ABSTRACT

KLEIN, EMILY THERESA. Lingering Hemlock Resistance to the Hemlock Woolly Adelgid
(Under the direction of Drs. Steven D. Frank and Robert M. Jetton).

Eastern hemlock (*Tsuga canadensis* Carrière) and Carolina hemlock (*Tsuga caroliniana* Englemann) are keystone species throughout their native range in North America. Eastern and Carolina hemlocks are in decline because of the hemlock woolly adelgid (*Adelges tsugae* Annand; Hemiptera: Adelgidae; HWA), an invasive pest native to Southeast Asia. HWA was first detected in the US in Virginia in 1951. HWA now infests hemlocks in at least 21 states and parts of Canada. HWA can kill hemlock trees in as little as 4 years. However, some eastern hemlocks survive in stands where most hemlocks have died. These healthy trees are called 'lingering hemlocks' and may be resistant to or tolerant of HWA feeding. It is unknown whether lingering hemlocks provide resistance to HWA.

Multiple management strategies have been implemented in attempts to manage HWA populations. Biological control, chemical control, silviculture, and host plant resistance are parts of HWA integrated pest management. Host plant resistance is under studied and lingering hemlocks may provide a source of resistance to HWA.

In Chapter 1, we conducted a literature review to identify mechanisms of resistance that lingering hemlocks could use to protect themselves from HWA feeding. We reviewed research on antibiosis, antixenosis, and tolerance, the three main mechanisms of host plant resistance, in hemlocks. We found that most research has focused on antibiosis as a mechanism of resistance and there was the least amount of information for antixenosis. We found that lingering hemlocks produce more defense compounds and grow more than susceptible eastern hemlocks. This means that lingering hemlocks show some signs of resistance to HWA. We then conducted our own research to learn more.

In Chapter 2, we conducted an observational study where we measured HWA density and fecundity, and tree growth on lingering hemlocks from 10 locations in North Carolina and Virginia. We randomly sampled 41 lingering and 6 hybrid genotypes by clipping branches to bring back to the lab. We assessed HWA density and fecundity over two HWA generations and assessed tree growth over one growing season. Six lingering hemlock genotypes reduced HWA densities over multiple generations. Nine lingering hemlock genotypes grew more than the wild-type control trees. This study suggests some lingering hemlock genotypes may have resistance to HWA feeding. An experimental approach would clarify our findings.

In Chapter 3, we conducted experiments to assess mechanism of resistance of lingering hemlock and hybrid genotypes compared to the wild-type control. We tested the three main types of host plant resistance by purposefully infesting branches with HWA and measuring the change in HWA density (antibiosis) and growth over time (tolerance). We also conducted preference tests to see if HWA prefer settling on control trees compared to lingering genotypes (antixenosis). We found 26 lingering hemlock genotypes that seem to exhibit antibiosis by reducing HWA density more than the wild-type control as they mature from nymphs to adults. One of these lingering hemlock genotypes also had HWA prefer settling on the control which suggests that it may be a poor host. We had seven lingering hemlock genotypes grow more than the wild-type control during the main growing season (February-October). With these experiments, we did identify lingering hemlock genotypes that exhibit mechanisms of resistance to HWA feeding.

The objective of this project was to identify lingering hemlock genotypes that exhibit resistance to HWA. Resistant hemlock trees can be propagated for reforestation and for breeders to use. Having resistant hemlocks in the field can work with HWA integrated pest management and help control HWA populations in the future.

Lingering Hemlock Resistance to the Hemlock Woolly Adelgid

by
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DEDICATION

This is dedicated to 22-year-old Emily who swore she would never go to graduate school.

BIOGRAPHY

Emily grew up in Delran, New Jersey; a small town 20 miles outside of Philadelphia. She went to Ursinus College to pursue a bachelor’s degree in biology. As a small, liberal arts school, Ursinus presented her with amazing opportunities, two of which introduced her to entomological research. First, she took a course called *Biology in the Neotropics* where she spent 3 weeks in Costa Rica conducting a research project on leafcutter ants. A year and a half later, Emily went to Sweden to assist on a collaborative agricultural entomology project with her professor, Cory Straub, and Mattias Jonsson at SLU. She graduated from Ursinus in 2020 and navigated the job scene during COVID. Emily was a Mosquito control technician in Burlington County, NJ for two years before deciding to go back to school. Her choice to study entomology came naturally because of her previous experiences and opportunities. A year into her graduate degree, Emily added Forestry as a co-major to expand her career options into the realm of forest health. Outside of class and research, Emily enjoys attending Wednesday night trivia, crocheting, and watching sports.

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CHAPTER 1

Assessing Mechanisms of Hemlock Resistance to Hemlock Woolly Adelgid

Abstract

Our goal is to review the evidence for host plant resistance in hemlocks using recent literature and to identify potential mechanisms of resistance against the hemlock woolly adelgid (*Adelges tsugae* Annand (Hemiptera: Adelgidae); HWA). Hemlock wooly adelgid is an introduced pest in Eastern North America which has devastated eastern hemlocks (*Tsuga canadensis* Carrière) and Carolina hemlocks (*Tsuga caroliniana* Englemann) throughout their native range. We focus on three types of host plant resistance: antibiosis, antixenosis, and tolerance. Most research has been conducted on antibiosis, although hemlocks likely use multiple mechanisms for HWA resistance. Few have studied multiple mechanisms of resistance simultaneously. We hope to analyze and draw conclusions from resistance mechanisms currently being used by hemlock species against hemlock woolly adelgid infestations to provide evidence that some eastern hemlocks have resistance to HWA.

Introduction

Hemlock woolly adelgid (*Adelges tsugae* Annand (Hemiptera: Adelgidae); HWA) is a major threat to eastern hemlocks (*Tsuga canadensis* Carrière) and Carolina hemlocks (*Tsuga caroliniana* Englemann) in the United States. For almost 75 years, eastern and Carolina hemlocks have succumbed to HWA feeding. Host plant resistance could be a valuable tool for managing this pest.

Hemlocks (*Tsuga* spp.) are native to two continents. There are ten recognized species with populations native to Southeast Asia and North America (Figure 1.1). The native range of eastern hemlocks in North America extends from Alabama, United States north to Nova Scotia, Canada (Havill et al., 2011). In the eastern United States, this range is primarily located within the Appalachian Mountain region and west along the Great Lakes region to Minnesota. Carolina hemlock is also native to the eastern United States but have a limited range as they are endemic to Southern Appalachia and prefer rocky outcroppings (Jetton, 2008). In addition, there are more than 240 ornamental cultivars of *Tsuga* spp. (Swartley, 1984). Hemlocks are popular ornamental trees and important to many forest ecosystems.

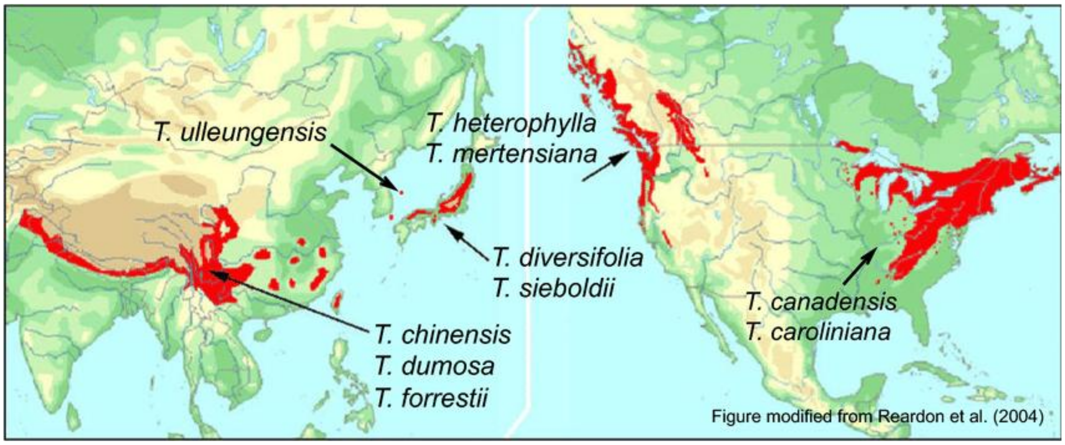

Figure modified from Reardon et al. (2004)

Figure 1.1. Worldwide distribution of *Tsuga* species. (Leppanen et al., 2019).

Hemlock woolly adelgids are found on all species of hemlock (Holman et al., 2017). They were first detected in the eastern U.S. in the 1950s, when a population of HWA was identified on ornamental hemlocks in Richmond, Virginia (Havill et al., 2014). In the 1960s, HWA was found in mature hemlock forests in the Blue Ridge Mountains (Souto, 1995). Since then, HWA has spread along the eastern hemlock range at an average rate of 9-15 km per year. As of 2023, HWA is present in forests from Alabama to Canada (Figure 1.2; Parker et al., 2023).

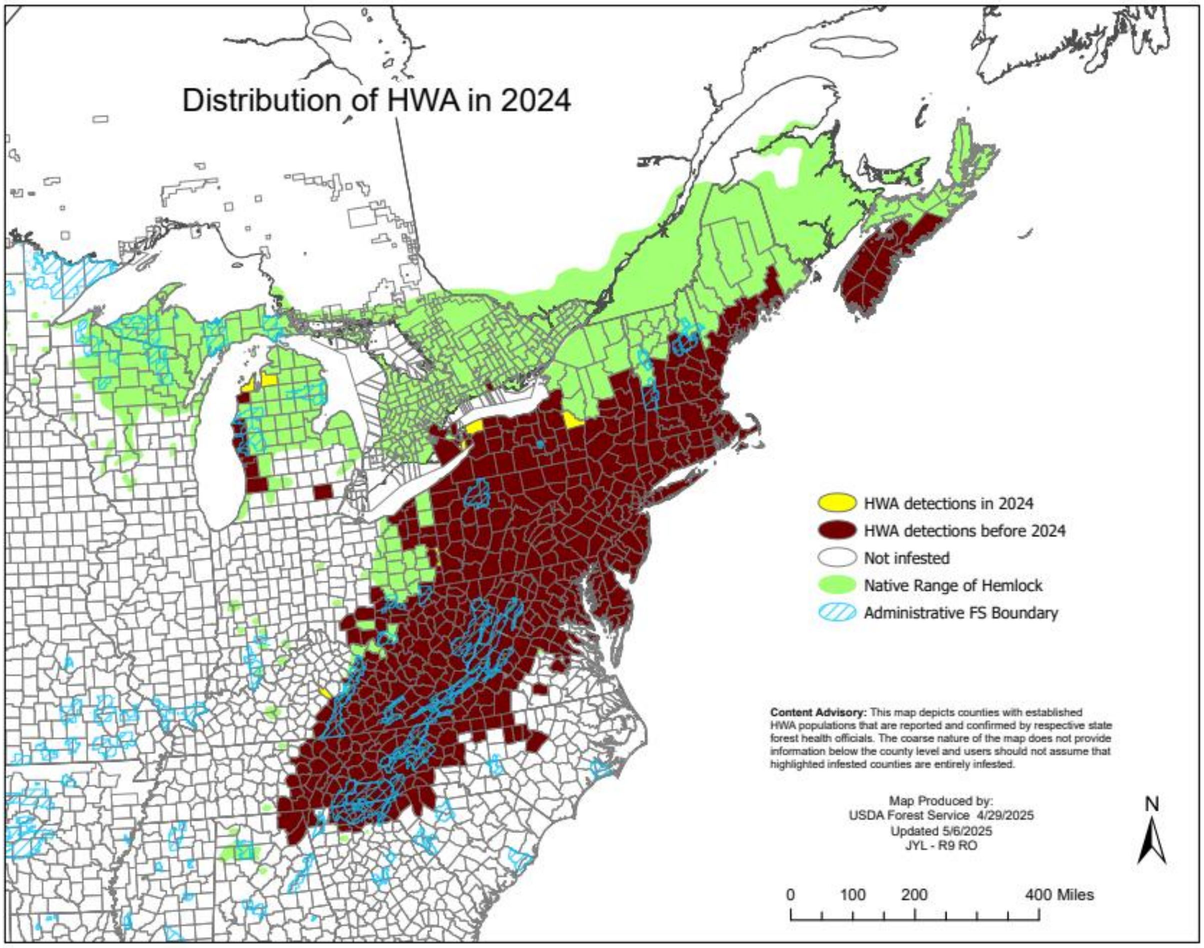

Figure 1.2. Range expansion of HWA from 1951 to 2024 (Hemlock Woolly Adelgid National Initiative, 2025).

In eastern North America, HWA has two asexual generations each year on hemlock trees (Figure 3). While HWA in Asia also complete two asexual generations on hemlock, they have a third sexual generation that reproduces on spruce. This segment of the life cycle is absent in North America (Havill et al., 2014). In North America, HWA produce 50-300 eggs per female, depending on the generation (McClure, 1991). After eclosing, HWA has a crawler stage that has eyes, legs, and antennae. Crawlers and eggs are passively dispersed and can be carried by wind or can attach to animals like birds and deer to get to new places (McClure, 1990). Hemlock woolly adelgids insert their piercing-sucking mouthparts at the base of hemlock needles, extracting nutrients from food reserves in the hemlock's storage cells (Havill et al., 2014; Young et al., 1995). Damage from HWA feeding results in discoloration, needle loss, and branch dieback which can kill mature trees in as little as four years (Havill et al., 2014; McClure, 1991).

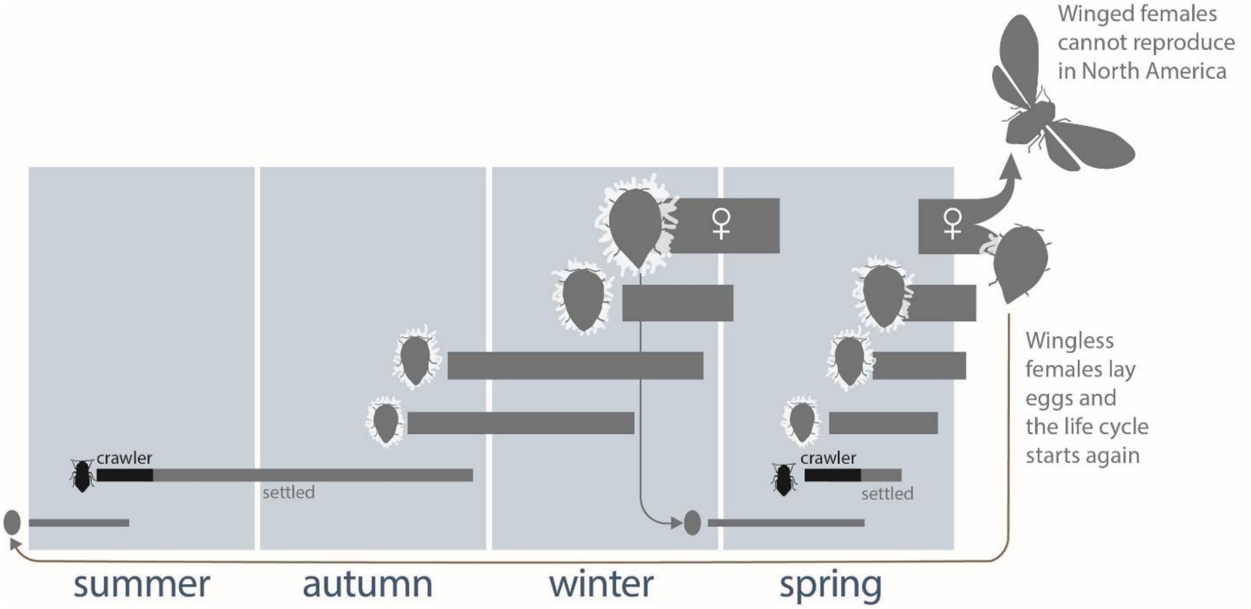

Figure 1.3. HWA life cycle in North America. Illustration by N. P. Havill and V. D'Amico (Havill et al., 2014)

In its native range, eastern hemlock is a foundation species and a significant component of eastern North American forests (Ellison et al., 2018). Reducing hemlock density in forests damages a unique ecosystem. The dense foliage of hemlocks moderates stream temperatures that are critical for trout and other aquatic animals (Ellison et al., 2018; McCarty and Addesso, 2019; Roberts et al., 2009; Webster et al., 2012) Eastern hemlocks shed branches to the forest floor, providing food and shelter for more than 120 vertebrates, such as deer, bird, and salamander species, as well as many invertebrates (Ellison et al., 2018; Parker et al., 2023).

Eastern forests have experienced a significant decrease in hemlocks following the invasion of HWA. After 22 years, hemlock forests in Virginia saw a 45% decrease in stand health with a 30% increase in hemlock mortality (McAvoy et al., 2025). This gradual rate of hemlock decline can also be seen in the Delaware Water Gap National Recreation Area where 15% of hemlocks were found dead after being infested with HWA for 8 years (Eschtruth et al. 2006). When mature hemlocks die, hemlock stands are typically replaced by species within *Rhododendron, Rubus* (blackberry)*, Acer* (maple)*, Fagus* (beech)*, Betula* (birch)*, Quercus* (oak), etc. (McCarty and Addesso, 2019; Ford et al., 2012; Abella, 2018; Birt et al., 2014). These species change the forest landscape by increasing sunlight, soil temperature, and the number of shade-intolerant species. The overall warming of these previously shaded and moist environments accelerates decomposition rates and decreases soil respiration affecting soil dwelling organisms (McCarty and Addesso, 2019; Ford et al., 2012; Abella, 2018; Birt et al., 2014). Hardwood species are unable to replicate the habitat provided by hemlocks, causing a permanent loss of a unique ecosystem.

Multiple management strategies have been implemented in hemlock ecosystems to reduce HWA populations in eastern North America. Insecticides, including the neonicotinoids imidacloprid and dinotefuran, are effective against HWA (Havill et al., 2014). They can protect hemlocks for up to six years but have negative effects on non-target species including canopy-feeding and soil-dwelling arthropods. In Asia and western North America, natural enemies control HWA populations on native hemlocks (Oten et al., 2014). *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) from the west coast of North America and *L. osakensis* Montgomery and Shiyake (Coleoptera: Derodontidae) from Japan have been released in eastern hemlock forests as biological control since 2003 and 2012, respectively. Adults and larvae feed on HWA eggs and nymphs during the fall and winter. Species of silver flies, *Leucotaraxis argenticollis* Zetterstedt (Diptera: Chamaemyiidae) and *L. piniperda* Malloch (Diptera: Chamaemyiidae), have also been released as their larvae feed on HWA in the spring and summer (Mayfield III et al., 2023). Cultural control using silvicultural techniques is a recent addition to HWA management. Used in conjunction with these other management strategies, silviculture is meant to help hemlocks by changing the landscape in which they grow. This could be by creating canopy gaps, planting more hemlocks, and breeding for resistance (Bentz et al., 2023; Mayfield et al., 2023). Chemical control, biological control, and silviculture are valuable tools in managing HWA. However, HWA is still spreading, and hemlock forests are declining, and these tools are difficult to employ on a large scale.

Host plant resistance shows promise as a complimentary tool that could be valuable for HWA management. Hemlock species that co-evolved with HWA tend to exhibit natural resistance, resulting in lower HWA abundance, limited stylet insertion, and continued production of new terminal growth (Tredici and Kitajima, 2004; Bentz et al., 2008; Leppanen et al., 2019). In contrast, HWA feeding typically causes severe damage and eventual mortality in most eastern and Carolina hemlocks. Nevertheless, individual eastern and Carolina hemlocks that appear healthy are occasionally found in forests where surrounding hemlocks have succumbed to HWA (Casewell et al., 2008; Ingwell & Preisser, 2011; Kinahan et al., 2020). Casewell et al. (2008) found that these healthy hemlocks, which we call ‘lingering hemlocks’, contain significantly fewer HWA than wild-type eastern hemlocks. Other research corroborates that lingering hemlocks show resistance to HWA (Montgomery et al., 2009; McKenzie et al., 2014; Oten et al., 2014; Klein, 2025). Conducting a review on resistance mechanisms in hemlocks, including lingering hemlocks, provides evidence that resistance to HWA exists in eastern hemlocks.

We review examples of the three mechanisms of plant resistance (antibiosis, antixenosis, and tolerance) to compare the extent of research and relative merits of each mechanism as a potential form of resistance used by lingering eastern hemlocks to combat HWA feeding.

Antibiosis

Antibiosis is a mechanism of resistance in which a resistant plant negatively affects a pest's biology (Painter, 1951). It can cause lower rates of insect fecundity, reduced size and weight, malformities, death, or a combination of these symptoms (López-Castillo et al., 2018).

Terpenes are the most common type of secondary metabolite that plants use as a defense mechanism against herbivores (Ninkuu et al., 2021). Terpenes physically deter or repel insect feeding. They change volatile organic compounds into defense emissions or antifeedants which reduce plant nutrients that can inhibit insect growth and development. Concentrations of terpenes can vary between populations of evergreens, within a tree, seasonally, and in response to herbivory or abiotic stress (Jackson et al., 1996).

Adelgids and relatives, like aphids, cannot tolerate high levels of monoterpenes. In a laboratory setting, aphid species that commonly fed on Sitka spruce (*Picea sitchensis*) were exposed to different doses of myrcene or piperitone. Myrcene and piperitone are monoterpenes that are found within new and old growth of Sitka spruce respectively. Exposure to these terpenes increased aphid mortality (Jackson et al., 1996). Similarly in hemlocks, monoterpene concentrations increase in response to HWA feeding. Broeckling et al (2003) found high levels of myrcene along with other defensive chemicals in eastern hemlock after being naturally infested with HWA. A combination of these chemicals may aid in defending hemlocks as they age since HWA has lower survival rates feeding on old growth (McClure, 1991).

In eastern hemlocks, HWA feeding increases the concentration of terpenes and other volatiles in plant tissues, indicating terpenes may act as an induced-defense (Pezet et al., 2013; Raven, 1983). Terpenes have been shown to provide effective defense against piercing-sucking insects like HWA in other hemlock species, such as Chinese (*Tsuga chinensis* (Franch.) E. Pritz.), Japanese (*T. sieboldii* Carrière and *T. diversifolia* Masters), and western (*T. heterophylla* Sargent) hemlocks. Eastern and Carolina hemlocks evolved to defend against chewing insects like the hemlock looper (*Lambdina fiscellaria* Guenée (Lepidoptera: Geometridae)) (Kinahan et al., 2020b; Kinahan et al., 2020a; Wilson et al., 2016). Eastern hemlocks with HWA present were found to be more susceptible to spongy moth (*Lymantria dispar* Linnaeus (Lepidoptera: Erebidae)) feeding, another chewing insect (Kinahan et al., 2020b). Attempting to defend against HWA feeding seems to weaken hemlock defense against chewing herbivores. To successfully defend against HWA, eastern hemlocks need to produce specific terpenes (α-pinene, β-caryophyllene, α-humulene, and germacrene D) which are already found in Asian, western, and some lingering hemlocks (Lagalante and Montgomery, 2003; McKenzie et al., 2014). Lingering hemlocks produce more terpenes in their twigs in September, when HWA breaks aestivation and resumes feeding, and December, when HWA start to reproduce, compared to susceptible eastern hemlocks (McKenzie et al., 2014). Lingering hemlock genotypes capable of producing the correct terpenes to reduce HWA infestations would be valuable for propagation and breeding programs.

In addition to terpenes, plant volatiles like benzyl alcohol and methyl salicylate are found in plants exposed to Hemiptera feeding. Benzyl alcohol causes mortality and lower fecundity in aphids in crop systems where methyl salicylate produces a hormone for systemic resistance (Pezet et al., 2013; Formusoh et al., 1997; Hardie et al., 1994; Quiroz et al., 1998). Both compounds are present in eastern hemlocks after HWA feeding and stay in circulation for the following growth year (Pezet & Elkinton, 2014). The increase in Benzyl alcohol can contribute to the reduction of HWA survival in lingering hemlocks to protect the tree another year from Hemiptera feeding.

Hydrogen peroxide (H_2O_2) is an influential plant defense because it increases the expression of defensive genes. High levels of H_2O_2 also damage the midgut of insects when they ingest the compound while feeding on plant material (Kmiec et al., 2018). H_2O_2 accumulation is localized to the damage caused by Hemiptera feeding. This localized accumulation is seen in Downy willows (*Salix lapponum*) after aphid feeding (Kmiec et al., 2018) and in eastern hemlocks after HWA and elongate hemlock scale (*Fiorinia externa* Ferris (Hemiptera: Diaspididae); EHS) feeding (Radville et al., 2011). Unlike aphids and EHS, the intense feeding by HWA caused higher levels of H_2O_2 to accumulate in the leaf tissue. Hydrogen peroxide was even found in needles that had no contact with HWA, indicating that the tree was activating a systemic defense response that could decrease the survival of HWA the following year.

Montgomery et al (2009) define antibiosis as slower growth and lower survival of HWA. In one case, HWA densities decreased by 35% on lingering hemlocks compared to wild-type eastern hemlocks (Kinahan et al., 2020a). In 2011, HWA density was 40% lower on lingering hemlocks compared to wild-type eastern hemlocks (Ingwell & Preisser, 2011). Although we do not know why HWA density decreased in HWA in these studies, we can predict that chemical compounds within the tree are contributing to hemlock resistance.

Lingering hemlocks appear to have a combination of terpenes distributed within the tree rather than relying on one specific terpenoid to protect the whole tree (McKenzie et al., 2014). Identifying compounds within a plant, especially a full-grown hemlock tree, is costly. The compounds can also change within a tree over time. This may be why researchers do not know every compound involved in hemlock chemical defenses. One study found 26 lingering hemlock genotypes from the southern Appalachians that seemed to exhibit antibiosis by reducing HWA density more than susceptible eastern hemlock controls (Klein, 2025). Specific terpenes were not tested but the natural decrease in HWA density from their boom-bust cycle was not evenly distributed throughout the genotypes suggesting that chemical compounds are contributing to the decrease in HWA survival. Research does show that terpenes and volatiles increase mortality and decrease fecundity of HWA. This demonstrates that antibiosis is a form of resistance likely utilized by lingering hemlocks but further studies should occur to confirm this.

Antixenosis

Antixenosis, or non-preference, is a mechanism of plant resistance in which pests prefer a susceptible genotype over a resistant genotype (Painter, 1951). Plants use physical characteristics like epicuticular waxes, trichomes, and thick tissues to deter feeding. Chemicals that deter feeding or decrease palatability are also classified as antixenosis. There can be overlap in the mechanisms antixenosis and antibiosis based on whether the chemical compounds negatively affect herbivory (antibiosis) or deter feeding (antixenosis) (Montgomery et al., 2009). Some terpenes are classified as deterrents for HWA feeding. High levels of α-pinene, α-humulene, β-caryophyllene, and germacrene D found in Asian, western and lingering hemlocks may act more as deterrents to HWA whereas isobornyl acetate, found in eastern and Carolina hemlocks, acts as an attractant which helps HWA find their susceptible hosts quicker (Lagalante & Montgomery, 2003; McKenzie et al., 2014).

Hemlock woolly adelgid can be deterred by the thickness and toughness of host plant material. Asian hemlock species (*Tsuga chinensis* (Franch.) E. Pritz.*,* *T. sieboldii* Carrière*, T. diversifolia* Masters), which co-evolved with HWA, developed thick cuticles and hard leaf cushions (Ayayee et al., 2014; Oten et al., 2012). These toughened plant tissues make it difficult for HWA to feed and use hemlocks as a host. Some hybrids of Chinese hemlocks with Carolina hemlocks have the thick cuticles where HWA insert their stylets (Oten et al., 2012). Wild type eastern hemlocks have relatively thin cuticles and soft leaf cushions compared to Asian species (Ayayee et al., 2014; Oten et al., 2012). This makes it easier for HWA to penetrate and feed on eastern hemlocks, making them a more susceptible host. We found no studies that examined whether lingering hemlocks differ in cuticle dimensions to non-lingering hemlocks. Identifying whether lingering hemlocks have physical barriers to HWA feeding would provide useful insight into this potential mechanism of resistance.

Hemlock woolly adelgid prefers feeding on new growth. One study found that 80% of nymphs colonize newest hemlock growth (McClure, 1991). Some hemlock species avoid HWA feeding by breaking bud earlier. Chinese hemlocks reach bud break two to three weeks earlier than eastern hemlocks (Montgomery et al., 2009), pushing out more delicate foliage during a point in the year when HWA are less active. This allows Chinese hemlocks to avoid HWA feeding on their new growth. Eastern hemlocks do not have the advantage of early bud break (Busov et al., 2015). As a result, their new growth is vulnerable at a time when HWA crawlers are active and looking for places to settle. Future studies could note whether lingering hemlocks have different budbreak phenology than from susceptible eastern hemlocks.

Eastern hemlocks are a suitable host for HWA because they lack resistance. In a limited preference test, Casewell et al (2008) compared seven lingering hemlocks against an eastern hemlock and a western hemlock. Five of the lingering hemlocks and the western hemlock had fewer HWA than the wild-type eastern. This resistance is likely due to antixenosis because HWA were given a choice on where to settle, and they avoided the resistant hemlocks. Similarly, Klein (2025) orchestrated preference tests with 10 lingering hemlocks and found HWA crawlers preferred wild-type controls over the resistant hybrid and one lingering hemlock genotype. These studies are useful, but there needs to be more preference studies conducted between hemlock species and lingering hemlock genotypes that show different susceptibility to HWA.

Antixenosis may be a mechanism of lingering hemlock resistance to HWA feeding based on thicker cuticles and hard leaf cushions found in resistant trees, the timing of bud break, or chemical deterrents within the tree. Future studies should focus on comparing plant tissue characteristics, hemlock phenology, and preference of HWA between lingering hemlocks and other resistant species and hybrids. These studies could help determine the extent to which antixenosis is exhibited by lingering hemlocks and whether it derives from anatomical factors like leaf cushions or physiological ones like phenology or chemical defenses.

Tolerance

Tolerance is a mechanism of resistance wherein a tree continues to grow and develop despite a pest infestation (Painter, 1951). This can be due to trees reallocating their resources for growth instead of using energy for defense (Eyles et al., 2010).

Asian hemlocks are known to be tolerant of HWA because they can continue growing with high infestations (Montgomery et al., 2009). Most Asian hemlocks co-evolved with HWA and have been accustomed to their feeding. Selecting and breeding lingering hemlocks which can grow well under heavy infestations of HWA could be a suitable management strategy.

Hemlocks tolerant of HWA may be able to outgrow HWA feeding. Seven lingering hemlocks from the southern Appalachians were found to exhibit tolerance by growing more than wild-type eastern hemlocks (Klein, 2025). This study only looked at one growing season but if hemlocks continue to grow and develop with HWA present, they could survive HWA feeding.

Silviculture can improve long-term survival of infested trees by providing hemlock seedings with direct sunlight to increase the tree growth rate (Brantley et al., 2017; Mayfield & Jetton, 2020). This helps trees produce new growth even while heavily infested with HWA (Mayfield et al., 2023; Miniat et al., 2020).

Hemlock woolly adelgids are not the only stressor eastern hemlocks have to face. Hemlocks infested with HWA decline faster with abiotic stressors such as living on steep slopes that have increased soil drainage or experiencing warmer winters as a result of climate change (Livingston et al., 2017). Biotic stressors like EHS, together with HWA, enhance hemlock decline by depleting resources especially in the seedling stage, making it hard for hemlocks to reproduce (Preisser et al., 2011). By improving environmental conditions for hemlocks, we can give each hemlock a higher chance of tolerating HWA infestations.

Conclusion

The goal of this review was to examine evidence for the three primary types of plant resistance, antibiosis, antixenosis, and tolerance, in eastern hemlocks. Understanding which of these mechanisms contribute to HWA resistance in different hemlock species could help select genotypes to breed for resistant eastern hemlocks. We found studies which identified higher production of defense compounds, like terpenes, in infested hemlocks. Interestingly, lingering hemlocks produce Germacrene D, a terpene, that is not found in susceptible eastern hemlocks but are found in resistant Chinese hemlocks (Lagalante & Montgomery, 2003; McKenzie et al., 2014; Table 1.1). We saw evidence that lingering hemlocks have high growth rates while infested with HWA, similar to Chinese hemlock, northern Japanese hemlock, and southern Japanese hemlock (Montgomery et al., 2009; Klein, 2025; Table 1). This suggests that like other hemlock species, lingering hemlocks may exhibit resistance mechanisms to survive HWA feeding.

There are only 10 hemlock species found worldwide (Leppanen et al., 2019; Figure 1.1). Testing all 10 species would give us the most accurate information on resistance in hemlocks. Although there is some evidence for resistance to HWA more research is needed to discern which mechanisms hemlock species are utilizing. Future studies should focus on growth rates and HWA preference of all hemlock species to provide comparisons between resistant and susceptible varieties. This beneficial information can give a baseline for hybrids and potentially resistant species. Hybrid varieties are also a way to preserve genes of susceptible hemlock species within their ecosystems, ensuring they are not lost forever.

When looking at lingering hemlocks, studies are unevenly distributed along eastern hemlocks' native range. Additional studies would potentially find new hemlock stands that vary in climate, phenology and other environmental factors that could be valuable for research and restoration programs. Identifying, and studying, a more diverse set of lingering hemlocks may provide stronger evidence for antibiosis and tolerance.

Investigating hemlock resistance to HWA is important. Hemlock woolly adelgid has destroyed eastern hemlock forests throughout the eastern United States, however, there still is hope. A forest simulator computer model predicted outcomes for different hemlock genotypes encountering HWA. It found that hemlocks with even partial resistance to HWA could remain a dominant forest species (Case et al., 2017). Host plant resistance will not be able to manage HWA populations on its own. In conjunction with biological control, chemical control, and silviculture, host plant resistance can aid in the balance of hemlock integrated pest management. If research can continue to support eastern hemlock IPM programs we could help restore a valuable ecosystem.

Table 1.1. Hemlock species with evidence of using mechanisms of host plant resistance.

***Tsuga* Species**	**Antibiosis**	**Antixenosis**	**Tolerance**	**Conclusions**
T. canadensis	Isobornyl acetate and α-pinene (Lagalante and Montgomery 2003), H_2O_2 (Radville et al., 2011)	Short trichomes, Thin cuticles (Oten et al., 2012), soft leaf cushions (Ayayee et al., 2014). More HWA than lingering trees (Casewell et al., 2008)	No information	Not resistant
T. canadensis Lingering	α-pinene, β-pinene, limonene and Germacrene D (McKenzie et al., 2014), Reduce HWA survival (Klein, 2025)	Less HWA than susceptible *canadensis* (Casewell et al., 2008), HWA crawlers prefer wild-type over one lingering hemlock genotype (Klein, 2025)	Increased growth rates compared to eastern (Klein, 2025)	Partial resistance
T. caroliniana	Isobornyl acetate, α-pinene and Germacrene D (Lagalante and Montgomery 2003)	Strip of trichomes, Hybrid: robust needle bases (Oten et al., 2012)	No information	Not Resistant, Hybrid: Partial resistance
T. chinensis	α-pinene, β-caroyophyllene, α-humulene, and Germacrene D (Lagalante and Montgomery 2003)	Strip of trichomes, Thick cuticles (Oten et al., 2012), hard leaf cushions (Ayayee et al., 2014). Break bud early (Montgomery et al., 2009)	High growth rates (Montgomery et al., 2009)	Resistant
T. diversifolia	Isobornylacetate, α-pinene, α-humulene (Lagalante and Montgomery 2003)	Short trichomes, Thick cuticles (Oten et al., 2012), hard leaf cushions (Ayayee et al., 2014)	High growth rates (Montgomery et al., 2009)	Resistant
T. dumosa	No information	No information	No information	Needs more information
T. forrestii	No information	No information	No information	Needs more information
T. heterophylla	Isobornylacetate, α-pinene, α-humulene (Lagalante and Montgomery 2003)	Long haired trichomes, Thick cuticles (Oten et al., 2012), hard leaf cushions (Ayayee et al., 2014)	still alive after HWA feeding (Rose et al., 2019)	Partial resistance
T. mertensiana	Germacrene D and α-pinene (Lagalante and Montgomery 2003)	Long haired trichomes (Oten et al., 2012), Hard leaf cushions in Autumn (Ayayee et al., 2014)	No information	Needs more information
T. sieboldii	Isobornylacetate, α-pinene, germacrene D (Lagalante and Montgomery 2003)	Thick cuticles at HWA insertion point (Oten et al., 2012)	High growth rates (Montgomery et al., 2009)	Resistant
***T. ulleungensis*	No information	No information	No information	Needs more information

***** Species are recently described and might not be considered in some of these studies***

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CHAPTER 2

Evaluating Hemlock Woolly Adelgid Density and Tree Growth of Lingering Hemlock Genotypes

Abstract

An invasive insect, the hemlock woolly adelgid (*Adelges tsugae* Annand; Hemiptera: Adelgidae; HWA), is killing eastern hemlocks (*Tsuga canadensis* Carrière), a keystone species, throughout its native range in North America. Yet in some long-infested hemlock stands, some trees persist that have not yet succumbed to HWA feeding. These 'lingering hemlocks' are thought to have natural resistance to HWA. To investigate this, we monitored 46 lingering hemlock genotypes from North Carolina and Virginia, propagated at the Mountain Research Station in Waynesville, NC. Our goal was to determine whether these genotypes reduce HWA abundance and fecundity, and whether HWA infestations affect hemlock growth. We randomly sampled branches from each tree and counted HWA nymphs, adults, and eggs to measure HWA density and fecundity. Tree growth was measured by marking and recording branch length in February and October 2024. Several genotypes showed early indications of resistance to HWA. Four lingering hemlock genotypes harbored consistently low numbers of HWA over a generation, six lingering hemlock genotypes reduced HWA populations across multiple life stages, and nine genotypes grew more than our wild-type control, four of which are lingering hemlocks. Further research is needed to confirm whether initial signs of resistance are sustained over multiple generations and to discern what mechanisms of resistance underly this resilience lingering hemlocks may be exhibiting.

Introduction

Eastern hemlock (*Tsuga canadensis* Carrière) is a foundation species in North American forests. Its range extends from Alabama north to Nova Scotia, Canada (Havill et al., 2011). In the

United States, this range is primarily within the Appalachian Mountain region and spreads west along the Great Lakes region to Minnesota. Eastern hemlocks have dense foliage that moderate stream temperatures and provide a hospitable environment for more than 120 vertebrates (Parker et al. 2023; Ellison et al. 2018; Sibley 2009). Unfortunately, hemlock forests are in decline because of an invasive insect, the hemlock woolly adelgid (*Adelges tsugae* Annand; Hemiptera: Adelgidae; HWA).

Hemlock woolly adelgid is an exotic pest native to Asia and was detected in the United States in the 1950s (Havill et al., 2014). They infest all 10 *Tsuga* species worldwide (Leppanen et al., 2019), but in the eastern United States it faces no specialized natural enemies. With a spread rate of 9-15 km per year, HWA has reached harmful densities. (Evans, 2016; Parker et al., 2023). Feeding results in discoloration, needle loss, branch dieback and eventually death (Havill et al., 2014; McClure, 1991). Aukema et al. (2011) deemed HWA the most damaging pest in its feeding guild and estimated that HWA causes $215 million ($254 million in 2025 dollars, per the Consumer Price Index) in damage each year.

In the eastern United States, HWA has two generations per year. The longer-lived sistens generation begins in summer and extends through early spring. Eggs eclose into a mobile crawler stage which are dispersed passively (McClure, 1991). Upon settling at the base of hemlock needles, sisten crawlers insert their stylets and enter aestivation from summer to fall. In the fall, they begin feeding and reproduce asexually, laying eggs around February. The resulting offspring, the progrediens generation, only lasts for a few weeks from spring into early summer. Progrediens develop uninterrupted, as this generation does not aestivate. Adults can produce 50-300 eggs. In the progrediens generation, some eggs develop into winged adults that cannot reproduce in the United States from a lack of an alternate host spruce (*Picea*). The rest of the eggs develop into wingless, sistens adults (Havill et al., 2014).

Multiple management strategies have been implemented to reduce the spread of HWA and the decline of hemlock forests. Biological control agents, including *Laricobius* beetles and silver flies (*Leucotaraxis argenticollis* and *L. piniperda*) have been released in eastern North America from native HWA ranges in Asia and Western North America (Havill et al., 2014). Although the Hemlock Woolly Adelgid National Initiative (2025) reports there have been over 4.5 million predators released, only 88 thousand have been recovered, which is approximately 2% of the released amount. This indicates that biological control numbers are not yet high enough to effectively decrease HWA populations. Chemical controls are also widely used to protect hemlocks. Imidacloprid and dinotefuran are systemic neonicotinoid insecticides used to protect individual hemlock trees and can be effective for 6 years (Havill et al., 2014). However, neonicotinoids persist in the environment for years and can harm non target organisms (Frank & Tooker, 2020). Both chemical control and biological control are difficult to employ on a large scale, because of this cultural control strategies are a recent addition to HWA management. Silviculture release by creating canopy gaps has been shown to increase the tolerance of hemlocks to HWA (Bentz et al., 2023; Mayfield et al., 2023). Host plant resistance is a possible addition to HWA integrated pest management. Selecting and breeding for resistant traits can work with biological control, chemical control, and silviculture as valuable tools in reducing HWA populations and keeping hemlocks in our ecosystems.

While eastern and Carolina hemlock are highly susceptible to HWA, some species exhibit resistance. Chinese hemlocks (*Tsuga chinensis* (Franch.) E. Pritz.) exposed to HWA showed no sign of HWA infestation and maintain healthy growth, even after four years. Eastern hemlocks under the same conditions hosted many HWA ovisacs and produced little new growth (Tredici & Kitajima, 2004). Similarly, western hemlocks (*Tsuga heterophylla* Sargent) exposed to HWA have significantly lower feeding densities and infestation rates than eastern hemlocks in the same studies (Oten et al., 2014). These observations have led efforts to develop hybrid crosses between resistant and susceptible hemlocks that combine resistance with the ecological value of native species.

Interestingly, some eastern hemlocks have survived in heavily infested stands where surrounding hemlocks have been killed by HWA. These survivors, called 'lingering hemlocks', may have resistance to HWA that susceptible hemlocks lack. Lingering hemlocks have been identified and studied before and are variably referred to as being 'putatively resistant' or having 'relative resistance'. Here we use the term 'lingering' to emphasize their persistence under high pest pressure. Lingering hemlocks have lower HWA densities, higher survival rates, better growth, and retain more foliage than wild-type eastern hemlocks (Casewell et al., 2008; Kinahan et al., 2020). In support of the hypothesis that lingering hemlocks may be resistant are similar findings in other tree species, such as green ash (*Fraxinus pennsylvanica* Marsh), where lingering individuals reduced survival of emerald ash borer (*Agrilus planipennis* Fairmaire) larvae compared to susceptible trees (Koch et al., 2020).

Lingering hemlocks could provide an effective strategy for reducing HWA populations and spread. The goal of this study is to determine whether identified lingering hemlock genotypes show resistance to HWA. We monitored HWA densities and tree growth in 46 genotypes. Our specific objectives were to determine whether lingering hemlock genotypes 1) affect HWA density and fecundity compared to each other and 2) exhibit increased growth compared to wild-type eastern hemlocks despite HWA infestations. Identifying and understanding resistant genotypes could help in the conservation and restoration of eastern hemlock forests.

Materials and Methods

Between 2014 and 2017, vegetative cuttings were collected from 41 lingering eastern hemlock individuals across 11 distinct locations in North Carolina and Virginia (Figure 2.1, Table 2.1) and propagated as rooted cuttings under nursery conditions for at least 6 years at the Mountain Research Station in Waynesville, NC. Rooted cuttings were transplanted into Trade 2 gallon pots (Nursery Supplies Inc., Kissimmee, FL) filled with aged southern pine bark fines amended with added limestone and maintained in a gothic arch cold frame with automated roll-up sides and an exhaust fan. Here the trees were exposed to natural infestations of HWA. Genotypes BLRI, SPSF, JRSP and SHHI were artificially infested in 2015 for a study starting in 2017. We fertilized cuttings with a resin-coated slow-release granule that contained nitrogen, phosphorus, and potassium in a ratio of 3:1:2 (Osmocote Blend 7-5-11, ICL, St. Louis, MO). In this study, we evaluated 48 total genotypes. 41 were lingering hemlock genotypes, one was an experimental cross of genotype CGR (*Tsuga canadensis*) with a Sargent's weeping hemlock (*Tsuga canadensis* 'Sargentii'), and six hybrid crosses with assumed resistance since one of their parents is known to resist or tolerate HWA (Table 2.2). Hybrids were genetically confirmed using SSR analysis. Crosses were grown from seed produced in 2012, 2014, 2015, or 2017. Tree heights ranged from 60 cm to 153 cm in 2023 at the onset of this study.

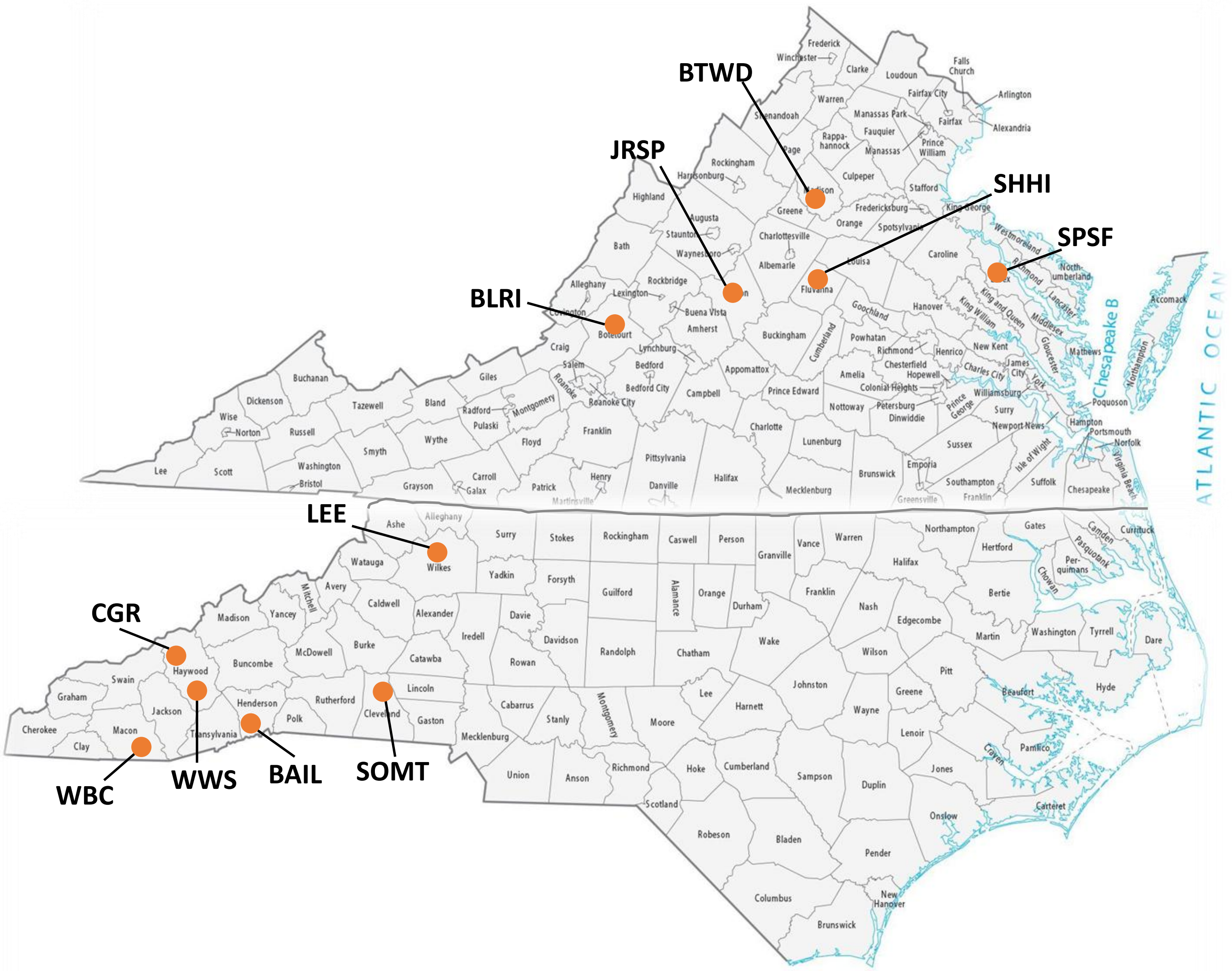

Figure 2.1. Lingering hemlock origin locations in North Carolina and Virginia. Location codes for Virginia: BLRI=Blue Ridge in Munford; VA, JRSP= James River State Park in Norwood, VA; BTWD= Brightwood, VA; SPSF= Sandy Point State Forest in Log Landing, VA; SHHI= Stage Junction, VA . Location codes for North Carolina: CGR= Maggie Valley, NC; LEE= Lee stand in Hays, NC; SOMT= South Mountains State Park in Casar, NC; BAIL= Bailey property in Flat Rock, NC; WWS= Waynesville Watershed; WBC= Williams Big Creek in Highlands, NC.

Table 2.1. Lingering hemlock location codes and origin locations.

Tree Code	Location	County	State	Coordinates
BAIL	Property in Flat Rock	Henderson	NC	35.27418341, -82.46172775
BLRI	Munford	Botetourt	VA	37.5801667, -79.33298333
BTWD	Brightwood	Madison	VA	38.387029, -78.182856
CGR	Maggie Valley	Haywood	NC	35.559865, -83.099644
JRSP	James River State Park	Nelson	VA	37.639016, -78.80378
LEE	Hays	Wilkes	NC	36.274418, -81.050194
SHHI	Stage Junction	Fluvanna	VA	37.812472, -78.145508
SPSF	Sandy Point State Forest	Essex	VA	37.680669, -76.922447
SOMT	South Mountains State Park	Cleveland	NC	35.58558, -81.65457
WBC	Highlands	Macon	NC	35.084262, -83.205355
WWS	Waynesville Watershed	Haywood	NC	35.41280119, -82.96853089

Table 2.2. Genetically confirmed hybrid genotypes and parentage evaluated in this study.

ID Code	Parentage (Maternal x Pollen)
TcarxTchin103	***Tsuga caroliniana* x *Tsuga chinensis***
TcarxTchin104	***Tsuga caroliniana* x *Tsuga chinensis***
TcarxTsieb19	***Tsuga caroliniana* x *Tsuga sieboldii***
TcarxTchin20	***Tsuga caroliniana* x *Tsuga chinensis***
TcarxTsiebD3.1	***Tsuga caroliniana* x *Tsuga sieboldii***
TcarxTsiebD3.2	***Tsuga caroliniana* x *Tsuga sieboldii***

May 2023 Progrediens Adult Density and Fecundity:

In May 2023, we measured HWA adult density on 22 lingering hemlock genotypes and one hybrid genotype. We collected samples from four genetically identical trees (ramets) from each genotype. From each tree, we cut one 4 cm branchlet from the tip of a branch in the middle of each tree. We placed all samples on ice and transported them back to the lab at North Carolina State University (NCSU), Raleigh NC. In the lab, we counted the number of adult HWA from the progrediens generation on the underside of each cutting using dissecting microscopes. After assessing 22 lingering hemlock genotypes and 1 hybrid for HWA adult density, we randomly selected three undamaged HWA ovisacs from each cutting and transferred them to a small petri dish filled with 70% ethanol. We dissected the three ovisacs and counted the total number of eggs within each one. This was repeated for each tree. The number of eggs from the three ovisacs were averaged to get the mean number of eggs per ovisac for each tree.

July 2023 Sistens Nymph Density:

In July 2023, we continued monitoring the same 22 lingering hemlock genotypes and one hybrid genotype (four ramets each). For this sampling event, two 4 cm branchlets were collected by counting 5 branches up from the bottom of the main stem and 5 branches down from the top of the main stem on the opposite side of the tree. We transported the cuttings back to the NCSU lab on ice where we counted settled HWA nymphs from the sistens generation on the underside of each cutting. The number of nymphs on the two cuttings from the same tree were averaged to calculate HWA nymph density per tree.

October 2023 Sistens Nymph Density:

In October 2023, our monitoring was expanded to 41 lingering hemlock genotypes, one experimental cross (*Tsuga canadensis* 'CGR' x *Tsuga canadensis* 'Sargentii'), and six hybrid genotypes to include more trees. For 10 lingering hemlock genotypes (WBC1, WBC3, WWS1, BAIL3, JRSP1, LEE42, LEE48, BAIL2, LEE51, WBC2) we expanded the number of ramets to eight, for two genotypes (SPSF4, BLRI11) we expanded the number of ramets to seven, and for five lingering hemlock genotypes (SHHI1, BLRI10, BLRI7, SHHI3, LEE24) we expanded the number of ramets to six. Four ramets were sampled from each of the remaining genotypes. Branchlets were harvested and HWA nymph densities for the sistens generation were calculated following the same protocol used in the July 2023 sampling.

February 2024 Sistens Adult Density and Fecundity:

In February 2024, we continued monitoring the same 48 genotypes and ramets as October 2023. Branchlets were harvested following the same protocol used in July 2023 and HWA adult densities for the sistens generation were calculated. Then we randomly selected three ovisacs from each cutting on the 46 lingering hemlock genotypes and 9 hybrids assessed in October 2023. Eggs per ovisac were counted and calculated following the same protocol used in May 2023.

Hemlock Genotype Growth:

In February 2024, we monitored the growth on 24 lingering hemlock genotypes, four hybrid genotypes, one experimental cross (*Tsuga canadensis* ‘CGR’ x *Tsuga canadensis* ‘Sargentii’), and a wild-type control. Overall genotypes decreased because of individual tree mortality. The lingering hemlock genotypes, hybrid genotypes, and experimental cross each had 4 ramets while we measured 8 individual susceptible eastern hemlocks to account for possible resistance in the future. We measured two branches from each tree. Branches were measured from main stem to tip. We recorded the number of HWA present on these branches during our measurements. Branches were marked with a twist tie and left for the main part of the growing season. In October of 2024, we remeasured the marked branches from trunk to tip and used this to calculate the growth increment for each branch. We averaged increments of branches taken from the same tree and recorded the difference in growth from February to October.

Data Analysis:

For each set of counts (density and fecundity), we fit generalized linear models (GLM) to test our null hypothesis that there is no difference between HWA counts on different genotypes. Both models showed overdispersion with a Poisson distribution, so our final models used a negative binomial distribution. All GLMs were conducted with the Fit Model procedure in JMP Pro 18 (JMP Statistical Discovery LLC, Cary, NC) on genotype and the respective HWA count we wanted to test. F-tests for fixed effects were considered significant when $P < 0.05$.

We fit a linear model (LM) to test our null hypothesis that each genotype grows the same amount every growing season. The LM was conducted with the Fit Model procedure in JMP Pro 18 (JMP Statistical Discovery LLC, Cary, NC) on growth increment and genotype. F-tests for fixed effects were considered significant when $P < 0.05$.

Results

May 2023 Progrediens Adult Density and Fecundity:

Mean HWA adult densities for the progrediens generation ranged from zero to 7.5 across 22 different genotypes (Figure 2.2). The GLM fit for May 2023 adults was significant ($F_{21,65}$= 2.6275097; Prob>F= 0.0016*). Mean eggs per ovisac ranged from zero to 17.9 across the same 22 genotypes (Figure 2.3). The GLM fit for May 2023 eggs per ovisac was also significant ($F_{21,63}$= 1.8406232; Prob>F= 0.0329*).

July 2023 Sistens Nymph Density:

In July 2023, settled nymph densities for the sistens generation ranged from 2.6 to 36 (Figure 2.4). Genotypes WBC1, LEE42, WBC3, SOMT1, and LEE24 had the highest counts of settled nymphs while JRSP1, BLRI10, SPSF4, SOMT2, and BLRI7 had the least. The GLM fit for July nymphs across 22 genotypes was significant ($F_{21,55.3}$= 3.4962851; Prob>F= <.0001*).

October 2023 Sistens Nymph Density:

Overall counts of HWA nymphs in the sistens generation that broke aestivation were lower than settled nymphs in July. Mean nymph counts ranged from zero to six (Figure 2.5). Genotypes WWS3,WBC2, LEE39, BAIL3, WBC3, and LEE51 had the most nymphs in October 2023. The GLM fit for October nymphs across 48 genotypes was significant ($F_{47,191}$= 1.7308767; Prob>F= 0.0054*).

February 2024 Sistens Adult Density and Fecundity:

Mean HWA adult densities for the sistens generation ranged from zero to 6.25 across 48 different genotypes (Figure 2.6). The GLM fit for February 2024 adults was significant ($F_{47,192}$= 1.5547976; Prob>F= 0.0205*). Mean eggs per ovisac ranged from zero to 79.7 across the same 48 genotypes (Figure 2.7). The GLM fit for February 2024 eggs per ovisac was also significant ($F_{47,83}$= 2.3446162; Prob>F= 0.0003*).

Hemlock Genotype Growth:

Mean growth increments across 30 different genotypes ranged from -1 cm to 8.4 cm between February 2024 and October 2024 (Figure 2.8). Genotypes TcarxTchin103, TcarxTsiebD3.1, TcarxTchin20, TcanCGRxTcanSarg, CGR1, BLRI1, TcarxTsiebD3.2, BAIL3, and LEE48 showed increased growth compared to the wild-type control. These genotypes included our four hybrid genotypes and the cross between lingering genotype 'CGR' and a weeping hemlock. Genotypes BLRI7 and SPSF4 reduced growth by -1 cm. The linear model fit for hemlock genotype growth was significant ($F_{29,90}$= 4.1893007; Prob>F= <.0001*).

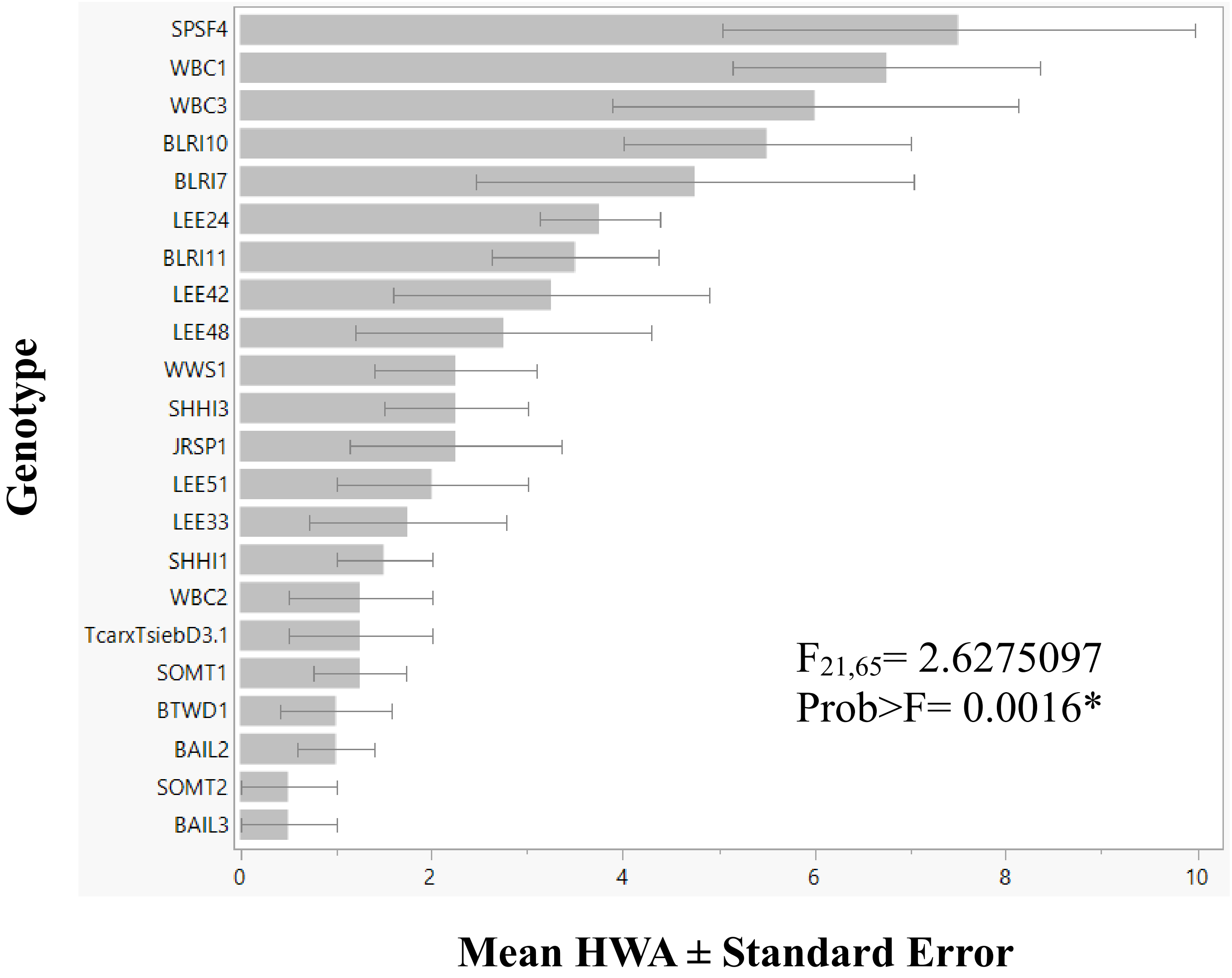

Figure 2.2. Mean HWA counts for progrediens adult density in May 2023 ± Standard Error.

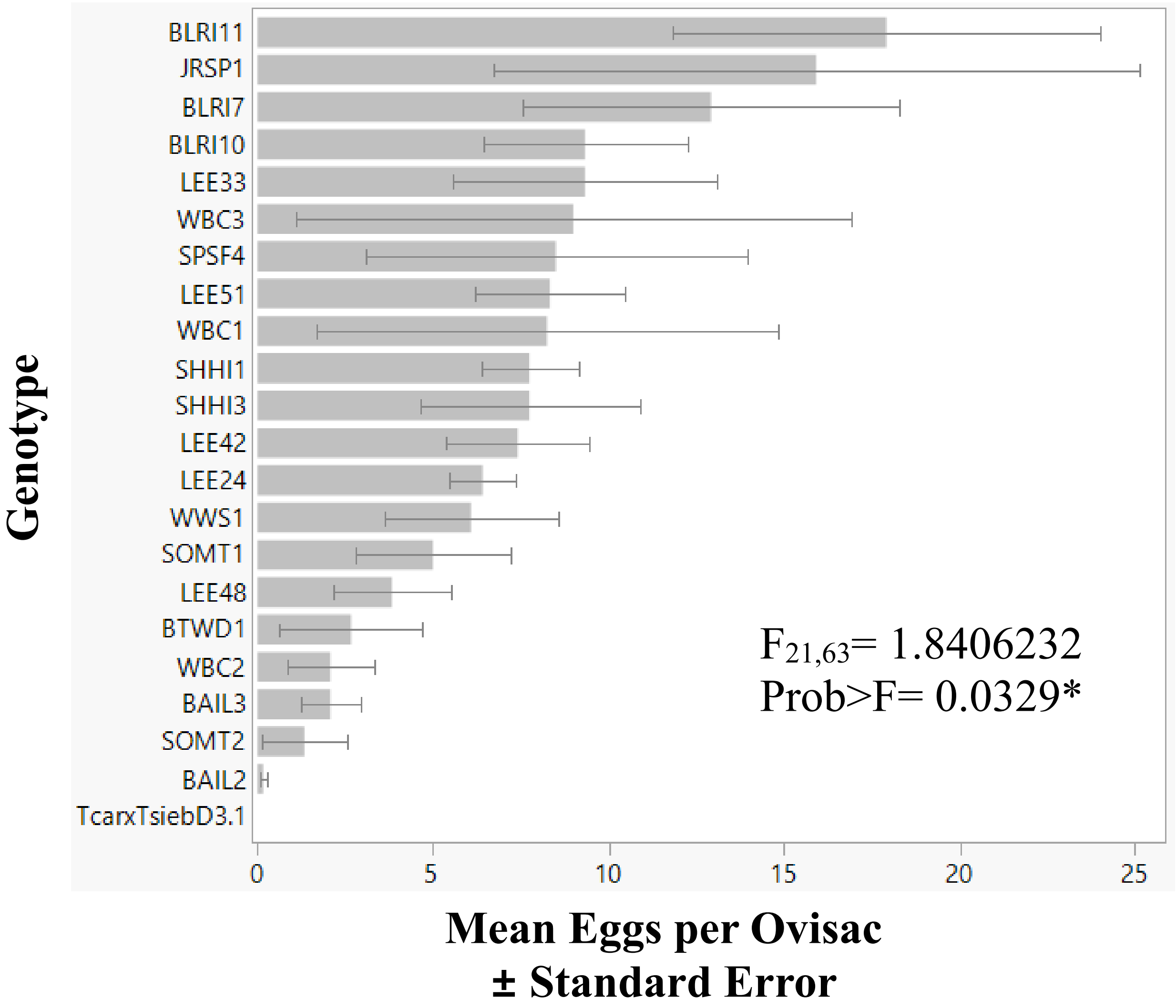

Figure 2.3. Mean HWA egg counts per ovisac for May 2023 sistens generation ± Standard Error.

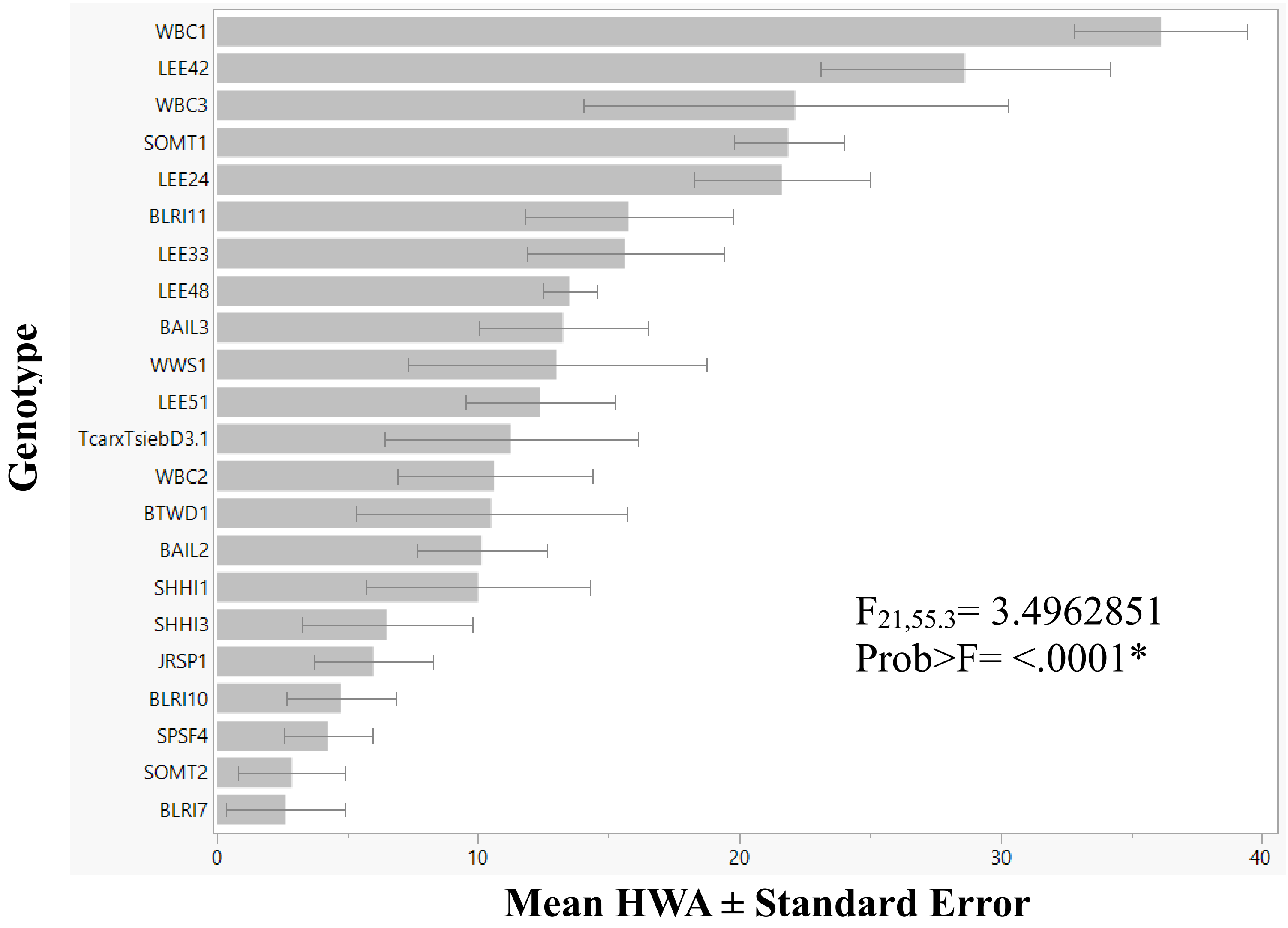

Figure 2.4. Mean HWA counts for settled sistens nymphs in June 2023 ± Standard Error.

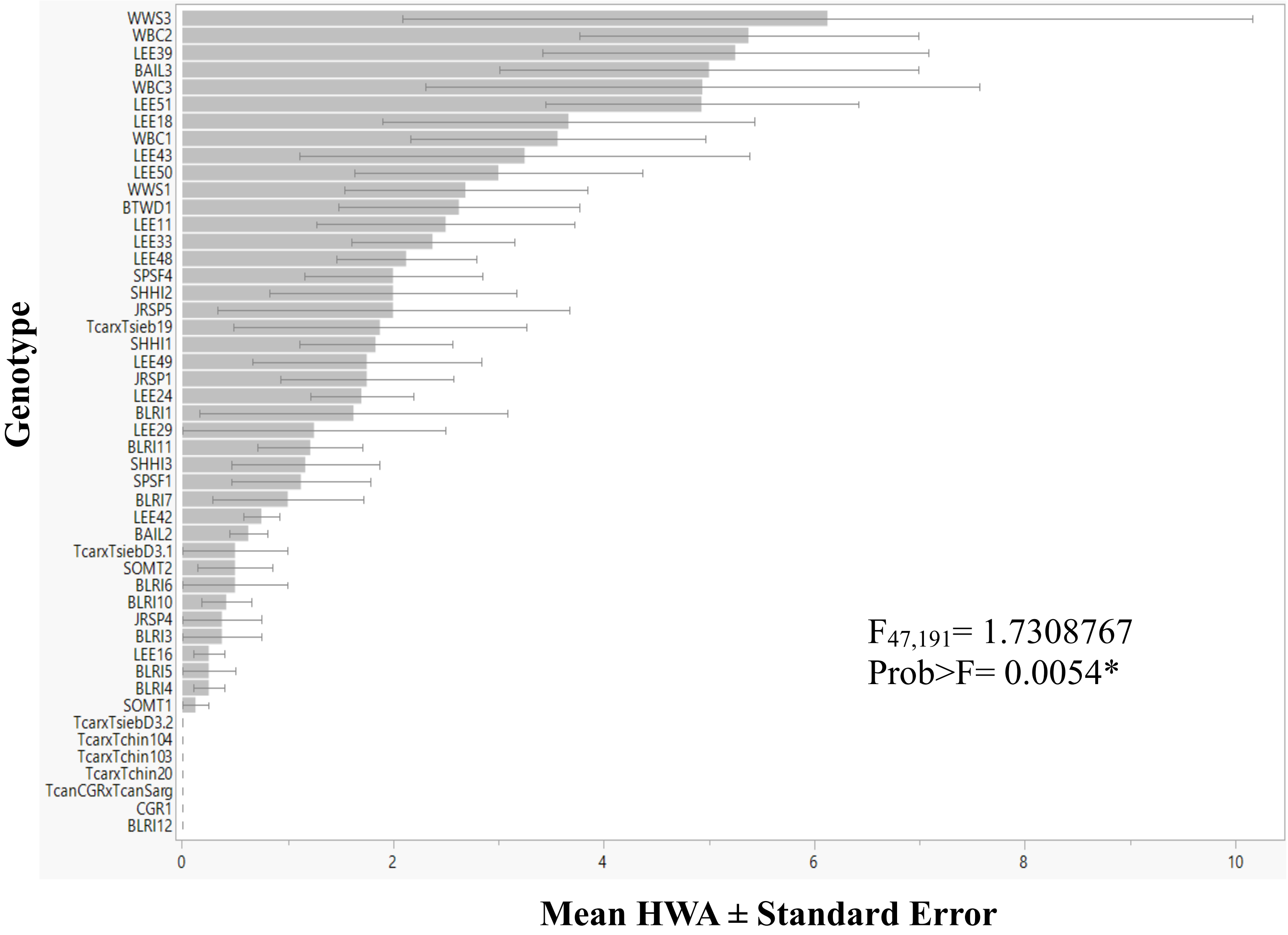

Figure 2.5. Mean HWA counts for nymphs in the sistens generation after breaking aestivation in October 2023 ± Standard Error.

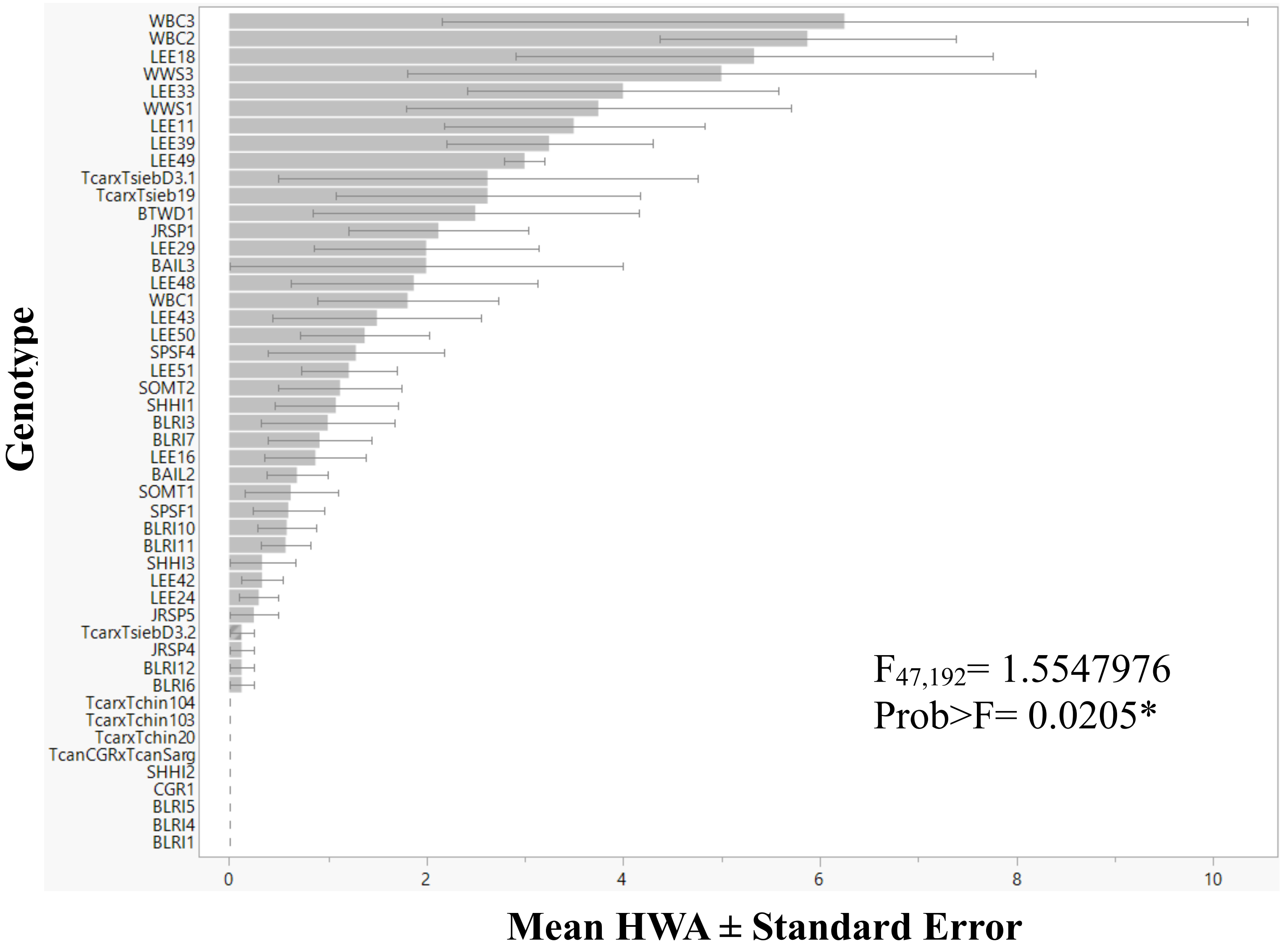

Figure 2.6. Mean HWA counts for sistens adult density in February 2024 ± Standard Error.

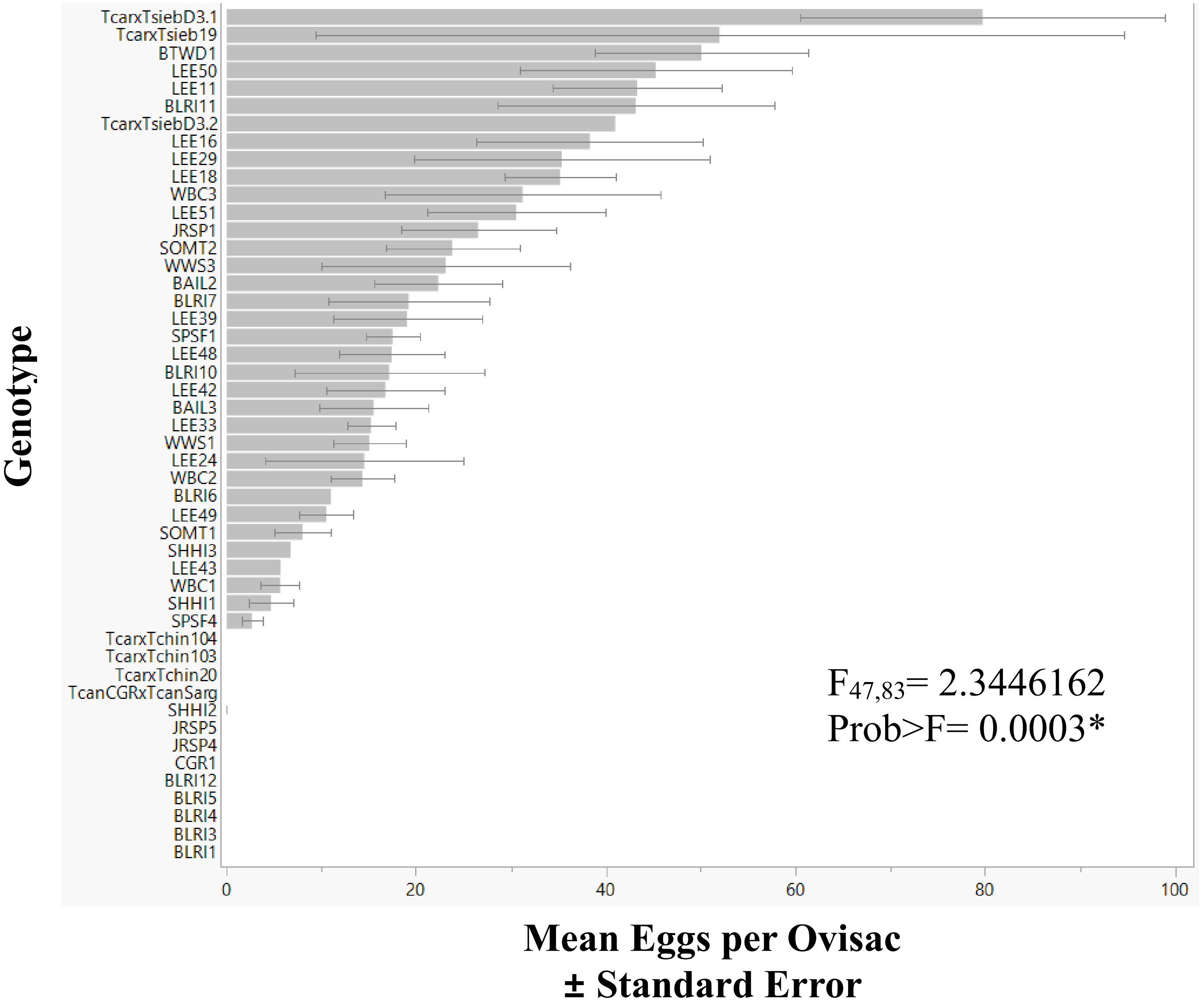

Figure 2.7. Mean HWA egg counts per ovisac for February 2024 progrediens generation ± Standard Error.

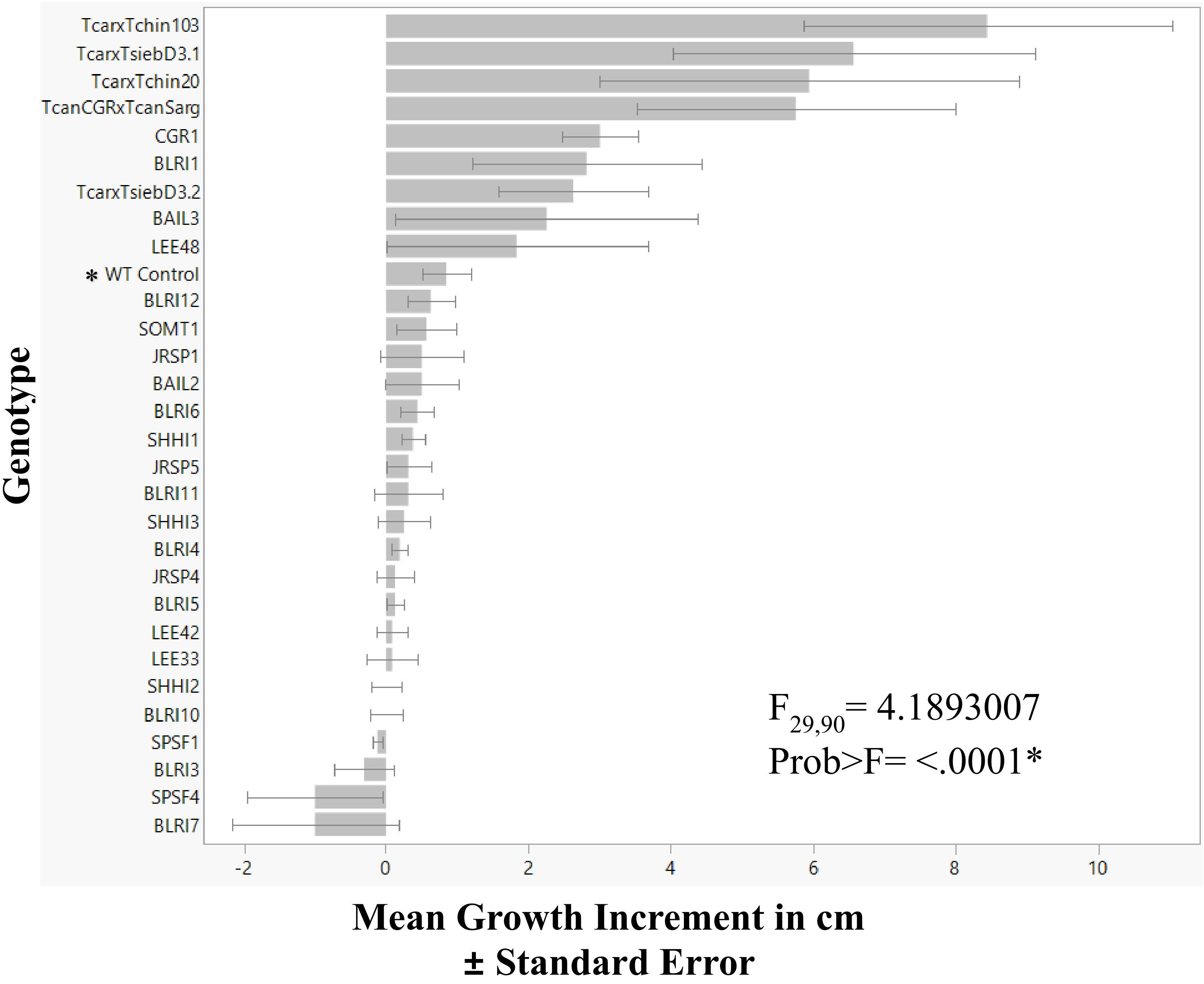

Figure 2.8. Average growth increments of genotypes over one growing season (February 2024-October 2024) in centimeters ± Standard Error.

Discussion

This study represents the first evaluation of lingering hemlock genotypes propagated entirely from surviving hemlocks in the Southern Appalachians. We found lingering hemlock genotypes that might indicate resistance to HWA though many more replications of this study need to be completed across several years to confirm this. Throughout this study, we considered potential resistance in genotypes that maintained consistently low HWA densities, genotypes that reduced HWA numbers over each generation, and genotypes which produced new growth while infested. Since this was an observational study, these genotypes are worth pursuing in the future to learn more about their potential resistance.

When looking for genotypes that maintained low numbers of HWA over the course of a year, we had to consider both HWA generations (progrediens and sistens). Genotype SOMT2, for example, had low HWA counts of adults and eggs per ovisac in May, and low populations of July nymphs; however, HWA counts increased in October nymphs and February adults. This change could be due to our sampling method. To count HWA in the lab, we removed individual branches. Once removed, the branch was unable to be used again later in the year. Other genotypes, like BAIL2, BTWD1, and BAIL3 harbored low numbers of progrediens adults, but had increased numbers of adults over the sistens generation. While our preliminary results over one year do not confirm resistance in any of the tested genotypes, they do illustrate the characteristic boom-bust cycle of HWA populations—an initial surge followed by a sharp decline, likely due to resource depletion or environmental pressures.

Hemlock woolly adelgid density is determined by needle base availability and thus is affected by a boom-bust cycle (Young et al., 1995). Following a new infestation, HWA density rapidly increases on a tree. Each crawler finds a place to settle and begins feeding at the base of a hemlock needle. During the first year, the infested hemlock does not produce much if any new growth, as its resources are depleted from HWA feeding. The next year, the new generation of HWA do not have anywhere to feed and they die off. The tree recovers quickly. It produces new growth that same year which attracts the next generation of HWA, and the cycle continues (McClure, 1991; Mayfield III et al., 2023).

We also aimed to identify genotypes associated with reduced HWA densities. Again, the boom-bust cycle is evident. Genotypes LEE39, BAIL3, and LEE50 had high counts of nymphs in October (progrediens generation), but these counts decreased when counting adults in February 2024 (sistens generation). Conversely, we saw WBC2, WWS1, and LEE33 with high numbers of adults in the sistens generation, but those adults produced few eggs for the progrediens generation.

Multiple factors could be contributing to the observed reduction in HWA density. One of these factors is the passive dispersal and single active stage of HWA, which may limit their ability to cover every branch on each tree (McClure, 1991). Another factor is HWA preference, which may be different between specific hemlocks. Eastern hemlocks have thin cuticles and soft leaf cushions that make them easier targets for HWA feeding (Ayayee et al., 2014; Oten et al., 2012); however, some genotypes may vary in the hardiness of their tissues. Another consideration is the exposure a given hemlock had to HWA. Some trees may have been exposed to HWA for longer or in closer proximity to a dense population of HWA which caused different levels of infestation that reduced our counts. Tree resistance factors could also reduce HWA density. Chemicals within the plant tissue, like terpenes, are toxic enough to reduce HWA populations by limiting nutrients they need for growth and development (Ninkuu et al., 2021).

Hemlock woolly adelgid is an organism that prioritizes high reproductive rates, thus a natural density-dependent mortality occurs at each life stage (Young et al., 1995). More monitoring will need to be done on the genotypes we studied to determine whether they are resistant to HWA feeding, or whether other factors are contributing to the observed reduction in HWA density.

We found nine genotypes that grew more than the wild-type control (*), suggesting they exhibit some form of tolerance to HWA feeding. One of the genotypes with high growth was the experimental cross between lingering genotype CGR and a Sargent's weeping hemlock (TcanCGR x TcanSarg). Sargent's weeping hemlocks are not known to exhibit resistance to HWA, but they can produce large amounts of new growth. Over the course of 80 years, one Sargent's weeping hemlock only grew 4 feet in height but grew 23.5 feet in width (Del Tredici, 1980). The abundant growth of this genotype may have helped these trees to outpace HWA feeding. Another of the four lingering hemlock genotypes that had more growth compared to the wild-type control was CGR1. Genotype CGR1 grew the most of all the lingering hemlocks, excepting its cross with the Sargent's weeping hemlock. Future testing of these promising genotypes is needed to determine whether there are any significant relationships between HWA and their growth.

We tested six genetically confirmed hybrids. Four of these hybrids survived for two full years of monitoring. The hybrids showed low counts of HWA and produced more growth compared to the wild-type hemlocks. Two hybrids are a cross of Carolina and Chinese hemlock while the other two are a cross of Carolina and southern Japanese hemlocks (*T. sieboldii*). Chinese hemlocks and southern Japanese hemlocks are known to have high growth rates, thick cuticles at HWA insertion points, and chemicals that make them resistant to HWA feeding (Lagalante & Montgomery, 2003; Montgomery et al., 2009; Oten et al., 2012). Hybridizing resistant hemlocks with native hemlocks allows native genes to be present in the ecosystem, while providing a measure of resistance to HWA. There are over 100 confirmed interspecific hybrids that are praised for their resistance to HWA and their growth characteristics (Bentz et al., 2023).

This project started with collecting and monitoring HWA on lingering hemlock genotypes to observe whether any genotypes showed potential for being resistant to HWA. Our future aims are to identify resistant genotypes that can be used in conjunction with integrated pest management strategies to decrease HWA populations. To properly identify resistant genotypes, a multi-year effort is needed which will involve testing for resistance and growing individual trees to full size to see whether they can consistently reduce HWA populations across generations. If successful, the results of such a study could be used to reestablish hemlock forests and conserve a unique and valuable ecosystem.

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CHAPTER 3

Variation in Hemlock Woolly Adelgid Survival, Settling Preference, and Hemlock Branch Growth Among Lingering Hemlock Genotypes

Abstract

Hemlock woolly adelgid (*Adelges tsugae*; HWA) is an invasive insect that kills eastern hemlock (*Tsuga canadensis* Carrière) and Carolina hemlock (*Tsuga caroliniana* Engelmann), which are ecologically imporatant species in their eastern North American range. 'Lingering hemlocks' can be found in stands where most hemlocks have died and may be resistant to, or tolerant of, HWA feeding. To test whether lingering hemlocks are resistant to HWA, we propagated 200 hemlock trees comprising 40 lingering hemlock genotypes from 10 locations and 8 hybrid genotypes. We tested the primary types of host plant resistance: antibiosis, antixenosis, and tolerance. To test for antibiosis, we measured change in HWA density on different hemlock genotypes by caging infested branches on each tree and counting HWA as nymphs and adults across two generations. We found that 26 lingering hemlock genotypes and the hybrid (resistant control) reduced HWA survival in the progrediens generation compared to wild-type eastern hemlocks (susceptible control). To test for antixenosis, we conducted a preference experiment in which HWA crawlers could choose between lingering and non-lingering hemlock cuttings. This experiment showed that HWA on one genotype demonstrated a preference for wild type genotypes compared to lingering trees. To test tolerance, we measured hemlock growth relative to HWA density. Seven lingering hemlock genotypes had more growth than our wild-type eastern hemlocks during the main growing season (February to October 2024). The results of this study should help determine which lingering hemlock trees exhibit resistance that can be propagated to help manage HWA populations.

Introduction

Eastern hemlock (*Tsuga canadensis* Carrière) is a foundation species in the Appalachian Mountain region of the United States (Ellison et al., 2018). Carolina hemlocks (*Tsuga caroliniana* Engelmann) are a rare endemic species restricted to steep, rocky slopes in southern Appalachia (Jetton et al., 2008). Hemlock's dense foliage moderates stream temperatures and provides a hospitable environment for more than 120 vertebrates (Parker et al. 2023; Ellison et al. 2018; Sibley 2009). Unfortunately, eastern and Carolina hemlocks are dying because of an exotic invasive pest, hemlock woolly adelgid (*Adelges tsugae* Annand; Hemiptera: Adelgidae; HWA).

Hemlock woolly adelgid was first detected in eastern United States in the 1950s and since then, hemlock forests in at least 20 states have been infested by this invasive pest (Havill et al., 2014). The adelgid inserts its feeding stylet near the base of hemlock needles to extract nutrients from xylem ray parenchyma cells, causing needle discoloration and loss, branch dieback, and tree death (Young et al., 1995). Eastern and Carolina hemlocks are the only *Tsuga* species worldwide susceptible to mortality from HWA feeding. Trees can succumb to HWA feeding in as little as four years, although many survive infestation for ten or more years (Holman et al., 2017).

In the eastern United States, HWA has two generations per year. The longer-lived sistens generation begins in summer and extends through early spring. Eggs eclose into a mobile crawler stage which are dispersed passively (McClure, 1991). Upon settling at the base of hemlock needles, sisten crawlers insert their stylets and enter aestivation from summer to fall. In the fall, they begin feeding and reproduce asexually, laying eggs around February. The resulting offspring, the progrediens generation, only lasts for a few weeks from spring into early summer.

Progrediens develop uninterrupted, as this generation does not aestivate. Adults can produce 50-300 eggs. In the progrediens generation, some eggs develop into winged adults that cannot reproduce in the United States from a lack of an alternate host spruce (*Picea* sp., most often *P. torano*). The rest of the eggs develop into wingless, sistens adults (Havill et al., 2016).

Multiple management strategies have been implemented to reduce the spread of HWA and the decline of hemlock forests. Biological control agents, including *Laricobius* beetles and silver flies (*Leucotaraxis argenticollis* and *L. piniperda*) have been released in eastern North America from native HWA ranges in Asia and Western North America (Havill et al., 2014). Although the Hemlock Woolly Adelgid National Initiative (2025) reports there have been over 4.5 million predators released, only 88 thousand have been recovered, which is approximately 2% of the released amount, indicating that biological control is not yet effective enough to decrease HWA populations. Chemical controls are also widely used to protect hemlocks. Imidacloprid and dinotefuran are systemic neonicotinoid insecticides used to protect individual hemlock trees and can be effective for 6 years (Havill et al., 2014). However, neonicotinoids persist in the environment for years and can harm non-target organisms (Frank & Tooker, 2020). Both chemical and biological control are difficult to employ on a large scale; because of this, cultural control strategies are a recent addition to HWA management. Silviculture release by creating canopy gaps can increase the tolerance of hemlocks to HWA (Bentz et al., 2023; Mayfield et al., 2023). Moreover, host plant resistance is a possible addition to HWA integrated pest management. Selecting and breeding for resistant traits can work with biological control, chemical control, and silviculture as valuable tools in reducing HWA populations and keeping hemlocks in our ecosystems.

Tree resistance occurs by three mechanisms: antibiosis, antixenosis, and tolerance (Painter, 1951). Resistance in the form of antibiosis negatively affects the pest's biology. Antibiotic resistance can be seen within the tree as the changing of chemical compounds to inhibit insect growth and development. For example, Chinese hemlocks (*Tsuga chinensis* (Franch.) E. Pritz.) are found to have high levels of sesquiterpenes that reduce HWA density and oviposition (Lagalante and Montgomery 2003; Tredici and Kitajima 2004). Resistance in the form of antixenosis means the tree has physical or chemical characteristics to deter pests from feeding. For example, Chinese, northern Japanese (*T. diversifolia* Masters*),* and southern Japanese (*T. sieboldii* Carrière) hemlocks have thicker cuticles and harder leaf tissue that make it difficult for HWA to insert their stylet (Oten et al. 2012; Ayayee, et al., 2014). Trees can also exhibit tolerance as a mechanism of resistance. Asian hemlocks and their hybrids with Carolina hemlock can tolerate HWA because they can continue growing even with high HWA infestations (Montgomery et al., 2009).

Eastern hemlocks have not demonstrated resistance to HWA. However, some eastern hemlocks are still standing and seemingly healthy in areas where most have been killed by HWA. These seemingly healthy trees are known as lingering hemlocks. They have been found to have more chemical defenses (terpenes), lower HWA densities, and more growth than susceptible eastern hemlocks (McKenzie et al. 2014; Casewell et al. 2008; Kinahan et al. 2020a). The current hypothesis is that lingering hemlocks have some form of resistance to HWA but the mechanisms of this resistance are unknown.

We evaluated 35 lingering hemlock genotypes from North Carolina and Virginia for resistance to HWA. We conducted experiments comparing southern Appalachian lingering hemlocks against wild-type and hybrid controls to determine if resistance mechanisms, antibiosis, antixenosis, tolerance, or a combination are used. With antibiosis in mind, we wanted to determine whether lingering hemlock genotypes reduce HWA density compared to the wild-type control. If resistant genotypes are utilizing antibiosis we would expect to see a larger decrease in HWA density on these genotypes. We also wanted to determine whether HWA prefer wild-type control genotypes over lingering hemlocks. Resistant genotypes utilizing antixenosis may not be fed upon compared to a susceptible eastern hemlock. We then wanted to determine whether lingering hemlocks grow more compared to wild-type controls while under HWA infestation. Genotypes growing and developing while infested may be exhibiting tolerance to HWA feeding. Previous observational studies (Casewell, 2008; unpublished Klein thesis chapter 2) have indicated some lingering hemlock genotypes demonstrate initial signs of resistance. Identifying which mechanisms of resistance are employed by lingering hemlocks can lead to enhanced traits in resistance breeding. This can provide a better selection of resistant propagation material for reforestation efforts and revitalizing the hemlock nursery industry.

Materials and Methods

Between 2014 and 2017, vegetative cuttings were collected from 41 lingering eastern hemlock individuals across 9 distinct locations in North Carolina and Virginia (Figure 2.1, Table 2.1) and propagated as rooted cuttings under nursery conditions for at least 6 years at the Mountain Research Station in Waynesville, NC. Rooted cuttings were transplanted into Trade 2 gallon pots (Nursery Supplies Inc., Kissimmee, FL) filled with aged southern pine bark fines amended with added limestone and maintained in a gothic arch cold frame with automated roll-up sides and an exhaust fan. Here, the trees were exposed to natural infestations of HWA. Genotypes BLRI, SPSF, JRSP and SHHI were artificially infested in 2015 for a study starting in

2017. We fertilized cuttings with a resin-coated slow-release granule that contained nitrogen, phosphorus, and potassium in a ratio of 3:1:2 (Osmocote Blend 7-5-11, ICL, St. Louis, MO). In this study, we evaluated 42 total genotypes; 35 were lingering hemlock genotypes, one was a group of eastern hemlocks grown from wild-collected seed by the North Carolina Forest Service State Nursery used as a wild-type control, another was an experimental cross of genotype CGR (*T. canadensis*) with a Sargent's weeping hemlock (*T. canadensis* 'Sargentii'), and five hybrid crosses with assumed resistance since one of their parents is known to resist or tolerate HWA (Table 2.2). Hybrids were genetically confirmed using SSR analysis. Crosses were grown from seed produced in 2012, 2014, 2015, or 2017. Tree heights ranged from 60 cm to 153 cm in 2023 at the onset of this study.

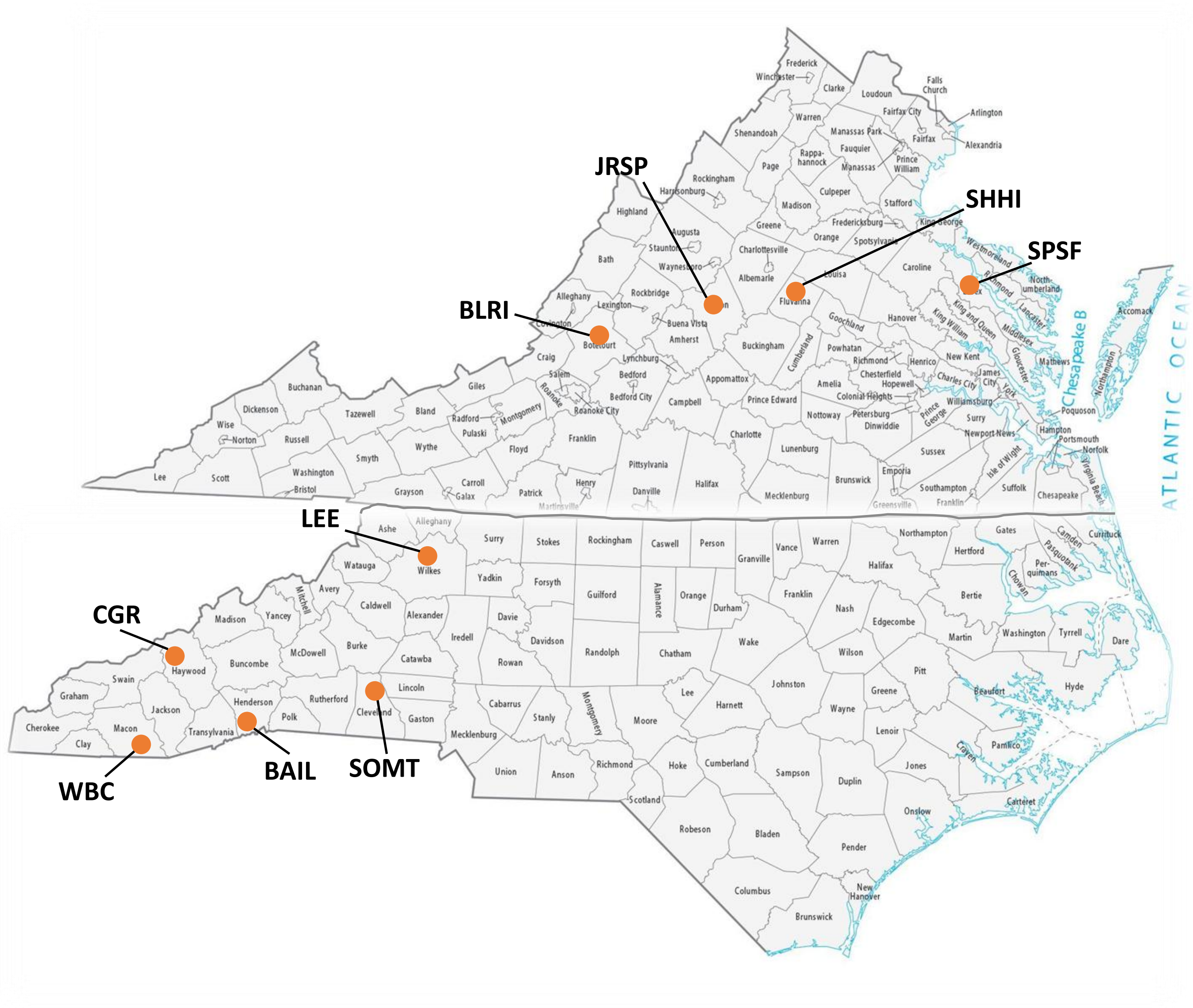

Figure 3.1. Geographic origins of lingering hemlocks in North Carolina and Virginia. Location codes for Virginia: BLRI=Blue Ridge in Munford; VA, JRSP= James River State Park in Norwood, VA; SPSF= Sandy Point State Forest in Log Landing, VA; SHHI= Stage Junction, VA . Location codes for North Carolina: CGR= Maggie Valley, NC; LEE= Lee stand in Hays, NC; SOMT= South Mountains State Park in Casar, NC; BAIL= Bailey property in Flat Rock, NC; WBC= Williams Big Creek in Highlands, NC.

Table 3.1 Lingering hemlock location codes and origin locations.

Tree Code	Location	County	State	Coordinates
BAIL	Property in Flat Rock	Henderson	NC	35.27418341, -82.46172775
BLRI	Munford	Botetourt	VA	37.5801667, -79.33298333
CGR	Maggie Valley	Haywood	NC	35.559865, -83.099644
JRSP	James River State Park	Nelson	VA	37.639016, -78.80378
LEE	Hays	Wilkes	NC	36.274418, -81.050194
SHHI	Stage Junction	Fluvanna	VA	37.812472, -78.145508
SPSF	Sandy Point State Forest	Essex	VA	37.680669, -76.922447
SOMT	South Mountains State Park	Cleveland	NC	35.58558, -81.65457
WBC	Highlands	Macon	NC	35.084262, -83.205355

Table 3.2. Genetically confirmed hybrid genotypes and parentage evaluated in this study.

ID Code	Parentage (Maternal x Pollen)
TcarxTchin103	***Tsuga caroliniana* x *Tsuga chinensis***
TcarxTsieb19	***Tsuga caroliniana* x *Tsuga sieboldii***
TcarxTchin20	***Tsuga caroliniana* x *Tsuga chinensis***
TcarxTsiebD3.1	***Tsuga caroliniana* x *Tsuga sieboldii***
TcarxTsiebD3.2	***Tsuga caroliniana* x *Tsuga sieboldii***

Antibiosis and Tolerance

In February 2024, we randomly selected four propagated trees (ramets) of 35 lingering hemlock genotypes, five hybrid genotypes, and one wild-type control group. We used eight individual trees in our wild-type control group to account for variation in natural resistance since they were not clones. We randomly selected and measured two, 30 cm (±5 cm) branches that had sufficient growth, minimal HWA infestation, and could be caged for 4-8 months. These measurements were recorded as initial growth data. We infested each branch with HWA collected from a hedgerow on the research station property (35.489167, -82.969167). We attached cuttings that contained 10 ovisacs to each experimental branch with a bobby pin and adjusted the number of ovisacs if the branch was already infested. We caged the two branches on each tree with an organza bag that was 42 cm long and 10 cm wide, so the crawlers would not escape. The open end of each bag was wrapped around the branch to seal it and clamped to the tree with a binder clip. We differentiated between the two cages with pink and yellow flagging tape to help with counting in the future.

In May 2024, we counted nymphs of the progrediens generation on each branch for initial HWA population density. To do this we removed the binder clip and slid the cage off the branch. We removed the dead infested material with the bobby pin and counted living HWA with optivisors on the top and bottom of the branch. We recorded counts for the two branches of each tree and put the cage back in place.

In June 2024, we returned to count HWA progrediens adults to assess change in HWA density and move our cages to new branches to repeat this experiment for the sistens generation. Genotypes that reduced HWA denisty more than the wild-type control were classified as exhibiting antibiosis. When moving cages, individual trees were discarded if they were dead or dying because they would not last for another 8 months. We returned in October 2024 to count sistens nymphs and re-measured the progrediens branches that were caged to calculated growth increments for each tree. This was used to assess new branch growth or dieback relative to HWA density during February 2024 to October 2024. We classified genotypes that grew more than the wild-type control as exhibiting tolerance. In February 2025, we returned to count adults of the sistens generation and re-measured the caged sistens branches to assess branch growth or dieback during June 2024 to February 2025.

Antixenosis

In June 2024, we categorized ten lingering hemlock genotypes as ‘high-potential’ from preliminary data gathered from 2024 antibiosis experiment. Genotypes BAIL2, BLRI1, BLRI12, JRSP1, JRSP4, SHHI2, SPSF1, and SPSF4 seemed to exhibit resistance by having low HWA adult counts. We took five, 15 cm cuttings from each tree and five cuttings from a hybrid genotype (TcarxTchin20) and 11 cuttings from five randomly selected wild-type control trees. Cuttings from each genotype were taken from a single healthy tree. There were no replications since all cuttings came from the same tree. Cuttings were wrapped in wet paper towels, bagged and brought back to North Carolina State University in Raleigh, NC in a cooler.

In the lab, we cleaned cuttings in a mixture of 15 mL dish soap per 1 gallon of water and rinsed in 1 gallon of water and left to dry. We modified 50 mL falcon tubes by cutting off the bottom to fit on top of a floral water pick and put a quarter sized hole in the lid so the cuttings could respirate. Each genotype cutting was paired with a wild-type control cutting. The paired cuttings were placed in 50 mL tubes and needles were stripped from the bottom stem so they could be inserted into the water pick. We sealed the hole at the bottom of the tube by wrapping the stems of the paired cuttings with cotton. Four cm of the stems were left sticking out of the bottom and inserted into the water pick (Figure 3.2). Two HWA ovisacs were placed in the middle of both cuttings inside the tube. We placed a five cm by five cm organza square on top of the tube before sealing it with the modified lid. The cotton and mesh were used so the crawlers would not escape. Water picks were filled with tap water. We checked water levels every two days for the duration of the experiment. Grids were made using string, skewers, and a Styrofoam base to hold the tubes and water picks in place for the duration of the experiment. Each grid held 9 tubes, so we used a random number generator to block the placement of each tube. We randomized 55 tubes and 8 blank spaces. After six days we ended the trial and counted the settled crawlers on each cutting. We classified genotypes as exhibiting antixenosis when HWA chose the wild-type over the lingering cutting.

We repeated the preference trial in February 2025 using ovisacs from the sistens generation. We took cuttings from the four genotypes with ‘high-potential’ (BAIL2, BLRI1, JRSP5, and SOMT1) and one genotype with ‘low-potential’ (BLRI12) from our analyzed antibiosis experiment in addition to our resistant control (TcarxTchin20) and our wild-type controls. Resistant genotypes were selected based on significant decreases in HWA counts from nymphs to adults in our progrediens antibiosis experiment 2024. We took one cutting from each of the four ramets of each lingering and hybrid genotype to get four replications per genotype. Simultaneously, we took five cuttings from the eight control trees to ensure we had enough plant material to pair with the lingering and hybrid genotypes. We used the random number generator to create a block design. After seven days, the trial ended, and we counted the settled crawlers on each cutting.

Data Analysis

For antibiosis, we fit a linear model (LM) to test our null hypothesis that there is no difference in reduction of HWA density across each genotype. The LMs were conducted with the Fit Model procedure in JMP Pro 18 (JMP Statistical Discovery LLC, Cary, NC) on proportion change in HWA density and genotype. We also fit a LM for tolerance to test our null hypothesis that each genotype grows the same under HWA infestation. This LM was conducted on growth increment and genotype. F-tests for fixed effects were considered significant when $P < 0.05$.

For antixenosis, a binomial was fit to test our null hypothesis that HWA equally choose susceptible and resistant hemlocks. We conducted the binomial with the Fit Model procedure in JMP Pro 18 (JMP Statistical Discovery LLC, Cary, NC) on proportion of HWA that chose the control and genotype. We added the total number of HWA present as the frequency to refine the proportions. ChiSquares for effect tests were considered significant when Prob>Chisq < 0.05.

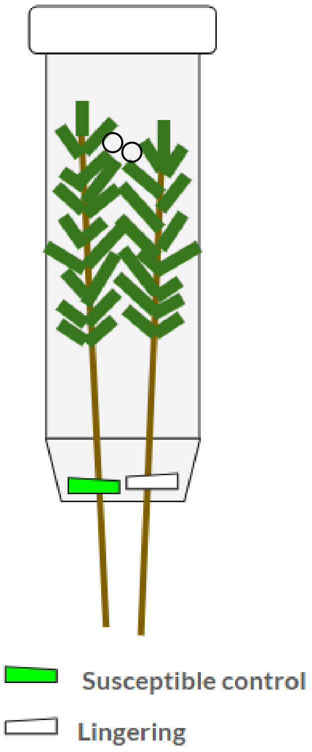

Figure 3.2. Example of 50 mL Falcon tube with infested control and lingering hemlock cuttings used for preference trials in June 2024 and February 2025.

Results

Antibiosis and Tolerance

February 2024-June 2024

The LM fit for proportion change in progrediens HWA density was significant ($F_{32,99}$=5.29; P <0.0001*). In the progrediens generation of the antibiosis experiment, the proportion change of HWA from nymphs to adults ranged from -1 to -0.3 (Figure 3.3). The hybrid control (TcarxTchin20) reduced HWA by 100%. The wild-type control reduced HWA the least by 28.9%. All other genotypes were between the range of the hybrid control (TcarxTchin20) and the wild-type control. Four hybrid genotypes, TcanCGRxTcanSarg, and 35 lingering genotypes reduced HWA density by more than 50%.

The LM fit for genotype growth increment was significant ($F_{29,89}$= 4.56; P <0.0001*). Mean growth increments across 30 different genotypes ranged from -2.4 cm to 84.8 cm between February 2024 and October 2024 (Figure 3.4). Genotypes TcarxTchin20, TcanCGRxTcanSarg, TcarxTsiebD3.1, CGR1, TcarxTsiebD3.2, BAIL3, TcarxTchin103, BLRI12, JRSP4, BLRI5, and SOMT1 showed increased growth compared to the wild-type control. These genotypes included all five hybrid genotypes, 6 lingering hemlock genotypes and the experimental cross between lingering genotype 'CGR' and a weeping hemlock. Genotype BAIL2 reduced growth by 2.4 cm.

June 2024-February 2025

The LM fit for proportion change in sistens HWA density was significant ($F_{29,89}$= 1.55; P= 0.0205*). In the sistens generation of the antibiosis experiment, proportion change of HWA nymphs to adults ranged from -0.9 to 0 (Figure 3.5). The wild-type control had the largest change and reduced HWA density by 87.7%. No genotypes reduced HWA more than the wild-type control. Three hybrid genotypes, TcanCGRxTcanSarg, and 12 lingering hemlock genotypes reduced HWA density more than 50%. Genotype BLRI6 did not reduce HWA density (0%) in the sistens generation.

The LM fit for genotype growth increment was significant ($F_{30,88}$= 1.70; P= 0.0289*). Mean growth increments across 30 different genotypes ranged from -4.67 cm to 6.375 cm between June 2024 and February 2025 (Figure 3.6). Genotypes TcarxTsiebD3.1 and TcarxTchin20 (Hybrid control) showed increased growth compared to the wild-type control. Genotypes JRSP4, SOMT1, LEE33, SHHI2, BLRI1, BLRI11, BLRI5, LEE48, BLRI7, SHHI3, and LEE42 experienced branch dieback ranging from -0.19 cm to -4.67 cm.

Antixenosis

In 2024 and 2025, HWA crawlers preferred the wild-type control over genotypes BAIL2 and Hybrid (Figures 3.7 and 3.8). The binomials fit for each year were significant (2024-ChiSquare= 77.08; Prob>ChiSq= <0.0001* and 2025-ChiSquare= 51.67; Prob>ChiSq= <0.0001*). In 2024, 91.9% of HWA chose the wild-type control over BAIL2 and 65.6% of HWA chose the wild-type control over Hybrid (TcarxTchin20). In 2025, 69.4% of HWA chose the wild-type control over BAIL2 and 87.4% of HWA chose the wild-type control over Hybrid (TcarxTchin20). In contrast, BLRI12 and JRSP1 were preferred over the wild-type control in 2024 and BLRI1 was preferred over the wild-type control in 2025.

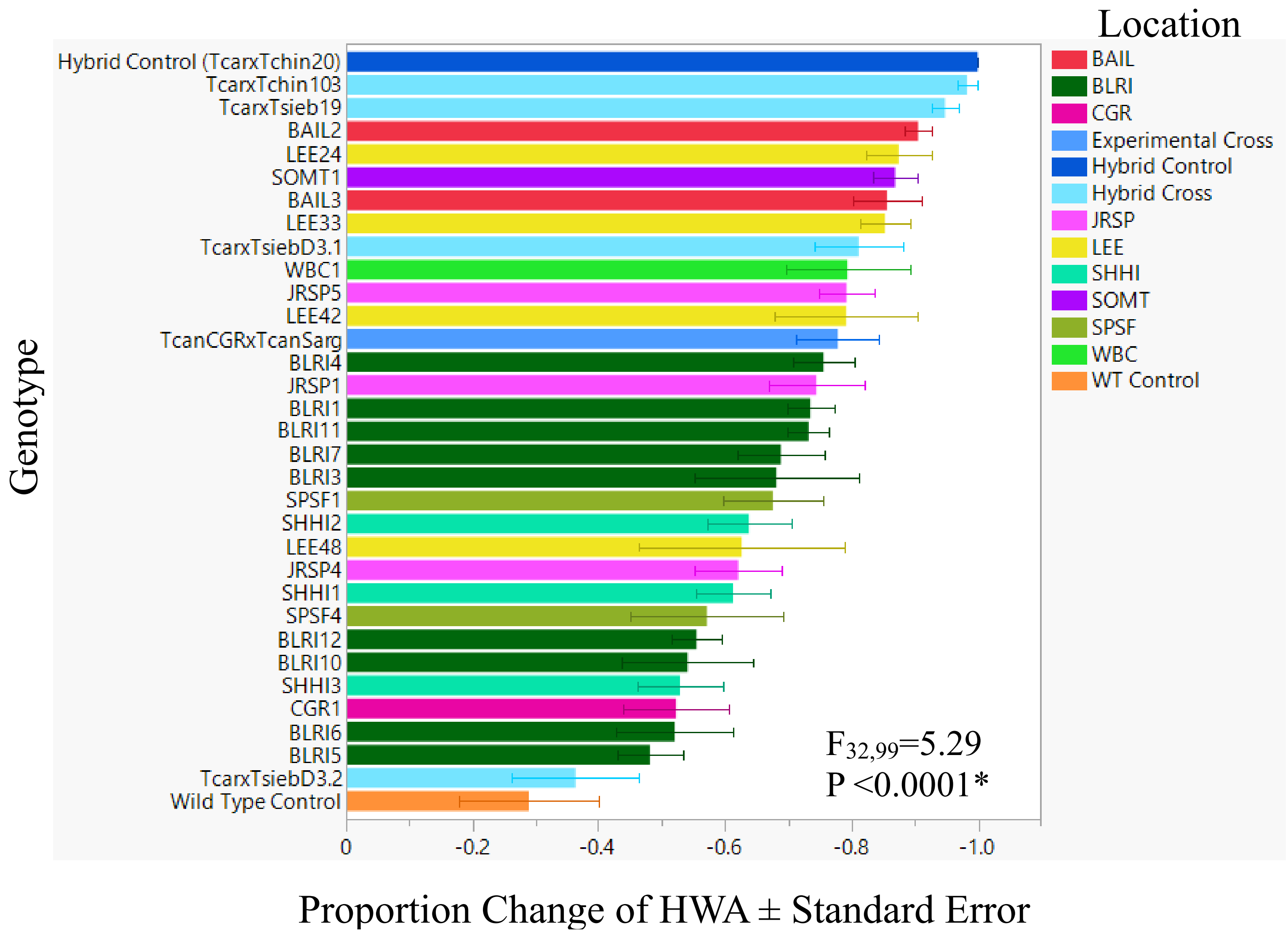

Figure 3.3. Average proportion change of HWA density (nymph to adult) during the progrediens generation (February 2024-June 2024) ± Standard Error.

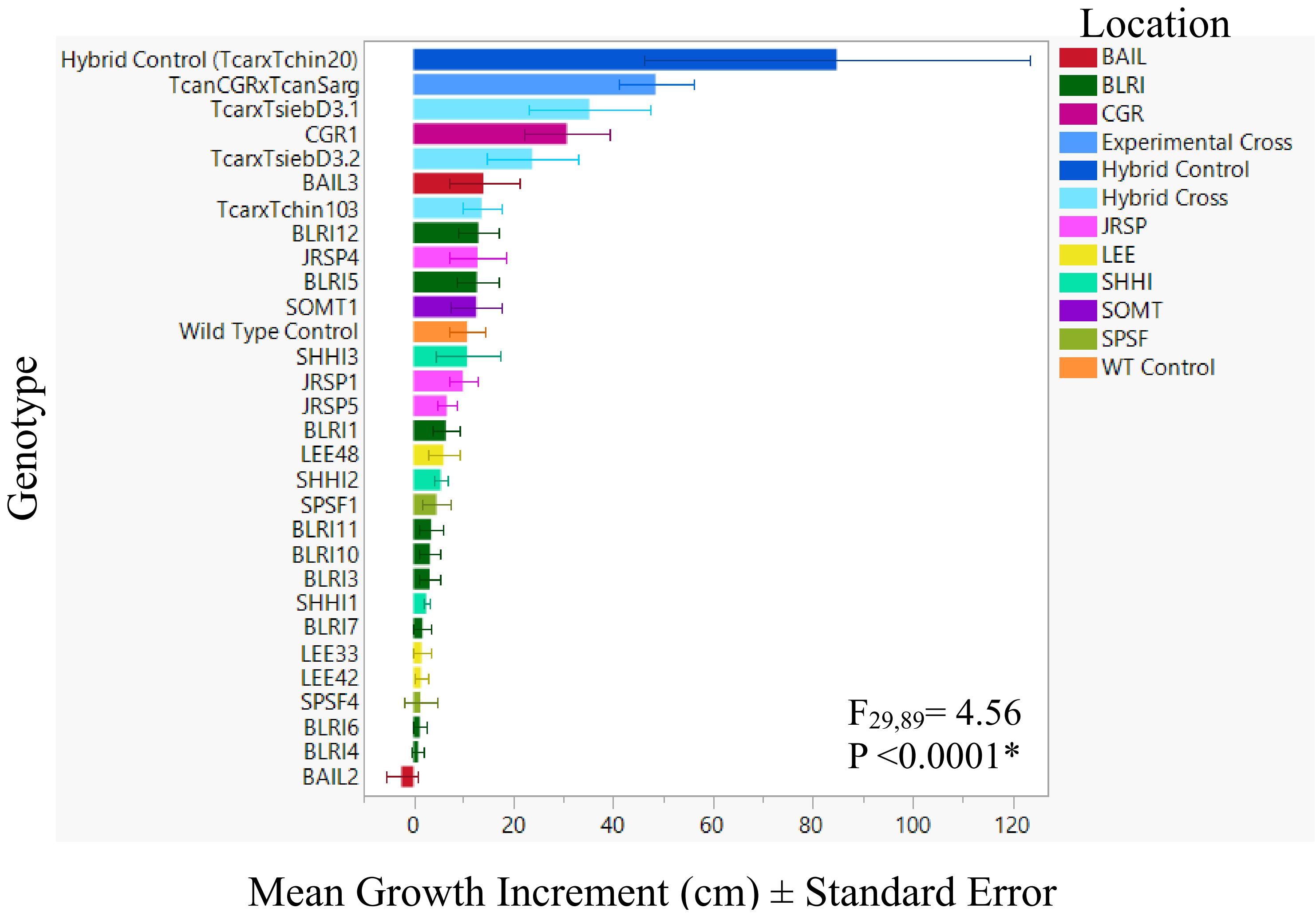

Figure 3.4. Average growth increment in cm per genotype infestation with ten HWA ovisacs (February 2024- October 2024) ± Standard Error.

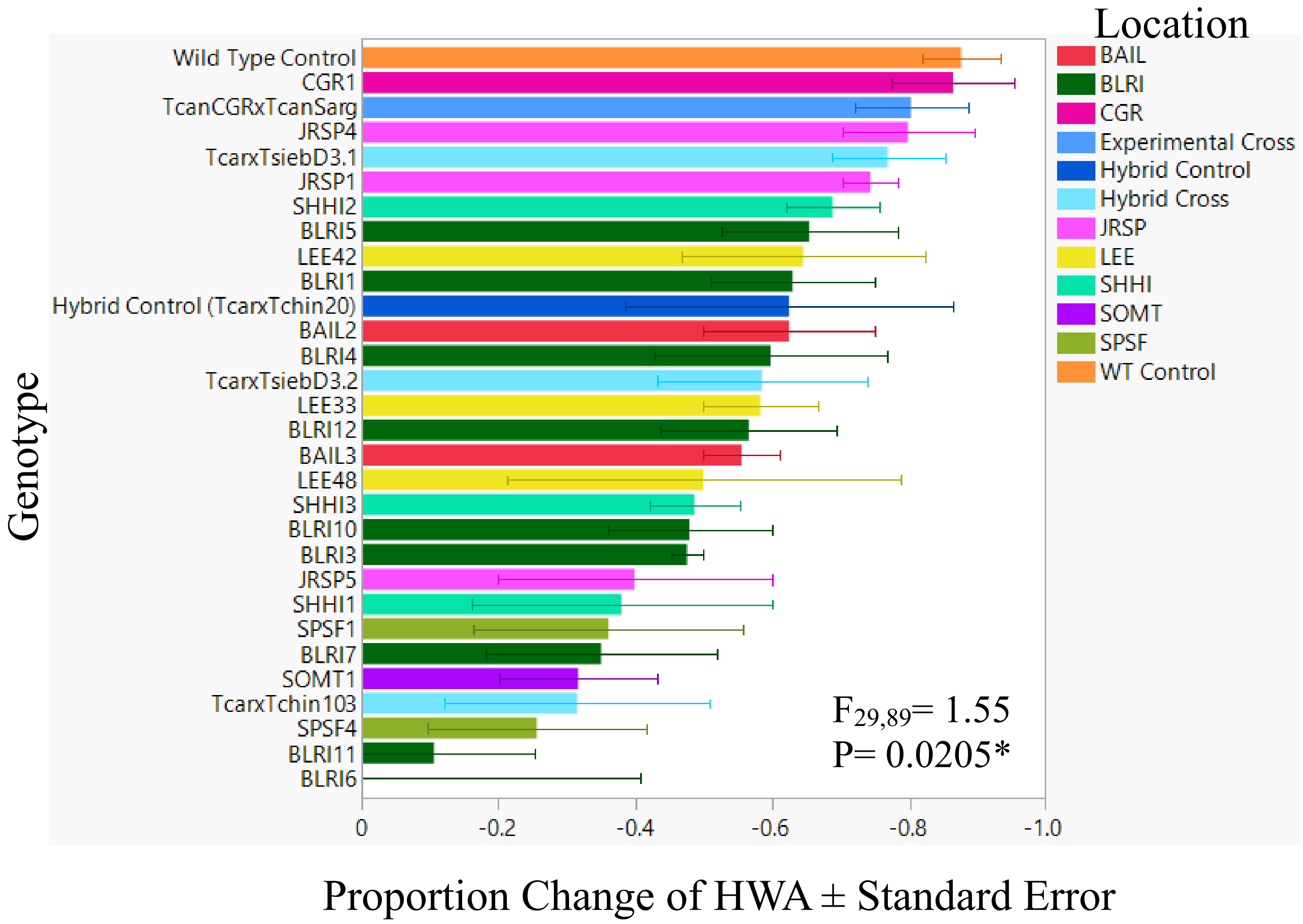

Figure 3.5. Average proportion change of HWA density (nymph to adult) during the sistens (overwintering) generation (June 2024-February 2025) ± Standard Error.

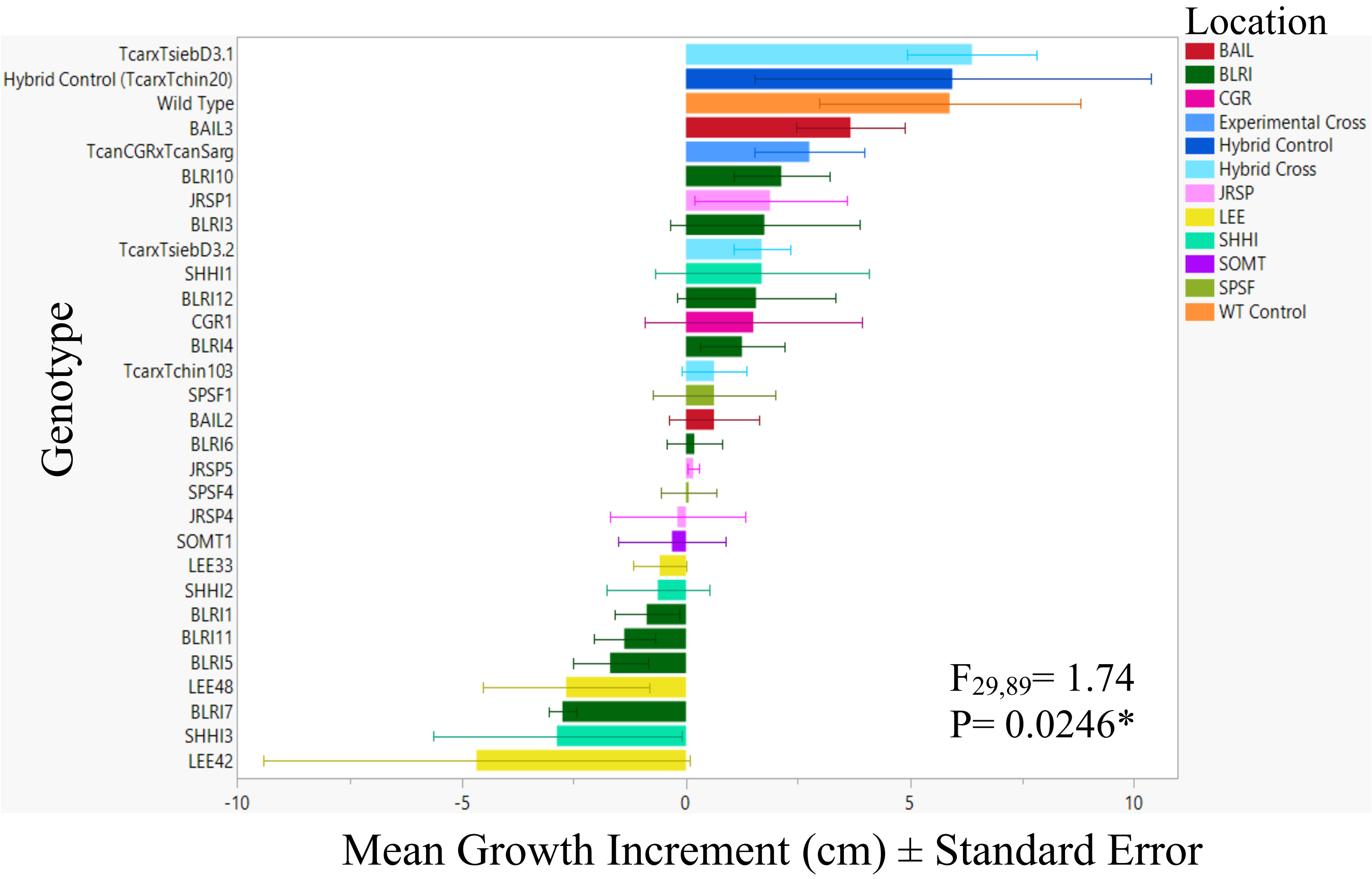

Figure 3.6. Average growth increment in cm per genotype infestation with ten HWA ovisacs (June 2024 - February 2025) ± Standard Error.

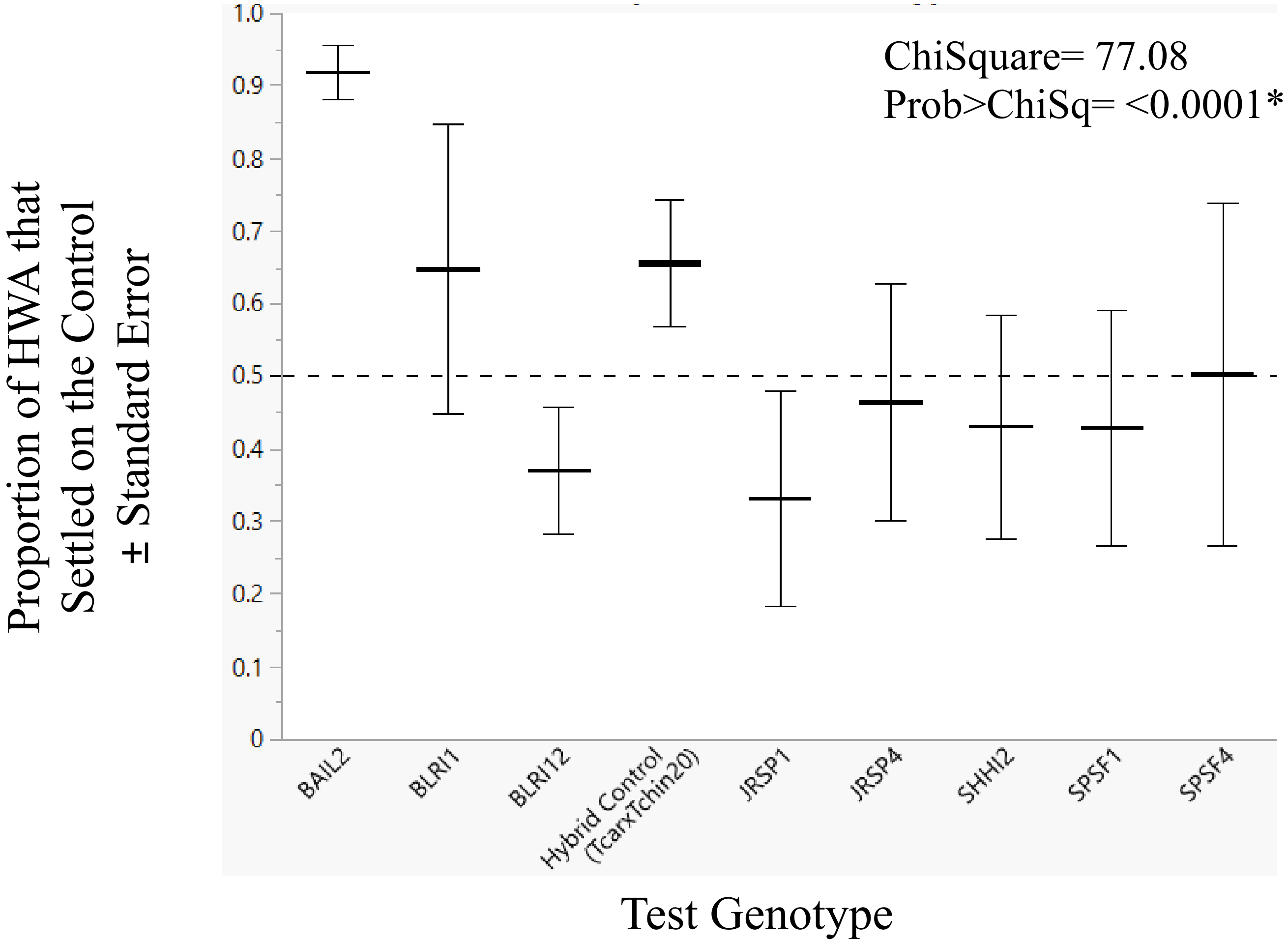

Figure 3.7. Average proportion of HWA crawlers that settled on the wild-type control during the June 2024 preference experiment ± Standard Error.

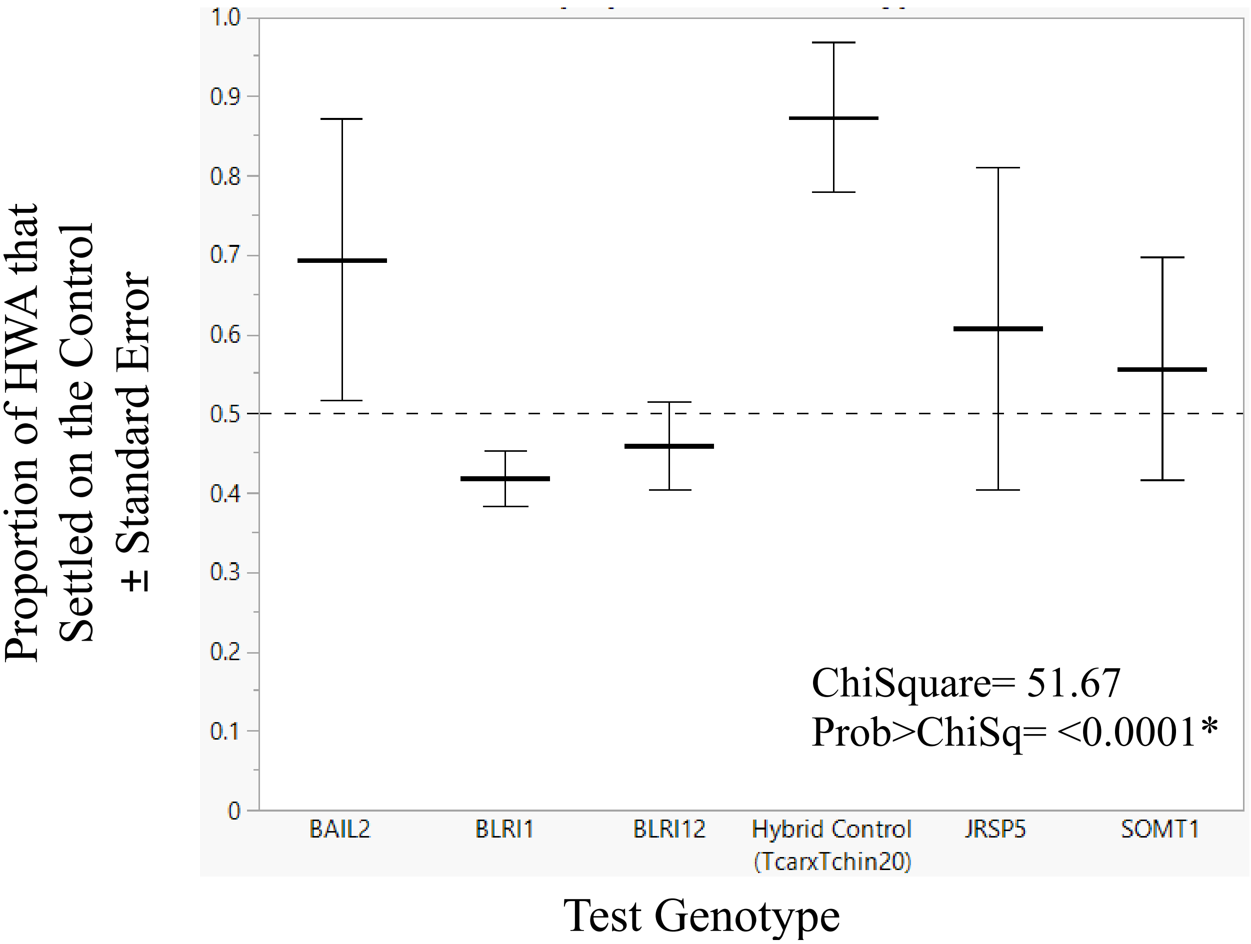

Figure 3.8. Average proportion of HWA crawlers that settled on the wild-type control during the February 2025 preference experiment ± Standard Error.

Discussion

This study tested lingering hemlock genotypes from the Southern Appalachians for three mechanisms of resistance: antibiosis, antixenosis, and tolerance. We found lingering hemlocks that may be utilizing each of the mechanisms of resistance (or a combination of mechanisms); however, more replications and trials are needed to gather further evidence of this. We considered resistance as antibiosis when lingering genotypes reduced HWA density more than the wild-type control, antixenosis when HWA preferred wild-type control over a lingering genotype, and tolerance when genotypes produced more new growth compared to the wild-type control while infested.

We found 26 of the 33 genotypes tested exhibited antibiosis, one lingering genotype exhibited antixenosis, and 7 lingering genotypes exhibited tolerance. All lingering hemlock genotypes, hybrid genotypes, and the experimental cross are outlined according to mechanism tested (Table 3.3). Hopefully this information provides useful when looking for resistant lingering hemlocks in the future. More tests should be conducted in a larger field setting to see if the trees are still exhibiting resistance mechanisms at full size.

When considering antibiosis as a mechanism of resistance in lingering hemlock genotypes, all genotypes reduced HWA density more than the wild-type in the progrediens generation but the wild-type showed the greatest reduction in the sistens generation. This may be evidence of the boom-bust cycle of HWA populations—an initial surge in population size followed by a sharp decline, likely due to resource depletion or environmental pressures.

Hemlock woolly adelgid density is partially determined by needle base availability and thus is affected by a boom-bust cycle (Young et al., 1995). Following a new infestation, HWA density rapidly increases on a tree. Each crawler finds a place to settle at a needle base and begins feeding. During the first year, the infested hemlock does not produce much, if any, new growth, as its resources are depleted from HWA feeding. The next year, the new generation of HWA does not have anywhere to feed and populations decline. The tree recovers, exhibited by new growth, which attracts the next generation of HWA, and the cycle continues (McClure, 1991).

We tried to control HWA populations on each tree by artificially infesting each with 10 ovisacs. Hemlock woolly adelgid is a species that prioritizes high reproductive rates with a range of 50 to 300 eggs in each ovisac; thus, a natural density-dependent mortality occurs at each life stage (Young et al., 1995; Havill et al., 2016). Trees which consistently reduce HWA across multiple life stages may indicate resistance. By artificially infesting and switching branches within each generation, we exposed some branches to HWA feeding for the first time. A hemlock's defense response develops and changes with HWA feeding (Pezet et al., 2013). Chemicals like sesquiterpenes also develop with tree age and differ between tree parts (Pezet et al., 2013; Pezet & Elkinton, 2014). Introducing HWA to a new part of the tree may not have allowed enough time for induced defenses to develop. A longer period of HWA establishment may have been required to observe a reduction substantial enough to classify the tree as resistant. Observing multiple generations of HWA on the same branch or tree may give better insight into which genotypes are exhibiting antibiosis as a mechanism of resistance.

Genotypes exhibiting greater growth than the wild-type control may possess greater tolerance to HWA feeding. In the progrediens generation, six lingering hemlock genotypes outgrew the wild-type control, suggesting potential for comensatory growth. In contrast, no lingering genotypes grew more than the wild-type in the sistens generation. Wild-type genotypes may take longer to react to HWA feeding which is why we see a spike in growth and HWA reduction in the overwintering sistens generation. Future studies should test whether they can outgrow HWA feeding.

When considering antixenosis as a mechanism of resistance, we looked for HWA preference for the wild-type control. Susceptible eastern hemlocks have thin cuticles and soft leaf cushions, facilitating stylet insertion and feeding (Oten et al., 2012; Ayayee et al., 2014). In contrast, resistant hemlocks, like the Chinese hemlock, have thick cuticles and firm leaf cushions, which makes it harder for HWA to feed (Oten et al., 2012; Ayayee et al., 2014). In both sistens and progrediens preference assays, HWA crawlers consistently chose the wild-type control over both the hybrid control (TcarxTchin20) and lingering genotype BAIL2. The hybrid, a cross between Carolina hemlock and Chinese hemlock, has previously demonsttrated robust needle bases though not as protected and well-defended as Chinese hemlocks, it is significantly stronger than susceptible eastern hemlocks.

Lingering hemlock cuticles and leaf cushions have not yet been studied. Further testing on genotype BAIL2 should look at its cuticles and leaf cushions to see if lingering hemlocks differ between resistant species and susceptible eastern hemlocks. This may detect why HWA prefer the wild-type control over certain genotypes. Lingering hemlock genotypes JRSP5 and SOMT1 should also be considered in future studies. While HWA did not demonstrate significant preference for the wild-type control over these genotypes in 2025, there was a trend in that direction deserving of further research. As a laboratory experiment, HWA were unable to feed and grow to adults. It may be worthwhile to monitor HWA crawler preference across multiple generations to track consistency and HWA growth and development. This may also show that lingering genotypes utilize more than one mechanism to survive HWA feeding.

Some lingering hemlock genotypes exhibited multiple mechanisms of resistance. Notably, the resistant hybrid (TcarxTchin20) was the only genotype that exhibited all three mechanisms of resistance. Genotype BAIL2 showed evidence of both antibiosis–through reduced HWA densities–and antixenosis, as it was less preferred than the wild-type control. Interestingly, BAIL2 did not have sufficient growth across both generations, suggesting a potential trade-off between defense and growth. This aligns with findings that investment in defensive traits can divert resources away from growth (Eyles et al., 2010). The presence of multiple resistance mechanisms may act synergistically to protect hemlocks from HWA damage, enhancing tree survival and longevity.

This project began as an effort to document lingering hemlock trees in the southeastern United States. Over time, we expanded our aims, ultimately indentifying promising candidates for future resistance breeding and propagation programs. By identifying possible resistance mechanisms that help these trees deal with HWA feeding, we can better understand what the tree needs to survive. To conclusively demonstrate resistance in any of these genotypes, years of continued testing over multiple generations are needed to see which genotypes consistently grow and reduce HWA density. In conjunction with biological control, chemical control, and silviculture practices, these resistant mechanisms can help to reestablish hemlock forests, restore the hemlock nursery industry, and conserve a valuable ecosystem.

Table 3.3 Tested genotypes and the mechanisms of resistance they presented.

Tree Code	Antibiosis	Antixenosis	Tolerance
TcarxTchin103	Reduced HWA density more than WT control in progrediens generation.	Not tested.	Grew more than WT control Feb 24-Oct 24.
TcarxTsieb19	Reduced HWA density more than WT control in progrediens generation.	Not tested.	Not tested.
TcarxTchin20 (Hybrid Control)	Reduced HWA density by 100% in progrediens generation.	HWA preferred control in progrediens and sistens generation.	Increased growth by 84 cm between Feb 24 and Oct 24 and 5.9 cm between June 24 and Feb 25 both were more than WT control.
TcarxTsiebD3.1	Reduced HWA density more than WT control in progrediens generation.	Not tested.	Grew more than WT control Feb 24-Oct 24 and June 24-Feb 25.
TcarxTsiebD3.2	Reduced HWA density more than WT control in progrediens generation.	Not tested.	Grew more than WT control Feb 24-Oct 24.
BAIL2	Reduced HWA density more than WT control in progrediens generation.	HWA preferred control in 2024 and 2025.	
BAIL3	Reduced HWA density more than WT control in progrediens generation.	Not tested.	
BLRI1	Reduced HWA density more than WT control in progrediens generation.	No preference in 2024. HWA preferred the	

		test genotype in 2025.	
BLRI3	Reduced HWA density more than WT control in progrediens generation.	Not tested.	Grew more than WT control Feb 24-Oct 24.
BLRI4	Reduced HWA density more than WT control in progrediens generation.	Not tested.	
BLRI5	Reduced HWA density more than WT control in progrediens generation.	Not tested.	Grew more than WT control Feb 24-Oct 24.
BLRI6	Reduced HWA density more than WT control in progrediens generation. Did not reduce HWA density in the sistens generation.	Not tested.	
BLRI7	Reduced HWA density more than WT control in progrediens generation.	Not tested.	
BLRI10	Reduced HWA density more than WT control in progrediens generation.	Not tested.	
BLRI11	Reduced HWA density more than WT control in progrediens generation.	Not tested.	
BLRI12	Reduced HWA density more than WT control in progrediens generation.	HWA preferred the test genotype in 2024. No preference in 2025.	Grew more than WT control Feb 24-Oct 24.
CGR1	Reduced HWA density more than WT control in progrediens generation.	Not tested.	Grew more than WT control Feb 24-Oct 24.
JRSP1	Reduced HWA density more than WT control	HWA preferred the test genotype	

	in progrediens generation.	in 2024. Not tested in 2025.	
JRSP4	Reduced HWA density more than WT control in progrediens generation.	No preference in 2024. Not tested in 2025.	Grew more than WT control Feb 24-Oct 24.
JRSP5	Reduced HWA density more than WT control in progrediens generation.	Not tested in 2024. No preference in 2025.	
LEE24	Reduced HWA density more than WT control in progrediens generation.	Not tested.	Not tested.
LEE33	Reduced HWA density more than WT control in progrediens generation.	Not tested.	
LEE42	Reduced HWA density more than WT control in progrediens generation.	Not tested.	
LEE48	Reduced HWA density more than WT control in progrediens generation.	Not tested.	
SHHI1	Reduced HWA density more than WT control in progrediens generation.	Not tested.	
SHHI2	Reduced HWA density more than WT control in progrediens generation.	No preference in 2024. Not tested in 2025.	
SHHI3	Reduced HWA density more than WT control in progrediens generation.	Not tested.	
SOMT1	Reduced HWA density more than WT control in progrediens generation.	Not tested in 2024. No preference in 2025.	Grew more than WT control Feb 24-Oct 24.

SPSF1	Reduced HWA density more than WT control in progrediens generation.	No preference in 2024. Not tested in 2025.	
SPSF4	Reduced HWA density more than WT control in progrediens generation.	No preference in 2024. Not tested in 2025.	
WBC1	Reduced HWA density more than WT control in progrediens generation.	Not tested.	Not tested.
TcanCGRxTcanSarg	Reduced HWA density more than WT control in progrediens generation.	Not tested.	Grew more than WT control Feb 24-Oct 24.

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