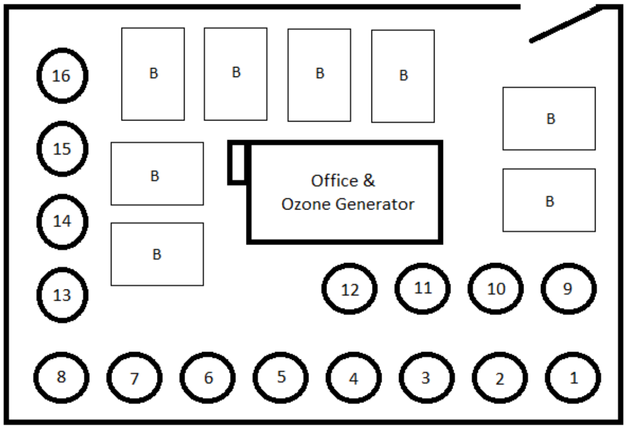

Figure 5. Diagram of greenhouse in which exposures were conducted. The computer monitoring station (office) and ozone generator are in the center of the greenhouse. Numbered circles represent each individual CSTR chamber, and rectangles designated with a "B" represent work benches used for growing plants.

Table 2. Two-way ANOVA test output for the 2010 exposure experiment. ANOVA was used to test for significant differences ($\alpha = 0.05$) of final (week 3) injury values among 6 seed sources and 3 ozone treatments.

Effect	Num DF	Den DF	F Value	Pr > F
Ozone	2	13	47.21	<.0001
Source	5	65	8.51	<.0001
Ozone*Source	10	65	1.91	0.0601

Table 3. Summary SAS output for multiple regression forward selection. This multiple regression analysis was developed using the mean final INJ_P values, mean ozone concentration, mean initial plant height (cm), and mean temperature (°C) for each chamber. Two interaction terms were also used in the model: the interaction between temperature and ozone (teoz) and the interaction between height and ozone (heoz).

Step	Variable Entered	Number Vars In Model	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
1	ozone	1	0.4295	0.4295	27.1527	10.54	0.0059
2	teoz	2	0.1999	0.6293	15.4372	7.01	0.0201
3	heoz	3	0.1244	0.7538	8.8966	6.07	0.0299
4	temp	4	0.0843	0.8381	5.1080	5.73	0.0356
5	height	5	0.0161	0.8543	6.0000	1.11	0.3173

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