University of Cincinnati

Date: 7/14/2015

I, Susan Yvonne Jaconis, hereby submit this original work as part of the requirements for the degree of Doctor of Philosophy in Biological Sciences.

It is entitled:
The effects of diesel exhaust and particulate matter on the growth, reproduction, and ecophysiology of plants

Student’s name: Susan Yvonne Jaconis

This work and its defense approved by:

Committee chair: Theresa Culley, Ph.D.
Committee member: Ishi Bufham, Ph.D.
Committee member: Timothy Keener, Ph.D.
Committee member: Mingming Lu, Ph.D.
Committee member: Jodi Shann, Ph.D.

16229
The effects of diesel exhaust and particulate matter on the growth, reproduction, and ecophysiology of plants

Susan Yvonne Jaconis
July 14, 2015
B.S., North Carolina State University, 2010

Ph.D. Dissertation
Department of Biological Sciences
McMicken College of Arts & Sciences
University of Cincinnati

Committee chair: Theresa M. Culley, Ph.D.
Committee member: Ishi Buffam, Ph.D.
Committee member: Timothy Keener, Ph.D.
Committee member: Mingming Lu, Ph.D.
Committee member: Jodi Shann, Ph.D.
Abstract

As environmental consciousness is increased worldwide, research efforts on ambient pollutants are extending from not only impacts on human health but also to effects of these pollutants on plants. This is especially important because many plants of economic and natural value grow in areas that experience heavy pollution; these plants must work to maintain their normal biological functions in spite of the influx of pollutants to which they are exposed. This series of interdisciplinary studies combines ecology with environmental engineering techniques to explore plant responses in the presence of common roadside pollutants. Specifically, field and lab techniques were used to examine the impacts of diesel exhaust and its particulate matter on the growth, reproduction, and ecophysiology of two plant species: (1) *Chicorium intybus* (chicory) and (2) *Glycine max* (soybean). Soybean and chicory are both of economic importance due to their use in food production and both are commonly found along traffic corridors. The first study examines chicory reproductive structures along roadways of varying traffic levels in Cincinnati, Ohio to understand impacts of particulate matter on reproductive processes. Physical hindrance of pollen deposition by accumulation of particulates on floral stigmas could have negative consequences on pollen germination and consequently, fertilization. Based on our results, there was little variation in the amount of particulate matter found on chicory flowers along roads of different traffic intensity and the reproductive effects of particulate matter due to road-type were not strong. However, a correlation between particle deposition counts and pollen deposition was detected as well as between particle deposition and pollen germination. In the subsequent two studies, open-top chamber experiments were conducted in the field to expose chicory and soybean plants to elevated diesel exhaust or ambient air. Prior to and following one week of daily five-hour treatments, all plants were measured for their growth, reproductive, and
ecophysiological responses (photosynthetic rate, stomatal conductance, water use efficiency, number of total and clogged stomata). These traits were again measured after the plants were given a recovery period. The two plant species varied in their ecophysiological response to the elevated diesel exhaust. In terms of growth and reproduction, both species showed minor differences between plants exposed to elevated exhaust and ambient air immediately after the treatment period. However, following the recovery period, soybean plants that had been exposed to elevated diesel exhaust responded with increased aboveground biomass but without increasing their reproductive output. These studies are both timely and valuable for understanding the consequences of anthropogenic air pollution on plants in order to better aid in urban and agricultural planning.
Acknowledgements

This work is dedicated to my mom, Yvonne, who died just months before my defense. Her boundless love and encouragement has given me the courage to surmount life’s journeys and find happiness in every moment.

My heart is filled with appreciation for all the people that have come in and out of my life throughout my graduate career—friends new and old, family, and peers. I especially thank my advisor, Theresa M. Culley, who I look up to as both an incredible person and a fantastic scientist. A big thank you to my committee member, Jodi Shann, who has been a wonderful support along with my other committee members Tim Keener, Mingming Lu, and Ishi Buffam, who have all been amazingly helpful during the development of my project and a huge part of its success. Help from Roger Ruff, David Lentz, and Paul McKenzie at The University of Cincinnati’s Center for Field Studies made my field experiments possible and thank you to Tim Keener and Marissa S. Liang for their help with the experimental generator. Thank you to everyone in my lab: Yamini Kashimshetty, Alina Avanesyan, Megan Philpott, Sunita Yadav, Allison Mastalerz, Ben Merritt, Rob Tunison, and Eric Tepe for daily support and friendship and also my indispensable field and lab help from: Ali Meier, Robert Elam, Kala Stephens, and Cameron Brown. Thank you also to Mitch Cruzan and his lab at Portland State University who welcomed me with open arms into their scientific community.

I give so much gratitude for my close girlfriends who have been with me through everything since high school: Molly Jones, Christine Smith, Cortney Dahlgren, Aditi Parmar, and Kyrie Hampton. Thank you also to my wonderful friends in the Cincinnati area: Elysam Raib, Ali Meier, Matthew Westbrook, Kate Johnson, Tashia Pierce, Kelsey Feser, and Jenny Arkle. I would not be the person I am today without these people by my side.
And finally, thank you to my remarkable father, Rocco, and my extraordinary sister, Maryanne. I love both of you, always. Thank you to everyone mentioned and the people I have yet to meet. May your hearts always be filled with happiness.
Table of Contents

Page Number

List of Tables ..............................................................................................................iv
List of Figures ...............................................................................................................v
Acknowledgements .....................................................................................................vii
Abstract .........................................................................................................................ix
Chapter 1 Introduction - The influence of airborne pollutants on plants............1
Chapter 2 Reproductive interference by particulate matter in Cichorium intybus: A comparison of interstates, U.S. highways, state highways, and county roads.................................................................20
Chapter 3 The effects of short-term exposure to diesel exhaust and particulate matter on the ecophysiology of Cichorium intybus and Glycine max........................................................................................................40
Chapter 4 Growth and reproduction of Cichorium intybus and Glycine max after short-term exposure to diesel exhaust.................................68
Chapter 5 Conclusion - A brief summary and future directions.......................94
List of Tables

Chapter 2

Table 2.1 Roadside chicory sampling sites - GPS coordinates and road names........31

Chapter 4

Table 4.1 Statistical results of the ANOVA examining the effect of treatment within each sampling period for chicory growth measures........................83

Table 4.2 Statistical results for the ANOVA analysis examining chicory reproductive measures within each treatment across sampling periods....83
# List of Figures

<table>
<thead>
<tr>
<th>Chapter 2</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2.1 Map of Cincinnati with roadside chicory sampling locations</td>
<td>32</td>
</tr>
<tr>
<td>Figure 2.2 Chicory flower (Left) with magnified stigma showing where particulate matter, pollen, and germination rates were measured (Right)</td>
<td>33</td>
</tr>
<tr>
<td>Figure 2.3 An image of the active stigmatic portion of a chicory flower showing particulate matter deposition and pollen grains (blue) with some pollen tube germination</td>
<td>33</td>
</tr>
<tr>
<td>Figure 2.4 Mean values of large PM (A), medium PM (B), and small PM (C) on chicory stigmas at all road types and geographic locations</td>
<td>34</td>
</tr>
<tr>
<td>Figure 2.5 Mean values of total pollen on chicory stigmas at all road types and Geographic locations</td>
<td>35</td>
</tr>
<tr>
<td>Figure 2.6 Mean values of germination rates on chicory stigmas at all road types and geographic locations</td>
<td>36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 3</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 3.1 Images of chicory plants with inflorescences (Left) and soybean plants (Right)</td>
<td>58</td>
</tr>
<tr>
<td>Figure 3.2 Experimental design showing the treatment chamber and generator layout in the field</td>
<td>58</td>
</tr>
<tr>
<td>Figure 3.3 Image of the experimental generator with exhaust manifold showing the air distribution equipment</td>
<td>59</td>
</tr>
<tr>
<td>Figure 3.4 Circular plant arrangement within the experimental chambers</td>
<td>59</td>
</tr>
<tr>
<td>Figure 3.5 Mean values of instantaneous photosynthetic rate (A), stomatal conductance (B), and water use efficiency (C) for chicory plants</td>
<td>60</td>
</tr>
<tr>
<td>Figure 3.6 Mean values for the total number of stomates on the top (A) and bottom (B) of chicory leaves</td>
<td>61</td>
</tr>
<tr>
<td>Figure 3.7 Mean values of instantaneous photosynthetic rate (A), stomatal conductance (B), and water use efficiency (C) for soybean plants</td>
<td>62</td>
</tr>
</tbody>
</table>
Figure 3.8  Mean values for soybean leaf top (A) and bottom (B) total stomata........63

Chapter 4

Figure 4.1  Mean values for chicory leaf height with statistical results........484
Figure 4.2  Mean values for chicory node number with statistical results........484
Figure 4.3  Mean values for chicory stalk number (A) and stalk height (B) with statistical results..................................................485
Figure 4.4  Mean values for soybean stalk height with statistical results........686
Figure 4.5  Mean values for soybean node number with statistical results........686
Figure 4.6  Mean above soybean aboveground biomass values with statistical results........................................................................487
Figure 4.7  Mean values for chicory pollen grains (A) and pollen germination rates (B) with statistical results..................................................488
Figure 4.8  Mean values of particulate matter on chicory plants with statistical results........................................................................489
Figure 4.9  Soybean average seed per pod values (A), average seed weights (B), and mean total pod number per plant (C) with statistical results........490
Chapter 1:

Introduction - The influence of airborne pollutants on plants

Air pollution has become an increasingly important global concern as the environment continues to be reshaped by human activity. When considering any pollutant, their impacts on human health are the priority. There have been countless studies on the effects of air pollution on human health and longevity (e.g. Lv et al., 2011), many of which detail negative consequences on the respiratory and cardiovascular systems (Lorenzo et al., 2011). However, there is a strong relationship between human physical and emotional health and the well being of the environment, which was documented even thousands of years ago (e.g. Lv et al., 2011). This connectedness between humans and the environment has made it necessary to extend our understanding of airborne pollutants on human health to its effects on plant life. For example, the U.S. Environmental Protection Agency (EPA) considers plants in their National Ambient Air Quality Standards (NAAQS). In the NAAQS, vegetation and crops are protected under secondary standards based on their contribution to public welfare. A substantial number of studies of air pollution on plants have focused on the effects of sulfur dioxide (SO$_2$), carbon dioxide (CO$_2$), ozone (O$_3$), and nitrogen oxides (NO$_x$) (e.g. Saxe, 1983a; Saxe, 1983b). The effects of particulate matter (PM) on plant life, however, have undergone relatively less examination. This introduction will address some of the known effects of SO$_2$, CO$_2$, O$_3$, NO$_x$, and PM on multiple aspects of plant health including their growth, reproduction, and ecophysiological processes.

Effects of gases on plant stomata and photosynthetic capabilities

When studying airborne pollutant effects on plants, the condition of stomata can be indicative of overall plant health because of the changes stomata incur due to environmental
factors. These changes can also influence the photosynthetic capabilities of the plant (Robinson et al., 1998). Stomata can be affected by even the slightest concentrations of pollutant exposure (McAinsh et al., 2001). Specifically, stomatal behavior regulates gas exchange (i.e. exchange of CO₂ and H₂O) and thus the degree that gases and other air pollutants are taken into the plant tissue. The reactions of stomata to pollutants will differ based on their age, the surrounding environmental state, plant species, exposure time, and concentration of pollutants (McAinsh et al., 2001). In addition, the degree of absorption is variable based upon the plant’s stomatal conductance (Nighat et al., 2000).

Airborne pollutants can have both direct and indirect effects on stomatal responses. Direct exposure of air pollutants to plants can reduce the speed of stomatal closure in leaves due to the disruption of calcium ion homeostasis, which reduces guard cell control (McAinsh et al., 2001). Indirect effects can include damaged subsidiary or epidermal cells and alterations in stomatal cell wall structure. These mechanical effects can lessen guard cell resistance and lead to dilation of the stomata (McAinsh et al., 2001).

In a study by McAinsh et al. (2001), the signaling transduction pathway of stomata was examined to further understand stomatal responses to air pollution. One of the key components of the pathway that generates the opening and closing of stomata is the action of cytosolic free calcium ions. McAinsh et al. (2001) showed that an increase in O₃ causes a corresponding increase in the cytosolic free calcium ion concentration in seedlings. In addition, increased levels of cytosolic free calcium in stomatal guard cells led to stomatal closing and the shift of calcium into guard cells, which interferes with the calcium homeostasis and signaling. Along with ozone induced reduction of stomatal conductance, there was an associated increase of the calcium levels in the guard cells (McAinsh et al., 2001). If calcium homeostasis is disrupted in these
guard cells, stomatal responses to environmental stimuli are compromised. McAinsh et al. (2001) also proposed that increases in cytosolic free calcium ion concentration might alter the potassium ion channel uptake necessary for stomatal opening.

In a different study, Nighat et al. (2000) observed stomatal and photosynthetic responses of *Ruellia tuberosa* L. to air pollution released by thermal power plants, including SO$_2$, CO$_2$, and nitrogen oxides. In this case, leaf chlorosis and necrotic spots were detected on plant leaves only in polluted locations, indicating that the thermal power plant smoke had affected the plants. Leaf quantity and size were also found to be significantly lower in plants exposed to pollution. As the plants aged, stomata of plants in the polluted areas did not experience consistent growth while stomata of control plants showed growth in both length and width. Furthermore, the stomatal pore length actually revealed a decreasing size trend after flowering in the polluted plants. Variations in stomatal index and stomatal density counts were observed based on flowering time of the plant. In addition, stomatal density tended to decrease in the upper part of the leaves before flowering in the presence of air pollution but this result was not significant. The stomatal indices showed significant increase on the upper leaf surface in pre-flowering with non-statistically significant increases in other plant life stages (Nighat et al., 2000).

Biochemically, Nighat et al. (2000) found no significant differences in chlorophyll *a* and *b* or carotenoid concentration based on the presence of pollutants. All traits showed a decrease with increasing plant age. In general, stomatal conductance and photosynthetic rates remained low in the polluted treatment, instead of increasing with plant age as might be expected for plants not exposed to pollution (Nighat et al., 2000).
Sulfur dioxide effects

Sulfur dioxide is one of the earliest air pollutants studied in terms of harm to plants and it is more prevalent in developed countries due to industry (Middleton et al., 1958; Miller, 1983). Even early studies from the 1950s comparing plant growth in ambient air to plants in air filtration chambers showed that plants growing in the absence of SO₂ exhibit superior growth than those exposed to the pollutant (Bell et al., 2011). Overall, the cost of plant exposure to SO₂ is a reduction in total plant productivity (Saxe, 1983b). There is a wide spectrum of harm from SO₂ to plants, with the majority of studies indicating a variety of deleterious effects related to increasing SO₂ levels (Matyssek et al., 1995). Most instances of short-term exposure to SO₂ encourage stomatal pore opening while long-term exposure promotes the opposite effect (McAinsh et al., 2001).

An early study by Saxe (1983a; 1983b) examined the long-term physiological effects of continuous low-level exposure to SO₂ in bean plants, Phaseolus vulgaris. An important factor of this study was its novel method of long-term rather than short-term exposure, as this is more indicative of natural conditions. The plants were grown in five separate chambers and each chamber was treated with a different level of SO₂ ranging from 10 μg m⁻³ to 950 μg m⁻³. As often demonstrated in other studies (e.g. Nighat et al., 2000), a reduction in photosynthetic capabilities and the opening of stomata was observed in P. vulgaris with increasing SO₂ levels. Plants within the two higher-level SO₂ treatments had significantly lower net photosynthetic and transpiration rates than plants in the ambient air control, while those treated with the lowest two SO₂ doses showed no difference in rates (Saxe, 1983a).

Both reversible and irreversible inhibition of photosynthesis was observed in P. vulgaris from SO₂ exposure, with irreversible inhibition caused primarily by minimization of the green
leaf area. It has also been shown in *in vitro* studies (Saxe, 1983a) that photosynthetic inhibition occurs both directly, affecting the light and dark processes, and also indirectly from membrane deterioration. As a result of direct inhibition, a plant may exhibit decreased ability to uptake CO$_2$ due to the stomatal closure caused by the SO$_2$, and therefore experience reduced photosynthetic capabilities (Saxe, 1983a).

Long-term effects of low-level exposure to SO$_2$ have also been shown to significantly reduce plant biomass (measured as both fresh and dry weight), the presence of chlorophyll in leaves, and starch amounts (Saxe, 1983b). Some of these effects are thought to correspond to the SO$_2$ induced decrease in photosynthetic capabilities. It was also found that at the higher exposure levels, the protein amino acid concentration increased in bean pods (Saxe, 1983b) with negative consequences for plant fitness. The increased amino acid concentration made plants more nutritionally beneficial to herbivores (Bell *et al.*, 2011). Furthermore, as exposure time to SO$_2$ increased, the concentration of sulfur in leaves and fruit increased as well (Saxe, 1983b). An increased concentration of SO2 within foliage is known to negatively affect plants; for example, by causing leaf browning (Carlson, 1974).

In addition to anthropogenic sources, there can be natural contributions of SO$_2$ in the atmosphere, commonly from volcanic emissions. SO$_2$ from volcanic activity is often found in the presence of CO$_2$, making it necessary to conduct field experiments in such a way that separates the effects of the two atmospheric pollutants. A study by Tanner *et al.* (2007) looked precisely at this question while focusing on effects on stomata. Stomatal frequencies in *Nephrrolepis exaltata*, the common swordfern, were measured at multiple sites in Hawaii. Most sites were located near the volcanic vents of Kilauea and plants therefore experienced long-term exposure to elevated gas levels. In areas nearest to certain craters where levels of SO$_2$ were
highest, the lowest stomatal frequencies were observed. Furthermore, plants in locations near
craters with high SO\(_2\) levels but low CO\(_2\) levels still exhibited significantly lower stomatal
indices than plants in control areas. The reduction in stomatal frequency is thought to be a
phenotypic response that can lessen the interference of SO\(_2\) on photosynthetic activity. In a
different study of SO\(_2\) effects (Winner and Mooney, 1985), potted plants were strategically
placed at varying distances from a Kilauea vent. In this case, the plants were able to seal stomata
due to SO\(_2\) exposure based on their tolerance to leaf injury (Winner and Mooney, 1985). Other
stomatal studies suggested that larger stomata and higher stomatal conductance in leaves resulted
in higher absorption of SO\(_2\) (Nighat et al., 2000).

**Nitrogen containing airborne pollutants**

Nitrogen oxides are one of the most significant traffic based phytotoxicants. Vehicular
emissions of NO\(_x\) include nitric oxide (NO), which can oxidize with ozone (O\(_3\)) to create
nitrogen dioxide (NO\(_2\)), and other nitrogenous compounds including nitrous oxide, ammonia and
nitrous acid (HONO). An overwhelming release of NO can leave portions un-oxidized, creating
a mixture of both NO and NO\(_2\) in the ambient air (Honour et al., 2009). NO\(_2\) is generally
considered a stronger phytotoxicant than NO based on nitrogen oxide studies. However, Saxe &
Christensen (1985) performed experiments of continuous exposure to NO in greenhouses over a
series of months and found visible damage to plants such as specks, yellowing, or substantial leaf
loss and growth hindrance, which may indicate a greater toxicity by NO (Saxe, 1994).

Based on evidence from Saxe (1986), the passage of NO through stomata as opposed to
the cuticles is thought to cause the damage to plants. However, NO’s lipid solubility suggests it
enters the plant through the cuticle more often than through the stomata (Saxe, 1994). In contrast
to Saxe’s explanation of NO\(_x\)’s pathway into the plant (1994), more recent studies (Van Hove et
al., 1991; Van Hove and Bussen, 1994) claim the majority of nitrogen containing gases enter the leaves through the stomata rather than cuticular entrance (Stulen et al., 1998). Plant internal resistance to \( \text{NO}_2 \) is generally low while resistance to \( \text{NO} \) is high and there may also be mesophyllic resistance to \( \text{NO}_2 \) (Stulen et al., 1998).

In general, nitrogen oxides are thought to have minimal effects on stomata (McAinsh et al., 2001). After accessing the apoplast by stomatal entrance, \( \text{NO}_x \) transfigures to \( \text{NO}_3^- \) or \( \text{NO}_2^- \) (Stulen et al., 1998). Stulen (1998) explains that after transfiguration and storage of \( \text{NO}_x \), it is assimilated into the plant. A fumigation experiment by Bell et al. (2011) observed the effects of \( \text{NO}_x \) on the biomass of seven plant species. The majority of their results were not statistically significant but there was a pattern of reduced dried aboveground biomass and dried root weight in plants fumigated with \( \text{NO}_x \) compared to the control (Bell et al., 2011).

The presence of nitrous acid (HONO) in the atmosphere can come from several sources including traffic emissions, conversion of \( \text{NO}_2 \), and a series of gas phase reactions. In terms of plants, this is significant because HONO can be both formed on a plant and taken up by plants, with uptake mostly occurring through the stomata (Schimang et al., 2006). To date, the effects of HONO on plants have not been studied to a large degree.

**Carbon dioxide and ozone specific studies**

Carbon dioxide and atmospheric ozone are often studied in conjunction although they demonstrate contrasting effects on plants. \( \text{CO}_2 \) is often the focus of study because it is rising at exorbitant rates both naturally and by anthropogenic causes. Ozone, particularly tropospheric \( \text{O}_3 \), also has a huge impact worldwide because it is a major stressor in more than 30% of all forests (Onandia et al., 2011). Its phytotoxicity can cause reduced photosynthesis, acceleration of leaf senescence, alterations to the partitioning of carbon, and in high doses, ozone can cause
plant necrosis (Onandia et al., 2011). Ozone enters through the stomata and prompts the creation of reactive oxidative molecules, which in turn trigger oxidative stress responses. This can damage the cell structure, molecules, and DNA of the plant (Onandia et al., 2011; Tai et al., 2010). Stomata are known to be highly sensitive to O₃ chronic exposure (Onandia et al., 2011).

Conversely, elevated CO₂ levels have a tendency to stimulate plant growth and escalate photosynthetic activity (Tai et al., 2010). For example, Wong (1979) demonstrated this in an early study by enriching cotton and maize plants with elevated CO₂ for 30-40 days. In both species, dry weights revealed increased plant growth for those plants administered with the higher CO₂ level. Responses to short term elevated carbon dioxide exposure have also shown positive growth and increased photosynthetic rates (e.g. Gaastra, 1959). Plant growth stimulation in the presence of elevated CO₂ is generally the result of an increase in photosynthetic rate. When comparing plants of different species and photosynthetic class (C3, C4, and CAM), naturally faster growing species generally have a stronger response to the gaseous enrichment than slower growing plants (Poorter and Navas, 2003).

Reductions in stomatal conductance have also been documented with elevated CO₂ and O₃ levels (Onandia et al., 2011). This decrease is likely the result of intracellular CO₂ increasing, leading to a narrowing of stomata. Reductions in stomatal conductance due to elevated CO₂ levels could be a defense strategy by minimizing O₃ intake. In cases where the guard cells of stomata are affected by elevated O₃, the narrowing may not affect photosynthesis. Ozone damage can also impede guard cell function due to ozone damage on the guard cell or local epidermal cells, which in turn causes a reduction in stomatal responses to water stress (Onandia et al., 2011). Drought induced closure capabilities of stomata can be reduced if a plant has experienced exposure to ozone (McAinsh, 2001).
In field experiments, leaves of *Betula papurifera* (paper birch) exposed to CO\(_2\) or O\(_3\) either separately or combined were found to have significantly lower stomatal conductance after a short-term exposure to increased levels, compared to unexposed plants. A short-term decrease of CO\(_2\) resulted in control leaves exhibiting significantly higher stomatal conductance responses with no significant results in a combined CO\(_2\) + O\(_3\) interaction (Onandia *et al.*, 2011). Photosynthetic capabilities, however, were not affected by the various treatments.

**Particulate matter**

Air pollutants considered the most harmful historically have been those produced from industry - often coal smoke and production of the gases described thus far. Particulate matter (PM) are particles in the ambient air created from chemical reactions between gases and primary particles. Sources of PM can be natural or through human activity (Schlesinger, 2007). Size is one of the most important characteristics of particulates because it helps determine how they will affect human and biological communities. A majority of studies have focused on aerodynamic diameters of 10 µm (PM\(_{10}\)) and PM of 2.5 µm (PM\(_{2.5}\) or fine PM) because particulates at sizes less than PM\(_{10}\) are known to be detrimental (de Oliveira Alves 2011). Human exposure to PM has resulted in respiratory problems, neurological ailments, asthma attacks, lung cancer, eye irritation and even mutagenicity (de Andrade *et al.*, 2011; Ohio Environmental Council, 2004). It is known that in humans, health conditions due to particulate matter cause approximately 70,000 deaths per year in the United States alone (Ohio Environmental Council, 2004). To date, research on particulate matter has been limited to that pertaining to human health with relatively little exploration of its effects on plants.

A pioneering study by de Oliveira Alves (2011) explored the genotoxic effects of particulates 10 µm and smaller, produced by vegetal biomass burning, on the species
Tradescantia pallida. By performing micronuclei assays in T. pallida between the time of year with highest and lowest particulate emission, genotoxic damage was observed to occur during the intervals of highest PM release (de Oliveira Alves, 2011).

Other particulates, such as those emitted from vehicles, have become of increasing concern as motor vehicle use increases across the globe (Bell et al., 2011). Particulates come from diesel combustion, which creates carbonaceous materials that absorb various surrounding compounds, both organic and inorganic, and then condense. Catalytic converters, car oils and metal-based additives give rise to the development and emission of these particulates (Sarvi et al., 2011). However, particulate matter is not limited to exhaust emissions. Instead, airborne particulates can be the product of resuspension, a process in which something as simple as driving a car down a dusty road distributes PM into the air. Paved roads have also been shown to contribute to airborne particulates (Escrig et al., 2011). Research carried out by Gauderman et al. (2007) revealed that living within 500 meters of highways can result in a reduction in human lung function. This, along with other studies, indicates that living closer to heavily used roadways increases a person’s likelihood to experience health problems (Carneiro et al., 2011).

In terms of roadway particulate studies on plants, minimal research has been done. A 2011 study by Carneiro et al. (2011) used pollen abortion rates of Bauhinia blakeana to assess the effects of particulates on plants, with a focus on using plants as a method to gauge air pollution toxicity to humans. Approximately 3,000 pollen grains from each of three sites at 0, 60, and 120 meters from the chosen major road were collected and analyzed according to size, form, and staining. Pollen abortion rates were found to be significantly higher next to the road than at either 60 or 120 meters away (Carneiro et al., 2011). In addition, needles of Pinus thunbergii exhibited clogged stomatal pores due to automobile exhaust particulates and fine dust
in heavy traffic locales (Takayama, 2005). The clogging was also dependent upon the age of the plant and its epicuticular condition; if the epicuticular wax has deteriorated, the particulates are more likely to be removed by rain. Furthermore, the needles exposed to the automobile air pollution experienced early defoliation (Takayama, 2005).

Although levels of PM are often measured to be below the World Health Organization’s recommended levels ($\text{PM}_{2.5} 10\mu g/m^3$ and $\text{PM}_{10} 20\mu g/m^3$ annual means), recent studies are showing that much lower levels are detrimental to human and environmental health (de Oliveira Alves, 2011; WHO, 2006). This is an area of continued and necessary research both in humans and plants.

**Airborne pollutants in combination**

In many cases, plant exposure to multiple pollutants requires lower concentrations of each individual pollutant to observe effects on stomata, caused by synergistic action (McAinsh et al., 2001). For example, studies of soybeans, *Glycine max*, by Carlson (1983) show that SO$_2$ and NO$_2$ in conjunction have higher reductions in stomatal conductance than exposure to the same concentrations of SO$_2$ and NO$_2$ singly (McAinish, 2001).

A recent study by Assadi et al. (2011) of forests of the river red gum, *Eucalyptus camaldulensis*, sought to describe both the morphological and physiological changes caused by general air pollutants leached from a nearby industrial company, including SO$_2$, nitrogen oxides, carbon oxides, and increased levels of heavy metals. The compounds were not only released into the atmosphere directly, but SO$_2$ and O$_2$ also reacted to form acid rain (Assadi et al., 2011). In this study, morphological characteristics of the red river gum including leaf area and length as well as petiole length and width were significantly reduced in areas of industrial air pollution. One explanation of this response is that the size reduction is a mode of resistance; the less the
leaf is exposed to the pollution, the less the phytotoxicant can affect it. However, these reductions could also be the result of physiological damage caused by the pollutants themselves (Assadi et al., 2011).

Although it is often easiest to detect air pollution in the field by morphological changes observed within the plant, earlier phytotoxic damage can cause physiological changes to the plant such as modifying chlorophyll content. Assadi et al. (2011) detected significantly higher levels of chlorophyll a and b, soluble sugar, carotenoids and proline in leaves of E. camaldulensis from polluted areas. All biochemical increases were explained to be common plant reactions for stress tolerance. The authors asserted that these early physiological changes could potentially be used as indicators of local air pollution.

**Plant tolerance to airborne pollution**

The studies described above give clear evidence that airborne pollutants, including gaseous and particulate types, are absorbed and affect exposed plants, especially the leaf tissue. Furthermore, evidence reveals that plants grown closer to areas of pollutant exposure exhibit deleterious effects. The ability of airborne pollutants to travel makes them a concern in most areas. However, it is also important to consider the plant’s ability to tolerate such pollutants. Some plants are not only capable of tolerating toxicants but are likewise able to detoxify some of them after absorption and thus can remove them from the atmosphere. A plant’s capacity to tolerate is based on the pollution to which it is exposed, the species, the severity of injuries incurred, and alterations to morphological characteristics (Gostin, 2009).

To understand this concept, Gostin (2009) carried out a study on several species of Fabaceae near a cement plant, exposing them to cement dust pollution and other anthropogenic created pollutants including those from nearby motor vehicles. Results indicated lowered foliar
lamina thickness in pollutant-exposed *Trifolium* plants versus the control. In contrast, *T. montanum*, *T. repens* and *L. corniculatus* leaves exhibited a significant increase in epidermal cell external wall thickness in polluted locations, although the plant typically experienced a decrease in leaf size. Stomatal density increases and stomatal size decreases were also noted in the lower epidermis of leaf tissue with pollutants in *T. montanum* and *T. repens*, thus controlling rate of pollution absorption into the leaves. Necrosis along with pollutant deposits in xylem and phloem vessel walls of the leaf midveins were also detected on leaves exposed to air pollution. Other symptoms included mass deposits in upper epidermal stomatal chambers, epidermal cell collapse, and detachment of the lower epidermis from the mesophyll layer (Gostin, 2009). Although these alterations and damages were observed, the *Fabaceae* plants were quite resilient, surviving to maturity. Overall, the results were indicative of adaptation to long-term exposure to the pollutants, specifically the deposit accumulation (Gostin, 2009).

**Significance**

Contemporary human activities are dramatically increasing the distribution of airborne pollutants worldwide. The known negative effects of pollutants on both humans and plants make this field of research of high importance. Although the U.S. and other developed nations are working towards minimizing pollutant emissions, some developing nations are experiencing unmonitored, escalating levels of atmospheric air pollutants (Bell *et al.*, 2011). These nations are rapidly increasing use of automobiles and industry, which has significant implications for human health and their local vegetation (Rai and Kulshreshtha, 2006). Plant yields and nutritional content could be negatively impacted by air pollution (Rai and Kulshreshtha, 2006). It is therefore necessary to understand effects of air pollution so that steps can be taken to reduce any serious negative consequences that may affect the wellbeing of societies worldwide. It is crucial
that scientists continue to work towards understanding pollutant effects on all biological communities. This understanding will help make informed choices when choosing plants for improving urban air quality while being mindful of more sensitive plants in agricultural decisions.

In the studies to follow, biologists and environmental engineers were brought together to examine air pollution in an ecological context. The purpose of these studies was to investigate and estimate the impact of anthropogenic air pollution on the growth, reproduction, and ecophysiology of plants, with an emphasis on particulate matter. The survey in Chapter 2 looks closely at the response of chicory, *Cichorium intybus*, to roadway particulate matter deposition on its floral stigmas. Roadways of varying traffic densities were chosen as study sites to determine if negative effects on reproductive processes in the chicory plants increased with more heavily used roads. Chapters 3 and 4 detail an interdisciplinary field experiment in which chicory and soybean, *Glycine max*, were grown in outdoor open-top chamber and exposed to diesel exhaust and control treatments. First, the ecophysiology of the two species is examined in Chapter 3 followed by Chapter 4, which takes an in-depth look at the growth and reproductive plant responses to the diesel exhaust. These studies are an important step in documenting and understanding the type and extent of damages due to air pollution and PM exposure that will help biologists, crop breeders, and horticulturalists optimize plant performance.
References


de Oliveira Alves, N., Matos Loureiro, A. L., Cavalcante Dos Santos, F., Nascimento, K. H.,
Dallacort, R., De Castro Vasconcellos, P., de Souza Hacon, S., Artaxo, P., Batistuzzo de
Medeiros, S.R. (2011). Genotoxicity and composition of particulate matter from biomass
burning in the eastern Brazilian Amazon region. Ecotoxicology and Environmental Safety
74, 1427-1433.

Escrig, A., Amato, F., Pandolfi, M., Monfort, E., Querol, X., Celades, I., Sanfelix, V., Alastuey,
Environmental Management 92, 2855-2859.

Gaastra, P. (1959). Photosynthesis of crop plants as influence by light carbon dioxide,
temperature and stomatal diffusion resistance. Meded Landbouwhogesch Wageningen 59,
1–68.

Gauderman, W. James, Vora, H., McConnell, R., Berhane, K., Gilliland, F., Thomas, D.,
traffic on lung development from 10 to 18 years of age: a cohort study. The Lancet 369,
571-577.

Notulae Botanicae Horti Agrobotanici Cluj-Napoca 37, 57-63.

herbaceous plants to urban air pollution: Effects on growth, phenology and leaf surface
characteristics. Environmental Pollution 157, 1279-1286.

autoregressive asymmetric stochastic volatility strategy to alert of violations of the air


Schlesinger, R. B. (2007). The health impact of common inorganic components of fine particulate matter (PM$_{2.5}$) in ambient air: A critical review. *Inhalation Toxicology* 19, 811-832.


Chapter 2:

Reproductive interference by particulate matter in *Cichorium intybus*: A comparison of interstates, U.S. highways, state highways, and county roads

Abstract

Roadside plants are constantly working to maintain normal biological processes despite the influx of airborne pollutants through their systems. While the effects of many gaseous pollutants on plant life have been studied, particulate pollutants are much less explored. This is especially true of field experiments where particulate dispersion is heavily influenced by factors such as meteorology and roadway use. Therefore, in this study we examined chicory (*Cichorium intybus* L.) flowers growing directly along roadsides in the Cincinnati, Ohio area to assess the influence of particulate matter on plant reproduction. Plants along interstates, U.S. highways, state highways, and county roads were compared because different road-types typically vary in their motor vehicle use and thus should have varying levels of particulate deposits on flowers. We examined floral stigmas for total number of particulates and pollen tube germination rates to determine the degree of particulate interference with reproductive processes. Our results suggest that there was minimal variation among road-types in the amount of particulate matter found on chicory flowers and the deposition of particulates on stigmas based on road-type does not show a strong link to variation in pollen deposition and pollen germination. Correlations between particulate levels and total pollen counts as well as between particulate levels and pollen germination suggest an effect of particulate deposition on floral reproduction that should be further investigated. Countries in which vehicle use is increasing and where pollutants are not regulated as strictly as in the United States may experience more negative effects from particle
pollution. Future studies should also investigate plants less resilient than chicory, including economic species and crops, which may be more severely affected.

**Keywords:** *Cichorium intybus, Glycine max*, stigma clogging, particulate matter, pollen germination

**Introduction**

As the use of motor vehicles increases worldwide and especially in developing nations where air pollution is less monitored, understanding the effects of vehicle emissions on air quality is becoming increasingly important. Historically, air pollutants produced from industry and automobiles were considered the most harmful to both humans and plants - these pollutants most often consisted of coal smoke and production of gases such as carbon dioxide (CO₂), sulfur dioxide (SO₂) and nitrogen oxides (NOₓ). As these gases are now more closely monitored, there is an increased focus on other potentially damaging airborne pollutants, such as particulate matter (PM). PM is also produced by industrial and vehicular activity (among other methods) and has negative health effects on humans ranging from eye irritation and respiratory problems to neurological ailments, and even lung cancer (de Andrade *et al.*, 2011; Ohio Environmental Council, 2004). Because of these primary detrimental human health effects, air pollution, and in particular PM, has become a ubiquitous national and global concern. Consequently, research has now begun to focus on secondary effects of PM on vegetation and crops.

Exhaust from vehicles is one of the principal anthropogenic sources of airborne PM (Rai *et al.*, 2010). Vehicular particulates are produced by incomplete fuel combustion, which creates a carbonaceous material that absorbs surrounding compounds (both organic and inorganic) and then condenses (Sarvi *et al.*, 2011). The use of vehicles on roadways not only produces PM by way of car and truck exhaust but also leads to localized dusts on road surfaces becoming airborne
while driving (Farmer, 1993). PM may also become airborne near motorways due to wear on vehicles including tire abrasion, brake linings and clutch plates (Thompson et al., 1984).

Living along roadsides and lacking voluntary locomotion, plants are highly susceptible to anthropogenic activity in their vicinity, including motorway exhaust and PM. Specifically, plants of economic (crops) and natural value are constantly bombarded with roadway pollutants in many areas. Although the effects of pollutant gases on plants have been heavily examined (e.g. Honour et al., 2009; Saxe, 1983a; Saxe, 1983b), the effects of PM from roadways are less well demonstrated in the field despite the potential of particulates to negatively impact crucial plant functions such as reproduction.

Several recent studies have described some of the effects of PM on reproduction such as a delay in flowering time in some species treated with daily doses of urban dust (Rai et al., 2010). Delayed flowering and lower rates of flowering were also observed in fumigation studies exposing several herbaceous plants to an urban roadside pollutant mixture (Honour et al., 2009). Reductions in fruit production were also observed in a variety of annual plant species following urban dust treatments (Rai et al., 2010). Air pollution including that of PM can also have detrimental effects on pollen, potentially leading to pollen inviability and other harmful consequences. For example, pollen collected from Lagerstroemia indica L. in highly polluted areas include smaller, misshapen and delicate pollen grains, with finer exine coatings along with decreased overall pollen production and in some cases, exocytosis (Rezanejad et al., 2003). Pollen subjected to high pollutant levels was also coated with greater quantities of PM (Rezanejad et al., 2003).

In this study, we examine chicory (Cichorium intybus L.) inflorescences collected along different types of roadways to estimate the impact of motorway PM on reproductive processes.
To do so, we examined stigmatic receptivity to pollen by quantifying the ability of PM to bind to floral stigmas and thus potentially hinder the adherence of pollen and interfere with pollen germination. Ultimately, this would reduce seed production and limit reproductive output in roadside populations. We hypothesized that larger, more heavily used roads would incur higher amounts of PM on chicory stigmas and lower counts of viable pollen and germination.

**Methods**

**Location**

Sampling took place in the greater Cincinnati, Ohio area during Summer 2012. Cincinnati is located at approximately 147 m elevation, with temperatures that averaged 25°C with 9.58 cm of cumulative rainfall during June and July 2012 when sampling was conducted. Four road-types based on the Ohio Kentucky Indiana Regional Council (OKI) classifications were selected for sampling because they have varying amounts of traffic usage: (1) interstate, (2) U.S. highways, (3) state highways and (4) county roads. Within the Cincinnati area, four geographic locations (A-D) were chosen as replicates, each containing all of the four road-types (1-4) for a total of 16 sampling sites (Fig. 2.1). Each sampling site is referred to by its geographic location and road-type (e.g. A1 for geographic location A, interstate; Table 2.1; Fig. 2.1). Annual average daily traffic (AADT) values were collected by OKI near each of the sampling sites. The average AADT across the four replicates within each road-type were 121,533 for interstates, 24,718 for U.S. highways, 13,843 for state highways, and 8,873 for county roads. Sampling sites at each road-type within each geographic location were located within a five-kilometer radius to minimize localized meteorological variations.

**Study Species**

In this study, we examined chicory (*Cichorium intybus* L.), an herbaceous and weedy
plant that is common along roadways especially in industrial areas (Fig. 2.2). Chicory is used in
food production, often as a sweetener or coffee substitute (Aksoy, 2008). Although it is native to
Europe, West Asia, and North Africa, it has become naturalized throughout the U.S. including
the Midwest region, making it a good choice for this study. Furthermore, it has been used as a
phytoindicator in other pollution studies (e.g. Misik, et al., 2006). Chicory was also chosen
based on its flowering time during summer months. Each chicory flower (technically a
composite head of florets, but referred to here as a flower) blooms for only a single day;
therefore flowers that were collected while open in the field had only been exposed to roadway
exhaust for a single day. Ideal for this study, chicory will continue to bloom for several days if
stems are collected from the field and placed in water in a lab setting, thus providing control
flowers from the same inflorescence that were previously exposed to roadways.

Data Collection

To examine potential reproductive effects of PM along roadways of varying traffic usage,
chicory inflorescences were collected for microscopic analysis of the flowers. Based on
availability, stems from 10 to 20 plant individuals were collected at each of the 16 sampling
locations (see above); 20 open flowers were randomly chosen and were immediately preserved
in formalin-acetic-alcohol (FAA) solution as field specimens. The remaining stems were then
allowed to bloom in a controlled laboratory setting for three days where five open flowers per
plant were collected each day over five days and preserved in FAA as controls of non-exposure
to roadside PM. All plants and flowers were collected on weekdays between 2:00 and 4:00 PM
EST within a two-week time frame with minimal variation in temperature and sunlight. No
rainfall occurred during sampling. Each field and control flower was dissected to collect and
stain five stigmas per flower. Each stigma was stained with aniline blue and examined
microscopically under 40x power to count the total number of viable pollen grains, pollen germination rate (percentage of pollen germinated to total pollen), and number of particulate matter pieces touching the active portion of the stigma (Figs. 2.2-2.3). To better assess stigma coverage by particulates, the PM was also classified into size categories in relation to the size of a single chicory pollen grain (0.08mm diameter): (1) small (defined as equal to or less than the size of a pollen grain), (2) medium (between the size of one and two pollen grains), and (3) large (greater than the size of two pollen grains).

Data Analysis

A two-way ANOVA with Type III sums of squares and post hoc Tukey tests was conducted in SAS vers. 9.0 (SAS Institute, Cary, NC) to separately examine the effects of the treatment (road-type) and geographic location (A-D) for each trait measured (pollen count, germination rate, and PM count). Both road-type and geographic location were considered fixed factors in the analysis because traffic patterns at different locations in the Cincinnati area may not be representative of all urban locations in the U.S. Pearson correlations were also conducted among PM counts and germination rates and among PM counts and pollen count.

Results

Particulate Matter

Particulates of all sizes (small, medium, large) were found at similar levels on flowers growing along interstates and U.S. highways. However, significantly lower levels of particulate matter of all sizes were detected on flowers growing along county roads than along other road-types (small: F_{[3, 266]}= 19.58, P=<0.0001, medium: F_{[3, 266]}=22.35, P=<0.0001, large: F_{[3, 267]}=15.17, P=<0.0001; Figs. 2.4A-C). Furthermore, flowers growing along state highways accumulated significantly less medium and large sized PM than interstates and U.S. highways,
but exhibited similar levels to the county roads. A significant interaction between road-type and geographic location was detected in each PM analysis (small: $F_{[9, 266]} = 10.18, P = <0.0001$; medium: $F_{[9, 266]} = 8.5, P = <0.0001$; large: $F_{[9, 267]} = 8.27, P = <0.0001$). PM was also detected in the controls, although at much lower levels than in the field and the lack of zero PM values may be attributed to the control setting being an active laboratory.

**Pollen**

At all locations sampled, the total number of pollen grains on stigmas did not differ among flowers growing along U.S. highways, state highways, or county roads. However, the total number of pollen grains on stigmas was significantly higher in flowers along interstates ($F_{[3, 266]} = 29.77, P = <0.0001$; Fig. 2.5). Because sample A1 had a much higher total pollen level than all other samples, it was removed in a separate analysis, but interstate flowers still had significantly higher pollen counts ($F_{[3, 247]} = 3.15, P = 0.0255$). Pollen germination rates were statistically highest in flowers growing along U.S. highways. Flowers along county roads demonstrated lower germination rates than U.S. highways but higher rates than along interstates. However, state highway germination rates were similar to both county roads and interstates ($F_{[3, 267]} = 17.21, P = <0.0001$; Fig. 2.6). The interaction between road-type and geographic location was significant in both the total pollen counts and germination rates (total pollen: $F_{[9, 266]} = 47.09, P = <0.0001$; germination rate: $F_{[9, 267]} = 9.39, P = <0.0001$). Significant correlations were also detected between the level of small particulate matter and pollen germination rates ($R = 0.377, P = 0.015$). Both small and medium PM also had significant correlations with total pollen counts (small PM vs. total pollen: $R = -0.640, P = <0.0001$; medium PM vs. total pollen: $R = -0.511, P = 0.0006$).
Discussion

Under the assumption that plant reproduction is negatively impacted by roadway particulates, we expected more heavily trafficked roads to (1) have higher amounts of airborne PM of all sizes with (2) lower pollen deposition and lower pollen germination rates because higher PM levels should inhibit pollen from landing directly on floral stigmas. With the exception of germination rates, our results were largely consistent with these hypotheses although they were less severe than we had expected. The fact that interactions between geographic location and road-type were present indicated that PM and pollen patterns associated with road-types were not consistent across all geographic locations.

Particulate Matter

Overall, the highest PM levels were always found on flowers sampled along interstates and in general, most of the deposited particulates observed on the chicory stigmas were small sized PM. However, among flowers found along interstates, U.S. highways, and state highways, the quantity of small PM on their stigmas did not significantly differ. Because the level of PM on county roads was significantly less, these results support our hypothesis that less PM would be detected on flowers of county roads. However, a corresponding difference among the other road-types was also expected but was not detected. There was also slight variation of the medium and large PM levels among different road-types, with averages lower than two particles per stigma. Therefore, even though significant differences in medium and large PM levels were found among the road-types, there was little variation in quantity of small PM.

Traffic patterns and meteorology, which can vary based on locale, are factors that may explain the observed lower levels of medium and large PM detected on roadside vegetation. In general, particles are distributed in the atmosphere based on their size and composition. For
example, larger, heavier PM is less likely to travel as far as smaller particulates and will also settle more quickly. However, the surrounding meteorological conditions, such as temperature, air density, wind speed, and wind angle, play a large role in where particles are deposited (Liang, 2014). Weather patterns can influence spatial and temporal variations in PM dispersion thus changing where PM accumulates on flora. Factors such as traffic flow and the presence of multiple traffic lanes on a road can play a role in local wind patterns (Eskridge and Rao, 1983; Liang, 2014). Furthermore, air turbulence from roadway traffic and even car idling can also have an effect on particle distribution.

Our results showed that all particulate sizes were consistently higher in geographic locations B and D than in A and C (Fig 2.4A-C). Although sampling was consistent based on road-type, geographic locations B and D were more central to Cincinnati’s city center and occurred at a slightly lower elevation than the other sites, which may explain these results. PM at higher elevations are known to disperse further (Pandis, 2004). Because results are variable even within one city, this emphasizes site specific variations of PM distribution are to be expected and results observed in Cincinnati could be very different from other cities.

Pollen

It was unexpected that the total amount of pollen on chicory stigmas would be highest on flowers collected along the interstate. Despite high traffic flow at this location, more pollinator visitation may be encouraged due to the open conditions of the site. Light availability and the amount of floral resource, especially in the case of roadside chicory, can play a role in pollinator visitation (Jackson et al., 2014). The fact that interstates offer large open areas may have also allowed pollen to disperse more readily by wind. Even though different road-types were examined, the lack of variation in pollen amounts on chicory at U.S. highways, state highways,
and county roads could be due to similar characteristics in the local habitat. Many of the same plant species were found at each of the sampling sites with minimal unpaved land area for growth. Local adaptation of chicory plants to air pollution at individual roadsides may also play a role in their ability to tolerate PM exposure. Despite limited variation in PM levels based on road-type, differences in pollen germination were detected, but with no clear pattern. However, disregarding roadtype, the correlation analysis suggests a relationship between small PM level and pollen germination as well as small PM level and total pollen. An effect of PM level on chicory reproduction based on PM level is likely and further investigation is warranted.

Although this study only quantified PM, atmospheric gases contributed by vehicle exhaust could also effect pollination and fertilization. In general, gases can have both positive and negative effects on plant growth depending on the gas and the plant species (for example, elevated CO₂ can stimulate overall growth while SO₂ can cause a reduction in plant productivity; Saxe, 1983b). However, a link of PM to reproductive stress has not yet been determined. Even though pollen exposed to pollutant gases can have malformed grains with finer exine coatings, results have not linked these effects to reproductive challenges in plants (Rezanejad et al., 2003). Furthermore, despite the ability of CO, SO₂, and NO₂ to pass through the porus exine coating of pollen to the intine with minimal effect on concentration of pollen proteins, further effects within the pollen may be deleterious and are relatively unexplored (Ruffin et al., 1983).

Ambient pollution could also have negative effects on stigmatic receptivity as shown in studies of acid rain deposition on Oenothera parviflora (Cox, 1984). The chemicals surrounding the particulates could also have an impact on plant reproduction through stigmatic interactions. Because the chemical composition of PM can change acidic levels, PM may interact with the stigmatic surfaces of plant to change its composition and receptivity to pollen (Cox, 1984;
Grantz et al., 2003). However, the effects of variation in PM chemical composition on plants is only minimally explored (Grantz et al., 2003). Furthermore, floral design and flower phenology play a role in pollutant exposure and accumulation (Cox, 1984). Certain topographical characteristics of the floral reproductive structures such as textured surfaces could aid in pollutants entering and lingering on the flowers. Similarly, stigmatic secretions may influence pollutant adherence. Plants growing in a roadside setting may also experience greater exposure to ambient pollutants if the timing of their floral opening occurs during high traffic volumes.

Conclusion

As a common roadside species, chicory did not show strong negative responses to roadway pollution. Based on our results, there is overall minimal variation of PM presence on stigmas based on road-type. Most of the particulate matter adhering to chicory stigmas was smaller than the size of a single pollen grain. There was no link detected between the PM levels on stigmas and total pollen counts or pollen germination rates based on road-type. Instead, a link was identified when analyzing PM levels and reproductive traits with disregard to road-type. The unique characteristic of floral opening and closing within a single day limits the ability of flowers of chicory to be physically impaired if particulates clog stigmas over a longer time period (although longer-lived leaves do not possess this benefit). In contrast, other species without this capability, including economically valuable and agricultural plants along roadways, may suffer from daily accumulation to PM and thus experience altered reproduction. Therefore, it is important that further field studies are conducted to understand potential effects of PM on the reproductive capacity of other economic species. Because of high variability in different locations, it will also be important to conduct studies in multiple sites to get a more complete understanding of the effects of PM on plant reproduction of chicory and other species.
### Tables

**Table 2.1.** The roads and GPS coordinates for each roadside chicory sampling sites in Cincinnati, OH.

<table>
<thead>
<tr>
<th>Sampling Site</th>
<th>Road Name</th>
<th>GPS Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Interstate 275</td>
<td>39°14'07&quot;N; 84°37'40&quot;W</td>
</tr>
<tr>
<td>A2</td>
<td>US Highway 27</td>
<td>39°13'35&quot;N; 84°35'16&quot;W</td>
</tr>
<tr>
<td>A3</td>
<td>State Route 126</td>
<td>39°13'39&quot;N; 84°36'55&quot;W</td>
</tr>
<tr>
<td>A4</td>
<td>Galbraith Road</td>
<td>39°13'15&quot;N; 84°36'32&quot;W</td>
</tr>
<tr>
<td>B1</td>
<td>Interstate 75</td>
<td>39°10'51&quot;N; 84°29'07&quot;W</td>
</tr>
<tr>
<td>B2</td>
<td>US Highway 42</td>
<td>39°10'09&quot;N; 84°28'10&quot;W</td>
</tr>
<tr>
<td>B3</td>
<td>State Route 126</td>
<td>39°12'39&quot;N; 84°29'11&quot;W</td>
</tr>
<tr>
<td>B4</td>
<td>Section Road</td>
<td>39°11'47&quot;N; 84°26'42&quot;W</td>
</tr>
<tr>
<td>C1</td>
<td>Interstate 71</td>
<td>39°16'04&quot;N; 84°21'16&quot;W</td>
</tr>
<tr>
<td>C2</td>
<td>US Highway 3</td>
<td>39°15'43&quot;N; 84°20'13&quot;W</td>
</tr>
<tr>
<td>C3</td>
<td>State Route 126</td>
<td>39°13'40&quot;N; 84°20'22&quot;W</td>
</tr>
<tr>
<td>C4</td>
<td>Spooky Hollow Road</td>
<td>39°13'22&quot;N; 84°19'46&quot;W</td>
</tr>
<tr>
<td>D1</td>
<td>Interstate 75</td>
<td>39°07'26&quot;N; 84°32'07&quot;W</td>
</tr>
<tr>
<td>D2</td>
<td>US Highway 27</td>
<td>39°09'06&quot;N; 84°32'14&quot;W</td>
</tr>
<tr>
<td>D3</td>
<td>State Route 264</td>
<td>39°06'47&quot;N; 84°33'45&quot;W</td>
</tr>
<tr>
<td>D4</td>
<td>Vine Street</td>
<td>39°08'06&quot;N; 84°30'34&quot;W</td>
</tr>
</tbody>
</table>
Figure 2.1. Map of Ohio with the major roadways surrounding the greater Cincinnati region is shown with the four geographic locations labeled. Geographic location A is shown including the four specific road-types A1, A2, A3, and A4.
Figure 2.2. (Left) A single chicory flower (technically a composite head of florets) on an inflorescence stem. (Right) A magnified photo of chicory reproductive parts highlighting the active stigmatic portion where PM, pollen, and germination rates were measured.

Figure 2.3. An image of the active stigmatic portion of a chicory flower showing particulate matter deposition and pollen grains (blue) with some pollen tube germination. The photo was taken microscopically under 40x power.
Figures 2.4. Mean values of large PM (A), medium PM (B), and small PM (C) on chicory stigmas at all road-types and geographic locations. Road-types are labeled as (1) interstate, (2) US highway, (3) state highway, and (4) county roads; Geographic location is indicated by A-D on each bar. Shown are results of an ANOVA examining the effects of road-type and geographic location on PM with Tukey test results and standard error.
Figure 2.5. Mean values of total pollen on chicory stigmas at all road types and geographic locations. Road types are labeled as (1) interstate, (2) US highway, (3) state highway, and (4) county roads; Geographic location is indicated by A-D on each bar. Shown are results of an ANOVA examining the effects of road type and geographic location on PM with Tukey test results and standard error.
Figure 2.6. Mean values of germination rates on chicory stigmas at all road types and geographic locations. Road types are labeled as (1) interstate, (2) US highway, (3) state highway, and (4) county roads; Geographic location is indicated by A-D on each bar. Shown are results of an ANOVA examining the effects of road type and geographic location on PM with Tukey test results and standard error.
References


Chapter 3:

The effects of short-term exposure to diesel exhaust and particulate matter on the ecophysiology of *Cichorium intybus* and *Glycine max*

Abstract

Living along traffic corridors, plants represent a hallmark example of the biological challenges faced from anthropogenic air pollution. In particular, plants of economic and agricultural importance are constantly exposed to vehicle exhaust and particulate matter (PM) in many areas. This interdisciplinary study combines ecological methods and environmental engineering to investigate the consequences of diesel exhaust and associated PM on plant ecophysiology using controlled open-top chamber experiments. *Cichorium intybus* (chicory) and *Glycine max* (soybean) were exposed to either diesel exhaust or ambient air along with a control daily for one week. Plants were measured for ecophysiological traits (photosynthetic rate, stomatal conductance, water use efficiency, number of total and clogged stomata) before and after treatment and also following a recovery period. Responses varied between species, suggesting further research is critical for future implementation of urban and agricultural planning.

**Keywords:** *Cichorium intybus*, diesel, ecophysiology, *Glycine max*, particulate matter

Introduction

Within their local environment, plants are often considered beneficial cleaners that reduce ambient pollutants and improve air quality (e.g. Manning, 2008). However, the consequence of airborne pollutants on plants themselves is relatively understudied; this information is necessary because of the economic value and ecological importance of various plant species. Numerous studies have detailed the effects of pollutant gases (e.g. Ainsworth *et
al., 2002; Mulchi et al., 1992), but there is a general lack of quantification of the effects of airborne particulate matter (PM) on plants, despite the potential of these pollutants to negatively impact basic physiological processes, such as photosynthesis.

A primary anthropogenic source of PM in urban areas is vehicular exhaust (Rai et al., 2010). PM from automobiles is a major concern as vehicle usage increases across the globe (Bell et al., 2011). Urban roadway PM is primarily contributed by diesel trucks, which produce 50 to 200 times more particulates than the average catalyst gasoline engine (Sebelius, 2011). Diesel exhaust also emits gases of interest including carbon dioxide (CO$_2$), carbon monoxide (CO), sulfur dioxide (SO$_2$), nitrogen oxides (NO$_x$), and ozone (O$_3$). Diesel PM deposition on plants, especially on leaf surfaces, can last for long periods of time because it is not easily removed by meteorological conditions such as wind and rain (Kulshreshtha et al., 1994). Furthermore, leaf characteristics can play a role in accumulation of PM. For example, viscid (sticky) leaves collect PM more easily and rough leaves affect the solubility of pollutants (Kumar et al., 2013).

Both gaseous and particulate pollutants have a variety of effects on plants (e.g. Caporn, 2013). This is because pollutants can interfere with basic plant gas exchange processes that are in constant flux depending upon the surrounding environment. For example, photosynthesis is highly dependent on the CO$_2$ and moisture in the air; any interruption to gas exchange could interfere with basic photosynthetic reactions. To date, studies of PM on plants indicate stunted growth, reduced photosynthetic capabilities, stomatal clogging, and stigma clogging (e.g. Honour et al., 2009; Thompson et al., 1984). Within a leaf, stomatal clogging can lead to elevated water loss because stomata are unable to respond by opening and closing (Wagner, 1939). PM and other airborne pollutants can have additional effects on plants such as
acceleration of epistomatal wax degradation (Sauter et al., 1987) and erosion of epicuticular waxes on leaf surfaces, increasing susceptibility to gaseous airborne pollutants (Durrani et al., 2004). These gases can also damage stomata, causing impairment to stomatal regulation, thus leading to further entry of harmful gases (Durrani et al., 2004; Kammerbauer and Dick, 2000).

Few studies have examined direct effects of roadside PM on a local scale. Investigations have documented negative effects of PM, such as reductions in twig growth, transpiration, and photosynthesis, associated with traffic exposure along roadsides (Kammerbauer and Dick, 2000). Rai et al. (2006; 2010) also demonstrated that leaves of plants grown in polluted locations have an elevated incidence of epidermal cells and small stomata.

In this study, our goal was to quantify ecophysiological effects in plants caused by PM from diesel exhaust. We hypothesized that the ecophysiology and stomatal capabilities of plants would be negatively affected by elevated exposure to diesel exhaust, specifically diesel PM. We subjected plants growing in outdoor open-top chambers to a low dose of diesel exhaust from a generator over several days and examined their responses in terms of photosynthetic rate, stomatal conductance and number, and water use efficiency (WUE). To determine if the observed effects were due to PM, we also quantified the physical presence of PM on leaf surfaces.

Materials and Methods

Location

Field experimentation took place during Summer 2013, at the University of Cincinnati’s Center for Field Studies (UCCFS) in Harrison, Ohio, approximately 22 miles west of the metropolitan area of Cincinnati (39°17’13.91”N; 84°44’25.62”W). The UCCFS is positioned adjacent to agricultural lands and a restored prairie within the Miami Whitewater Forest, owned
by Great Parks of Hamilton County. This site experiences low traffic levels, resulting in limited exposure to traffic emissions. From June to August of 2013, temperatures averaged 22°C (range: 6.1°C-36.5°C) with a total of 25 cm of rainfall.

**Study Species**

Two angiosperm plant species were examined during this study: soybean (*Glycine max*) and chicory (*Cichorium intybus*) (Fig. 3.1). Soybean, an annual herb in the Fabaceae, is a major food crop produced internationally; in the U.S. and particularly Ohio, it is one of the top agricultural crops and is often planted along roadsides. Soybean actively grows during spring and summer, when it produces economically valuable fruits (e.g. Libault *et al.*, 2010). Soybean has been used in studies of gaseous pollutants (e.g. Ainsworth *et al.*, 2002; Heagle *et al.*, 1973), and O₃ is known to reduce crop yields (Fishman *et al.*, 2010). Negative effects of PM originating from roadways could similarly have serious ramifications on soybeans, especially because of the close proximity of many agricultural fields to roads.

Chicory is an herbaceous and weedy herb within the Asteraceae family and which is native to Europe, West Asia and North Africa. It is used in food production, often as a sweetener or coffee additive. Chicory commonly grows wild along roadsides in the U.S. and worldwide, particularly in industrial zones (Aksoy, 2008). Chicory has been used in other air pollution studies, often as a phytoindicator (e.g. Misik *et al.*, 2006), and is a particularly good choice for this study because it's naturalized range extends throughout the Midwestern U.S.

**Experimental Design**

To test the effects of diesel exhaust on plants, outdoor open-top chambers and control chambers (Fig. 3.2) were constructed to perform the following three treatments: (1) elevated exposure to diesel exhaust within a chamber (Elevated), (2) exposure to ambient air within a
chamber (Ambient), and (3) ambient air without a chamber (Control). There were three replicates per treatment type, which were arranged for practicality based on access to the generator (Fig. 3.2). The non-sided Control chambers, which have no protection from the elements, were positioned south of the Elevated treatment chambers to prevent wind contamination from the exhaust source.

Each open-top chamber was constructed using vertical stakes of 2.54 cm diameter galvanized steel, electrical conduit to create a 2 m diameter pentagonal frame. Each of the five corners of conduit was pounded 0.3 m underground, leaving 1.3 m above ground. The vertical stakes were enclosed with translucent PVC plastic film (Plastic Film Corporation Romeoville, Illinois) that allows transmission of ultraviolet light (Fig. 3.3). The open-top design ensured that the conditions within the interior of the chamber stayed relatively consistent with the surrounding meteorological conditions while allowing pollutants to linger. To test for possible chamber effects, the non-sided Control was composed of a 2 m diameter pentagonal area, consistent with the experimental chambers, but without the PVC film enclosure. The Controls were placed within a deer exclosure to minimize herbivory.

To generate PM, a diesel generator (ETQ-DG4LE 4000Watt/3500Watt HP Diesel Portable Generator), running under full load, was modified with three aspirator pumps attached at 120 degrees at the same elevation on an extended stack to dilute and combine the diesel exhaust with compressed ambient air from two air compressors (Porter-Cable 3.5 gallon Pancake) (Fig. 3.3). The aspirator pumps were designed such that the jet fluid (compressed air) was drawing in the raw diesel exhaust via the aspirator pump vacuum line, which was inserted into the exhaust duct. In this manner, the raw exhaust containing the diesel PM was quickly cooled and diluted. The raw diesel exhaust induction tubes were placed as close as possible to a
central location within the circular exhaust duct. Diluted exhaust gas from the three dilution tubes were tested according to EPA Method 5 and emitted an average of 0.0370 g/m$^3$ total PM. Each of three outlet tubes from the aspirator pumps ran into a separate Elevated treatment chamber to discharge the modified air at the top central point. For the Ambient treatment chambers, one of three tubes ran directly from a second air compressor to each chamber in the same manner.

Each chamber contained 15 plant individuals of each study species. Soybeans were germinated from seed (Organic Soybean, Living Whole Foods, Inc.) in the University of Cincinnati greenhouse and transferred to 5 liter volume treepots after their first true leaves appeared. Similar sized chicory plants were collected from a single natural roadside population (to account for local adaptation) in May of 2013 and potted in 5 liter treepots. All chicory and soybean plants were potted using soilless growing media (Pro-Mix, High Porosity Mycorrhizae Professional Growing Medium). Plants were kept potted during the experiment to minimize underground root competition and soil variation. Plants were placed outdoors for one week followed by an additional week in the chambers to acclimate. The pots were arranged in two circles within each chamber: an outer circle of 10 alternating individuals of each species and an inner circle of 5 alternating individuals with a small central circular area to allow for drainage (Fig. 3.4). Plants were watered daily throughout the acclimatization period and the duration of the experiment. To minimize interference with chamber air circulation, pots were inserted into the ground with each rim 3 cm above ground level.

Data Collection

All plants underwent the same set of initial measurements (referred to as "Initial"; see below) one day prior to beginning treatments. Treatments began on June 18, 2013 and ended on
June 25, 2013 ("Final"). During this time, air was pumped into the chambers daily from 9:00 AM until 2:00 PM EST when maximum photosynthetic activity occurs. This continued for a total of five weekdays, after which the experiment was terminated due to the unforeseen loss of the generator. Plants were then allowed a 5½ week recovery period before the soybean plants underwent another round of measurements beginning August 6, 2013 (“Recovery”). Soybeans were chosen over chicory for this last sampling period based on time constraints in measurement processing.

To quantify the ecophysiological responses, individual plants were measured for instantaneous photosynthetic rate ($A; \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), stomatal conductance ($g; \text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and water use efficiency (WUE; mmol·mol$^{-1}$; calculated as $A/g$) using a LI-COR 6400 infrared gas analyzer (IRGA, Li-Cor Inc., Lincoln, Nebraska) during each sampling period. We chose to use $A/g$ to estimate WATER USE EFFICIENCY because it takes into account the rate of gas entry to the stomata and not movement within the leaf interior (as when the transpiration rate is used for the denominator) because we were specifically interested in the function of the stomata.

Measurements were taken between 9:00 AM and 3:00 PM EST before photosynthetic processes tapered off for the day. The IRGA was recalibrated daily with the following settings: air flow, 500 $\mu$mol/s; sample CO$_2$ concentration, 370 $\mu$mol; leaf temperature, 26ºC; stomatal ratio, 0.05; and PAR, 1200 $\mu$mol photons m$^{-2}$s$^{-1}$. Relative humidity was kept between 45 and 65% to minimize variation between plants. One attached leaf per plant was placed in the sampling chamber and allowed to reach photosynthetic equilibrium before measurements were taken. For soybean, the second to distal-most leaflet was measured. Chicory leaves were selected from the upper most basal rosette level. Leaves smaller than the sampling chamber area of 6 cm$^2$ were collected and scanned using a LI-3100C area meter (Li-Cor Inc., Lincoln, Nebraska);
measurements were then adjusted based on this leaf size. Some rabbit herbivory occurred prior to the experiment in which soybean plants in the non-sided Control chambers were damaged; a mesh fence was then installed around the chambers, preventing further damage.

To estimate stomatal density and PM accumulation on leaves, leaf peels of individual plants were collected during the Initial and Final sampling periods. Clear nail polish was applied to the top and bottom of one leaf surface. After drying, the paint was covered with a clear piece of tape, peeled off and placed on a microscope slide. For both top and bottom peels, a circular area of $3.14\text{mm}^2$ (the maximum microscope view in which stomatal clogging could be ascertained) was examined for total stomata count and the proportion of clogged stomata to total stomata (i.e., percentage of clogged stomata). A stomate was classified as clogged if it had a particulate attached to any portion of the structure.

Data Analysis

For analysis of the ecophysiological data, photosynthetic rate, stomatal conductance, and water use efficiency were analyzed separately for each species and sampling period using a nested ANOVA and *post hoc* Tukey tests to examine effects of treatment (Elevated, Ambient, and Control) with chamber nested within treatment. A nested ANOVA was also used to examine the effects of time for each of the ecophysiological measurements (photosynthetic rates, stomatal conductance, water use efficiency) with the chamber effect nested within time. In this analysis, the time variables were Initial and Final sampling periods (for chicory), and also the Recovery period (for soybean only). All data conformed to the assumptions of normality and heterogeneity required for the analyses.

The leaf peel data were analyzed in two ways. First, total stomatal counts for the leaf top and bottom were analyzed separately using a nested ANOVA as described above. In this
analysis, each species was examined separately for the effects of treatment and chamber nested within treatment for each sampling period, followed by Tukey tests. Another nested ANOVA focused on time and chamber nested within time for only the Initial and Final periods, followed by Tukey tests. The percentage of clogged stomata did not follow a normal distribution and were therefore analyzed using a rank sum test based on the Wilcoxon rank sum test White modification (Ambrose et al., 2007).

Results

Chicory

In most cases, Control plants did not differ significantly from plants in the Ambient treatment in terms of photosynthetic rate, stomatal conductance, water use efficiency, and stomatal counts (Fig. 3.5). The remainder of the analysis includes all three treatments but focuses primarily on the comparison of Ambient and Elevated treatments. At the beginning of the experiment during the Initial sampling period, there were no differences in photosynthetic rate ($F_{[2,122]}=0.85$, $P=0.4306$; Fig. 3.5A) and stomatal conductance ($F_{[2,120]}=2.74$, $P=0.068$, Fig. 3.5B) in plants across treatments. In the main analysis that examined the effect of these treatments within each sampling period, photosynthetic rates for chicory plants in the Ambient treatment were significantly higher at the Final sampling period than plants of the Elevated treatment ($F_{[2,117]}=21.57$, $P<0.0001$, Fig. 3.5A). Stomatal conductance of chicory in the Ambient and Elevated treatments was also higher in the Final sampling period compared to the Initial period; but there were no statistical differences between the two treatments at the Final period ($F_{[2,117]}=1.39$, $P=0.2525$, Fig. 3.5B). In all treatments, water use efficiency did not differ significantly between Ambient and Elevated treatments during the Initial or Final sampling periods, although values for both treatments were lower at the Final sampling period. Only a
marginal difference in water use efficiency was detected at the Initial sampling period ($F_{[2, 120]}=2.98, P=0.0547$, Fig. 3.5C). At the Final sampling period, the water use efficiency in the Control treatment was significantly lower than the Ambient and Elevated treatments, but the more conservative Tukey test did not detect a difference between treatments ($F_{[2, 117]}=3.61, P=0.0302$, Fig. 3.5C).

In the analysis within each treatment (Ambient, Elevated, Control) across sampling periods, there was a significant increase from the Initial to Final sampling period for photosynthetic rate (Control: $F_{[1, 75]}=6.37, P=0.0137$; Ambient: $F_{[1, 80]}=52.48, P<0.0001$; Elevated: $F_{[1, 84]}=18.51, P<0.0001$) and stomatal conductance (Control: $F_{[1, 75]}=20.97, P<0.0001$; Ambient: $F_{[1, 76]}=52.55, P<0.0001$; Elevated: $F_{[1, 84]}=12.78, P=0.0006$). In contrast, water use efficiency decreased for chicory in all treatments; however the decrease was only significant for the Ambient and Control treatments (Control: $F_{[1, 75]}=18.86, P<0.0001$; Ambient: $F_{[1, 80]}=25.02, P<0.0001$). This decrease in WATER USE EFFICIENCY was not significant for plants in the Elevated treatment ($F_{[1, 84]}=3.16, P=0.0791$).

In the analysis within individual sampling periods, the total number of stomata on the top and bottom of chicory leaves (TS-top stomates; BS-bottom stomates) at the Initial sampling were significantly lower in the Elevated treatment than in the Control and Ambient treatments (TS: $F_{[2, 124]}=13.79, P<0.0001$; BS: $F_{[2, 125]}=5.78, P=0.004$). At the Final sampling period, TS of plants in the Elevated treatment was similar to plants in both the Ambient and Control but the Ambient TS was significantly lower than the Control ($F_{[2, 120]}=3.91, P=0.0226$). BS did not show a significant difference across treatments ($F_{[2, 121]}=1.23, P=0.2957$).

Analysis of plants within each treatment across sampling periods revealed that TS of plants in the Ambient and Control treatments remained similar from the Initial to Final sampling
periods while in the Elevated treatment there was a significant increase in TS (Control: $F_{[1, 79]} = 0.02, P=0.8911$; Ambient: $F_{[1, 84]} = 3.76, P=0.056$; Elevated: $F_{[1, 81]} = 10.02, P=0.0022$, Fig. 3.6A). In contrast, BS in the Ambient treatment significantly decreased from the Initial to Final sampling periods while plants in the Elevated and Control treatments did not experience any significant change (Control: $F_{[1, 79]} = 3.77, P=0.0558$; Ambient: $F_{[1, 84]} = 10.24, P=0.0019$; Elevated: $F_{[1, 83]} = 0.42, P=0.5179$, Fig. 3.6B). The proportion of clogged to total stomata for both top and bottom of leaves exhibited no significant differences in the analyses within or across sampling periods ($P>0.05$).

**Soybean**

Contrary to the results for chicory, soybean Control plants performed significantly better than plants in the Ambient treatment in several cases, such as for photosynthetic rate and stomatal conductance (Fig. 3.7). This may have been due to the herbivory experienced by the Control plants prior to the experiment; in some plant species, a common response to herbivory involves hormonal release and a consequent surge in growth. In the analysis within sampling periods of Elevated and Ambient treatments, soybean photosynthetic rates were progressively lower in both treatments in the Final sampling period, compared to the Initial period and again after the Recovery period (Fig. 3.7). The photosynthetic rates for soybean in the Control treatment were statistically higher than plants in both the Ambient and Elevated treatments at all sampling periods (Initial: $F_{[2, 92]} = 15.69, P<0.0001$; Final: $F_{[2, 126]} = 32.94, P<0.0001$; Recovery: $F_{[2, 85]} = 29.04, P<0.0001$, Fig. 3.7A). Stomatal conductance in the Elevated treatment was statistically higher than that of the Ambient treatment at both the Final and Recovery sampling periods (Initial: $F_{[2, 92]} = 3.44, P=0.0362$; Final: $F_{[2, 126]} = 42.96, P<0.0001$; Recovery: $F_{[2, 85]} = 22.53, P<0.0001$, Fig. 3.7B). The WATER USE EFFICIENCY of plants in the Ambient treatment was
statistically higher than in the Elevated treatment at each sampling period (Initial: $F_{[2, 92]}=7.14$, $P=0.0013$; Final: $F_{[2, 126]}=2.28$, $P<0.0001$; Recovery: $F_{[2, 85]}=16.05$, $P<0.0001$, Fig. 3.7C).

Analyzing within each treatment across sampling periods, photosynthetic rates of soybean plants in all treatments decreased from the Initial to Final sampling period and again from the Final to Recovery periods. The only significant differences detected in photosynthetic rates were in the Ambient treatment at the Recovery period, which was significantly lower than the Initial and Final sampling periods; and in the Elevated treatment, where photosynthetic rates after the Recovery period were significantly lower than in the Initial sampling period (Control: $F_{[2, 84]}=0.31$, $P=0.7318$; Ambient: $F_{[2, 110]}=14.74$, $P<0.0001$; Elevated: $F_{[2, 109]}=3.8$, $P=0.0253$). In the Ambient treatment, stomatal conductance increased significantly from Initial to Final sampling followed by a significant decrease from Final to Recovery (Control: $F_{[2, 84]}=37.08$, $P<0.0001$; Ambient: $F_{[2, 110]}=3.67$, $P=0.0286$). Plants within the Elevated treatment, however, experienced an increase in stomatal conductance among all sampling periods (Initial, Final, Recovery) but only the increase from the Initial to Final period was significant ($F_{[2, 109]}=13.4$, $P<0.0001$). Water use efficiency tended to decrease consecutively from the Initial to Final sampling periods and again at the Recovery period for plants in the Ambient and Elevated treatments, but only the reductions in the Elevated treatment were significant (Ambient: $F_{[2, 110]}=2.33$, $P=0.1016$; Elevated: $F_{[2, 109]}=21.75$, $P<0.0001$).

When the data was analyzed within sampling periods, TS at the Initial sampling period was significantly lower for plants in the Elevated treatment than in the Ambient and Control treatments ($F_{[2, 120]}=57.74$, $P<0.0001$); TS in the Ambient treatment was also significantly lower than in the Control. By the Final sampling period, TS in the Ambient treatment was significantly lower than in the Elevated treatment ($F_{[2, 126]}=6.23$, $P=0.0026$). At the Initial sampling period,
BS was significantly higher in plants in the Control treatment than plants in the Ambient treatment; both were significantly higher than the BS of plants in the Elevated treatments ($F_{[2, 120]} = 27.44, P<0.0001$). By the Final period, BS of plants in the Ambient treatment was significantly lower than in both the Elevated and Control treatments ($F_{[2, 126]} = 12.3, P<0.0001$).

Within each treatment, TS of soybean leaves tended to increase from the Initial to Final sampling periods in the Ambient and Elevated treatments, but only significantly so in the Elevated treatment (Control: $F_{[1, 81]} = 19.57, P<0.0001$; Ambient: $F_{[1, 84]} = 1.76, P=0.1879$; Elevated: $F_{[1, 81]} = 73.73, P<0.0001$, Fig. 3.8A). BS decreased significantly in the Ambient treatment from Initial to Final sampling periods while it increased significantly in plants of the Elevated treatment (Control: $F_{[1, 81]} = 1, P=0.3191$; Ambient: $F_{[1, 84]} = 7.58, P=0.0072$; Elevated: $F_{[1, 81]} = 46.23, P<0.0001$, Fig. 3.8B). No significant differences within or across sampling periods were detected for the percentage of clogged stomata for either the top or bottom of the leaves of soybean plants ($P>0.05$).

**Discussion**

If diesel exhaust negatively impacts the ecophysiology of plants, we would expect plants exposed to elevated diesel exhaust to exhibit decreased ecophysiological activity compared to plants in the Ambient treatment. If plants are able to overcome reduced ecophysiology from pollutant exposure, we would also anticipate a recovery in which subsequent measurements in the absence of the pollutant should rebound to previous values. Although decreased activity with pollutant exposure was observed, it was highly variable between species and across sampling periods, and often depended on the ecophysiological measure (photosynthetic rate, stomatal conductance, water use efficiency, or stomatal clogging) considered. To understand the overall
effects of exposure to elevated diesel exhaust, we focus on the comparison of the Ambient and Elevated treatments in more detail below.

**Photosynthetic Rates**

For chicory plants, the photosynthetic rates increased in both the Elevated and Ambient treatments after the one-week treatment period. However, exposure to the elevated diesel exhaust resulted in a lower relative increase than in the Ambient treatment. In general, CO$_2$ is known to increase photosynthetic activity (e.g. Tai *et al.*, 2010); therefore CO$_2$ present in the exhaust could be contributing to the increase we observed. This increase could also be associated with normal growth and the increased need for photosynthates as the summer growing season progresses and fruit production begins. The lower relative increase observed in the Elevated treatment is not surprising because other diesel gases and PM are known to decrease photosynthetic capabilities (e.g. Rai and Kulshreshtha, 2006; Saxe, 1985). In contrast, soybean plants in both the Ambient and Elevated treatments experienced only slight and nonsignificant reductions in photosynthetic rates from the Initial to Final sampling period and again after the Recovery period. This suggests that there was no substantial impact of elevated diesel exhaust on photosynthetic rates in soybean plants, under the conditions of our study.

There are several reasons for the differential performance of chicory and soybean. Because soybean plants were first exposed to treatments as seedlings, their naturally rapid growth may have resulted in their photosynthetic rates per leaf area decreasing as more leaves were being produced. Additionally, soybeans produce many compound leaves, thus having greater leaf area per plant, while chicory plants have a smaller, basal rosette of leaves with few thin, serrated leaves on the stem (Fig. 1). These vegetative differences may help explain the contrasting results between chicory and soybean if photosynthetic rate is measured per unit area.
of the leaf. Photosynthetic rates of soybeans over the entire plant may be higher than in chicory individuals, and deserves further study.

Moreover, chicory may not experience many of the deleterious effects of air pollution because as a common roadside plant often flourishing along traffic ways, it may already be adapted to polluted conditions. Dwivedi and Tripathi (2007) investigated the air pollution tolerance index (APTI) of various plant species surrounding brick industrial plants based on their tolerance to SO₃, NO₂ and PM found that plants of lower APTI inhabited less polluted locations. Although soybean or chicory were not assessed specifically, plants of the Fabaceae had a lower average APTI than those of the Asteraceae family. If soybean and chicory act similarly to species of their respective families, chicory would be expected to have a greater threshold to air pollution, which is consistent with our results.

Stomatal Conductance, Counts, and Clogging

The lack of a clear treatment effect on stomatal conductance in chicory plants was unexpected. As plants grow and age, their stomatal conductance tends to increase, which was observed in chicory plants in both the Ambient and Elevated treatments. However, previous air pollution studies focusing on PM and gases, such as SO₂, O₃, and CO₂, are often associated with conductance reductions (e.g. McAinsh, 2001; Nighat et al., 2000; Onandia et al., 2011), which was not consistent with our findings.

In contrast, the significant increase observed in stomatal conductance in soybean in the Elevated treatment from the Initial to Final sampling period may have been a product of PM presence. Stomatal conductance is expected to increase with the growth of a plant, but for soybean plants in our Elevated treatment, PM may trigger greater increases in conductance to compensate for PM clogging. The higher stomatal conductance may allow for greater intake of
CO$_2$ in response to certain conditions and may have also increased in response to elevated CO$_2$ in the diesel exhaust. The observed increase in stomatal conductance may also explain the lack of a treatment effect on photosynthetic rate in soybeans. Furthermore, after the Recovery period, stomatal conductance in soybeans in the Ambient treatment had decreased to a value similar to their Initial measurement while in the Elevated treatment, there was a slight but nonsignificant increase. This suggests that the lack of particulates in the Elevated chamber during the Recovery period may have allowed the plants to stabilize.

The total number of stomata on the top and bottom of soybean leaves and on the top of chicory leaves increased in the Elevated diesel treatment, relative to the Ambient and Control treatments. This pattern was also noted previously by Rai et al. (2010) in which the frequency of stomata in annual plant species increased when treated with a daily 5g dose of urban dust. An explanation for this plant response has not yet been described. Physical clogging of stomata may encourage stomate production or inhibition of optimal gas exchange may make it necessary for the plant to produce more pores. Additionally, stomates may increase in number in response to elevated CO$_2$ levels in diesel exhaust. The fact that both soybean and chicory had greater number of stomata on leaves produced during exposure to diesel exhaust support these latter hypotheses.

**Water Use Efficiency**

The two species examined exhibited different water use efficiency responses. The fact that chicory plants in all treatments experienced similar reductions in water use efficiency over time suggests that diesel exhaust was not the primary cause. This is consistent with chicory’s common occurrence along the roadsides, where it may have undergone adaptation to pollutant exposure, and therefore it is unsurprising that plants in the various treatments would respond similarly. In contrast, the consistent and significant reduction in water use efficiency in soybeans
exposed to diesel exhaust suggests that soybeans may be less resilient to diesel exposure than chicory. However, it is also possible that high temperatures and other ambient conditions within the chambers may have stressed the plants, most specifically the soybeans. Soybeans in the Elevated treatment did respond with slightly more noticeable reductions in water use efficiency between each sampling period which also suggests that soybean may be more sensitive to air pollution.

Was PM the cause of the results observed?

Our results indicate that there is the potential for diesel exhaust to negatively impact the ecophysiology of some plant species, but we cannot say with certainty that the results described above are due solely to PM deposition on leaves. Results showing no effect of diesel exhaust on the percentage of clogged stomata in chicory and soybean suggests PM was not accumulating on the leaves during the duration of the project. Because open-top chambers allow plants to experience natural meteorological conditions, it is possible that PM deposited on plants was washed or blown from leaf surfaces before allowing for longer term stomatal clogging. In addition, the results observed could be heavily influenced by the gaseous components of the diesel exhaust (e.g. CO₂, CO, SO₂, NOₓ, and O₃). Because our experiment was designed only to focus on PM, the gases present in the Elevated treatment were not quantified. Future investigations should test the individual components of diesel exhaust on plant ecophysiology.

Conclusion

Differential effects of elevated diesel exhaust were detected between chicory and soybean plant species. Photosynthetic rates were only reduced in chicory, but stomatal conductance increased in soybeans after exposure to diesel exhaust, with differential species responses in water use efficiency. This ability to react to the surrounding environment by soybeans may be
beneficial for survival but over time it may put stress on leaf tissue and have more serious long-term effects that warrant investigation. This could have implications on biomass and fruit production in this economically important species (see Chapter 4). Because of the experimental design and the exposure to natural outdoor conditions, it is possible that the results observed were a combination of PM and gaseous pollutants present in the diesel exhaust. Further research is needed to investigate the effects of PM in real world urban conditions, especially at higher doses of airborne vehicle pollution. Given the observed variation in ecophysiological responses across species, this is especially important for both mitigating air pollution for human health while being aware of unwanted effects on plants of economic value during urban planning and decision making processes.
**Figures**

**Figure 3.1.** *Cichorium intybus* plants with inflorescences (Left) and *Glycine max* plant displaying compound leaves (Right).

**Figure 3.2.** Experimental design displaying the layout of the three chamber treatments and their location in reference to the generator. The non-sided Control chamber was located in a fenced in area away from the generator.
Figure 3.3. Generator with exhaust manifold and air compressors distributing elevated exhaust air to treatment chambers.

Figure 3.4. Circular plant arrangement within treatment chambers with 15 potted plants each of chicory and soybean alternating in two circles with a central drainage area.
Figure 3.5. Mean values of instantaneous photosynthetic rate (A), stomatal conductance (B), and water use efficiency (C) for chicory plants. Shown are results of an ANOVA examining the effects of treatment and chamber nested within treatment for Initial and Final sampling periods with Tukey test results and standard error.
Figure 3.6. Mean values for the total number of stomates on the top (A) and bottom (B) of chicory leaves. Shown are ANOVA results examining change within each treatment (Control, Ambient, and Elevated) from Initial to Final sampling with Tukey test results and standard error.
Figure 3.7. Mean values of instantaneous photosynthetic rate (A), stomatal conductance (B), and water use efficiency (C) for soybean plants. Shown are results of an ANOVA examining the effects of treatment and chamber nested within treatment for Initial, Final, and Recovery sampling periods with Tukey test results and standard error.
Figure 3.8. Mean values for soybean leaf top (A) and bottom (B) total stomata. Shown are ANOVA results examining change within each treatment (Control, Ambient, and Elevated) from Initial to Final sampling with Tukey test results and standard error.
References


Misik, M., Solenska, M., Micieta, K., Misikova, K., & Knasmuller, S. (2006). In situ monitoring of clastogenicity of ambient air in Bratislava, Slovakia using the *Tradescantia*
micronucleus assay and pollen abortion assays. *Genetic Toxicology and Environmental Mutagenesis* 605, 1-6.


Chapter 4:

Growth and reproduction of *Cichorium intybus* and *Glycine max* after short-term exposure to diesel exhaust

Abstract

As air pollution has become an increasingly ubiquitous national and global concern, plants have become important tools to cleanse the atmosphere of airborne pollutants. Given that many plant species of economic and natural value are now grown in urban areas and traffic corridors, it is impossible to disregard the effects of airborne pollutants on the plants themselves. Using an interdisciplinary approach combining environmental engineering methods with ecology, we examined the effects of diesel exhaust and its associated particulate matter on two plant species: (1) *Glycine max* (soybean) and (2) *Cichorium intybus* (chicory). Plants were grown in outdoor open-top chambers and exposed to either diesel exhaust or ambient air along with a chamber control for one week. We analyzed their reproductive and growth traits before and after treatment, and again after a recovery period to better understand the impact of diesel exhaust on plant health. In both species, only minor growth and reproductive differences were detected between plants exposed to elevated exhaust and ambient air immediately after treatment. However, given a recovery period, soybean plants that had been exposed to elevated diesel exhaust responded with increased aboveground biomass without increasing their reproductive output. This research is a valuable step towards urban and agricultural planning in the face of anthropogenic air pollution.

**Keywords:** *Cichorium intybus*, diesel, *Glycine max*, growth, reproduction
Introduction

As the human population grows and nations further develop, roadways are being used more heavily than ever before. In light of this, anthropogenic pollution along roadways continues to be a cause for discussion and mediation. Air pollution in particular has been a major cause for concern due to the serious human health implications that affect the respiratory and cardiovascular systems (Lorenzo et al., 2011). Some countries have been working to regulate airborne pollutants for many years, including the U.S. with its Clean Air Act of 1963.

One way of cleansing the atmosphere is by using plants as purifiers to reduce ambient pollutants, therefore improving air quality (e.g. Manning, 2008). For example, the large surface area of leaves in urban woodlands help filter atmospheric particles (Beckett et al., 1998). Urban trees and shrubs within the U.S. alone have been estimated to remove 711,000 metric tons of air pollutants annually, including ozone (O₃), particulate matter (PM), nitrogen oxides (NOₓ), sulfur dioxide (SO₂), and carbon monoxide (CO) (Nowak et al., 2006). Diesel emissions are a major contributor of these gases and particulates to urban roadways (Sebelius, 2011). Diesel exhaust has also been shown to resemble the urban atmospheric pollutant mixture (Honour et al., 2009).

While using plants as biological cleansers is an extremely beneficial and natural antidote to ambient pollution, the agricultural and economic value of many plant species makes it imperative that we consider the effect of pollution on the plants themselves. Even though plants as a whole are resilient to the many anthropogenic challenges they encounter, some plant families are less tolerant (Dwivedi and Tripathi, 2007). From the plant perspective, increased levels of certain pollutants such as carbon dioxide (CO₂) can actually stimulate their growth. Although this may initially seem beneficial for crops and other plants grown for human use, it is also important to understand how this growth will affect fruit production. Although plants may
respond to elevated CO₂ with stimulated growth, CO₂ in conjunction with other pollutants have shown growth reductions at roadside locations in comparison to a control setting (e.g. Kammerbauer and Dick, 2000). To date, there have been relatively few studies focusing specifically on the effects of PM on plants. Stunted growth and physical clogging of stomates and stigmas has been observed (e.g. Honour et al., 2009 and Thompson et al., 1984). Stigma clogging could act as a physical block to pollination and therefore hinder fertilization in flowers.

To examine the growth and reproductive responses of plants in an elevated airborne pollutant setting, we subjected plants growing in outdoor open-top chambers to repeated doses of diesel exhaust from a diesel generator. Although some elevated gases were expected to stimulate plant growth, we hypothesized that there would be overall negative responses to growth and reproduction.

**Materials and Methods**

**Location**

All experimentation and sampling took place at the University of Cincinnati’s Center for Field Studies (UCCFS) during the summer of 2013. UCCFS is located about 22 miles west of Cincinnati’s city center in Harrison, Ohio (39°17’13.91”N; 84°44’25.62”W). Because the site is bordered by farmlands and the Great Parks of Hamilton County’s Miami Whitewater Forest, it experiences low traffic and associated pollutant emissions. During the summer months of June to August 2013, the temperature ranged between 6.1°C-36.5°C with an average of 22°C and rainfall totaled 25 cm.

**Study Species**

For this study, two angiosperm plant species were used based on human uses and their typical proximity to roads: (1) soybean (*Glycine max*) and (2) chicory (*Cichorium intybus*).
Soybeans are one of the major crops produced internationally and by the U.S. for their economically valuable fruit pods (e.g. Libault et al., 2010). In Ohio specifically, soybean is one of the major crops produced and is often grown along roadsides in agricultural fields. Soybean is an annual herb of the Fabaceae family that has an active growth period during spring and summer months (e.g. Libault et al., 2010). Soybeans have also been used in previous studies of atmospheric pollutants (e.g. Ainsworth et al., 2002; Heagle et al., 1973).

Chicory, of the Asteraceae family, is often found thriving along heavily used roadways in the United States. It is an herbaceous and weedy herb, often having multiple stalks and a basal rosette of leaves. Each chicory flower is technically a composite head of florets, and will bloom during the summer months. Each flower blooms for a single day so that flowers collected while open in the field only had their reproductive parts exposed to a single day of the surrounding conditions. Chicory is economically important in agriculture as a sweetener in foods and teas. Although the species is native to Europe, West Asia, and North Africa, chicory is naturalized in the Midwestern states of the U.S., including Ohio. Prior studies have used chicory as a phytioindicator of air pollution (e.g. Misik et al., 2006).

**Experimental Design**

Field methods for this experiment follow the open-top chamber design outlined in Chapter 3. Six outdoor, open-top chambers and three non-sided control chambers were built to expose study plants to three treatments with three replicates of each. The treatments were (1) elevated exposure to diesel exhaust within a chamber (Elevated), (2) exposure to ambient air within a chamber (Ambient), and (3) ambient air without a chamber (Control). The open-top design allows the interior of the chamber stay consistent with the surrounding meteorological conditions while providing protection from the elements and allowing pollutants within the
chambers to linger. Chambers were constructed as 1.3 m high and 2 m diameter vertical pentagonal spaces framed by 2.54 cm diameter galvanized steel, electrical conduit. This frame was enclosed with translucent PVC plastic film (Plastic Film Corporation Romeoville, Illinois). The unenclosed Control treatments were kept within a fenced deer exclosure as protection from herbivory and these were placed south-wind of the other treatment chambers to prevent exhaust contamination from the other treatments.

A diesel generator (ETQ-DG4LE 4000Watt/3500Watt HP Diesel Portable Generator) was specially customized to provide the treatment chambers with diluted diesel exhaust (Elevated treatment) or ambient air (Ambient treatment). An extended stack was created for the generator with three aspirator pumps fixed around it at 120 degrees, which combined raw diesel exhaust with ambient air for the elevated treatment. Compressed ambient air was provided to the aspirator pumps by two air compressors (Porter-Cable 3.5 gallon Pancake). One outlet tube from each of the three aspirator pumps ran to the top central point of the elevated treatment chambers to discharge the modified air. Using the EPA Method 5 test, diluted exhaust gas from the three dilution tubes were found to emit an average of 0.0370 g/m³ total PM. In the open-top chambers in the Ambient treatment, tubing ran directly from the air compressors to discharge untarnished surrounding air to the same location within the chambers.

Fifteen plant individuals of each of the two species were arranged in an alternating pattern to create two circles within each chamber. The inner circle had five plants and the outer circle contained ten plants for a total of 30 plants in each chamber and 135 total plants per species across all chambers. To keep underground root competition to a minimum and prevent variation in soil composition, plants were grown in 5 liter volume treepots using soilless media (Pro-Mix, High Porosity Mycorrhizae Professional Growing Medium), which were then buried
with 3 cm left above ground. Prior to transplantation into these pots, soybeans were germinated from seed (Organic Soybean, Living Whole Foods, Inc.) in a controlled greenhouse setting at the University of Cincinnati until their first true leaves emerged and then allowed to acclimate outside for one week. Chicory plants were collected from a single roadside field population in May 2013 and immediately transferred to the 5 liter treepots. All plants were watered daily.

Data Collection

All plants were first measured as an “Initial” sampling period prior to beginning treatment on June 18, 2013. During treatment, air was delivered to the chambers from 9:00 AM to 2:00 PM EST, daily for approximately one week. The unforeseen loss of the generator ended the treatment phase early and measurements were again taken after the last treatment day, June 25, 2013 (referred to as “Final”). On August 6, 2013 a series of “Recovery” measurements were taken to assess the plants after a 5½ week recovery period.

To quantify the growth responses at each measurement period, individual chicory plants were measured for stalk height, leaf height, stalk number, and node number at the Initial, Final, and Recovery sampling periods. Stalk height was measured from the soil level to the highest point of the plant on any stalk. Leaf height was measured as the highest natural height of the basal rosette of leaves. Stalk and node number consisted of counts of the number of stalks and nodes on each individual plant, respectively. Soybean plants were measured at the Initial and Final sampling periods for stalk height and node number in the same manner as chicory. Due to exorbitant growth, these measurements were not taken for soybean at the Recovery period but replaced by aboveground biomass. We were unable to harvest chicory plants for aboveground biomass measurements.
Successful pollination requires that pollen is able to adhere to available stigmatic surfaces within the flower. As a measure of reproductive responses, chicory flowers that were exposed in the chambers were collected for microscopic analysis of stigmas. During the Initial and Final sampling, five flowers per plant were collected and preserved in formalin-acetic-alcohol (FAA) solution. Three stigmas of each flower were then dissected out in the laboratory, and the stigmas were then stained with aniline blue on microscope slides. Each stigma was then examined microscopically under 40x power to quantify the total number of viable pollen grains, the pollen germination rate as a percentage of pollen germinated to total pollen, and number of particulate matter pieces touching the active portion of the stigma. Soybean fruits were also collected from each plant at the Recovery period and used to calculate the total number of pods per plant, average seed weight, and average number of seeds per pod.

Data Analysis

To analyze chicory and soybean growth, the characters of stalk height, leaf height, stalk number, and node number were analyzed separately for each species and sampling period in a nested ANOVA, with chamber nested within time. All data conformed to the assumptions of normality and heterogeneity required for the analyses, except for chicory stalk number, which was transformed using the natural log. Post hoc Tukey tests were then done to examine effects of treatment (Elevated, Ambient, and Control) with chamber nested within treatment. A nested ANOVA was also used to examine the effects of time for each of these growth measurements, with the chamber effect nested within time. In this analysis, the time variables were Initial and Final sampling periods (for soybean), in addition to the Recovery period (for chicory only). Aboveground biomass of soybean plants was analyzed using a one-way ANOVA with Type III
sums of squares and *post hoc* Tukey tests to examine the effects of the treatment during the Recovery sampling period.

Chicory reproductive measurements (number of pollen grains, percent germination, and total particulate matter) were also analyzed separately for each sampling period (Initial and Final) using a nested ANOVA and *post hoc* Tukey tests to examine effects of treatment with chamber nested within treatment. Percent germination and total particulate matter were log\(_{10}\) transformed to meet the assumptions of heterogeneity and all other chicory reproductive data met the assumptions without adjustment. A nested ANOVA was also used to examine the effects of time (Initial and Final sampling periods) for each of these growth measurements with the chamber effect nested within time.

For the analysis of soybean reproductive measures, the average seed number, average seed weights, and total pod numbers of soybean plants were analyzed individually using a one-way ANOVA with Type III sums of squares and *post hoc* Tukey tests to examine the effects of the treatment during the Recovery sampling period. The assumptions of normality were met by all soybean reproductive measures, except average seed weights which was natural log transformed.

**Results**

**Growth of Chicory**

In general, there were no significant differences (specific statistical values provided in Table 4.1) in chicory growth in terms of leaf height, node number, and stalk number among Control, Ambient, and Elevated treatments within each sampling period (*P* > 0.05; Table 4.1; Figs. 4.1-4.3A). Chicory stalk height showed no difference among treatments at the Initial sampling period (*F*\(_{2,51}\) = 0.22, *P* = 0.8014; Fig. 4.3B), but plants grown in the Elevated treatment...
had significantly higher stalk height than both the Control and Ambient treatments at the Final sampling time ($F_{[2, 75]}=7.11, P=0.0015$; Fig. 4.3B). At Recovery, the Elevated chicory stalk height was significantly higher than the Control treatment plants ($F_{[2, 69]}=4.86, P=0.0106$) but the Ambient treatment had similar heights to both the Control and Elevated (Fig. 4.3B).

For the analysis within each treatment (i.e. Ambient, Elevated, Control) across sampling periods, results varied for the chicory growth measures. In several cases, Control and Elevated treatments responded similarly. Control and Elevated treatment plants showed an increase in stalk height from Initial to Final sampling and maintained the same height at Recovery (Control: $F_{[2, 66]}=4.26, P=0.0184$; Elevated: $F_{[2, 67]}=32.94, P<0.0001$). In the Ambient treatment, stalk height at the Initial sampling was significantly lower than after the Recovery period but at the Final sampling period, heights were similar to both the Initial and Recovery values ($F_{[2, 66]}=5.27, P=0.0075$). Similarly, leaf height measurements increased from the Initial to the Final sampling period and maintained the same height at Recovery (Control: $F_{[2, 126]}=5.36, P=0.0059$; Elevated: $F_{[2, 126]}=5.86, P=0.0037$). Within plants of the Ambient treatment, leaf height did not change significantly across sampling periods ($F_{[2, 126]}=2.8, P=0.0643$). No significant differences were detected for stalk number of any treatment (Control: $F_{[2, 126]}=0.82, P=0.4415$; Ambient: $F_{[2, 126]}=2.2, P=0.1146$; Elevated: $F_{[2, 126]}=2.11, P=0.1257$). In all three treatments, node number increased significantly from Initial to Final sampling and maintained the same node number at Recovery (Control: $F_{[2, 72]}=9.19, P=0.0003$; Ambient: $F_{[2, 72]}=7.59, P=0.001$; Elevated: $F_{[2, 73]}=8.68, P=0.0004$).

**Growth of Soybean**

Differences between soybean Ambient and Elevated treatments occurred infrequently. In the analysis within individual sampling periods, stalk height was significantly lower in the
Control treatment than in both the Ambient and Elevated treatments at the Initial and Final sampling periods (Initial: $F_{[2, 126]}=65.18, P<0.0001$; Final: $F_{[2, 126]}=131.04, P<0.0001$; Fig. 4.4). Similarly, node number on soybean plants was significantly higher in the Ambient and Elevated treatments than the Control treatment at the Initial sampling period ($F_{[2, 126]}=9.65, P=0.0001$; Fig. 4.5). At the Final sampling period, node numbers were significantly higher in the Elevated treatment than the Control; however, the Ambient levels were similar to both the Elevated and Control treatments ($F_{[2, 126]}=6.24, P=0.0026$; Fig. 4.5).

Within each treatment, significant increases from Initial to Final sampling period were detected in soybean stalk height and node number (stalk height Control: $F_{[1, 75]}=438.02, P<0.0001$; stalk height Ambient: $F_{[1, 80]}=812.46, P<0.0001$; stalk height Elevated: $F_{[1, 74]}=215.11, P<0.0001$; node number Control: $F_{[1, 75]}=589.31, P<0.0001$; node number Ambient: $F_{[1, 80]}=410.36, P<0.0001$; node number Elevated: $F_{[1, 74]}=373.28, P<0.0001$). In the analysis of aboveground biomass at the Recovery sampling period, the Elevated treatment was significantly higher than both the Control and Ambient treatments ($F_{[2, 81]}=3.74, P=0.028$; Fig. 4.6).

**Reproduction of Chicory**

In the main analysis of treatment effects on plants within each sampling period, the number of pollen grains and percent germination showed no statistical difference between the Ambient and Elevated treatments at the Initial and Final sampling periods (Figs. 4.7A-B). However, plants in the Control group exhibited a significantly lower number of pollen grains while percent germination was significantly higher than both the Ambient and Elevated treatments at the Initial and Final sampling periods (pollen grains Initial: $F_{[2, 64]}=82.56, P<0.0001$; pollen grains Final: $F_{[2, 26]}=16.07, P<0.0001$; percent germination Initial: $F_{[2, 64]}=67.71, P<0.0001$; percent germination Final: $F_{[2, 26]}=12.84, P=0.0001$; Figs. 4.7A-B). At the Initial
sampling, total particulate matter was significantly higher on plants in the Ambient treatment but at the Final sampling, all treatments were similar (Initial: $F_{[2, 64]} = 9.82, P=0.0002$; Final: $F_{[2, 26]} = 1.39, P=0.2663$; Fig. 4.8).

In most cases, no statistical differences (specific values provided in Table 4.2) were detected in the analysis within each treatment across sampling periods for the number of pollen grains, percent germination, or total particulate matter on plants ($P>0.05$; Table 4.2). The only significant change detected was an increase from the Initial to Final sampling period of total particulate matter for plants grown in the Control treatment ($F_{[1, 32]} = 9.6, P=0.0046$).

**Reproduction of Soybean**

Values for average seed number per pod were similar in plants of all treatments ($F_{[2, \ 126]} = 0.96, P=0.3843$; Fig. 4.9A). Soybeans in the Control treatment exhibited higher average seed weights and total pod numbers than in the Ambient treatment (Figs. 4.9B-C). However, the Elevated treatment plants had values similar to both the Ambient and Control (average seed weight: $F_{[2, 126]} = 3.72, P=0.0271$; total pods: $F_{[2, 126]} = 3.19, P=0.0444$; Figs. 4.9B-C).

**Discussion**

Based on our hypothesis that elevated diesel exhaust would have a negative impact on overall plant growth and reproduction, we expected plant responses to the Elevated treatment to be lower compared to the Ambient treatment. Additionally, if plants can recover from such reduced growth and reproduction, we would predict a rebound after the Recovery period. In general, neither soybean nor chicory experienced severe growth or reproductive impediments upon exposure to elevated diesel exhaust within a week long period. Here we will focus on the comparison between the Ambient and Elevated treatments to understand the overall effects of exposure to elevated diesel exhaust.
Growth of Chicory

In most cases, there was no significant difference in growth responses of chicory relative to the elevated exhaust treatment. Following the Initial sampling period, chicory plants in all treatments experienced a slight growth spurt, which could be associated with normal growth for the summer growing season and the initiation of fruit production. After this early growth, little change from the given Recovery period was detected in both stalk number and node number. This similarity in response between the Ambient and Elevated treatments was unexpected. Certain gases of diesel exhaust are usually associated with growth hindrance, such as NO$_x$, SO$_2$, and O$_3$ (e.g. Bell *et al.* 2011; Saxe 1994). CO$_2$, however, generally elevates plant growth and has been shown to cause an increase in plant mass across multiple species, especially if administered for short-term durations (Makino and Mae, 1999; Wong, 1979). However, in our experiment the effect of CO$_2$ may have balanced the negative growth consequences of the other gases, resulting in similar growth between Ambient and Elevated treatments. In contrast, leaf height measurements decreased slightly after the Initial period but there was no difference in response among treatments; therefore this trait may not have been affected by elevated exhaust. Stalk height was the only growth response of chicory to respond differently in the Elevated treatments. After the Initial increase in growth, plants within the Elevated treatment grew more than the other treatment during the subsequent sampling periods. As a general growth measurement, stalk height may be the most sensitive and reactive to the elevated CO$_2$ in the chamber, as compared to other traits measured.

Growth of Soybean

In soybean, early growth was also observed after the Initial sampling period and as in the case of chicory, it was most likely due to the normal summer growing season. This is especially
likely as soybean plants were started from seed and were small seedlings at the first measurement. Unlike chicory, soybean plants in the Elevated treatment did not grow at a faster pace than plants within the Ambient treatment. As a species, soybeans may be more sensitive to air pollution and may not be able to utilize the benefits that may be associated with higher levels of CO₂ like other more resilient species. Chicory and other plants in its taxonomic family already inhabit and flourish in polluted locations like roadways and industrial areas (Dwivedi and Tripathi, 2007) and are likely adapted to these conditions. We did find that even though no differential growth measures were observed immediately after treatment, soybean plants that underwent exposure to elevated diesel exhaust had higher biomass than plants in the Ambient treatment after the Recovery period. Although plants did not show major growth differences by treatment during the week of treatment, we do not know if this would persist with more continuous treatment. Evaluation of photosynthetic rates in this same open-top chamber study suggests that the photosynthetic activity of soybeans was not intensified during the duration of the treatment (see Chapter 3). Soybean plants exposed to the Elevated treatment may have had to work more intensely to keep their photosynthetic rates at the same level as plants within the Ambient treatment. However, during the Recovery period, these plants may have continued at this elevated work rate. In this scenario, without the elevated exhaust during this period, the soybeans would be working hard to compensate for non-existent strains and the energy could have gone towards biomass production. In future studies, it will be important to expose plants to longer-term treatments to understand what may happen over the entire growing season.

**Reproduction of Chicory and Soybean**

In most cases, PM levels on chicory stigmas increased slightly after treatment exposure and were consistent across treatments. The expected higher PM values on plants in the Elevated
exhaust treatment were not observed. Based on our results, chicory does not show signs of reproductive challenges due to physical hindrance of pollen to stigmas. Pollen grain counts and pollen germination rates were similar for the Ambient and Elevated treatments before and after the treatment period. This combined with the consistent PM values suggests that this level of atmospheric PM pollution does not clog stigmas. The unenclosed control chamber had lower pollen grain counts and higher germination rates, which may be due to greater exposure to wind and also to generally healthier plants. The chambers tended to retain heat and enclosed plants had a higher incidence of leaf chlorosis. Even in the case of soybean reproductive output, no treatment effect was observed. Average seed weight, average seed number, and total number of seed pods were not affected by the elevated exhaust. Again, the dosage and short-term duration of the elevated exhaust exposure may play a large part in this lack of response. In addition, the fact that chicory flowers open and close within one day limits the ability of chicory to be physically impaired by PM stigma clogging. Other species, however, lack this capability and instead may experience negative consequences due to daily accumulation to PM if their flowers remain open for several days. Because many of these species include economically valuable and agricultural plants, such as major US crops (e.g., corn and cotton), it is important to continue studies of PM on the reproduction of other economic species.

Conclusion

Although previous studies have suggested that plant yields and even nutritional content could be negatively impacted by air pollution (Rai and Kulshreshtha, 2006), reproductive and fruit yields of chicory were not affected by elevated diesel exhaust in our study. However, the significant results in aboveground biomass of soybeans after the Recovery period suggest there is some differential growth activity. Some economic species are valued for their fruit while other
species are important because of their foliage. Because of this, future studies should address the effects of air pollution on various species and their economically valuable output (e.g. fruit or leaves). Knowing and understanding the type and extent of damages due to gaseous and particulate air pollutant exposure will help biologists, crop breeders, and horticulturalists optimize plant performance. Furthermore, this same concept can be applied to choosing the best species for mitigating air pollution. If certain species are known to have high tolerance to air pollution, they can be chosen above plants less tolerant for planting in areas where natural atmospheric cleansers are desired.
Tables

Table 4.1. Statistical results of the ANOVA examining the effect of treatment within each sampling period for chicory growth measures.

<table>
<thead>
<tr>
<th>Chicory Growth Measure</th>
<th>Sampling Period</th>
<th>d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf height</td>
<td>Initial</td>
<td>2, 126</td>
<td>1.33</td>
<td>0.2679</td>
</tr>
<tr>
<td>Leaf height</td>
<td>Final</td>
<td>2, 123</td>
<td>1.36</td>
<td>0.2611</td>
</tr>
<tr>
<td>Leaf height</td>
<td>Recovery</td>
<td>2, 122</td>
<td>1.09</td>
<td>0.3386</td>
</tr>
<tr>
<td>Node number</td>
<td>Initial</td>
<td>2, 57</td>
<td>0.18</td>
<td>0.8346</td>
</tr>
<tr>
<td>Node number</td>
<td>Final</td>
<td>2, 83</td>
<td>0.22</td>
<td>0.8051</td>
</tr>
<tr>
<td>Node number</td>
<td>Recovery</td>
<td>2, 71</td>
<td>0.05</td>
<td>0.9529</td>
</tr>
<tr>
<td>Natural log of stalk number</td>
<td>Initial</td>
<td>2, 126</td>
<td>0.02</td>
<td>0.8220</td>
</tr>
<tr>
<td>Natural log of stalk number</td>
<td>Final</td>
<td>2, 123</td>
<td>0.01</td>
<td>0.9923</td>
</tr>
<tr>
<td>Natural log of stalk number</td>
<td>Recovery</td>
<td>2, 122</td>
<td>0.00</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

Table 4.2. Statistical results for the ANOVA analysis examining pollen grains, log transformed percent germination, and log transformed total particulate matter within each treatment across sampling periods.

<table>
<thead>
<tr>
<th>Chicory Reproductive Measures</th>
<th>Treatment</th>
<th>d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollen grains</td>
<td>Control</td>
<td>1, 32</td>
<td>1.53</td>
<td>0.2272</td>
</tr>
<tr>
<td>Pollen grains</td>
<td>Ambient</td>
<td>1, 35</td>
<td>2.18</td>
<td>0.1487</td>
</tr>
<tr>
<td>Pollen grains</td>
<td>Elevated</td>
<td>1, 29</td>
<td>1.5</td>
<td>0.2310</td>
</tr>
<tr>
<td>Log transformed percent germination</td>
<td>Control</td>
<td>1, 32</td>
<td>0</td>
<td>0.9775</td>
</tr>
<tr>
<td>Log transformed percent germination</td>
<td>Ambient</td>
<td>1, 35</td>
<td>0.01</td>
<td>0.9303</td>
</tr>
<tr>
<td>Log transformed percent germination</td>
<td>Elevated</td>
<td>1, 29</td>
<td>0.33</td>
<td>0.5681</td>
</tr>
<tr>
<td>Log transformed total particulate matter</td>
<td>Control</td>
<td>1, 32</td>
<td>9.6</td>
<td>0.0046</td>
</tr>
<tr>
<td>Log transformed total particulate matter</td>
<td>Ambient</td>
<td>1, 35</td>
<td>0.55</td>
<td>0.4630</td>
</tr>
<tr>
<td>Log transformed total particulate matter</td>
<td>Elevated</td>
<td>1, 29</td>
<td>1.38</td>
<td>0.2497</td>
</tr>
</tbody>
</table>
**Figures**

**Figure 4.1.** Mean value results for chicory leaf height including results of an ANOVA examining the effects of treatment and chamber nested within treatment for Initial and Final sampling periods with Tukey test results and standard error.

**Figure 4.2.** Mean value results for chicory node number including results of an ANOVA examining the effects of treatment and chamber nested within treatment for Initial and Final sampling periods with Tukey test results and standard error.
Figure 4.3. Mean value results for chicory (A) stalk number (B) and stalk height including results of an ANOVA examining the effects of treatment and chamber nested within treatment for Initial and Final sampling periods with Tukey test results and standard error.
Figure 4.4. Mean value results for soybean stalk height including results of an ANOVA examining the effects of treatment and chamber nested within treatment for Initial and Final sampling periods with Tukey test results and standard error.

Figure 4.5. Mean value results for soybean node number including results of an ANOVA examining the effects of treatment and chamber nested within treatment for Initial and Final sampling periods with Tukey test results and standard error.
Figure 4.6. Mean values of soybean aboveground biomass at the Recovery sampling period with results of ANOVA and Tukey tests examining the effects of the treatment.
Figure 4.7. Mean value results for chicory (A) pollen grains and (B) pollen germination rates including results of an ANOVA examining the effects of treatment and chamber nested within treatment for Initial and Final sampling periods with Tukey test results and standard error.
Figure 4.8. Mean value results for particulate matter on chicory plants including results of an ANOVA examining the effects of treatment and chamber nested within treatment for Initial and Final sampling periods with Tukey test results and standard error.
Figure 4.9. Mean values of soybean (A) average seed number per pod, (B) average seed weight, and (C) total pod number per plant at the Recovery sampling period with results of ANOVA and Tukey tests examining the effects of the treatment.
References


Chapter 5:

Conclusion - A brief summary and future directions

Summary

Overall, this series of studies investigated the response of *Cichorium intybus* (chicory) and *Glycine max* (soybean) plants to air pollution commonly found along roadways. This was accomplished by carrying out two main experiments: (1) a field survey of chicory flowers produced along roads that varied in traffic intensity to quantify particulate pollution and deposition on stigmatic surfaces as a measure of reproductive processes (Chapter 2); and (2) an outdoor open-top chamber field experiment that exposed both species to diesel exhaust as a representative of ambient roadside gases and particulate matter (PM) to investigate the growth, reproductive, and ecophysiological responses of the plants (Chapters 3 & 4).

In general, chicory pollen deposition and pollen germination as measures of reproduction did not show significant reductions due to exposure to larger roads and did not show significant stigmatic clogging due to particulates (Chapter 2). This was the case for the different road-types across Cincinnati including interstates, U.S. highways, state highways, and county roads. A relationship based on PM levels no matter the road-type was detected. This suggests PM may still be affecting chicory reproduction. When examining the reproductive impacts of PM on chicory plants with short-term exposure to diesel exhaust in open-top chambers (Chapter 4), we saw little effect on stigma clogging, pollen deposition, and pollen germination. Because chicory is commonly found flourishing along roadways, these results may be due to this species in particular being well suited and adapted to particle pollution. Similarly, soybean reproductive output did not seem to suffer with short-term exposure to the diesel exhaust. Chicory plants did show some increased growth in the presence of diesel exhaust while soybean plants did not share
this effect. Again, the acclimatization of chicory to roadway conditions may explain these growth results. When the chamber-grown soybean plants were given the recovery period after their treatment exposure to diesel exhaust, they responded with significantly more aboveground biomass than soybean plants that did not receive the diesel exhaust treatment. In addition, the fact that there was no significant difference in soybean reproductive output between the two treatments after the recovery period suggests that the increase in biomass did not occur due to fruit production. When assessing the ecophysiological responses of chicory and soybean plants exposed to diesel exhaust, differential effects were observed between the two species (Chapter 3). Soybean plants increased their stomatal conductance after diesel exposure while chicory did not. In contrast, photosynthetic rates of soybean did not differ based on treatment, but chicory exhibited reduced photosynthetic rates after exposure to diesel exhaust.

Future Directions

The fact that roadways are a continuous source of ambient air pollution make it imperative that research continues to further understand impacts of gaseous and particulate pollutants on the environment. Although many efforts are ongoing in the United States and worldwide to mitigate air pollution, it is unrealistic to assume that ambient pollution will be completely removed. Therefore, it is and will continue to be crucial to understand current and future pollutants in the environment, and their effect on humans and plants, as an extension of human livelihood. Plants play a huge role in the well being of humans because they are the basis of our food chain and provide the foundation for many items produced commercially - ranging from clothing and medicine to buildings and fuel. While the studies described here provide timely and valuable insight to the effects of these pollutants on terrestrial plant life, they also show that more research on the subject is warranted, especially in regard to particulate matter
found in diesel exhaust. As previously described, the effects of many gaseous pollutants emitted via vehicular exhaust (e.g. SO\(_2\), CO\(_2\), and NO\(_x\)) have been documented over the years. However, particulate matter is an increasingly ubiquitous concern and our study provides early insight of how these particles might affect plant life in natural and field settings. To date, most studies focusing on the effects of particulate matter on plants use laboratory experiments where particulates are manually distributed on the plants. Our goal was to begin research in an innovative and interdisciplinary direction that focuses on real world ambient PM in natural and field settings.

To best understand plant responses to diesel exhaust, our initial plan was to expose our study species to daily diesel exhaust and control treatments for the full summer growing season (Chapters 3 & 4). As is often the case with field-based projects, the experimentation did not go as originally planned. Due to the unforeseen loss of our generator used in our experiments, we modified our study from a long-term exposure to the one-week exposure experiment described previously. Despite this change in experimental design, our results provide important information on the experimental design of open-top chamber studies with a modified diesel generator that is beneficial for future research. Furthermore, the results from the short-term experiment are useful for understanding the initial responses of plants to exposure from an early growth stage. Future research examining full growing season exposure would be the next step in generating long-term results. This will reveal if there are long-term effects that are undetectable during shorter durations and especially in regard to pollutant accumulation over time.

Additionally, the loss of the generator also limited our ability to completely quantify the composition of gases in the diesel exhaust. Particulates themselves also vary in composition because their components include various particles and chemicals. Our focus on PM based on
quantity and size may not tell the complete story and continued research including the separation of the exhaust chemicals in a field setting could link specific gases and PM composition to individual plant responses.

In the current political climate and public debate, focusing on the topic of pollution can be a sensitive subject. While we initially hypothesized there would be negative consequences on plants due to gaseous and particulate roadway pollutants, our findings on the responses of plants can be useful for urban and agricultural planning regardless of whether the plant responses were affected negatively or positively. For instance, we found that chicory plants were more tolerant to exhaust and roadway particulate air pollution than soybean plants. Future research can work towards identifying more species that have high tolerance and assess their ability to uptake pollutants, thus removing them from the atmosphere. These species could potentially be used to cleanse the air and mitigate ambient pollution in a natural way. Investing in studies to pinpoint plant species that provide these beneficial services would ultimately save resources on attempting to use less than optimal species.

In terms of agriculture and economic species, future studies could identify plants that have difficulty growing in the presence of roadside exhaust. With this information in hand, agricultural areas can be designed to keep less tolerant crops further from the road and those more tolerant, closer. Results from Chapter 4, focusing on plant growth and reproductive processes, suggest that given a recovery period after short-term diesel exhaust exposure, soybean plants produce more vegetative biomass than they would have naturally. This biomass is in the form of stem and leaf tissues rather than reproductive output (i.e. fruit). This could be a negative consequence if the crop was grown for its fruit; however, if the harvestable portion is the foliage, knowledge of this outcome could help in agricultural planning. Furthermore, many landscaping
plants valued for their aesthetics are grown along roadways. These plants may also be sensitive to air pollution making understanding the effects of roadway pollutants on these species another important research direction. Plants of different habits (e.g. shrub, herbs, and trees) or photosynthetic method (CAM, C3, and C4) also have potential for varying responses to roadway pollution and should be examined further. For example, trees are likely to be affected more than shrubs because they have greater storage for chemicals and are often longer lived. In addition, the ability of CAM plants to keep their stomata closed night and day may present different implications for gas exchange and PM accumulation and clogging compared to C3 and C4 plants, which should also be explored. Our research provides a foundation for continuing in this line of research. Future studies that include longer exposure time to diesel exhaust and exploration of a variety of species could reveal further variations in plant responses to air pollution and favorable methods for optimal farming practices and urban planning.