Expression of Oxidative Stress Regulators by Plants Used as Vegetative Noise Barriers upon Exposure to Road Transport Anthropophony

Jordan C. C'Dealva-Lenik

Follow this and additional works at: https://digitalcommons.esf.edu/honors

Part of the Plant Sciences Commons, and the Transportation Engineering Commons

Recommended Citation
C'Dealva-Lenik, Jordan C., "Expression of Oxidative Stress Regulators by Plants Used as Vegetative Noise Barriers upon Exposure to Road Transport Anthropophony" (2017). Honors Theses. 119.
https://digitalcommons.esf.edu/honors/119

This Thesis is brought to you for free and open access by Digital Commons @ ESF. It has been accepted for inclusion in Honors Theses by an authorized administrator of Digital Commons @ ESF. For more information, please contact digitalcommons@esf.edu, cjkoons@esf.edu.
Expression of oxidative stress regulators by plants used as vegetative noise barriers upon exposure to road transport anthropophony

by

Jordan C'Dealva-Lenik
Candidate for Bachelor of Science
Division of Environmental Science
With Honors
May 2017

Thesis Project Advisor: Lee A. Newman, Ph.D.

Second Reader: Jaime Mirowsky, Ph.D.

Honors Director: William M. Shields, Ph.D.

Date: May 8, 2017
Abstract

Recently, noise pollution has been recognized as a profound global issue with serious consequences for ecological, human, and animal health. Only one study has documented a health impact of noise on plants, noting expression of oxidative stress regulators to combat reactive oxygen species (ROS) generation in plant cells. Anthropophony (human-generated sounds) is the major cause of noise pollution, particularly road transport anthropophony (RTA) from vehicles and traffic congestion. Two plant species, *Buxus microphylla* (Wintergreen boxwood) and *Juniperus squamata* (Flaky juniper), were selected based on their use as vegetative barriers to control RTA. A field recording of RTA and corresponding sound pressure level measurements were taken alongside the U.S. 101 freeway in Woodland Hills, CA. This recording was looped to exposed plants (n=5) through a studio monitor at 73.5 dBA (±1%) for 24 hours in a soundproof room. Control plants (n=5) were left in silence in the soundproof room for 24 hours. Leaf tissue was harvested and analyzed via colorimetric UV/Vis spectrophotometry for total polyphenols, an oxidative stress regulator. It was hypothesized that RTA-exposed plants would express increased total polyphenols levels compared to non-exposed plants. The mean net total polyphenols content was not significantly different between exposed and control boxwoods (p = 0.998) or exposed and control junipers (p = 0.201). This indicates that RTA-exposed plants did not express increased total polyphenols levels. However, this study was the first to investigate potential adverse impacts of environmentally-relevant sound levels on plant species that may have a high daily noise pollution burden.
TABLE OF CONTENTS
Acknowledgements...................................................................................... i
Introduction.................................................................................................. 1
Materials and Methods................................................................................ 10
Results.......................................................................................................... 15
Discussion.................................................................................................... 16
Conclusion.................................................................................................... 24
References.................................................................................................... 26
Appendices................................................................................................... 31
Acknowledgements

It truly does take a village to get a research project completed. I am forever grateful to all the people who assisted me on this journey, without whom none of this would have been possible. First and foremost, I must express my deepest thanks to my advisor, Dr. Lee Newman, for her wisdom, guidance, and patience. I never believed it would be possible to carry out an independent research project related to noise pollution, but Dr. Newman never wavered in her support for making sure I was successful at doing just that. Her commitment to her students is unparalleled, as she helped me steer around the numerous obstacles I encountered along the way. Finally, Dr. Newman also provided additional financial resources to help procure the reagents needed to carry out the analyses in this study, for which I am incredibly thankful.

Next I must thank Dr. Bill Shields and the SUNY-ESF Honors Program for providing the bulk of the funding for this project. This project would never have been possible if it wasn’t for Bill. It cannot be understated how special it is for undergraduate students to be able to access financial resources to carry out an independent research project like this. There are numerous other professors who assisted me along the way. Thanks to Dr. Susan Parks and Dr. Jaime Mirowsky for giving me advice on which type of field recorder and sound level meter to consider using, respectively. Thanks to Dr. Mary Collins for helping me figure out how to input the equation to solve for A-weighted equivalent continuous sound levels in Excel. I especially want to thank Dr. Ivan Gitsov and Joy Logan of the SUNY-ESF Chemistry department for lending me use of a Vernier spectrophotometer and other needed analytical resources. Thanks to Dr. Diane Kiernan for advice and guidance for statistical analyses.
So many graduate and undergraduate students helped me in innumerable ways, and I am very thankful for their support and encouragement all along the way. I would like to thank Dr. Newman’s graduate students, Wenjun Cai, Nikolai Ivanov, and Daniel Collins for helping me at the most inopportune hours. To the undergraduates who assisted me with plant procurement and transportation, watering, tissue harvesting, and statistical analyses: Max Sosa, Noah Garwood, Sarah Cruz, Julia Bernhardt, Jessica Cobb, and Jet’aime Lewis, thank you. Kai Troge deserves special thanks and my utmost gratitude for his commitment to assisting me for the bulk of the polyphenols extraction and analysis, and also for watering my plants for a month.

Finally, I would like to express my deepest appreciation to my family, especially my parents and grandparents, for believing in me even when I did not believe in myself. I would like to thank my mother, Hope, for thinking of the ideal location to make my field recording, and for also taking photos that day. I would like to thank my father, Keith, for helping me narrow down which plant species to use. To both of my parents, thank you for letting me feed my curiosity all these years and for instilling in me a wondrous love for this planet we call home. Your love and support means the world to me.
Introduction

The rapid pace of human population growth and development has led to a transformation in what our world sounds like today. As rural, agrarian societies have shifted to industrialized ones, the sounds of human machines have become omnipresent, and have radically altered many of the world’s soundscapes. A soundscape is the acoustic structure of any environment (Krause, 2016), and represents the collection of biological, geophysical, and anthropogenic sounds emanating from a landscape that vary over space and time (Pijanowski et al., 2011). Soundscapes have three components: biophony, geophony, and anthropophony (Krause, 2016). Biophony represents all non-human biological sounds, whereas geophony represents all non-biological natural sounds (Krause, 2016). The final component of a soundscape is anthropophony, which represents all human-generated sounds, regardless of whether they come from human-made objects or from the human voice (Krause, 2016).

Each of these soundscape components exist together in discrete proportions that vary depending on the extent of human disturbance in a given place. As natural areas become progressively more disturbed as they are converted into urban areas, anthropophony will increase at the expense of biophony (Farina, 2014). This is problematic because anthropophony is the major cause of noise pollution, particularly road transport anthropophony (RTA) from vehicles and traffic congestion, where such vehicular sound can travel up to 4 km in distance depending on the type and volume of the traffic (Farina, 2014). It is important to distinguish between anthropophony and noise. While both terms are often used interchangeably in the context of noise pollution, they mean very different things. Anthropophony is objectively quantifiable, because as
previously mentioned, it is a distinct type of sound that has recognizable characteristics: this type of sound clearly comes from human origins. Noise, on the other hand, is mostly subjective and is harder to quantify. While noise can be described in objective terms, such as an unintentional, random, or degraded sound or the summation of various sounds which create a confused pattern, noise is often defined as any unwanted or undesirable sound (Farina, 2014). Which sounds are "unwanted" or "undesirable" are completely dependent upon an individual's perception and preferences, and thus certain types of anthropophony may or may not be considered noise to an individual, just as certain types of natural sounds (biophony and geophony) may be considered noise to the same individual. While it is likely that many people would consider road transport anthropophony (RTA) to be noise, this study will avoid the use of the term "noise" when describing RTA because noise is predominantly a subjective term.

Noise pollution, regardless of the type of sound that causes it, is now being recognized as a profound global issue, even though it has long been underestimated. Research proliferating at a rapid pace within the last few decades has shown that noise pollution has dangerous consequences for ecological health, human health, and the health of other animals (Barber et al., 2010; Farina, 2014; Slabkoorn and Peet, 2003). The consequences of noise pollution do not just extend to terrestrial animals, as marine animals such as whales are known to be highly vulnerable to underwater noise pollution (National Marine Fisheries Service, 2001). The World Health Organization has defined seven categories of adverse health effects in humans from noise pollution: 1) hearing impairment, 2) interference with communication, 3) sleep disturbances, 4) cardiovascular disturbances, 5) disturbances in mental health, 6) impaired task performance, and 7)
negative social behavior and annoyance reactions (Farina, 2014). Kight and Swaddle (2011) highlighted other potential health impacts in humans and animals from noise pollution, including neuroendocrine impacts, reproduction and development impacts, metabolic impacts, impacts on the immune system, and impacts on DNA integrity and genes. As urban areas continue to expand, leading to more extensive intrusion of anthropophony, research on noise pollution health impacts needs to better represent organisms that have not been adequately studied, such as plants.

To date, most research on plant exposure to sound has centered on applications in biotechnology and agriculture, focusing on harnessing the ability of certain sound frequencies at a given intensity to improve plant growth, resistance against pests and disease, and nutritional value, and thus improve crop yield and quality (Hassanien et al., 2014). There has been no concerted effort in attempting to the elucidate impacts of noise pollution in plants, likely because the concept of sound mechanoperception in plants has been grossly underappreciated and has long been met with disbelief. Humans have long viewed the ability of an organism to sense and perceive sound as having to conform to the conventional auditory structures (e.g. presence of eardrums or cochlear structures) of certain animals (Gagliano et al., 2012). However, remarkably diverse morphological structures exist within animal species that are capable of sensing sound and do not conform to conventional auditory structures, showing that conventional structures are not prerequisites for sound mechanoperception in any organism (Gagliano et al., 2012).

If the ability to sense and respond to physical stimuli is key to all living livings, and plants are known to respond to physical stimuli such as touch and gravity (Telewski, 2006), then plants should also be able to sense sound. This notion led to the beginning of
serious efforts to study whether plants could perceive sound, and if so, how this could occur. While it is known today that plants perceive and respond to sound in a myriad of ways (Gagliano et al., 2012; Appel and Cocroft, 2014), debate centered on the “whether plants can perceive sound” question for so long that research into the “how plants perceive and transduce sound” remains in its infancy (Mishra et al., 2016). It remains unclear exactly what the mechanism of the signal-transduction pathway for plant response to sound is and how it works. However, what is known about the pathway shares many similarities to the touch-signaling pathway in plants (Mishra et al., 2016).

The stretch-activated ion channel model proposed by Telewski (2006) seems the most promising candidate based on synthesis of current knowledge (Mishra et al., 2016). The idea behind this model is that the signal (i.e. sound waves) will elicit a transduction event in the plant cell through alteration of the cell’s membrane potential, caused by ions fluxing into the cell through a stretch-activated mechano-sensitive ion channel (Mishra et al., 2016). It has been hypothesized that sound waves impinging upon plant cells can cause the cell’s membrane to stretch from resting tension to a tension of at least 1 mN/m, which activates the cell’s mechano-sensitive ion channels (Telewski, 2006). Two types of these ion channels are believed to be activated: MscS-like (MSL) channels, which are non-ion specific, and Mid 1-complementing activity (MCA) channels, which are Ca$^{2+}$ ion channels (Mishra et al. 2016). Ca$^{2+}$ ions are believed to be the critical messenger ion in the pathway, and are thought to possibly convey the message to calcium-dependent protein kinases (CDPKs) and/or other Ca$^{2+}$ sensors (Mishra et al., 2016). From here, the message is thought to be conveyed from the CDPKs to different signaling proteins and transcription factors, which leads to expression of genes that cause the cell to produce
antioxidant enzymes and stress-responsive proteins, among other types of proteins (Mishra et al., 2016). Since the Ca$^{2+}$ signature generated in plant cells has been shown to be unique dependent on the characteristics of the sound waves the plant was exposed to, it is possible that this is the basis for which plants can distinguish between different sounds to generate particular responses (Mishra et al., 2016).

Li et al. (2008) were the first group to document the expression of ROS-scavenging antioxidant enzymes in response to oxidative stress (membrane lipid peroxidation) in a plant upon exposure to sound waves of a given intensity and frequency. Prior to this, ROS were known to be enhanced in plants due to drought stress and desiccation, salt stress, cold and heat stress, heavy metal exposure, UV radiation exposure, exposure to air pollutants, high light stress, nutrient deprivation, and pathogenic attack (Mittler, 2002). Reactive oxygen species (ROS) are mostly byproducts of regular cellular metabolism, the result of incomplete reduction of O$_2$ to H$_2$O, generated in plants mainly within the organelles that perform highly oxidizing metabolic activities (Van Breusegem et al., 2001). In particular, chloroplasts are considered to be the most powerful source of ROS in plants (Dat et al., 2000). The concentrations of ROS are low under normal conditions, and for good reason, since excessive levels of ROS are known to be phytotoxic to plants, since all ROS are capable of causing oxidative stress within plant cells through unrestricted oxidation and destruction of various cellular components (Mittler, 2002). ROS have been shown to cause membrane lipid peroxidation, protein oxidation, enzyme inhibition, and DNA and RNA damage, all of which, if extensive enough, can induce cell death (Mittler, 2002). Excessive levels of ROS can also damage
the plant’s photosynthetic apparatus, leading to extensive cellular damage and chlorosis of the leaves (Van Breusegem et al., 2001).

During exposure to stress conditions (such as sound), the cellular homeostasis of a plant’s cells is disturbed, such as through leakage of electrons to O$_2$ in the electron transport systems in chloroplasts and mitochondria (Dat et al., 2000). This leads to increased levels of ROS within the cells. It is thus imperative that plants have an effective system in place to regulate excessive ROS through scavenging in order to protect cells from oxidative stress. Plants possess an efficient antioxidant defense system, whereby specific antioxidants are produced in distinct subcellular locations in certain organs of the plant to regulate different ROS spatially and temporally (Vranova et al., 2002). A plant’s antioxidant defense system consists of enzymatic antioxidants and non-enzymatic antioxidants (Vranova et al., 2002). Non-enzymatic antioxidants include compounds such as polyphenols.

Plant phenols (aka. polyphenols) are a broad class of compounds characterized as aromatic metabolites that contain one or more acidic phenolic hydroxyl groups (Grace, 2005). The major classes of polyphenolic compounds in plants are hydroxycinnamic acids (HCAs), flavonoids, anthocyanins, and tannins (Grace, 2005). Polyphenolic compounds were long thought to not be part of a plant’s antioxidant defense system, until it was observed that their biosynthesis was activated by induction of diverse stresses in a plant, resulting in increased intracellular levels of polyphenols under stress conditions (Grace, 2005). ROS are directly scavenged by polyphenolic compounds because the one-electron reduction potential of phenols is lower than that of ROS, enabling preferential oxidation of phenolic compounds over other cellular components (Grace, 2005).
Polyphenols, which are produced in the cytoplasm and stored in vacuoles, vary considerably with respect to their tissue and subcellular localization depending on the compound, and this directly impacts their ability to scavenge certain ROS because they are often spatially separated from the main sites of ROS production (chloroplasts and mitochondria) (Grace, 2005). However, if severe stress causes ROS levels in chloroplasts and mitochondria to overwhelm the scavenging capacity of antioxidants in those organelles, ROS will leak and diffuse into other cellular compartments to be scavenged by polyphenolic compounds (Grace, 2005).

All plants are exposed to sound regardless of their environment, but certain plants are distinctly exposed to noise pollution from road transport anthropophony. These plant species are those used as vegetative noise barriers to reduce levels of road transport anthropophony in urban areas. Investigation of the ability of plants to attenuate sound started with pioneering research in the 1970s and 1980s (Cook and Van Haverbeke, 1971; Aylor, 1972), and the efficiency of vegetative noise barriers near roadsides has been a popular topic for study worldwide ever since (Karbalaei et al., 2015). Vegetative noise barriers are considered to be one of the cheapest methods to combat noise pollution, particularly in developing countries (Karbalaei et al., 2015). The idea of using plants to control sound in urban environments is also substantially popular, as indicated by Yang et al. (2011), where individuals questioned overwhelmingly believed that vegetative noise barriers are the most effective type of noise barrier compared to manmade noise barriers. These individuals also overestimated the physical potential of vegetative noise barriers to reduce sound levels (Yang et al., 2011).
The ability of vegetative noise barriers to reduce sound levels in an environment is remarkably multifaceted. Solely considering physical characteristics, the most effective vegetative noise barriers can generally reduce sound levels by 4-8 dBA (Fang and Ling, 2005; Yang et al., 2011). Plants accomplish this sound level reduction by means of excess attenuation, which refers to the scattering, reflection, refraction, and absorption of sound waves caused by something obstructing a sound source (Herrington, 1976). Significant factors that shape the amount of excess attenuation of a vegetative noise barrier include barrier length, width, height, and density (Cook and Van Haverbeke, 1971); arrangement of plants and use of different plant species in the barrier (Yang et al., 2010); the size, shape, weight, and area of a plant’s leaves (Aylor, 1972); and the branching characteristics of the plant (Aylor, 1972).

Highlighting the multifaceted nature of vegetative noise barriers further, they are also able to achieve additional noise reduction than would be obtained by physical properties of the plants and barriers alone. This extra noise reduction is subjective to the listener and comes via psychological means (Yang et al., 2011). In essence, the power of an individual’s belief that vegetative noise barriers are the most effective type of noise control method, buoyed by overestimating the actual potential for vegetative noise barriers to reduce sound levels, seems to make the individual perceive less sound in a noisy environment (Yang et al., 2011). Additionally, visual appreciation for the beauty of vegetative noise barriers in an urban environment impacts the sound level individuals perceive in these environments (Yang et al., 2011). The positive emotions individuals tie to seeing vegetation in urban environments counteracts the negative emotions associated
with noise, resulting in individuals having significantly calmer minds in the presence of vegetation and noise, which results in perceiving less sound (Yang et al., 2011).

Plants are capable of reducing sound levels at distinct frequencies and at different intensities depending on the species. This is critical when deciding which plants to use in a vegetative noise barrier, as the sounds of vehicles and traffic on roads are predominantly in the middle and low frequencies (Yang et al., 2010). Shrubs are the most effective plants in reducing sound levels because their dense foliage and branches enables extensive scattering of sound waves (Fang and Ling, 2003). Therefore, shrubs used as vegetative noise barriers serve as ideal plants to study for potential health impacts of exposure to road transport anthropophony.

While the work of Li et al. (2008) was instructive in indicating that plant cells are susceptible to oxidative stress upon exposure to sound waves, their study on a health impact of sound on plants was limited in two ways. First, the study involved exposing their plant of choice to a single frequency (1000 Hz), single intensity (100 dB) sound for 60 minutes each day. Second, the study involved use of a plant (*Dendrobium candidum*) that does not encounter road transport anthropophony, one of the most significant sources of noise pollution, on a daily basis. Plants are never exposed to single frequency, single intensity sounds for any length of time, regardless of the environment they inhabit. Environmentally relevant sounds that plants may be exposed to will be of multiple frequencies at ever-changing intensities, and will vary in duration. Road transport anthropophony from highways in urban areas may persist indefinitely (or nearly indefinitely). Plants that inhabit these areas have a heavy burden from anthropophony.
that may adversely impact their health in addition to any health impacts these plants may experience from air pollutants associated with vehicles.

This research attempted to address the deficiencies of the Li et al. (2008) study by exposing two shrub species to environmentally-relevant sounds (e.g. road transport anthropophony) at the intensities, and for the duration, likely to be encountered by plants that inhabit environments impacted by noise pollution. The objective of this study was to measure polyphenols, an oxidative stress regulator, in plants exposed to an environmentally-relevant intensity and duration of anthropophony and in plants that were not exposed to anthropophony. For this objective, it was hypothesized that plants exposed to an environmentally-relevant intensity and duration of road transport anthropophony would express increased levels of polyphenols compared to non-exposed plants. Shedding light on this question will help paint a clearer picture of how plants respond to noise pollution.

Materials and Methods

Field Recording Site

RTA from U.S. 101 freeway was selected as a target for field recording and corresponding sound level measurements. The ideal location to record anthropophony from the freeway was in a place as close to the freeway as possible that was also unobstructed by any major barriers such as a wall or trees. This location was in the Los Angeles, CA neighborhood of Woodland Hills, on a sidewalk on Del Valle Street west of Fallbrook Avenue, near a cul-de-sac, and adjacent to a pedestrian bridge over the U.S. 101 freeway that led to Avenue San Luis (34°9'48.94"N, 118°37'37.28"W) (Figure 1). At
this point of the freeway, there were four lanes going in each direction. The sole obstruction was a chain-linked fence, and the location was approximately 52-53 feet from the edge of the U.S. 101 North. The approximate average annual daily traffic (AADT) of the U.S. 101 North at this point was 204,000 vehicles in 2014 (California State Transportation Agency, 2014).

A 45 minute field recording was taken of the freeway using a Zoom H1 Handy Recorder equipped with a microphone windscreens muff. Sound pressure level measurements were taken simultaneously during the entire duration of the field recording using a PCE-322A sound level meter (PCE Instruments) set to dBA, slow, and a sampling rate of 0.5 seconds (Figure 2). Datalogged sound level measurements were saved and exported to Excel after the field recording was completed for determination of the A-weighted equivalent continuous sound level ($L_{eq}$ (dBA)) (Appendix A). The temperature at the site was documented during field recording, along with a general qualitative observation of wind speed.

Plant Materials

Plants selected for this study were those shrubs which have been investigated in the past as having potential to attenuate sound when planted as a vegetative barrier alongside roads and highways. Two genera of evergreens were chosen: *Juniperus* and *Buxus*. *Juniperus* species have been investigated for their efficacy as vegetative noise barriers as far back as 1971 (Cook and Van Haverbeke, 1971), whereas *Buxus* species have been investigated for the same purposes as recently as 2010 (Smyrnova et al., 2010). Ten junipers (*Juniperus squamata* var. *parviflora*) (The Home Depot, Camillus, NY) and
ten boxwoods (*Buxus microphylla* var. *Wintergreen*) (The Home Depot, East Syracuse, NY) were obtained and were regularly tended to in the SUNY-ESF Greenhouses (Appendix B). Five plants of each species were assigned as Control or Experimental using Excel’s RAND function and sort tool. Different ambient sound conditions in the greenhouse where the plants were kept were measured using the sound level meter, and data logged measurements were saved and exported to Excel for determination of the A-weighted equivalent continuous sound levels ($L_{eq}$ (dBA)) (Appendix A). Height measurements of the plants were taken prior to the exposure (Appendix C).

**Soundproof Room Set-Up**

The soundproof room utilized during the exposure was 2.44 m tall, 3.09 m wide, and 4.24 m long. A JBL LSR305 Studio Monitor was placed at the center edge of a table located at the back of the room. The studio monitor was 0.82 m off the ground. A laptop was connected to the studio monitor via a 1 m Hosa CMP-103 ¼ in TS to 3.5 mm TRS cable. The studio monitor volume was calibrated to be within 1% of the A-weighted equivalent continuous sound level calculated for the field recording by adjusting the volume levels and measuring the output sound levels using the sound level meter until the desired A-weighted equivalent continuous sound level was reached (Appendix A). Located in the center of the room was an adjustable shelf rack where five plants at a time were placed for the exposure. The plants on the shelf rack were placed at roughly the same height above the ground as the studio monitor. The distance of the studio monitor to the edge of the shelf rack was 1.14 m (Figure 3). The ambient sound level of the room was measured using the sound level meter, and data logged measurements were saved.
and exported to Excel for determination of the A-weighted equivalent continuous sound level (\(L_{eq}\,\text{dBA}\)) (Appendix B).

**Equivalent Continuous Sound Level Calculations**

All sound level measurements taken, including for the field recording, ambient greenhouse sound levels, and ambient soundproof room sound level, had an A-weighted equivalent continuous sound level calculated in Excel using the data that was exported from the sound level meter to Excel. The following equation was used to calculate the A-weighted equivalent continuous sound level:

\[
L_{eq}\,\text{dBA} = 10\text{log} \left( \frac{1}{T} \sum_{i=1}^{N} 10^{\frac{\text{SPL}_i}{10}/\Delta t} \right),
\]

where \(L_{eq}\) = equivalent continuous sound level;
\(\Delta t\) = sampling rate (s);
\(\text{SPL}_i\) = sound pressure level recorded at time interval \(i\);
\(T\) = total time period over \(L_{eq}\) to be measured (s);
\(N\) = number of sound pressure level measurements made during time period \(T\).

This summation equation is the discrete form of the equivalent continuous sound level, and serves as an approximation of definite integral found in the traditional equation for the equivalent continuous sound level (Trani and Roa, 2013). The integration required to manually solve the traditional equation is very complex and time-consuming and is usually avoided. Use of the discrete form of the equation is a way to bypass the integration, and allows for easier manual calculation of the equivalent continuous sound level. The equation was input into Excel to generate a value that was then verified by
breaking down the equation into segments and solving the equation one part at a time to ensure that no errors were made when the entire equation was input all at once.

*Exposure to Anthropophony from Field Recording*

Plants were transferred from the greenhouse to the shelf rack in the soundproof room one set at a time for a total of four different scenarios (two treatment groups per species). A 16/8 hour day/night light cycle was implemented using a Brinks Grounded 24-Hour Mechanical Timer Type 42-1023, and all plants were watered in the soundproof room as necessary. Each set of plants was left to acclimate to the ambient sound levels of the soundproof room for 48 hours prior to sound exposure in order to enable levels of polyphenols to come to a relative baseline, since the plants were housed in a greenhouse with moderate levels of background anthropophony.

For exposed plants, treatment was defined as exposure to the field recording at 73.5 dBA (±1%) for 24 hours, using Windows Media Player on a laptop to continuously loop the field recording and project it through the studio monitor to the plants (Figure 4). For control plants, treatment was defined as leaving the plants in silence in the soundproof room for an additional 24 hours on top of the 48 hour acclimation (Figure 5). All treatments were carried out at the same time of day. In order to capture the net concentration of polyphenols in the plants (ΔC) over time, leaf tissue was harvested immediately prior to treatment (C_initial), and immediately after treatment (C_final). Leaf tissue from all plants was harvested, flash frozen using liquid nitrogen and stored in a -80°C freezer until extraction. All leaf tissue harvesting was carried out at the same time of day.
Extraction and Analysis of Total Polyphenols

For each plant, total polyphenols content was determined using the method described by Maizura, Aminah, and Wan Aida (2011). Prior to extraction, leaf tissue samples were ground into smaller pieces, and then 0.1 g of leaf tissue was thoroughly mixed with 5 mL of 10% (v/v) Folin-Ciocalteu reagent in water. After 5 minutes, 4 mL of 7.5% (w/v) sodium carbonate (Na₂CO₃) solution was added and the mixture was incubated at room temperature for 2 hours (Figure 6). The supernatant was carefully removed to prevent uptake of the leaf tissue, and absorbance was measured at 765 nm using a spectrophotometer (Vernier SpectroVis Plus). A blank consisting of a mixture of 5 mL of 10% Folin-Ciocalteu reagent and 4 mL of 7.5% sodium carbonate was used to calibrate the spectrophotometer. Net total polyphenols content was quantified using a standard curve made from gallic acid solution (25, 50, 75, 100, 122.5 mg/L) (Figure 7), and concentrations were expressed as mg gallic acid equivalents (GAE) per gram of leaf tissue fresh weight (mg GAE/g FW).

Statistical Analysis

All analyses for total polyphenols content were performed in triplicate for each plant and each time point. Statistical analysis was performed using Minitab. Two-sample t-tests were used to compare mean net total polyphenols content between exposed and control plants for each plant species. Statistical significance was set to $p < 0.05$.

Results

The mean net total polyphenols content for exposed and control boxwoods was not significantly different ($\mu=0.00$; 95% CI [-3.80 to 3.79]; $p = 0.998$) (Figure 8). For
junipers, the mean net total polyphenols content was not significantly different between treatment groups ($\mu = 4.22; 95\% \text{ Cl } [-3.14 \text{ to } 11.59]; p = 0.201$). There was high intraspecies variability in net total polyphenols content for both species regardless of the treatment group (Appendix D). Of the boxwoods, the single largest net increase in total polyphenols content (3.01 mg GAE/g FW) and single largest net decrease in total polyphenols content (-5.10 mg GAE/g FW) occurred in the exposed treatment group. Of the junipers, the single largest net increase in total polyphenols content (7.00 mg GAE/g FW) and single largest net decrease in total polyphenols content (-8.03 mg GAE/g FW) occurred in the control treatment group. Net increases and net decreases in total polyphenols content existed in every treatment group for each species.

**Discussion**

The objective of this study was to measure polyphenols, an oxidative stress regulator, in plants exposed to an environmentally-relevant intensity and duration of anthropophony and in plants that were not exposed to anthropophony. It was hypothesized that plants exposed to an environmentally-relevant intensity and duration of road transport anthropophony would express increased levels of polyphenols compared to non-exposed plants. Since the mean net total polyphenols content was not significantly different between the exposed and control plants for either species, plants exposed to road transport anthropophony for 24 hours at 73.5 dBA did not express increased levels of polyphenols. However, this study advanced the nascent and practically nonexistent field of noise pollution health impacts research on plants in a number of ways.
First and foremost, a review of the general literature appears to indicate that this study is only the second to ever broach the question of what health impacts noise pollution has on plants, besides the work of Li et al. (2008). Second, this study aimed to tackle the issue of environmentally-relevant exposures in health impacts research, which tends to obscure the usefulness of the knowledge gained from this type of research. Plants in this study were exposed to multi-frequency sound from a freeway at an equivalent continuous sound level that attempted to be as close of a reflection as possible to the sonic conditions in which plants would encounter on a daily basis. Expression of oxidative stress regulators was then assessed at this level of exposure, rather than at the unrealistic exposure conditions carried out by Li et al. (2008). Consideration of environmentally-relevant sound exposures had never been investigated before. Seeing as no plants are ever naturally exposed to a single intensity, single frequency sound for a prolonged period of time, this study should set the precedent for using environmentally-relevant levels and frequencies of sound for all future noise pollution health impacts research on plants.

Third, this study was unique in that the plants chosen for study were plants that have an increased noise burden owing to their use as vegetative noise barriers. These plant species were chosen because they serve a relatively new and emerging role for humans to control noise pollution in urban areas. Should these types of plants be adversely impacted by noise or should they be resistant to noise, this will have implications on the efficacy of vegetative noise barriers as a noise pollution control method. Without proper consideration of a species that is likely to encounter elevated levels of noise on a day-to-day basis, the results of research in this field of study will
have limited relevance. Fortunately, this study avoided that pitfall, unlike Li et al. (2008), who selected the Chinese medicinal herb *Dendrobium candidum* as their plant species of choice for their exposure study. This study should thus set a precedent for future research in this field of study with regards to choosing plant species that are most likely to encounter a high noise pollution burden on a daily basis.

Fourth, this study presented a novel way to expose plants in a controlled environment to road transport anthropophony from a freeway. To control for other sources of noise pollution, and to control for other types of pollution that may have induced ROS generation in plants used as vegetative noise barriers (i.e. air pollution), the sound exposure was conducted in a soundproof room. Since it is not clear whether Li et al. (2008) conducted their study in a soundproof room, this study may be the first in this line of research to have used a soundproof room to control for interfering sounds and other types of stressors that could induce ROS generation in plants. The need to use a soundproof room was necessitated in part by the fact that vehicular air pollution generates ozone, which is known to increase ROS levels in plants (Dat et al., 2000). It would be impossible to tease apart whether increased ROS levels in plants used as vegetative noise barriers were caused mostly by air pollution or by noise pollution if a soundproof room was not used.

In order to expose plants in this controlled environment, it was necessary to be able to obtain and project a high-quality field recording to the plants at a sound level as close to the equivalent continuous sound level of the field recording as possible. The methodology for these parts of the study was thus generated independently of the general literature, and according to the financial limitations imposed. The quality of the
equipment used to obtain and project the field recording was unexpectedly much higher than anticipated, especially considering how relatively inexpensive the equipment was. A major benefit of this methodology was the use of a studio monitor to project the field recording in the soundproof room. A studio monitor was used instead of a generic speaker because it provides a richer quality of tone and less static for the sounds being emitted from it, which is why professional sound mixers prefer to use them for their work. In terms of this study, this prevented the field recording from sounding grainy when projected to the plants. Graininess would have imparted interfering sound on the plants, which this study successfully minimized as much as possible. The novel methodology created in this study can be used as a guide for future research in this area that involves the need to expose plants to different sources of sound in a controlled environment.

Fifth and finally, polyphenols, the antioxidant class of choice for this study, had never been evaluated before in this line of research. Li et al. (2008) solely investigated expression of various enzymatic antioxidants in plants exposed to sound. This study thus modestly enhanced the current body of knowledge regarding what happens to expression of different oxidative stress regulators in plants in response in sound exposure, as non-enzymatic antioxidants (i.e. polyphenols) were considered for the first time. Hopefully, future research in this area will continue to expand upon the steps made in this study to investigate the response of plant antioxidants that have been previously unstudied when it comes to sound exposure in plants.

Upon looking at the ambient levels of anthropophony that existed in the greenhouse where the plants were left for the majority of the study (Appendix A), one
might wonder if the plants became partially attenuated to elevated sound levels prior to exposure in the soundproof room. After all, the $L_{eq}$ of the field recording and of the studio monitor field recording calibration (73.5 dBA and 72.8 dBA, respectively) seem very close to the $L_{eq}$ of the start of a greenhouse misting event (68.6 dBA). However, it is unlikely that the ambient levels of anthropophony in the greenhouse caused the plants to become partially attenuated to elevated sound levels prior to their exposure, which would have impacted the analysis of total polyphenols. This is because the decibel (dB) is a logarithmic unit of measurement. A difference of 10 dB (i.e. from 60 dB to 70 dB) is not trivial, but is rather a ten-fold difference in the intensity, or perceived loudness, of the sound. Even though 68.6 dBA appears to be very close to 72.8 dBA, the latter sound level is actually substantially louder than the former. Additionally, the duration of the start and end of a misting event was so short (on the order of ten seconds or less), and the frequency of these events was so sporadic, that the plants likely did not have the time to attenuate to sound at those levels. The predominant ambient anthropophony in the greenhouse was when the greenhouse lights were either on or off. In these conditions, the sound levels the plants were exposed to were one to two orders of magnitude quieter than the sound level they were exposed to from the field recording.

It is premature to say whether plants experience no oxidative stress upon exposure to sound, for a variety of reasons. First, the sample size for exposed and control plants (n=5 per each species) was likely not robust enough to account for the individual variability in total polyphenols content between plants. During the spectrophotometric analysis, it was observed that the absorbance measurements were highly variable between plants, even if the plants were part of the same treatment group. If any differential
expression of total polyphenols actually exists between treatment groups, the small sample size of the experiment may have prevented its detection.

Second, the plants were exposed to anthropophony for 24 hours. It is possible that the duration of exposure was too short to detect significant differences between treatment groups. As this type of experiment had never been undertaken before (exposing plants to environmentally-relevant levels of anthropophony), it was unknown how long to expose the plants. Future work may include varying the exposure lengths of the study. The environmentally-relevant exposure duration question is complicated because of the assumptions made during the field recording and sound level measurements phase of the experiment. It was assumed that a looped 45 minute field recording at 73.5 dBA would be representative of a 24 hour exposure at environmentally-relevant sound levels. Of course, this is not true due to the inherent temporal and spatial variability of sound. Freeways will be substantially louder at different times of the day, at different points along the freeway, in different seasons, and among different freeways. However, if logistical limitations exist that prevent researchers from having access to a protective shelter and power supply for their data logging sound level meter, sound level measurements would likely not be feasible to take over a 24 hour period.

Future researchers may be somewhat aided by trying to make the longest field recording they can, given the maximum amount of points they are capable of data logging with their sound level meter. This could capture more of the temporal variation in sound levels throughout a day. At the end of the day, the question of what exposure duration is environmentally-relevant becomes a Goldilocks situation: finding an exposure duration that is short enough to remain relevant to how long plants used as vegetative
noise barriers are exposed, while also being long enough to detect differential antioxidant expression between exposed and non-exposed plants, if such differential expression actually exists.

Third, polyphenols may not be the best antioxidants with which to assess the question posed by the study. Considering how prevalent polyphenols are in plants compared to other antioxidants, it may be possible that polyphenol concentrations are so large that detection of subtle differences in expression between exposed and non-exposed plants would be very challenging to notice even with a large sample size and accurate analytical equipment. It is thus critical to analyze other plant antioxidants, which may be better indicators of oxidative stress from sound than polyphenols. Future research should analyze antioxidants such as proline (Hayat et al., 2012), and enzymatic antioxidants such as catalase (Dat et al., 2000).

Fourth, it may be possible that the two plant species chosen are tolerant to sound stress at environmentally-relevant levels. Future research warrants investigating antioxidant expression among other plant species which may be more sensitive to sound than the species chosen for this study. Cook and Van Haverbeke (1971) described many plant species that are suitable for use in vegetative noise barriers. These other species mentioned should be the starting point for further investigation into potential differential antioxidant expression due to sound exposure.

Fifth, it is possible that the intensity and frequencies of the road transport anthropophony field recording were not within the range that would trigger oxidative stress in plants. Li et al. (2008) noted antioxidant expression at a 100 dB, 1000 Hz
exposure, but they unfortunately did not study whether those values were threshold values for causing oxidative stress in the plant species they studied. Additionally, they did not investigate exposures at other intensities and frequencies. No research to date (outside of this study) has made further insight into the question of intensity and frequencies. A sound level meter capable of performing one-third octave band frequency analysis would have enabled the field recording to be broken down into a frequency spectrum, highlighting which frequencies were the most prominent (intense) in the recording. This was a limitation of the study, and thus the frequencies the plants in this study were exposed to remain unknown. Future research should consider the frequencies of the sound exposed to plants, as frequency is arguably more important in determining a plant's response to sound than the intensity of the sound (Mishra et al., 2016).

It is important to note another key limitation and weakness of this study. Any analysis of antioxidant expression in sound-exposed plants should be cognizant of the time-response from exposure to peak expression of whatever antioxidant is being studied. This would enable the researcher to assess the best time to harvest the plant tissue after exposures. Due to the time constraints under which this experiment was carried out, this factor was not studied. Finally, it is possible that further research may indicate that plants do not experience a stress response or any other adverse health impacts when exposed to environmentally-relevant levels and frequencies of sound. This answer would be fascinating from a biological perspective, as it would raise important questions such as why plants are tolerant to sound stress at environmentally-relevant levels whereas humans and other animals are sensitive at these same levels. A neutral response to environmentally-relevant levels and frequencies of sound would also be good news from
a health perspective, as it would indicate that if plants in areas with very high levels of anthropophony were to experience some adverse health impact, it would be from other factors rather than sound. More research is needed to expand on this study in order to obtain a clearer picture of what happens to plants exposed to environmentally-relevant levels and frequencies of sound.

Conclusion

This study was the first to investigate potential adverse impacts of sound on plants exposed to environmentally-relevant sound levels. The results showed that there was no significant difference in expression of total polyphenols between exposed and non-exposed plants of two species for a 24 hour exposure at 73.5 dBA. However, the study was novel in 1) its consideration of environmentally-relevant sound exposures, 2) its use of plants that may have a high daily noise pollution burden, 3) its assessment of the response of a non-enzymatic plant antioxidant to sound exposure, 4) its use of a controlled environment to conduct sound exposure, and in 5) the methodology used to obtain and project a field recording of road transport anthropophony to plants. While it appears that the hypothesis of this study was not supported, replication of this study is warranted as many factors may have accounted for lack of detection of differential antioxidant expression between exposed and non-exposed plants. There are numerous questions that still need to be answered when it comes to antioxidant expression to combat oxidative stress in plants exposed to environmentally-relevant levels and frequencies of sound.
Future research endeavors should not be limited to study of this one health impact only, but should investigate other potential biologically plausible mechanisms by which sound may adversely impact plant health. Much of this work will be aided by further advances in our understanding of how plants perceive sound, and thus it is imperative that research on sound mechanoperception in plants continues to be pursued and funded. Research on plant health and sound has lagged far behind that of research on human/animal health and sound. As urbanization continues, noise pollution is an issue that is not likely to go away in the future. It is therefore imperative to deepen our understanding of how sound can negatively impact all types of life, especially those organisms that have long been underrepresented in noise pollution health impacts research.
References


Appendices

Appendix A. A-weighted equivalent continuous sound levels.

<table>
<thead>
<tr>
<th>Location and/or Conditions</th>
<th>$L_{eq}$ (dBA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S. 101 Field Recording</td>
<td>73.5</td>
</tr>
<tr>
<td>Soundproof Room</td>
<td>39.0</td>
</tr>
<tr>
<td>Studio Monitor Field Recording Calibration</td>
<td>72.8</td>
</tr>
<tr>
<td>Greenhouse (lights off, fan on)</td>
<td>55.4</td>
</tr>
<tr>
<td>Greenhouse (lights on, fan on)</td>
<td>60.8</td>
</tr>
<tr>
<td>Greenhouse (start of misting event)</td>
<td>68.6</td>
</tr>
<tr>
<td>Greenhouse (end of misting event)</td>
<td>63.9</td>
</tr>
</tbody>
</table>

Appendix B. Greenhouse conditions.

Temperature Conditions

<table>
<thead>
<tr>
<th>Time</th>
<th>Temperature (°F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:00 AM - 6:00 PM</td>
<td>78-82</td>
</tr>
<tr>
<td>6:00 PM - 7:00 AM</td>
<td>74-76</td>
</tr>
</tbody>
</table>

Lighting Conditions

<table>
<thead>
<tr>
<th>Time</th>
<th>Indoor Lighting (On/Off)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:00 AM - 9:00 PM</td>
<td>On&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>9:00 PM - 5:00 AM</td>
<td>Off&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>: Lamps turned off if light measured in greenhouse went above 900 W/m$^2$ for over 20 minutes
Appendix C. Plant height measurements.

<table>
<thead>
<tr>
<th>Boxwoods</th>
<th>Height (cm)(^a)</th>
<th>Junipers</th>
<th>Height (cm)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-1</td>
<td>68.1</td>
<td>J-1</td>
<td>30.1</td>
</tr>
<tr>
<td>B-2</td>
<td>72.5</td>
<td>J-2</td>
<td>30.2</td>
</tr>
<tr>
<td>B-3</td>
<td>76.0</td>
<td>J-3</td>
<td>28.3</td>
</tr>
<tr>
<td>B-4</td>
<td>64.0</td>
<td>J-4</td>
<td>29.5</td>
</tr>
<tr>
<td>B-5</td>
<td>58.4</td>
<td>J-5</td>
<td>29.2</td>
</tr>
<tr>
<td>B-6</td>
<td>64.8</td>
<td>J-6</td>
<td>27.4</td>
</tr>
<tr>
<td>B-7</td>
<td>76.4</td>
<td>J-7</td>
<td>24.3</td>
</tr>
<tr>
<td>B-8</td>
<td>55.9</td>
<td>J-8</td>
<td>24.4</td>
</tr>
<tr>
<td>B-9</td>
<td>60.3</td>
<td>J-9</td>
<td>21.7</td>
</tr>
<tr>
<td>B-10</td>
<td>69.9</td>
<td>J-10</td>
<td>30.6</td>
</tr>
</tbody>
</table>

\(^a\): Boxwood height measured from length of longest shoot
\(^b\): Juniper height measured from length of longest vertically-oriented shoot

Appendix D. Net total polyphenols content (NTPC).

<table>
<thead>
<tr>
<th>Exposed</th>
<th>NTPC(^a)</th>
<th>Control</th>
<th>NTPC(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-1</td>
<td>0.20</td>
<td>B-3</td>
<td>-0.90</td>
</tr>
<tr>
<td>B-2</td>
<td>3.01</td>
<td>B-4</td>
<td>0.45</td>
</tr>
<tr>
<td>B-5</td>
<td>-0.79</td>
<td>B-7</td>
<td>-2.58</td>
</tr>
<tr>
<td>B-6</td>
<td>-2.38</td>
<td>B-8</td>
<td>-2.91</td>
</tr>
<tr>
<td>B-9</td>
<td>-5.10</td>
<td>B-10</td>
<td>0.90</td>
</tr>
<tr>
<td>J-3</td>
<td>5.61</td>
<td>J-1</td>
<td>-1.61</td>
</tr>
<tr>
<td>J-4</td>
<td>-1.55</td>
<td>J-2</td>
<td>-5.70</td>
</tr>
<tr>
<td>J-6</td>
<td>-0.08</td>
<td>J-5</td>
<td>-3.14</td>
</tr>
<tr>
<td>J-8</td>
<td>3.44</td>
<td>J-7</td>
<td>-8.03</td>
</tr>
<tr>
<td>J-10</td>
<td>2.21</td>
<td>J-9</td>
<td>7.00</td>
</tr>
</tbody>
</table>

\(^a\): NTPC reported in mg gallic acid equivalents/g leaf tissue fresh weight (mg GAE/g FW)
Figure 1. Line-of-sight to the U.S. 101 freeway at around 3 PM on 12/29/16, Woodland Hills, CA. Field recording/sound level measuring equipment was set up directly from this point.

Figure 2. Monitoring the data logging of the sound level measurements as the field recording was being taken.
Figure 3. Calibration of the studio monitor in the soundproof room. Shelf rack is separated from the studio monitor by 1.14 m.

Figure 4. Five junipers preparing to be exposed to the looped field recording in the soundproof room.
Figure 5. Five control boxwoods left in the silence of the soundproof room for 24 hours.

Figure 6. Extraction of total polyphenols from leaf tissue. Polyphenols reduce the yellow Folin-Ciocalteu reagent (10% (v/v)), which then turns dark blue upon addition of 7.5% (w/v) sodium carbonate solution. The dark blue solution is left to incubate for 2 hours at room temperature prior to spectrophotometric analysis.
Figure 7. Standard curve for net polyphenols content using gallic acid as the reference standard.

Figure 8. 95% confidence interval for the mean net total polyphenols content of the wintergreen boxwoods and flaky junipers.