THE ROLE OF TERPENOIDS AND PHENOLICS IN CONTROLLING ECOLOGICAL IMPACTS OF HEMLOCK WOOLY ADELGID IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK

By

Emily Renaee Branum

A professional paper submitted in partial fulfillment of the requirements for the degree

of

Master of Science

in

Land Resources and Environmental Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

July 2021

ACKNOWLEDGEMENTS

I would like to show appreciation to the MSU LRES professors whose courses I have taken. This includes Dr. Maria Costas, Dr. Timothy Seipel, Mr. Nicholas Fox, Dr. John Long, and many others. Thank you for such interesting and challenging content. I would especially like to thank Dr. John Townsend-Mehler for taking me under his wing and providing feedback and guidance as his first student for the professional paper course. It's worth noting that his Chemical Ecology course was among my favorite courses taken as a student in the program and was the inspiration behind my professional paper topic. Thank you to the Graduate Committee, especially Dr. Scott Powell for providing feedback and an initial path forward for my paper.

I would like to thank my friends for being patient with me while I completed this degree. They provided many free therapy and proofreading sessions that I'm very grateful for. Thank you, Mom and Dad for always encouraging me to stay the course. Lastly, thank you to my boss, Scott Morie, who has always encouraged academic and professional development and has allowed me to devote needed time to coursework.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

Abstract

The hemlock woolly adelgid (*Adelges tsugae*) is an invasive pest in the eastern United States where they have been colonizing and feeding on eastern hemlock oleoresin, initiating death and stand decline. Eastern hemlock stand reductions are important in the Great Smoky Mountains National Park because they host a notoriously biodiverse ecosystem, which is popular among tourists and economically supports the neighboring communities of Pigeon Forge and Gatlinburg, Tennessee. Rapid decline of eastern hemlock and their associated microclimates affect many abiotic and biotic aspects of the surrounding ecosystem. Although dependent on abiotic factors and metabolically costly to produce, terpenoids and phenolics have repellency potential in sufficient concentrations to prevent the loss of eastern hemlock and their associated ecological impacts. Therefore, I conducted a comprehensive, interpretive literature review to assess which terpenoids and phenolics are released after hemlock woolly adelgid herbivory, the extent to which they are produced, and if they lead to herbivory reductions. That knowledge is then related to ecological impacts caused by their release. Although not actually performed, I present sampling and analysis methods to achieve a representative terpenoid and phenolic profile followed by probable results, a discussion of current and potential management strategies, and subjects of future study. Overall, my paper is informative in nature and could provide insight and direction in the development of resistance-breeding, mitigation, and conservation programs. Regardless of which strategy is chosen, adequate monitoring, management, and restoration is needed to preserve eastern hemlock. Otherwise, the hemlock woolly adelgid population will continue to expand, negatively affect hemlocks, and degrade the Great Smoky Mountain National Park ecosystem.

Background Information

Introduction to Hemlock Woolly Adelgid

Adelges tsugae, better known as the hemlock woolly adelgid (HWA), is an invasive aphid-like insect that is suspected to have originated from Asia about 1924 (Broeckling & Salom, 2003). After first observation in Virginia in the 1950s, their populations exploded and they currently occupy a large portion of the Appalachian Mountains, from northern Georgia to southern Maine (Albani et al., 2010). HWA have become a serious concern in the southern Appalachians and were first identified in the Great Smoky Mountains National Park (GSMNP) in 2002. It is unknown when they first arrived in the park and perhaps they went unnoticed until 2002. Regardless, they have quickly spread since then due to a lack of natural predators and many modes of transportation including birds, wind, humans, and deer (He et al., 2017; Koch, 2005).

HWA chronically infest eastern hemlock (*Tsuga canadensis*) for many years, temporally distinguishing them from other exotic pests (Tingley et al., 2002). They use long piercing-sucking mouth stylets to feed on sap sugars found in xylem ray parenchyma cells of twigs and needles thus robbing them of nutrients, regardless of the age and size of trees (Broeckling & Salom, 2003). As such, they are typically located at the base of needles, on the bark of the twig. Upon finding a suitable site and inserting their stylet, they become dormant and produce a distinctive white, waxy, filamentous ovisac that is used to protect them and their eggs against predators and desiccation (Figure 3). These "cottony" protective coverings are used to identify HWA and persist over the female throughout the rest of her life (termed aestivation); even after eggs have hatched, offspring are gone, and death has occurred (Hoover, 2004). HWA are parthenogenetic meaning that they are all female and reproduce asexually. They undergo two generations and six development stages: egg, four nymphal instars, and adult; the winter generation is called sistens and the summer generation is called progrediens. Their life history begins when eggs are laid by an overwintering female during late winter through June and hatch, on average, in April. Hatching can continue through June and survival can continue until summer of the following year (Figure 1) (Williams, 2017). HWA females are black, oval-shaped, and 2-mm long; eggs are brownish-orange, oblong, and 0.25-mm long; nymphs are reddish-brown until they slowly transition into an adult (Williams, 2017; Hoover, 2004). Information on life history becomes important later in the paper when understanding the rationale of time of sampling, which is performed to identify and quantify HWA populations.

Effects on Hemlock

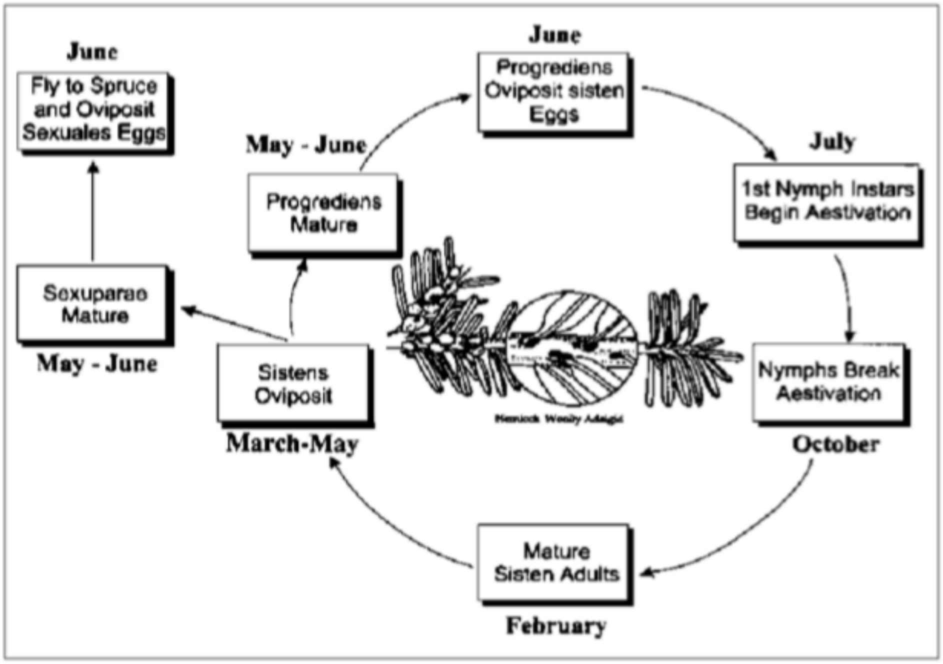

Figure 1: Hemlock woolly adelgid life cycle (Williams, 2017).

The physical effects of HWA on eastern hemlocks, henceforth termed ‘hemlock’, include decreased branch growth, branch mortality, and needle desiccation, a combination of which typically kills the entire tree in four years or less (Pezet et al., 2013). HWA is indiscriminate of hemlock age and thus attacks life stages ranging from seedlings and saplings to mature trees, which they tend to prefer, ultimately exhausting seed banks and preventing hemlock regrowth (Tingley et al., 2002). Feeding causes an increased supply of nitrogen to sites of new growth and water stress with associated decreases in stomatal conductance, nutrient transport efficiency, and photosynthetic productivity (Pezet et al., 2013; Gonda-King, 2014). Since their discovery, HWA have caused the deaths of thousands of eastern hemlocks in the GSMNP (Hakeem et al., 2010). Trees that survive infestation are very susceptible to other diseases, invasive species, and herbivores (U.S. Department of the Interior, 2018). Conifers like hemlock are particularly vulnerable to HWA because many of their sequestered resources are distributed to foliage, whereas deciduous trees put many of their resources into tissues that are inaccessible to sap feeders like roots and stems (Gonda-King, 2014).

Ecological Importance of Eastern Hemlock

Hemlock is designated as a foundational and forest cover species, filling a unique niche and serving an integral ecological role in the GSMNP ecosystem, especially in avian, aquatic and riparian habitats (Pezet et al., 2013). The loss of foundational tree species can change ecosystem processes like decomposition rates, primary productivity, nutrient dynamics, food webs, biodiversity, and hydrology (Ellison et al., 2005). Hemlock can also add stability to slopes, prevent erosion, contribute to stream channel reshaping, and decrease sediment loads in streams due to runoff (Evans et al., 1996). Hemlock is the most shade tolerant, longest-lived conifer in the U.S. and their organic matter inputs create moist, cool microclimatic habitats with unique soil chemistry and processes, nutrient-poor soil, and slow nitrogen cycling rates; especially in hemlock-dominated stands due to its acidic needle-chemistry profile (Ellison et al., 2005; Pezet et al., 2013; Evans et al., 1996). The decline in hemlock stands necessarily implies an initial loss of biomass in the form of slow-decaying organic matter inputs, which are valuable during winter months when deciduous organic matter inputs decrease; this condition persists until successive vegetation appears (Nuckolls et al., 2008).

In addition to soil moisture promotion, hemlock stands reduce diel stream temperature variations, stabilize stream base-flows, and perform thermoregulation. Because of this, streams that travel through hemlock stands are capable of supporting approximately 10% greater numbers of fish, freshwater invertebrates, and salamanders than would otherwise be supported in seasonally dry streams in areas lacking hemlock. This is partly due to the microclimate hemlock provides, but also because hemlock woody debris decays slower than hardwood, on the order of decades to centuries, catching stream organic matter and creating small, novel stream habitats (Ellison et al., 2005). Because hemlocks tend to be found near streams, aquatic communities in streams kept cool by the shade of hemlock forest canopies could be severely affected by their decline. Native brook trout, other fish species, and invertebrate diversity could plummet potentially leading to higher competitive advantages for invasive species like brown trout (Snyder et al., 2005).

Hemlock canopy characteristics are structurally unique and have been found to, "support moderate levels of avian diversity, including several species that are largely restricted to hemlock stands…as well as several species of mammals and amphibians (Tingley et al., 2002). Indeed, it is the most important conifer species in the eastern U.S. for game species' cover and winter shelter, such as white-tailed deer, turkey, ruffled grouse, rabbit, and the snowshoe hare (Deal, 2007). Tingley et al. (2002) examined avian impacts of hemlock loss due to HWA during a breeding season in canopies and understories with varying severities of HWA infestation. Results showed that avian composition was significantly associated with the severity of overstory hemlock mortality. Some birds prefer a more open canopy, due to HWA-induced mortality, while others prefer an intact canopy. The change that favors gap-dependent species is only temporary but it displaces species that require a full canopy, which is a concern for threatened bird species such as black-throated green warblers, which are closely associated with hemlock and would likely exhibit population decline with hemlock reduction, potentially leading to eventual extinctions (Tingley et al., 2002). Essentially, as hemlock is replaced, the ecosystem becomes increasingly homogeneous, which entails a decrease in avian, fish, stream invertebrate, and other species richness (Tingley et al., 2002).

Vegetation Succession

The destructive effects of HWA have only been documented on large scales since the 1980s so the "empirical evidence regarding the long-term future species composition of formerly hemlock-dominated forests" does not exist (Albani et al., 2010). Regardless, it is known and expected that the mortality of hemlock stands can cause both long and short-term ecological changes, partly due to a change in structure and composition of understory vegetation (Brooks, 2001). Hemlocks typically create understory environments that are inhospitable to other plants due to low light infiltration through its canopy and acidic needle deposition, but if there is an understory it is usually comprised of starflower, common woodsorrel, woodfern, goldthread, clubmoss, and false lily-of-the-valley (Eastern Hemlock, n.d.). As hemlocks are replaced by other plant species, plant composition and forest communities shift (Nuckolls et al., 2008). One of the effects of HWA infestation is the loss of needles, which allows more light transmission to the forest floor, affecting soil moisture and promoting the growth of opportunistic and invasive successional plants (U.S. Department of the Interior, 2018). As soil temperatures increase due to the influx of sunlight, their nutrient cycling patterns and chemistry change to favor early successional trees that grow quickly, which alters the entire landscape (Pezet et al., 2013).

There is a lack of scientific consensus regarding the degree of influence of hemlock decline on carbon dynamics. Multiple studies suggest that carbon dynamics will decline with a decrease in hemlock. However, it has been shown that the restoration of carbon uptake ability is determined by successional dynamics. Previously, the ecosystem demography model and a stochastic model of HWA distribution were used to determine future effects of HWA on forest carbon concentrations in the eastern U.S. (Albani et al., 2010). This model can account for various soil types, disturbance histories, climates, and HWA spread and may be particularly helpful in the determination of long-term ecological consequences of hemlock loss. Due to successional species, it was concluded that the loss of hemlock was not a significant detriment to carbon uptake and fluxes (Albani et al., 2010).

Unfortunately, hemlocks do not naturally regenerate and when they die *Rhododendron* spp. and other shrub species are likely to repopulate the areas of hemlock mortality if they are already present in the understory; this replacement would cause low water use and nutrient cycling rates. Conversely, if shrubs are not present, yellow poplar, sweet birch, sour wood, northern red oak, red maple, and scarlet oak have been found to replace hemlock, which causes higher water usage and nutrient cycling rates. Therefore, the presence and invasion of potential understory and surrounding tree species determine long-term ecosystem impacts in the GSMNP (Vose et al., 2013). It is due to the ecological decline discussed in the previous section and successive vegetation that it is important to understand the mechanisms that control and exacerbate HWA herbivory such as biological controls and the growth-differentiation balance hypothesis.

Secondary Compounds in Conifers

It is normal for conifers such as hemlock to biosynthesize, accumulate, and release species-specific oleoresin-based defense chemicals, namely terpenoids and phenolics, that are present in sufficient concentrations to deter pathogens and herbivorous insects (Michelozzi, 1999). Physiologically, these chemicals deter deleterious insects by interrupting ATP production in developmental pathways, cell membrane formation, and the functioning of the nervous system (Pezet et al., 2013). Terpenoids are derivatives of terpenes, both of which can be added to each other in cytosol via the mevalonate pathway or in plasmids via the methylerythritol phosphate pathway to produce various compounds and are classified as secondary defense compounds (Pezet et al., 2013). Secondary defense compounds are generated from secondary metabolic processes involving compounds located in specialized cells that are necessary for plant survival but not directly essential for respiratory or photosynthetic metabolism. Conversely, primary metabolism is the metabolic processes that perform cell maintenance and proliferation (Lattanzio et al., 2008). I will use various terms to describe secondary compounds throughout my paper and they include but are not limited to secondary defense compound(s), defense compound(s), and terpenoids/phenolics; these compounds are constitutive or induced. Constitutive defense compounds are always present regardless of threat and inducible defense compounds are initiated upon attack or infection (Villari et al., 2014).

Previous studies have found increased concentrations of mono- and sesquiterpenoids including β-caryophyllene, α-pinene, α-humulene, and germacrene D relative to concentrations of other terpenoids associated with reduced susceptibility to HWA like isobornyl acetate (Pezet et al., 2013). This may be due to an imbalance of growth-defense chemicals or a higher carbon content in HWA-resistant hemlocks leading to an increased allocation of carbon directed towards the generation of defense compounds (McKenzie et al., 2014). Additionally, phenolic compounds can be released as the result of HWA infestation. However, abiotic stressors like ultraviolet radiation and drought have also been known to cause their release (Kinahan et al., 2020). Phenolic compounds are not only important to defend against herbivory but are integral to conifer needle development and toughness, tree metabolism, growth, reproduction, and protection from ultraviolet radiation, an excess of which can cause mutagenesis and cell death (Kinahan et al., 2020; Lattanzio et al., 2008). They are created by polymerization and some act by covalently bonding to cell walls, effectively strengthening tissue and preventing further access to recently damaged sites, while others reside on the external surfaces of organs or in waxes (Kinahan et al., 2020; Lattanzio et al., 2008).

Growth-Differentiation Balance Hypothesis

Obtaining the metabolic costs of defense compound generation could provide insight to decreased hemlock growth, which could be exacerbated by HWA infestation and further have the same ecological effects as hemlock loss described above. Tree resource allocation follows the growth-differentiation balance hypothesis (GDBH), which postulates that trees will assign a hierarchal allocation of carbon to growth, storage, and defensive compounds based on resource availability and phenotypic plasticity. Because resource allocation to defense compounds relies on repeated evolutionary responses, repeated trade-offs may occur between various defensive pathways, like the methylerythritol phosphate pathway. Abiotic factors like precipitation frequency and associated soil infiltration and moisture and aspect and associated light availability are able to influence resource investment into terpenoid and phenolic production and must be accounted for when attempting to predict resource allocation to defensive compounds along with plant metabolism and the genetic control of that metabolism (Villari et al., 2014). In general, constitutive defenses are more metabolically costly since they are produced even in the absence of herbivores. Therefore, it is relevant to the goals of my paper that if the compounds released upon HWA feeding are constitutive, the metabolic costs to create them are higher than induced defenses, and it can be assumed based on the GBDH that there is some compromise in growth, which can impart the same ecological consequences as previously discussed. HWA infestation will only exacerbate these effects (Heil, 2002). Fortunately, it can be presumed that if a tree has constitutive defenses, "they are not expected to allocate resources to induced defenses against that same agent" (Villari et al., 2014). In short, if hemlock secondary compounds are primarily constitutive then they are constantly present and do not require extra energy costs to deter HWA and therefore does not initiate a growth limitation nor ecological impacts from growth limitation; impacts from the possibility of limiting feeding could still exist though.

The metabolic costs of HWA feeding can be determined by examining the, "phenotypic, resource-derived trade-offs on an individual-based sampling approach" (Villari et al., 2014). To do this, one must calculate "the inducible variation of each metabolite, defined as the difference between induced concentration and constitutive concentration in the same tree" and explore "trade-offs between constitutive concentration and inducible variation by regressing these two variables" (Villari et al., 2014). To differentiate defense compounds, twigs and needles harvested from uninfested trees would need to be evaluated according to Villari et al., who analyzed for constitutive and induced terpenoids and phenolics in Scots pine. The ecological impacts due to metabolic costs could be negligible as the study on Scots Pine showed that the production of defense compounds may not be exceptionally metabolically costly due to potential resource remobilization and, "multi-functionality of specialized metabolites" (Villari et al., 2014).

Previous studies have shown that individual trees can produce different degrees of induced responses and thus release varying amounts of secondary compounds. This is likely due to differences in genotype, life history, age, and environmental factors among individuals (Karban & Myers, 1989). Accordingly, the maturity and growth speed of needles also has an effect on the amount of phenolics released with young needles releasing less than mature needles and fast-growing species investing more resources in them (Kinahan et al., 2020; Endara & Coley, 2011). However, if abiotic stress is high, phenolic concentrations can be expected to be high (Kinahan et al., 2020). Terpenoids can vary seasonally and are highest during the peak growing season, late April to early July, and phenolics are most active outside of this spring season. Conifer phenology should be considered during the sampling period and is defined as, "seasonally varying developmental events driven by environmental cues" (Kinahan et al., 2020). These cues can be abiotic or biotic and the development of stress resistance initiated by them can be affected by changes in terpenoids and phenolics (Kinahan et al., 2020).

Abiotic Factors

The temporal length of stand resilience depends on environmental and climatic variables (Pontius et al., 2006). The most limiting abiotic stressor for growth and resource allocation to conifer defense compounds is soil moisture. Hemlock tends to grow in cool, moist areas on north and east facing aspects, in valleys, or in narrow riparian areas (Eastern Hemlock, n.d.; Ellison et al., 2005). They prefer nutrient-poor, well-drained soil, although they have been found in multiple soil types indicating that this is not a major influence on tree health, and are likely to be found from 2,000 to 5,000 feet in elevation in the Southern Appalachians (Evans et al., 1996; Eastern Hemlock, n.d.). The climatic conditions of GSMNP are classified as humid and subtropical and the area experiences cool winters, frequent thunderstorms, and hot and humid summers; however, the climate is prone to change. The primary form of precipitation is rainfall, which annually ranges from 55 inches in the valleys to 85 inches at higher altitudes and is fairly evenly distributed through the year. October is usually the driest month of the year while July is the wettest (U.S. Department of the Interior, 2018). Some studies have shown that susceptible hemlocks have lower concentrations of phosphorous and calcium and higher concentrations of nitrogen and potassium compared to other HWA-resistant hemlock species. Nitrogen, which is positively correlated to twig death and foliar transparency, and potassium may make hemlocks more palatable and nutritious to HWA making them more susceptible to invasion (Pontius et al., 2006).

The Resource Availability Hypothesis

The resource availability hypothesis can be used to predict herbivory based on growth patterns and vice versa. It postulates that slow-growing species like hemlock will have a low opportunity cost for defense compound production and be strongly affected by herbivory (Endara & Coley, 2011). Opportunity costs can be defined as decreases in competitive ability with surrounding plants for resources due to arrested growth in order to direct resources to defense compound production (Heil, 2002). A meta-analysis on the resource availability hypothesis in woody species concluded that slow growers are typically found in resource-poor environments and exhibit greater resource investments in constitutive defenses while fast growers tend to use inducible defenses, are found in resource-rich environments, and have less repellency strength since they direct resources toward growth even at the cost of high herbivory risk (Endara & Coley, 2011). Essentially, this hypothesis can predict implications of slow-growth and resource allocation on defense compound production, HWA susceptibility, and related ecological impacts in comparison to the same in deciduous, fast-growing species.

Problem Description

The goal of my paper is to conduct a synthetic literature review to successfully evaluate if, and which, constitutive and induced terpenoids and phenolics are released by hemlocks upon HWA feeding since these are the two, “primary classes of defensive metabolites present in conifer needles” (Kinahan et al., 2020) while taking abiotic influences like precipitation, soil moisture, and light availability into account, to determine if these compounds stop or reduce HWA herbivory. I will include a description of the sampling and analytical methods that could be used to achieve a representative terpenoid and phenolic composition, customized to meet project goals, based on previous studies’ analytical methods. Furthermore, these compounds and their effects, or lack thereof, on HWA will be related to ecological processes and impacts within the GSMNP ecosystem as a result of hemlock mortality.

The results of my proposed study are ecologically important in all environments but the GSMNP is a major recreational destination being the most visited national park in the U.S. with an estimated 10 million visitors per year (Deal, 2007). The most popular park activities include fishing, backcountry hiking and camping, horseback riding, picnicking, auto touring along the Blue Ridge Parkway, and fall color viewing. The Great Smoky Mountains as a whole are one of the oldest mountain chains on the planet and famously support rich biodiversity and it is the largest managed wilderness area in the eastern U.S. (U.S. Department of the Interior, 2018; Deal, 2007). It also contains some of the largest and oldest eastern hemlocks in the world with approximately 726 acres of old-growth hemlock, which is not a large amount considering the park is 525,000 acres (Deal, 2007). Nevertheless, if hemlock stands and their uniquely associated microclimatic conditions, which further contribute to and affect the surrounding ecosystem, are lost, it is possible that the whole vegetative community and ecosystem of the park, including wildlife, will change. This by itself is a sufficiently important reason to understand the effects of secondary defense compounds and HWA herbivory but it also affects budgetary management decisions and other resource allocation pertaining to HWA control due to the value placed on maintaining ecosystem integrity in this particular park. Indeed, the park generates over $734 million each year for the surrounding communities of Gatlinburg and Pigeon Forge, Tennessee (U.S. Department of the Interior, 2018). Overall, this condensed information may help resource managers make the most appropriate decisions when it comes to HWA population reduction and management in the GSMNP.

Survey Methods

For the purposes and goals of my paper, the following survey and analysis procedures were synthesized from various experimental studies and could be used to obtain terpenoid and phenolic concentrations if an experiment to achieve answers to the above problem statement was performed. Sampling locations could be identified by a map previously developed by a NASA Development Team (Figure 2), which depicts areas of hemlock distribution within GSMNP using Enhanced Vegetation Index and Normalized Difference Vegetation Index datasets (He et al., 2017). Based on Figure 2, sampling areas would be located in the southwest, northeast, and along the ridgeline at the center of the park because these areas have the densest hemlock stands. The collection of twigs should take place during HWA-active months (McKenzie et al., 2014). The results from a previous study conducted in Tennessee indicate that sampling should occur in July to allow the hatching and settlement of nymphs on the underside of needles and twigs. Life stages in Appalachia are slightly different than the standard life cycle time frames discussed above, which are based on New England locations, due to warmer temperatures. Sampling at this time also increases the probability of capturing both generations leading to a more inclusive, accurate estimate of HWA populations (Deal, 2007). Based on my research, I recommend that sampling mature twigs from 50 healthy trees in each area, with approximately 50 feet between them, and five branches per tree taken from equal height intervals would be representative of each sample population. Ten terminal twig tips, 10 cm in length, is assumed to yield a representative terpenoid composition per tree (McKenzie et al., 2014). After sampling, twig samples are placed on ice until analysis, which has been shown to not affect terpenoid and sample integrity (Lagalante & Montgomery, 2003). Mature twigs and needles should be chosen from mature trees because HWA preferentially feed on them and mature trees provide a higher ecological benefit compared to young trees (Kinahan et al., 2020).

Twigs of healthy trees, as evidenced by new growth, can be equally divided for each sample location, half of which can be examined for constitutive secondary defense compound composition and half of which can be exposed to HWA and examined to reveal any compounds and concentrations released by the induction effect since, “the difference between damaged and control plants is the best metric of induced resistance…” (Villari et al., 2014). This should also provide some idea of the relative metabolic costs of manufacturing the compounds.

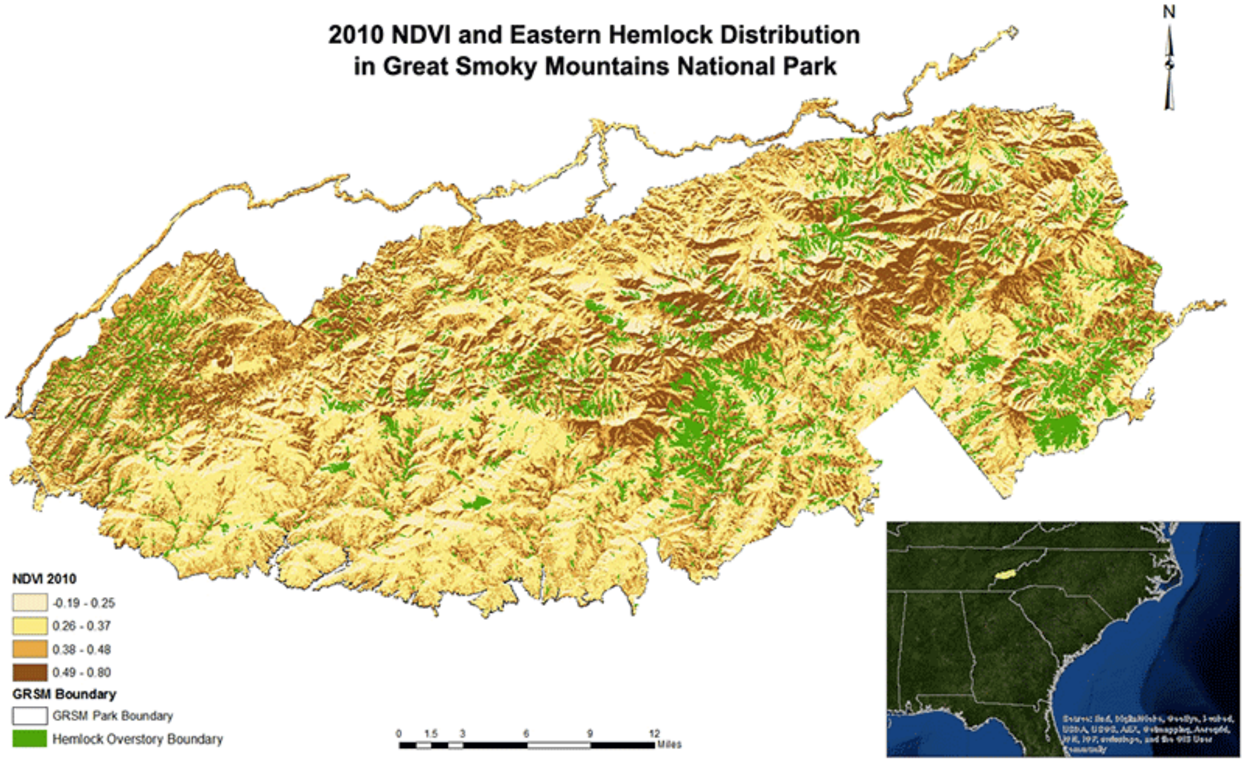

Figure 2: Map illustrating areas of hemlock overstory and Normalized Difference Vegetation Index values used to identify representative sampling locations (He et al., 2017).

Sample Analyses

Based on protocols used by Lagalante and Montgomery (2003), terpenoid extraction and identification can be accomplished using solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). A higher concentration of terpenoids can be found in hemlock twigs, therefore the twig and needle sample homogenization can permit access the resin canals; again, in this case, half before HWA exposure and half after (Costa, 2005). SPME involves the adsorption of plant volatiles onto a coated fiber and subsequent heating by the GC-MS injection port. The homogenized sample material is then collected in vials and heated in a water-jacketed beaker and maintained at 50°C. Because terpenoids differ in their volatility, adequate time must be given for all terpenoids to be brought to equilibrium; Lagalante and Montgomery (2003) used 1, 3, 5, 7, 10, 15, 20, and 30-minute exposure times to accomplish this. GC-MS and related laboratory equipment can then be used to further separate essential oil terpenoids followed by identification using a mass spectrum terpenoid database.

Previous analysis of seven *Tsuga* species identified 50 terpenoids using this technique (Table 1) (Lagalante & Montgomery, 2003). It should noted that not all of these compounds are necessarily present in eastern hemlock. Possible inaccuracies of terpenoid analysis could come from insect cross-contamination and unidentified abiotic stress. Also, any apparently uninfested trees that have high outlying concentrations of terpenoids, for constitutive compounds, should be removed from analyses because they are likely due to some other type of pre-existing stress condition or infection (Villari et al., 2014). A brief description of phenolic analysis involves protocols developed by Rigsby et al. (2020) which first exposes extract to supernatant and is then immediately used for HPLC-UV analysis. The use of multiple Greiner UV-Star well plates are used to perform spectrophotometric analysis and phenolics are quantified and separated using different solvents (Rigsby et al., 2020).

Table 1. Terpenoids Analyzed for in *Tsuga Canadensis*

tricyclene	myrcene	*β*-phellandrene	linalool	*trans*-piperitol
α-pinene	α-phellandrene	*cis*-ocimene	*cis*-p-menth-2-en-1-ol	piperitone
camphene	α-terpinene	*trans*-ocimene	*trans*-p-menth-2-en-1-ol	isobornyl acetate
sabinene	*o*-cymene	*γ*-terpinene	borneol	sabinyl acetate
β-pinene	limonene	terpinolene	ethyl octonoate	δ-elemene
α-cubebene	geranyl acetate	*β*-gurjunene	*β*-selinene	*γ*-cadinene
citronellyl acetate	*β*-bourbonene	Z-*trans*-α-bergamotene	viridiflorene	*δ*-cadinene
neryl acetate	*β*-elemene	α-humulene	α-farnesene	*E*-*γ*-bisabolene
α-ylangene	longifolene	*γ*-muurolene	*β*-bisabolene	germacrene D-4-ol
α-copaene	*β*-caryophyllene	Germacrene D	*cis*-*γ*-bisabolene	*τ*-cadinol

Potential Management Practices and Areas of Future Study

Existing strategies of HWA control on hemlock includes biological and chemical control, host gene conservation, cultural treatments, and host resistance approaches (Vose et al., 2013). The optimal time for HWA management is after their first generation, the progrediens, from September through October (Hoover, 2004). Several strategies have been proposed as potential components of an integrated pest management plan for HWA including the application of *Beauveria bassiana*, *Verticillium lecanni*, entomopathogenic fungi, and the release of natural enemies like *Sasajiscymnus tsugae* and *Laricobius nigrinus* (Costa et al., 2005).

Figure 3: *Sasajiscymnus tsugae* beetles feeding on hemlock woolly adelgid (Williams, 2017).

Of all management options, the release of *Sasajiscymnus tsugae* (Figure 3) is among the most popular and effective as they are natural predators of HWA in its native Japan and have been shown to be successful in mitigating their populations in the U.S.; approximately two million of them have been released across 16 states from Georgia to Maine. They are the only biological natural predator commercially available and they are reared on HWA. Their success is primarily due to their high fecundity and life cycle, of which egg laying, hatching, and new generations occur concurrently with HWA. They are also able to feed on all life stages of HWA, which make dormant young HWA easy meals and contribute to *Sasajiscymnus tsugae* success (Hakeem et al., 2010). Previous field experiments have shown that the release of 2,400 to 3,600 beetles over an unspecified area resulted in a 47-88% reduction in HWA over the course of only five months.

Interestingly, 350,000 *Sasajiscymnus tsugae* adults were released in GSMNP from 2002 to 2007 across 150 locations (Hakeem et al., 2010). Later, in 2008 and 2009, sampling for *Sasajiscymnus tsugae* across 33 sites in the GSMNP revealed that adults and larvae were present in seven (22%) of the release sites (Figure 4) (Cheah & McClure, n.d.; Hakeem et al., 2010).

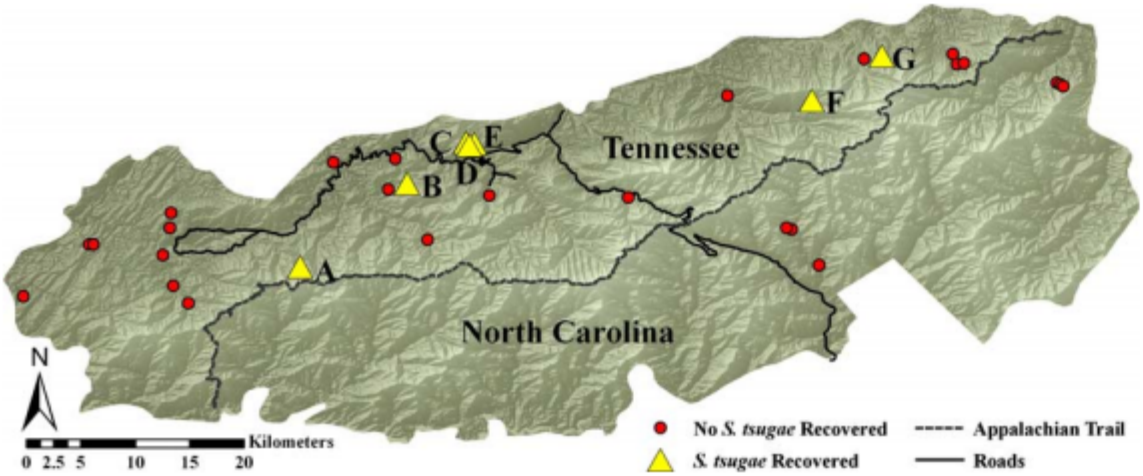

Figure 4: Recovery and release sites of *Sasajiscymnus tsugae* from hemlock in the Great Smoky Mountains National Park. Sampling locations include: A, Anthony Creek; B, Buckthorn Gap; C, Laurel Falls 1; D, Laurel Falls 2; E, Laurel Falls 3; F, Ramsey Cascades; and G, Buckeye Creek (Hakeem et al., 2010).

Most of these recoveries were found in sites B, C, and D, which are among the oldest release sites and leads to the conclusion that *Sasajiscymnus tsugae* populations take years to develop and become established, thus results from their release should be given adequate time to appear (Hakeem et al., 2010). Given the fast spread and effectiveness in HWA reduction shown by previous studies, one could assume that populations currently far surpass what they were twelve years ago. Standard U.S. Forest Service Forest Inventory Analysis (FIA) crown health assessment protocols can be used to assess hemlock health or decline following *Sasajiscymnus tsugae* release. To perform this evaluation, a statistical comparison is performed between sites of release, non-release, and baseline. In the past, this method has shown significantly less foliage transparency in areas with *Sasajiscymnus tsugae* release (Cheah, 2011). The results of hemlock crown health analysis can assist in the assessment of ecological damage severity caused by HWA and even the relative effectiveness of terpenoids as secondary defense compounds. Regardless of non-discrimination of life stage, it is important to note for biological control release programs that predators should be released around May for the best chance of survival due to favorable temperatures (Deal, 2007).

Laricobius nigrinus beetles are another potential option for HWA control and like *Sasajiscymnus tsugae*, they have synchronous life cycles with HWA. More specifically, they emerge from aestivation at the same time as HWA and lay eggs during the same time as HWA sistens. *Laricobius nigrinus* eggs are laid in HWA ovisacs and the larvae feed on HWA eggs upon hatching. Demonstrating effectiveness, one study found that *Laricobius nigrinus* females in British Columbia express an ovipositional preference for HWA ovisacs compared to other adelgid and non-adelgid species. This is probably because they can only survive to adult-stage development if they are laid and feed on HWA (Zilahi-Balogh et al., 2002). *Laricobius nigrinus* populations have been developing in the southern Appalachians since a 2003 release and can be found at several locations (Costa et al., 2005). As with any species release in a non-native area, significant risk assessment protocols will need to be completed to ensure that any predator release is a prudent, risk-adverse decision with a net benefit.

There are also systemic insecticides that can be used via soil or trunk injection but their effectiveness depends on the presence of sufficient soil moisture, therefore ground irrigation may be required before application in certain areas (Hoover, 2004). Soil nitrogen also needs to be considered when using insecticides as high concentrations can limit effectiveness (Pontius et al., 2006). Injection treatments have been successful post-rain and in riparian areas and are the most common form of control but can lead to adaptive resistance in HWA (U.S. Department of the Interior, 2018; Deal, 2007). Chemical and silvicultural controls have thus far proven to be impractical or ineffective when implemented on large scales (Costa et al., 2005). A more eco-friendly option compared to insecticides and logging practices is the use of induced defense compounds. Essentially, if any compounds identified from herbivory are induced, their production can potentially be artificially triggered by the, “application of ‘elicitors’, and plants can be genetically engineered to express these traits constitutively…” (Heil, 2002). This strategy has proven to be effective in agricultural and horticultural systems by using Actigard or Bion, which are trade names for the synthetic chemical acibenzolar-*S*-methyl that is based on induced defense compounds (Eyles et al., 2010). Eyles et al. suggested that the volatile organic compounds, “involved in induced indirect defences or characterization of the signaling molecule pathways in trees could provide potential targets for the commercial development of bioactive small metabolites. These discoveries could, in the long term, be patented, developed and marketed for application in forest systems worldwide…” (Eyles et al., 2010).

A helpful future study would be to identify which compounds are responsible for HWA resistance in other hemlock species and what their concentrations are. Results from this study could assist in the creation of HWA-resistance breeding programs and reforestation efforts, “aimed at not only restoring eastern hemlocks, but also maintaining the vital, broad-scale ecosystem functions that this tree provides” (Kinahan et al., 2020). It may be possible that an increase in plant response rate to HWA herbivory or a reduction in response decay rate could increase the likelihood of producing a resistant affect towards HWA (Karban & Myers, 1989). Along these same lines, it would also be beneficial to investigate the potential development of, or possible continual evolution of, HWA counter measures to hemlock defense compounds and their concentrations. Maybe an increase in carbon inputs and availability can invoke the production of defense compounds and reduce HWA feeding and hemlock mortality. Perhaps if hemlocks were also supplemented with phosphorous and calcium they could produce higher concentrations of terpenoids and phenolics, since susceptible hemlocks have been associated with decreased concentrations of these nutrients, and/or make themselves less palatable and nutritious for HWA, decreasing invasion risk. Whatever management decisions are made to reduce hemlock mortality, they will be influenced by budgetary demands and opportunity costs from competing programs like water and wildfire protection (Vose et al., 2013).

Discussion of Probable Experimental Results

Based on the above background information and proposed sampling and analysis, I hypothesize that the projected hemlock canopy decline in the GSMNP will initially increase the amount of organic matter nutrients and habitats available in streams but the inputs and their benefits will eventually completely disappear. Plant diversity due to succession will also be affected. Those plants that have become accustomed to growing in the presence of hemlock, and hemlock itself, will decrease and become replaced by an influx of successional species, altering forest ecosystem dynamics. There will also be an associated change in soil chemistry and moisture, which will affect photosynthetic productivity due to increased sunlight. Based on personal observation, hemlocks occur at varying elevations and in subsequently various ecosystems, likely with different abiotic elements like soil moisture, aspect, sunlight, and nutrient availability. Because of this, it is difficult to determine what the specific successional community will be comprised of and therefore if water usage and nutrient cycling rates will be high or low. Use of the ecosystem demography model would be helpful in evaluating the changes in ecosystem dynamics and abiotic factors because it can incorporate abiotic elements and HWA spread. So, once abiotic effects are accounted for, the contribution of terpenoids in regulating HWA can be analyzed. This is shown in a previous study where dominant soil textures were assigned to individual polygons (Albani et al., 2010). A study focused on the long-term ecological impacts of hemlock loss in the GSMNP is out of the scope of my paper and can only be speculated about but should be conducted because it could reveal areas of focus for mitigation and conservation.

Precipitation and other abiotic conditions are adequate where hemlock persist. If a tree has reached maturity, like those proposed to be sampled, then it can be assumed that they currently do not experience abiotic stress sufficient enough to kill them, however this can change in the future as climate change progressively worsens, affecting precipitation patterns soil moisture availability, and surrounding stream and terrestrial ecological damage. Since the proposed sampling occurs in July, it can be assumed that adequate soil moisture and low-light conditions will exist due to precipitation; fallen hemlock needles creating preferred conditions for soil water retention and acidity; and shade provided by other trees' full canopies and ridges shading valleys and north-facing slopes. This means that any increase in terpenoids and phenolic concentrations is likely due to HWA infestation. Studies have found that plants experiencing drought will increase production of terpenoids so while terpenoid concentrations will be present, they are not likely to be as high as they would be under drought conditions. Elevations in the GSMNP range from 1,509 to 5,709 feet, which is only a slight deviation from the most desirable range for hemlock, so elevation would not be expected to exert any effect on defense compound production (Deal, 2007; Isah, 2019).

Metabolic costs are expected to be high in hemlock since they favor nutrient-poor environments and are slow-growing (Endara & Coley, 2011). The high costs of defense compound production coupled with HWA herbivory is likely to cause higher tree stress than herbivory alone leading to more pronounced physiologic damage than deciduous trees. Thus, high metabolic costs and infestation are likely to lead to more pronounced ecological changes due to the rapidity of declining hemlock stands and the services they provide. Any induced defenses that tax metabolism further will add to the issue of rapid damage. Because hemlock is slow-growing the resource availability hypothesis predicts that they do not sacrifice any competitive ability or experience a reduction in opportunity costs in order to make their constitutive compounds and are at less risk of herbivory than fast growing species (Endara & Coley, 2011). However, HWA is a specialist species and does not feed on fast-growing species, so hemlock herbivory risk remains unchanged; the resource availability hypothesis only has limited relevancy to the hemlock-HWA relationship.

I suspect that if the previously described experiment were conducted, terpenoid defenses released from hemlock upon HWA herbivory would not differ significantly from those emitted and identified in previous studies including α-pinene, camphene, and isobornyl acetate, which are, “the three dominant terpene species of hemlock foliage and constitute ≥75% of all terpenes identified in eastern hemlock” (Pezet et al., 2013; Kinahan et al., 2020). A previous study showed that concentration of MeSA emitted from hemlocks increased by 10 to 100 times in the presence of HWA (McKenzie et al., 2014). Other terpenoids found in previous studies include linalool and α-phellandrene; those are likely to be found again here as well (Rigsby et al., 2020). Based on literature, hemlocks primarily produce constitutive compounds, so regardless of concentrations, they constantly expend energy to create them, which will cause the trees to die sooner when invaded by HWA than if they used induced defenses. Because sampling in the proposed experiment would occur in July, terpenoids would be at peak output and phenolics would be decreased compared to the overwintering season. However, some of the difference may be made up for because the twigs sampled would be mature, so they would likely produce greater phenolic results compared to young needles. Also, since hemlocks prefer nutrient-poor soil conditions, phosphorous concentrations are likely to be low, which research has shown to limit resource investment in constitutive defenses and increase foliar phenolic concentrations (Sampedro et al., 2011). One contributing factor that potentially limits the effectiveness of phenolics in the role of defense are HWA possess salivary enzymes, peroxidases and polyphenol oxidases, which are capable of metabolizing phenolics and converting them into relatively harmless substances (Lattanzio et al., 2008).

Despite evidence showing that the release of terpenoids and phenolics upon HWA herbivory does not prevent infestation, the existence of rare, resistant hemlocks indicate that they could be valuable in breeding and management strategies therefore influencing the degree of damage incurred and ecological impacts. This claim is supported by continuing investigative studies on HWA infestation, its magnitude, and future trends of hemlock loss (Figure 5).

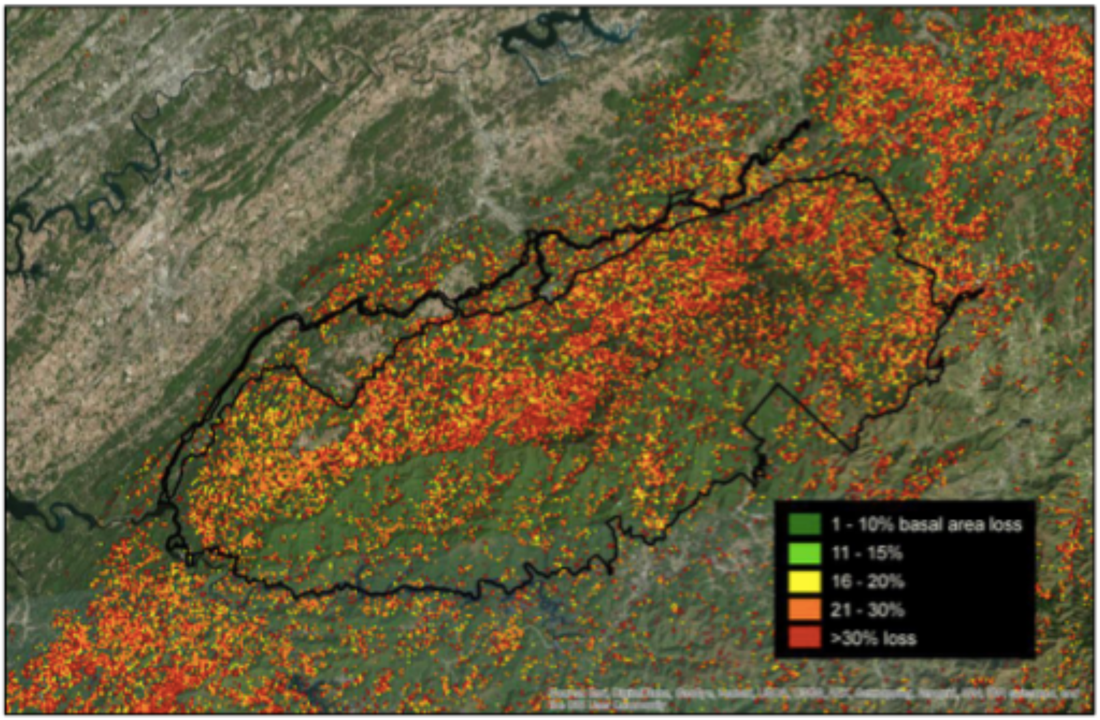

Figure 5: Models predict that hemlock woolly adelgid infestation will reduce hemlock basal area by 18% between 2013 and 2027. Source: U.S. Forest Service, 2013 (U.S. Department of the Interior, 2018).

Indeed, eastern hemlocks lack the mechanisms for host plant adaptive resistance unlike Asian and western hemlock species whose terpene profiles significantly differ from eastern hemlock. However, it is unknown which individual secondary compounds and their concentrations create this differentiation in repellency ability (Kinahan et al., 2020). Some studies have recently found evidence of HWA resistance in rare hemlocks growing in otherwise HWA-ravaged stands. When compared to susceptible hemlocks, it was found that resistant hemlocks possess greater constitutive concentrations of terpenoids. Therefore, the concentrations of these compounds are thought to determine level of host resistance (McKenzie and Elkinton, 2014). Obviously, the lack of HWA resistance also applies to hemlocks within GSMNP, which would make sense because HWA has only been documented in the GSMNP since 2002.

It appears that *Sasajiscymnus tsugae* release did not occur in one of the most hemlock-dense areas: the central area of the park (Figure 2). Interestingly, when comparing the maps in Figure 2 and Figure 4, the highest recoveries of *Sasajiscymnus tsugae* were not made in areas of high hemlock density. Given the time since release, *Sasajiscymnus tsugae* populations have had longer to become established and based on their population growth dynamics, they would likely at least be recovered from areas near Point G, Buckeye Creek; Point F, Ramsey Cascades, and surrounding release sites since hemlock stands are the densest in these areas and therefore would have the most HWA available for consumption. If *Sasajiscymnus tsugae* has not reduced HWA populations then it is likely that hemlock loss has caused and continues to cause the effects mentioned above including reduced avian, wildlife, and native aquatic species populations and increased short-term organic matter input to streams, primary productivity, soil erosion and sediment loads, soil nitrate-nitrogen availability, and biomass due to stream algae with an accompanying decrease in dissolved oxygen (Evans et al., 1996). If HWA is causing an increase in organic matter soil input and that matter contains high phenolics, the soil nutrient cycling and decomposition rates are likely to be affected based on impacts to decomposer community activity and composition (Lattanzio et al., 2008).

Conclusion

Studies have shown that hemlocks are on the decline in GSMNP (Figure 5), causing notable changes in forest composition. Ecological processes and features such as hydrology; decomposition rates; and riparian slope, vegetation, and stabilization will begin to deteriorate in areas where hemlock stands begin to disappear. With the loss of hemlock, the food web and some of the biodiversity that GSMNP is known for may dwindle as populations of birds, deer and small mammals, fish and stream invertebrates, and others are displaced and decrease in number. The services hemlock provides for neighboring streams like thermoregulation and organic matter inputs will decline in the long-term, decreasing vertebrate and invertebrate stream communities that rely on the unique microclimate and increasing the risk of invasion by invasive species.

The continued herbivory by HWA indicates a lack of evolutionary history between HWA and hemlock and no recoveries of hemlocks, individually or at the stand level, have ever been documented following HWA infestation without the assistance of chemical insecticides. However, in the GSMNP where hundreds of acres of hemlock exist and are unevenly distributed, the application of insecticides is not feasible or realistic (Deal, 2007). The hemlocks that survive HWA invasion, unless resistant, will be “immunocompromised” in a sense and be more vulnerable to diseases, herbivores, and other invasive species. Specialist herbivores like HWA may also use secondary compounds to locate their host plants. Therefore, it may actually be that an increased release of defense compound concentrations upon feeding attracts other HWA to the invaded tree (Heil, 2002).

It is possible, and likely, that hemlock population loss in the GSMNP is reversible and has not crossed an irreversible restoration threshold (U.S. Department of the Interior, 2018). That being said, restoration efforts are only as good as the resources put into them such as public support and funding. If budgets and public support are adequate to support HWA removal in the future, then the future is bright for hemlocks in the GSMNP. This has happened in other forests. For example, oak forests degraded by invasive understory in Chicago’s woodlands were saved by “legions of motivated volunteers, well-funded natural resource agencies, and a wealth of local expertise”, which was able to remove ground cover (Miller and Bestelmeyer, 2016). Volunteers could also be helpful in locating and sampling rare, resistant hemlocks, which could be used in breeding programs to create hybrids between resistant and susceptible trees for hemlock population restoration (Ingwell & Preisser, 2011).

In the future, adequate monitoring is needed to ensure control efforts are successful. This will require efforts using remote sensing and geographic information system (GIS) maps of satellite imagery using elevation, topographic relative moisture index, and riparian area imagery among other map variables and assessment techniques (Koch, 2005). As further research and monitoring gathers data on restoration success, project managers can better allocate resources.

The summarized literature review presented in my paper was synthesized to assess the composition and concentration of terpenoids and phenolics released from hemlocks upon HWA herbivory, while accounting for abiotic influences; determine if they had an effect on herbivory; what any effects meant for hemlock fitness; and the subsequent surrounding ecological impacts in the GSMNP. Despite the repeated identification of major terpenoid and phenolic constituents released upon HWA herbivory, they remain largely unstudied which something the proposed study in this paper could contribute to (Kinahan et al., 2020). This work is important for the GMSNP area because it supports a plethora of species and imposes large economic benefits for the surrounding communities. If my conclusions and assumptions in this paper are correct, I recommend that releases of *Sasajiscymnus tsuage*, supplemented with *Laricobius nigrinus*, continue to occur and any additional funding be allocated toward research for HWA-resistance breeding programs. There has already been some success with the use of *Sasajiscymnus tsugae* biological control and their populations may have continued to increase in the GSMNP, thereby decreasing surrounding HWA populations. Prevention of further hemlock loss is important to preserve unique habitat used by the many animals that are reliant on them. I predict that regardless of any emitted terpenoids and phenolics, unless restoration and conservation measures are taken, HWA will continue to decimate hemlocks and seed banks in the GSMNP. Ideally, it is my hope that my paper will improve the understanding of the importance of this ecological problem and provide guidance for hemlock restoration and treatment efforts within the study area.

References

Albani, M., Moorcroft, P., Ellison, A., Orwig, D., & Foster, D., 2010. Predicting the impact of hemlock woolly adelgid on carbon dynamics of eastern United States forests. *Canadian Journal of Forest Research*, 40(1), p.119-133.

Böttger A., Vothknecht U., Bolle C., & Wolf A., 2018. Terpenes and Terpenoids. In: Lessons on Caffeine, Cannabis & Co. Learning Materials in Biosciences. Springer, Cham. https://doi.org/10.1007/978-3-319-99546-5_10.

Broeckling, C. & Salom, S., 2003. Volatile emissions of eastern hemlock, Tsuga canadensis, and the influence of hemlock woolly adelgid. *Phytochemistry*, 62(2), p.175-180.

Brooks, R., 2001. Effects of the removal of overstory hemlock from hemlock-dominated forests on eastern redback salamanders. *Forest Ecology and Management*, 149(1-3), p.197-204.

Cheah, C., 2011. Chapter 4: Sasajiscymnus (Pseudoscymnus) Tsugae, A Ladybeetle from Japan. [online] Windsor, CT: The Connecticut Agricultural Experiment Station, Valley Laboratory, pp.43-50. Available at: https://www.researchgate.net/profile/Carole-Cheah/publication/235323502_Sasajiscymnus_Pseudoscymnus_tsugae_A_ladybeetle_from_Japan/links/54217b6d0cf2ce3a91b7870f/Sasajiscymnus-Pseudoscymnus-tsugae-A-ladybeetle-from-Japan.pdf#page=56.

Cheah, C. & McClure, M., n.d. *Sasajiscymnus (formerly Pseudoscymnus) tsugae (Coleoptera: Coccinellidae)*. [online] Biological Control. Available at: https://biocontrol.entomology.cornell.edu/predators/sasajiscymnus.php.

Costa, S., 2005. Sampling for Detection and Monitoring of Hemlock Woolly Adelgid Within Hemlock Stands. Asheville, NC: Forest Health Technology Enterprise Team, p.57-62.

Costa, S., Parker, B., Gouli, V., Brownbridge, M., Skinner, M., & Gouli, S., 2005. Insect-Killing Fungi as a Component of Hemlock Woolly Adelgid Integrated Pest Management. Asheville, NC: Forest Health Technology Enterprise Team, p.155-159.

Deal, I. K., 2007. Life history of hemlock woolly adelgid, Adelges tsugae Annand, on eastern hemlock, Tsuga canadensis (L.) Carriere, in the southern Appalachians and assessment of egg releases of Sasajiscymnus tsugae (Sasaji and McClure) for its management. University of Tennessee.

Eastern Hemlock. N.d. Srs.fs.usda.gov. [online] Available at: https://www.srs.fs.usda.gov/pubs/misc/ag_654/volume_1/tsuga/canadensis.htm.

Ellison, A., Bank, M., Clinton, B., Colburn, E., Elliott, K., Ford, C., Foster, D., Kloeppel, B., Knoepp, J., Lovett, G., Mohan, J., Orwig, D., Rodenhouse, N., Sobczak, W., Stinson, K., Stone, J., Swan, C., Thompson, J., Von Holle, B., & Webster, J., 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment*, 3(9), p.479-486.

Endara, M. and Coley, P., 2011. The resource availability hypothesis revisited: a meta-analysis. *Functional Ecology*, 25(2), pp.389-398.

Evans, R., Johnson, E., Shreiner, J., Ambler, A., Battles, J., Cleavitt, N., Fahey, T., Sciascia, J., & Pehek, E.,1996. Potential impacts of hemlock woolly adelgid. *Technology Transfer Hemlock Woolly Adelgid: Proceedings of the First Hemlock Woolly Adelgid Review: Charlottesville, Virginia, October 12, 1995*, *96*(10), p.42.

Eyles, A., Bonello, P., Ganley, R., & Mohammed, C., 2010. Induced resistance to pests and pathogens in trees. *New Phytologist*, *185*(4), p.893-908.

Gonda-King, L., Gómez, S., Martin, J. L., Orians, C. M., & Preisser, E. L., 2014. Tree responses to an invasive sap-feeding insect. *Plant Ecology*, *215*(3), p.297-304.

Hakeem, A., Grant, J. F., Lambdin, P. L., Buckley, D., Hale, F. A., Rhea, J. R., & Taylor, G., 2010. Recovery of Sasajiscymnus tsugae, released against hemlock woolly adelgid, Adelges tsugae, in the southern Appalachians. *Biocontrol Science and Technology*, *20*(10), p.1069-1074.

He, J., Weaver, S., Kling, C. & Ucar, Z., 2017. *Great Smoky Mountains Ecological Forecasting II*. [online] Develop.larc.nasa.gov. Available at: https://develop.larc.nasa.gov/2014/spring/GreatSmokyMountainsEcologicalForecastingII.html.

Heil, M., 2002. Costs of induced resistance – what do we know and what can they explain?. *Induced Resistance in Plants against Insects and Diseases*, [online] 25(6), pp.89-93. Available at: <http://www.iobc-wprs.org/pub/bulletins/iobc-wprs_bulletin_2002_25_06.pdf#page=113>.

Hoover, G., 2004. *Hemlock Woolly Adelgid*. Entomological Notes. [online] Pennsylvania State University. Available at: https://www.maine.gov/dacf/php/gotpests/bugs/factsheets/hem-wool-adelgid-penn.pdf.

Ingwell, L. L. & Preisser, E. L., 2011. Using citizen science programs to identify host resistance in pest-invaded forests. *Conservation Biology*, *25*(1), p.182-188.

Isah, T., 2019. Stress and defense responses in plant secondary metabolites production. *Biological research*, *52*.

Karban, R. & Myers, J. H., 1989. Induced plant responses to herbivory. *Annual review of ecology and systematics*, *20*(1), p.331-348.

Kinahan, I., Rigsby, C., Savage, S., Houseman, N., Marsella, A., Oppong-Quaicoe, A., DeBoef, B., Orians, C., & Preisser, E., 2020. Seasonal changes in eastern hemlock (Tsuga canadensis) foliar chemistry. *Canadian Journal of Forest Research*, 50(6), p.557-564.

Koch Jr, F. H., 2005. Spatial Tools for Managing the Hemlock Woolly Adelgid in the Southern Appalachians.

Lagalante, A. & Montgomery, M., 2003. Analysis of Terpenoids from Hemlock (Tsuga) Species by Solid-Phase Microextraction/Gas Chromatography/Ion-Trap Mass Spectrometry. *Journal of Agricultural and Food Chemistry*, 51(8), p.2115-2120.

Lattanzio, V., Kroon, P. A., Quideau, S., & Treutter, D., 2008. Plant phenolics—secondary metabolites with diverse functions. *Recent advances in polyphenol research*, *1*, p.1-35.

McKenzie, E. A., Elkinton, J. S., Casagrande, R. A., & Mayer, M., 2014. Terpene chemistry of eastern hemlocks resistant to hemlock woolly adelgid. *Journal of chemical ecology*, *40*(9), p.1003-1012.

McKenzie, A., Elkinton, J., Casagrande, R., Preisser, E. and Mayer, M., 2014. Understanding and Developing Resistance in Hemlocks to the Hemlock Woolly Adelgid. *Southeastern Naturalist*, [online] 40(9). Available at: <https://digitalcommons.uri.edu/cgi/viewcontent.cgi?referer=https://scholar.google.com/scholar?hl=en&as_sdt=0%2C43&q=terpenes+hemlock+trees&btnG=&httpsredir=1&article=1044&context=bio_facpubs>.

Michelozzi, M., 1999. Defensive roles of terpenoid mixtures in conifers. *Acta botanica gallica*, *146*(1), p.73-84.

Miller, J. R., & Bestelmeyer, B. T., 2016. What's wrong with novel ecosystems, really?. *Restoration Ecology*, *24*(5), p.577-582.

Nuckolls, A., Wurzburger, N., Ford, C., Hendrick, R., Vose, J. and Kloeppel, B., 2008. Hemlock Declines Rapidly with Hemlock Woolly Adelgid Infestation: Impacts on the Carbon Cycle of Southern Appalachian Forests. *Ecosystems*, 12(2), p.179-190.

Pezet, J., Elkinton, J., Gomez, S., Mckenzie, E., Lavine, M. and Preisser, E., 2013. Hemlock Woolly Adelgid and Elongate Hemlock Scale Induce Changes in Foliar and Twig Volatiles of Eastern Hemlock. *Journal of Chemical Ecology*, 39(8), p.1090-1100.

Pontius, J., Hallett, R. and Jenkins, J., 2006. Foliar Chemistry Linked to Infestation and Susceptibility to Hemlock Woolly Adelgid (Homoptera: Adelgidae). *Environmental Entomology*, 35(1), pp.112-120.

Rigsby, C. M., Body, M. J., May, A., Oppong, A., Kostka, A., Houseman, N., & Preisser, E. L., 2021. Impact of chronic stylet-feeder infestation on folivore-induced signaling and defenses in a conifer. *Tree Physiology*, *41*(3), p.416-427.

Sampedro, L., Moreira, X., & Zas, R., 2011. Costs of constitutive and herbivore-induced chemical defences in pine trees emerge only under low nutrient availability. *Journal of Ecology*, *99*(3), p.818-827.

Snyder, C., Young, J., Ross, R. & Smith, D., 2021. Long-Term Effects of Hemlock Forest Decline on Headwater Stream Communities. Asheville, NC: Forest Health Technology Enterprise Team, p.53.

Tingley, M., Orwig, D., Field, R. & Motzkin, G., 2002. Avian response to removal of a forest dominant: consequences of hemlock woolly adelgid infestations. *Journal of Biogeography*, 29(10-11), p.1505-1516.

United States Department of the Interior, 2018. *Natural Resource Condition Assessment - Great Smoky Mountains National Park*. Fort Collins, CO: National Park Service, p.8-10 & 228-230.

Villari, C., Faccoli, M., Battisti, A., Bonello, P. and Marini, L., 2014. Testing phenotypic trade-offs in the chemical defence strategy of Scots pine under growth-limiting field conditions. *Tree Physiology*, 34(9), pp.919-930.

Vose, J. M., Wear, D. N., Mayfield III, A. E., & Nelson, C. D., 2013. Hemlock woolly adelgid in the southern Appalachians: control strategies, ecological impacts, and potential management responses. *Forest Ecology and Management*, *291*, p.209-219.

Williams, S., 2017. *What is Hemlock Woolly Adelgid?*. [online] Online Pest Control. Available at: https://www.onlinepestcontrol.com/hemlock-woolly-adelgid/.

Zilahi-Balogh, G., Kok, L., & Salom, S., 2002. Host specificity of Laricobius nigrinus Fender (Coleoptera: Derodontidae), a potential biological control agent of the hemlock woolly adelgid, Adelges tsugae Annand (Homoptera: Adelgidae). *Biological Control*, 24(2), p.192-198.