5-22-2021

Salicylic Acid Response to Simulated Herbivory in Geographically Distinct T. heterophylla and H. discolor Populations

Amy E. Castle
Seattle Pacific University

SALICYLIC ACID RESPONSE TO SIMULATED HERBIVORY IN GEOGRAPHICALLY DISTINCT *T. heterophylla* AND *H. discolor* POPULATIONS

by

AMY E. CASTLE

FACULTY MENTOR:
DR. RYAN FERRER

HONORS PROGRAM DIRECTOR:
DR. CHRISTINE CHANEY

A project submitted in partial fulfillment of the requirements
for the Bachelor of Arts degree in Honors Liberal Arts
Seattle Pacific University
2021

Presented at the SPU Honors Research Symposium
Date: May 22nd, 2021

Abstract

It is commonly known that plants may produce salicylic acid as a chemical defense response to wounding, although the phenomenon has usually been observed with regard to insect herbivory. Stem and leaf tissue of two species, *Tsuga heterophylla* and *Holodiscus discolor*, which are often eaten by deer, were extracted in methanol and analyzed by HPLC to quantify salicylic acid concentration in experimentally wounded or control samples. No salicylic acid response was detectable in *T. heterophylla*, suggesting it is a less useful candidate species for future study. Some but not all *H. discolor* samples had a measurable salicylic acid response, which raises further questions about why only some samples produced detectable amounts of salicylic acid in response to the same simulated herbivory wound. However, it also establishes that *H. discolor* produces salicylic acid in response to wounding under some circumstances, thereby marking *H. discolor* as a possible species for further study. Understanding how chemical responses form in response to large mammal grazing may provide insight into how humans indirectly affect plants and their interactions with herbivores, which could have applications for public lands or conservation efforts. An appendix includes the transcript of the presentation of this project at the 2021 SPU Honors Research Symposium, as part of the panel "Considering Research as Ethical and Inclusive Storytelling."

Key Words: induced resistance, acclimatization, wounding, high performance liquid chromatography

Introduction

Salicylic acid signaling

For plants that are not poisonous, armed physically, or out of reach, animal or insect herbivory is common. One way that these plants signal and respond to such wounding is through chemical signals and phytohormones (Schultz, 1988; Erb et al., 2012). Salicylic acid (SA) is one of the main hormones involved in signaling (Ohse et al., 2017), in part because it produces induced resistance to insect herbivory (Schultz, 1988; Bi et al., 1996; Bostock et al., 2001; Ollerstam & Larsson, 2003). Species in which such signaling has been observed include *Solanum dulcamara* and *Salix viminalis,* as well as some squash and tomato species (Walling, 2000; Ollerstam & Larsson, 2003). In these studies, SA has appeared as a signal for increased pathogen-resistance mechanisms. Because SA is a signal for pathogen-resistance mechanisms, among other effects, herbivory can therefore be said to affect the plant's allocation of resources and energy long after the herbivore itself has moved on.

Although plant chemical responses to insects are well documented, less is known about the effects of large mammal grazing. Plants may tailor responses to different types of insects, suggesting that a similar flexibility may be employed when dealing with large mammals (Heidel & Baldwin, 2004; Poelman et al., 2008). It is important to understand how grazing animals like black-tailed deer (*Odocoileus hemionus columbianus*) affect a plant population, since top-down consumption is one of the most significant factors limiting plant growth in deciduous forests (Kuijper et al., 2010; Ohse et al, 2017). In *Fagus sylvatica* (European beech) and *Acer pseudoplatanus* (sycamore maple) trees, wounding with the addition of deer saliva produces a stronger salicylic acid response than wounding alone (Ohse et al., 2017).

Human interference in deer populations

Human interference may significantly increase or decrease deer populations, both directly and indirectly. On Blakely Island, one of the San Juan Islands in Washington State, logging throughout the late 1800s and early 1900s caused increased brush growth, which in turn supported increased deer populations (SPU4121, 2018). In combination with the absence of predators like coyotes and cougars, the Blakely Island deer population has reached carrying capacity and is regulated solely by bottom-up factors like resource availability (Kharitonova, 2018). Because of this, deer may have an even stronger effect on plant growth and development than noted in an undisturbed environment. In contrast, Seattle area deer populations have decreased greatly or become locally extinct over a similar time period, mostly due to urbanization and human influence, and therefore deer may not affect plant growth and development as in an undisturbed environment. Over time, increased or decreased deer herbivory levels may lead to acclimation in local plant populations (Schmitz et al., 2000; Mooney et al., 2010). Plants regularly use hormone responses to acclimate to cold (Welling & Palva, 2006), heat stress (Kotak et al., 2007), and salinity (Suzuki et al., 2016), which suggests that hormone responses to wounding could provide a similar opportunity for acclimation.

Identifying species for future study

Wounding *Tsuga heterophylla* (western hemlock) and *Holodiscus discolor* (oceanspray) by simulating herbivory is expected to produce a detectable salicylic acid response, as has been documented in other species. If *T. heterophylla* or *H. discolor* does show a salicylic acid response, it is also expected that the magnitude of the response will differ between sampling locations as the plant populations experience different intensities of deer herbivory. The Blakely Island populations have been exposed to very dense deer populations for the past century, while the Seattle parks have had greatly reduced deer populations (or no deer) for about the same time, and the forests in the Cascades mountain range have relatively undisturbed deer populations. In addition, the Blakely Island and Seattle park deer populations are free of most natural predators, while the Cascade deer populations are regulated by predators such as wolves, coyotes, bears, and mountain lions. If the salicylic acid response is partially dependent on deer in *T. heterophylla* and *H. discolor* as in other species (Ohse et al., 2017), then the plants may very well have acclimated to different intensities of deer grazing.

Methods

Sample Collection

Five samples were collected for each treatment (control and damaged) for both species across three sampling locations in Washington state (USA): Blakely Island, Discovery Park and Schmitz Preserve Park in Seattle, and Mount-Baker Snoqualmie National Forest Road 9020. Mature *H. discolor* plants were identified for sampling by the presence of dead flowers, while *T. heterophylla* seedlings were identified for sampling by measuring DBH (0-2 cm).

Samples were collected according to methods described by Ohse et al. (2017), although modified to suit our sample species. For the ocean spray samples, the terminal bud or leaf was clipped with scissors, discarded, and the time recorded. After about 2 hours, the sample, consisting of the next 4 leaves from the branch tip, was clipped from the same branch, placed in a labeled plastic bag, and immediately frozen. Control samples consisted of the terminal leaf and the next four leaves down the branch. They were selected at the same time as the other samples, then clipped and frozen at the end of the 2-hour period. The same procedure was followed for the western hemlock samples, but the initial wounding was measured to 2 cm off the branch tip, and the sample was measured to the next 10 cm. All samples were bagged separately in plastic freezer bags and frozen at -20^{o}C until tissue extraction and HPLC analysis.

Tissue Extraction and HPLC

Leaf and stem tissue from each sample were ground in liquid nitrogen, suspended in methanol, centrifuged, and the supernatant filtered. An equal volume of 2N HCl was added to the filtrate and boiled. Extracts were separated in ether, resuspended in methanol and filtered to 0.2 μm. Extracted samples were frozen at -20^{o}C until high performance liquid chromatography (HPLC) analysis. Standard solutions were created using known concentrations of salicylic acid and 0.05 mM caffeine as an internal standard. Caffeine (0.05 mM) was also added to extracted samples as an internal standard to facilitate later comparison to the standard solutions.

HPLC analysis was performed using methanol and 1% aqueous acetic acid solvents. Salicylic acid and caffeine were compared along concentration and HPLC absorbance peak area and a calibration curve was derived from the line of best fit. All samples, both standard and experimental, were run at least twice to confirm replicability of results, and methanol blanks were run between each sample to avoid cross-contamination in the instrument.

Results

Standard solutions produced a calibration curve with the equation y=0.1274x – 0.1873 and R^2=1.00 (Fig. 1). The R2 is so high because values at each SA concentration were averaged before being used for calculations. In addition to providing the calibration curve, the standard solutions verified that the HPLC did not detect other compounds, as shown by the lack of peaks other than the injection, caffeine, and SA (Fig. 2). The average retention time for caffeine was 1.833 min and the average retention time for SA was 5.386 min.

Two damaged *T. heterophylla* samples were run, both of which did not have a salicylic acid peak. The nearest peak was no earlier than 5.72, but was too late to represent salicylic acid (Fig. 3). Of the *H. discolor* samples, the damaged sample from the national forest consistently showed salicylic acid (Fig. 4). When compared to the calibration curve, the average SA concentration from all runs was 0.092 mM±0.0026 (mean±standard deviation). In one instance, a control sample from the national forest showed a likely salicylic acid peak corresponding to a concentration of 0.281 mM. However, subsequent chromatograms from the same sample did not show a salicylic acid peak at all.

Discussion

This study attempted to replicate the plant phytohormone response to wounding noted by Ohse et al. (2017) in two additional species. *T. heterophylla* saplings and *H. discolor* are both common targets of black-tailed deer, so they were considered likely candidates for a similar hormone response. However, *T. heterophylla* failed to produce a clear salicylic acid response to wounding. It is possible that this is because *T. heterophylla* is an evergreen tree, rather than a deciduous tree such as the ones observed in previous studies. Perhaps *T. heterophylla* did not produce SA in response to wounding because seasonal or physiological changes make another hormone response more likely, or decreased the magnitude to an undetectable level. However, another hemlock species, *Tsuga canadensis,* showed a similar mechanism of SA response to insect herbivory as has been previously studied in deciduous plants (Rigsby et al., 2019). This suggests that *T. heterophylla* has the ability to produce detectable levels of SA in response to herbivory. It is possible that the slightly later peaks in the *T. heterophylla* chromatograms did include SA, but that it was bound to another compound. While these results are discouraging, it is important to note that they are based on a very small number of samples, so additional samples should be evaluated before confirming them.

The variety of responses in the *H. discolor* samples raises several questions. Only the samples obtained from the national forest, where deer populations are kept in check by natural predators, showed the predicted salicylic acid response. While it is possible that the samples from the other locations also produced SA, albeit in concentrations so small as to be undetectable by HPLC, that still does not follow the trend that would be expected if salicylic acid response had acclimatized to deer population density.

One explanation is that the lack of detectable salicylic acid response in the urban *H. discolor* samples, in contrast to the national forest samples, was due to the different intensities of deer grazing in those locations. However, that does not explain why the Blakely Island samples didn't show an SA response either. The results from Ohse et al. (2017) strongly suggest that the presence of herbivores is one of the most powerful predictor of the intensity of SA response to wounding, although it must be noted that that study used deer saliva on damaged samples and this one did not. If these results do represent a larger phenomenon, they suggest that although herbivore presence (represented by deer saliva) has been shown to prompt a stronger salicylic acid response, it is not necessarily sustained when *H. discolor* plants are merely wounded without indicators of herbivore presence. From there, the question of what factors predict SA response intensity in the absence of herbivores remains.

Despite the lack of clarity around why samples did not follow the expected trends, it was observed that *H. discolor* as a species can produce salicylic acid in response to simulated herbivory, so it can be identified as a promising species for further study. More samples should be processed first and foremost to confirm the replicability of these results. After that, the addition of deer saliva applied to the wounds of damaged samples would be a potential addition to this protocol, in order to more directly assess if *H. discolor* regulates its salicylic acid response while in the presence of herbivores.

Acknowledgements

This project could not have been completed without the support of Dr. Ryan Ferrer, who provided lab space, extraction and HPLC protocols, troubleshooting, and guidance throughout. Many thanks also to Dr. Karisa Pierce for use of the HPLC instrument and Shun-Je Bhark for preparing laboratory supplies. Finally, thanks to Dr. Eric Long for his advice about deer population densities and sampling locations. All funding and supplies were provided by Seattle Pacific University (Seattle, WA, USA).

Literature Cited

Bi, J.L., Murphy, J.B. and Felton, G.W. 1996. Does salicylic acid act as a signal in cotton for induced resistance to *Helicoverpa zea*?. *Journal of Chemical Ecology 23*(7): 1805-1818.

Bostock, R.M., Karban, R., Thaler, J.S., Weyman, P.D. and Gilchrist, D. 2001. Signal interactions in induced resistance to pathogens and insect herbivores. *European Journal of Plant Pathology 107*:103-111.

Erb, M., Meldau, S. and Howe, G.A. 2012. Role of phytohormones in insect-specific plant reactions. *Trends in Plant Science 17*(5): 250-259.

Heidel, A.J. and Baldwin, I.T. 2004. Microarray analysis of salicylic acid- and jasmonic acid-signaling in responses of *Nicotiana attenuata* to attack by insects from multiple feeding guilds. *Plant, Cell, & Environment 27*(11): 1362-1373.

Kharitonova, A. 2018. Blakely's deer dilemma. Retrieved from https://blakely.spu.edu/2018/06/01/blakelys-deer-dilemma/.

Kotak, S., Larkindale, J., Lee, U., von Koskull-Döring, P., Vierling, E. & Scharf, K.D. 2007. Complexity of the heat stress response in plants. *Current Opinion in Plant Biology 10*(3): 310-316.

Kuijper, D.P.J., Cromsigt, J.P.G.M., Jędrezejewska, B., Miścicki, S., Jędrzejewski, W., Churski, M. and Kweczlich, I. 2010. Bottom-up versus top-down control of tree regeneration in the Białowieża Primeval Forest, Poland. *Journal of Ecology 98*(4): 888-999.

Mooney, K.A., Halitschke, R., Kessler, A., and Agrawal, A.A. 2010. Evolutionary trade-offs in plants mediate the strength of trophic cascades. *Science 327*(5793): 1642-1644.

Ohse, B., Hammerbacher, A., Seele, C., Meldau, S., Reichelt, M., Ortmann, S. and Wirth, C. 2017. Salivary cues: simulated roe deer browsing induces systemic changes in phytohormones and defense chemistry in wild-grown maple and beech saplings. *Functional Ecology 31*: 340-349.

Ollerstam, O. and Larsson, S. 2003. Salicylic acid mediates resistance in the willow *Salix viminalis* against the gall midge *Dasineura marginemtorquens*. *Journal of Chemical Ecology 29*(1): 163-174.

Poelman, E.H., van Loon, J.J.A. and Dicke, M. 2008. Consequences of variation in plant defense for biodiversity at higher trophic levels. *Trends in Plant Science 13*(10): 534-541.

Rigsby, C.M., Shoemaker, E.E., Mallinger, M.M., Orians, C.M. & Preisser, E.L. 2019. Conifer responses to a stylet-feeding invasive herbivore and induction with methyl jasmonate: impact on the expression of induced defenses and a native folivore. *Agricultural and Forest Entomology 21*(2): 227-234.

Schmitz, O.J., Hambäck, P.A. and Beckerman, A.P. 2000. Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. *The American Naturalist 155*(2): 141-153.

Schultz, J.C. 1988. Plant responses induced by herbivores. *Tree 3*(2): 45-49.

SPU4121. 2018. A brief history of Blakely Island and the San Juans. Retrieved from https://blakely.spu.edu/2018/05/29/a-brief-history-of-blakely-island-and-the-san-juans/.

Suzuki, N., Bassil, E., Hamilton, J.S., Inupakutika, M.A., Zandalina, S.I., Luo, Y., Dion, E., Fukui, G., Kumazaki, A., Nakano, R., Rivero, R.M., Verbeck, G.F., Azad, R.K., Blumwald, E. & Mittler, R. 2016. ABA is required for plant acclimation to a combination of salt and heat stress. *PLOS One 11*(1): e0147625.

Walling, L.L. 2000. The myriad plant responses to herbivores. *Journal of Plant Growth and Regulation 19*: 195-216.

Welling, A. & Palva, E.T. 2006. Molecular control of cold acclimation in trees. *Physiologia Plantarum 127*(2): 167-181.

Figures

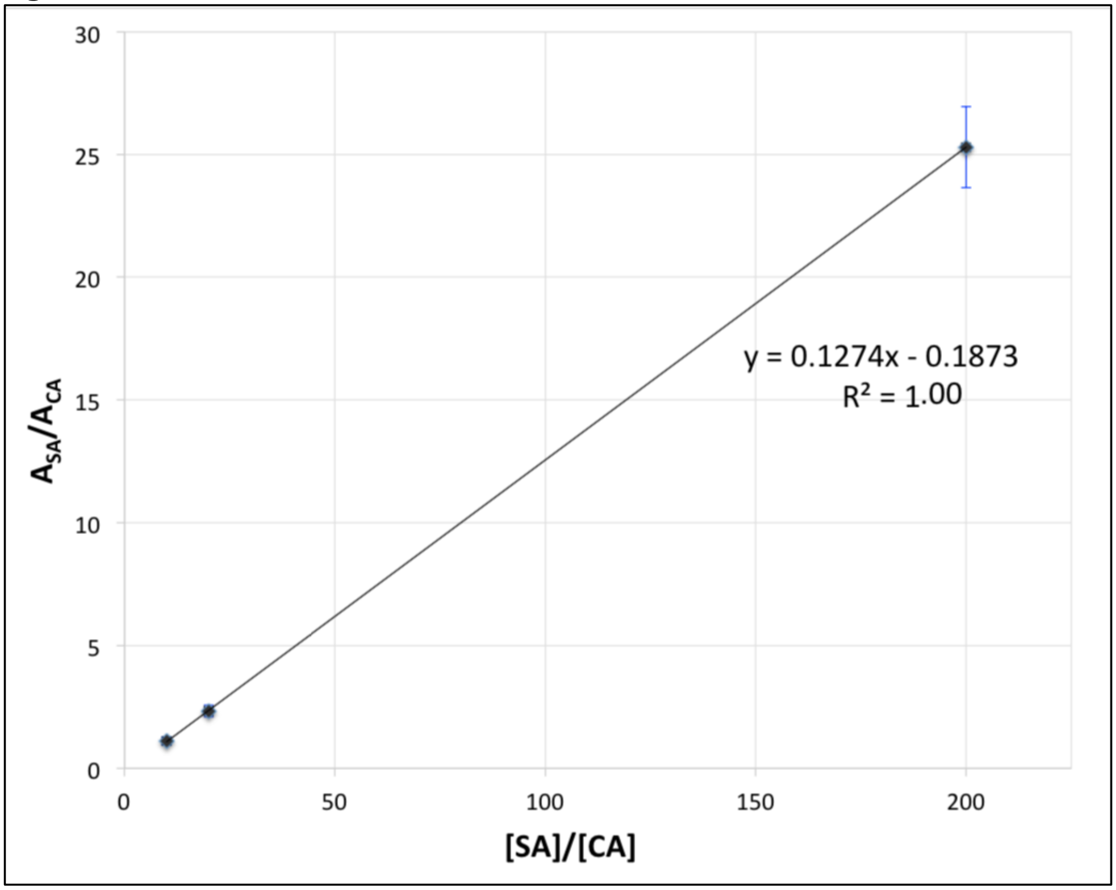

Figure 1: Calibration curve obtained from standard samples with known SA and caffeine concentration. The ratio of the area under the peak (A_{SA}/A_{CA}) and the ratio of concentration ([SA]/[CA]) were calculated for each standard run, and then the average points for each SA concentration were plotted. The equation of the best fit line is used to calculate the SA concentration of unknown samples.

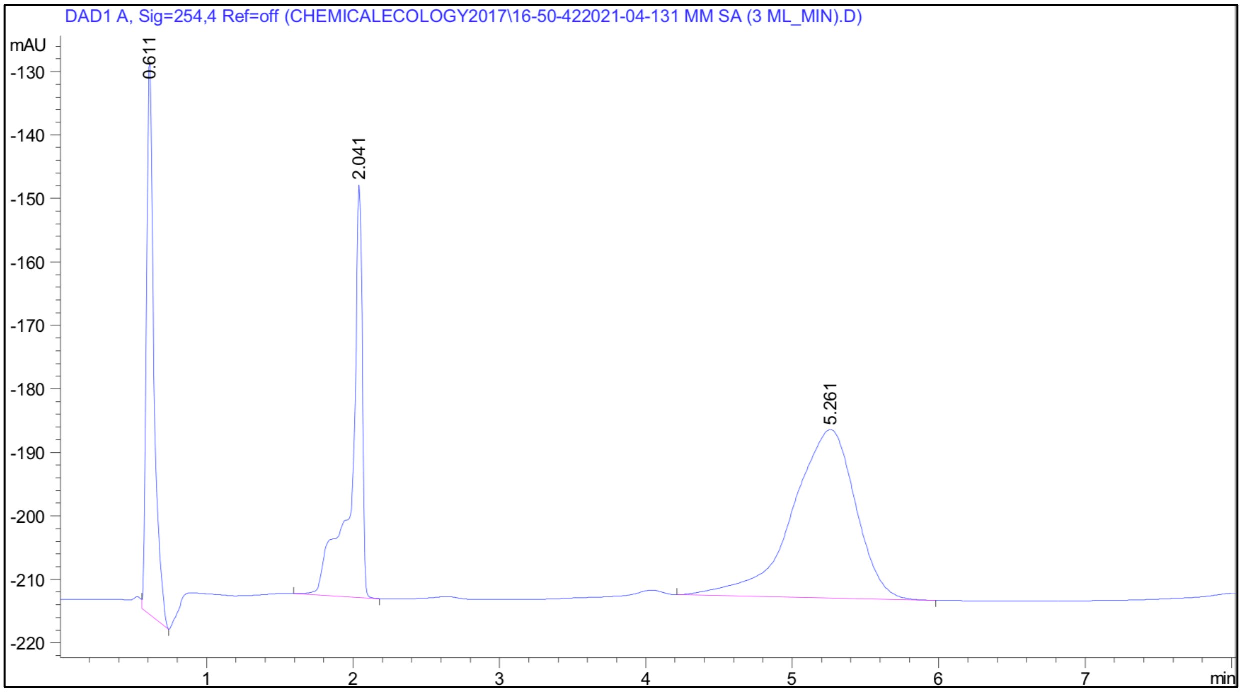

Figure 2: Example of a standard solution chromatogram. The peak at 2.041 min represents caffeine (0.05 mM) and the peak at 5.261 min represents salicylic acid (1 mM). The initial peak at 0.611 min is due to the injection of the sample and does not represent any compound.

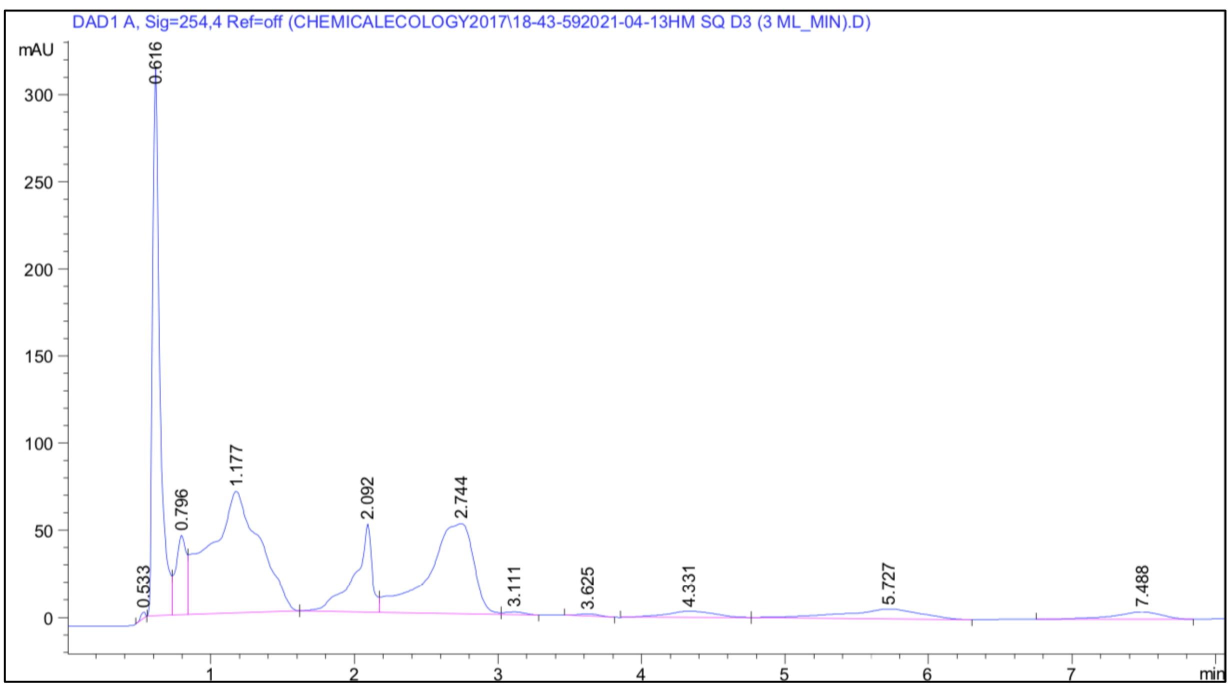

Figure 3: Chromatogram of a *T. heterophylla* sample from Mt. Baker-Snoqualmie National Forest that received the damaged treatment. The peak at 2.002 min represents the caffeine standard (0.05 mM). The peak at 5.727 min was retained slightly too long to represent SA.

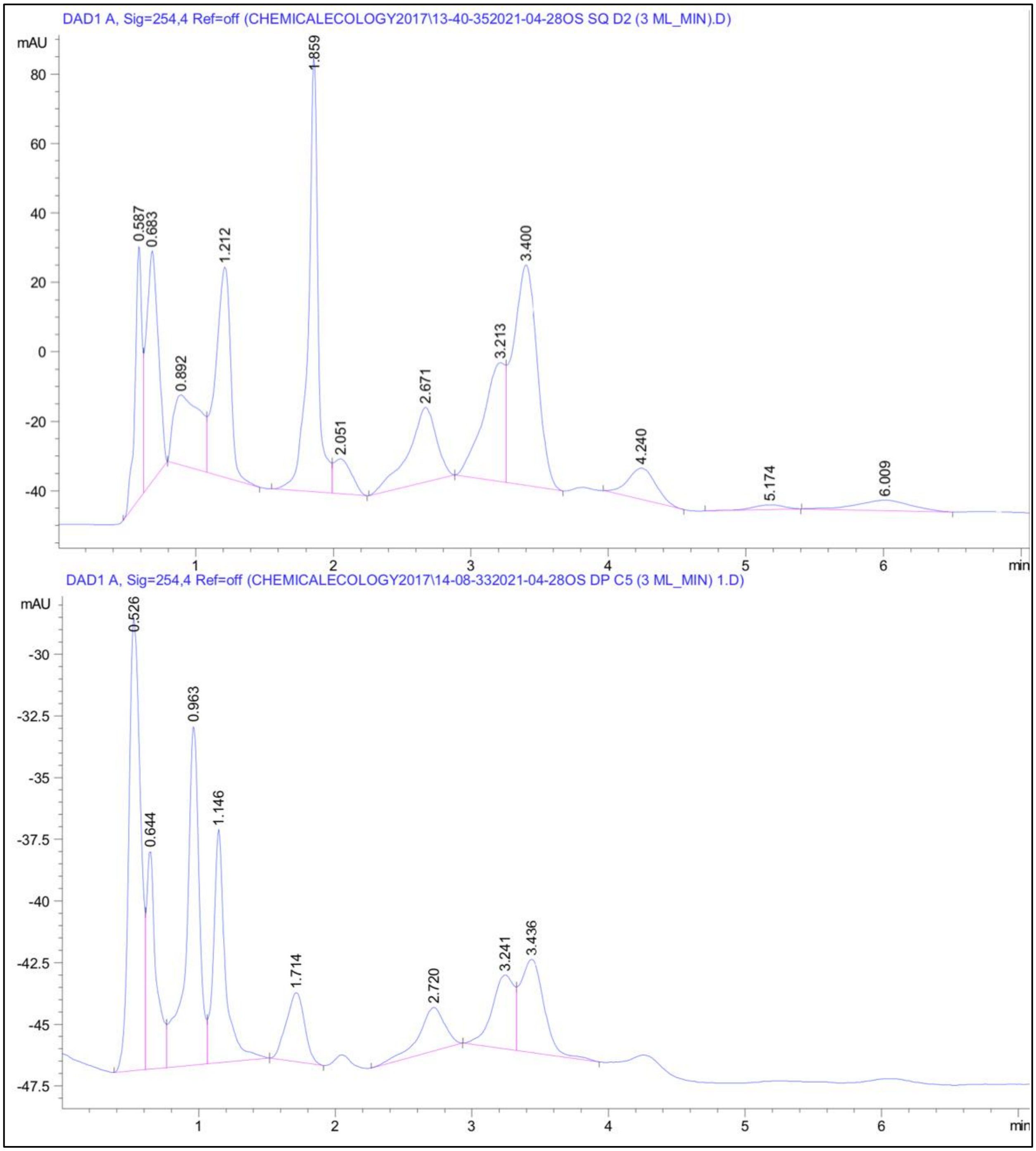

Figure 4: Chromatograms of a damaged and control *H. discolor* sample from the Mt. Baker-Snoqualmie National Forest compared. The damaged sample (above) shows a caffeine peak at 1.859 min and a SA peak at 5.174 min. The control sample (below) shows a caffeine peak at 1.714 min, but SA was not detected in the sample.

Appendix I: Honors Research Symposium Presentation

Overview

For my project, I looked at the salicylic acid response to simulated herbivory in geographically distinct *T. heterophylla* and *H. discolor* populations. As a bit of background information, plants that are eaten by herbivores, especially insects, often form salicylic acid in response to wounding. Salicylic acid is a signaling hormone that is used as a chemical defense mechanism and trigger for other pathways, such as pathogen resistance responses, and has been shown to give induced resistance to insect herbivory. Salicylic acid response to wounding by insects has been well documented across many plant species, usually due to agricultural concerns, but less is known about how large mammal herbivory may or may not result in similar responses. Large mammal grazing usually results in more tissue trauma and plant matter removed, simply because the herbivore must consume more to fuel itself.

The inspiration for my project was a 2017 study by Ohse et al. that has been instrumental in expanding our knowledge of the effects of large mammal herbivory. It showed that when deer saliva was applied to simulated grazing wounds in two species of deciduous tree, the concentration of salicylic acid present in leaf tissue increased. My research aims to identify other plant species that could be used in similar studies by evaluating baseline salicylic acid response to wounding without the application of deer saliva in an evergreen tree and deciduous shrub. I chose *Tsuga heterophylla* and *Holodiscus discolor* because both are native to the Pacific Northwest and often eaten by deer.

T. heterophylla, shown in this picture on the left, is commonly known as western hemlock and is an evergreen tree. *H. discolor*, shown on the right, is a deciduous shrub commonly known as oceanspray, named for the drooping clusters of flowers at branch tips. New growth or leaves are the most common targets of deer, which on this side of the Cascades are usually black-tailed deer. For that reason, I sampled *T. heterophylla* saplings in particular.

Because the presence of deer, represented by deer saliva applied to simulated wounds, resulted in an increased concentration of salicylic acid in the species from the 2017 study, I suspected that variations in local deer population density could result in acclimatization. If that were the case, plants sampled in a location with lots of deer would produce more salicylic acid in response to simulated large-mammal herbivory than plants in an undisturbed location with normal amounts of deer would. Similarly, plants sampled in a location with few or no deer would be predicted to produce less salicylic acid than an undisturbed location. I chose three different sampling regions so that I could examine this possibility. The first was on Blakely Island, where black-tailed deer populations are much denser and not regulated by predators. Currently, the Blakely Island deer population is at carrying capacity and is regulated by bottom-up factors like resource availability-deer often starve to death as food sources are completely exhausted. The second region was Seattle itself, which is urban enough that even uncultivated park areas like Discovery Park do not have any resident deer. Finally, the third set of samples was collected from Mount Baker-Snoqualmie National Forest, which maintains a balance of undisturbed deer populations that are regulated by natural predators, thus representing a control location.

For *T. heterophylla*, grazing was simulated by cutting 2cm off a branch tip, and the next 10 cm were collected for chemical analysis. For *H. discolor* samples, mature plants were identified as those that had previously flowered. The terminal floret or leaf was clipped to simulate wounding, and the next 4 leaves were collected along with the corresponding branch length. All samples from both species were collected in pairs of damaged and control samples during the fall of 2020.

To quantify salicylic acid concentration in the samples, a calibration curve was determined using high-performance liquid chromatography, or HPLC, on 4 standard solutions with known concentrations of salicylic acid. A constant concentration of caffeine was also added to each as an internal standard for comparison. In an HPLC chromatogram, such as the ones seen here, each peak represents a different compound that passed through the instrument. The time it takes a particular compound stays more or less the same, which allows retention time to be used to identify the peaks. My standard chromatograms indicated the average retention time for caffeine was about 1.83 minutes and the average retention time for salicylic acid was about 5.39 minutes. For the unknown samples, caffeine was also added as an internal standard before HPLC analysis so that if salicylic acid peaks occurred, the concentration could be calculated.

Two damaged *T. heterophylla* samples were run, both of which did not have a salicylic acid peak. The nearest peak was no earlier than 5.72 min but was still too late to represent salicylic acid, as indicated by the red arrow in Figure 1. The blue arrows in both chromatograms represent the caffeine peak. Of the *H. discolor* samples, the damaged sample from the national forest consistently showed salicylic acid, as shown by the green arrow in Figure 2. When compared to the calibration curve, the average SA concentration across all runs was 0.092 mM

Both species were originally considered likely candidates for a similar hormone response to that observed by Ohse et al. in 2017. However, *T. heterophylla* failed to produce a clear salicylic acid response to wounding, as noted by the lack of peak at 5.1-5.3 minutes on the chromatogram. It is possible that this is because *T. heterophylla* is an evergreen tree, rather than a deciduous tree such as the ones observed in previous studies. It is also possible that the slightly later peaks, marked by a red arrow in Figure 1, in the *T. heterophylla* chromatograms did include SA, but that it was bound to another compound that extended retention, although an entirely new study would be necessary to confirm that. While my results are rather discouraging, it is important to note that they are based on a very small number of samples, so additional samples should be evaluated before confirming them.

The variety of responses in the *H. discolor* samples raises several questions. Only the samples obtained from the control location showed the predicted salicylic acid response at all. While it is possible that the samples from the other locations also produced SA, albeit in concentrations so small as to be undetectable by HPLC, that still does not follow the trend that would be expected if the salicylic acid response had acclimatized to deer population density, which would have resulted in an even greater SA response in the Blakely Island samples. The results from Ohse et al. (2017) strongly suggest that the presence of herbivores is one of the most powerful predictors of the intensity of SA response to wounding, although that study used. If those results do represent a larger phenomenon, they suggest that although herbivore presence has been shown to prompt a stronger salicylic acid response, it is not necessarily sustained when *H. discolor* plants are merely wounded without indicators of herbivore presence. From there, the question of what factors predict SA response intensity in the absence of herbivores remains.

Despite the lack of clarity around why samples did not follow the expected trends, it was observed that *H. discolor* as a species can produce salicylic acid in response to simulated herbivory, so it can be identified as a promising species for further study. More samples should be processed first and foremost to confirm the replicability of these results.

Application and Synthesis

One indirect factor notable to this study was the influence that humans have on deer population sizes. On Blakely Island, which is one of the San Juan Islands in Washington State, logging throughout the late 1800s and early 1900s caused increased brush growth, which in turn supported increased deer populations. In combination with the absence of predators like coyotes and cougars typical of such islands, the Blakely Island deer population has been able to exceed the size that can be supported by the food available. Because of this, deer may have an even stronger effect on plant growth and development than noted in an undisturbed environment. In contrast, Seattle area deer populations have decreased greatly or become driven out completely over a similar period, mostly due to urbanization and human influence, and therefore deer would not be expected to affect plant growth and development as in an undisturbed environment. Therefore, even though humans don't usually participate in regular plant wounding like herbivores do, in a sense of cause and effect similar to a food chain, humans extend top-down regulation over most animals, which then more directly affect plants. Over time, increased or decreased deer herbivory levels could lead to acclimation in local plant populations, indirectly caused by influences that humans have on their surroundings.

Research examining the effects of humans on the environment and potentially aiming for change is tricky because of the different set of priorities ecologists may hold compared to the general population. Is it most important to preserve a completely undisturbed natural environment? That almost always interferes with the human need for housing and other trappings of society. In this case, both positions come with merits and difficulties. Whatever the situation in the past, currently Seattle is a major metropolitan area, and attempting to introduce either deer or deer predators to regulate the environment for plants would not go well with the surrounding residents. Although areas like parks have been set aside for preservation, they clearly do not perfectly match what a natural environment would look like. The struggle of public lands is highlighted in the contrast between the Seattle park sampling sites and the national forest. Both are uncultivated and set aside as "wild," yet only the national forest is able to support a full predator-herbivore food chain. Yet even uncultivated parks preserve more of the original natural environment than a gardened, paved park would. Sometimes the only thing that scientists and conservationists can do, at least in the present, is to gather data on what an undisturbed environment looks like and how the disturbed environment differs. If problems arise in the disturbed population, it may be easier to identify solutions with more knowledge available. In addition, if the opportunity comes to make changes to better replicate the original environment, a more comprehensive picture of how plants, animals, and inorganic factors interact would be necessary. Therefore, even though research such as this may not necessarily prompt attempts to decrease the effect of humans on natural environments such as parks because of the strength of human interests in the area, it is nonetheless worth examining how plants respond to different types and strengths of herbivory.

In general, more and broader knowledge of an area of study is preferable. However, one of the most important parts of constructing a research project involves knowing when to accept that not everything can be included. The constraints of time and resources are often the most influential, as I found throughout this project. A truly comprehensive analysis could have included sample sets collected both in the spring and fall to analyze new growth, larger data sets, or more replication during HPLC processing. Even further, I could have gone straight ahead with the application of deer saliva during sampling. Some factors could not be measured, such as the degree of human contact experienced by plants at each sampling location or how salicylic acid response levels might change over time. In the end, though, proceeding with a more simplified study allows it to focus on a few specific aspects of the hormone response and serve as a steady building block for further research.

This kind of advice is often given to writers when constructing new worlds or characters: Don't focus so much on the big picture that you forget the details. Every study like the 2017 Ohse et al. paper relies on precursor studies to conduct preliminary analysis and construct the set of assumptions that the researchers may use. Identifying species that exhibit salicylic acid responses at all paves the way for more specific analysis of how those responses are formed and to what magnitude. It also is valuable so that precious resources are not wasted on attempting to determine the particulars of a salicylic acid response in a species that might not even form the same kind of salicylic acid response, or any at all.

As a panel, our goal is to consider how research tells a story, pulling together bits and pieces of knowledge to construct an argument or narrative. Such stories are not comprehensive in and of themselves, but they contribute to a larger body of scholarship, in which all aspects should be included to form an accurate picture of the world. In the overall story of salicylic acid responses to wounding, or even specifically salicylic acid responses to large-mammal herbivory, my project represents the introduction and backstory: not the main arc, but an important part of the setting that our collective scientific knowledge exists in.