Chapter 1: Hemlock Woolly Adelgid and its Hemlock Hosts: A Global Perspective

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INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hempitera: Adelgidae), threatens the health and sustainability of the native eastern North American hemlocks, *Tsuga canadensis* (L.) Carrière and *T. caroliniana* Engelman. The lineage of HWA that was introduced to the eastern United States came from Japan sometime prior to 1951, and did not co-evolve with eastern North American forest ecosystems (Havill et al. 2006). As a result, eastern hemlock species cannot adequately resist or tolerate the impacts of adelgid feeding, and the native community of natural enemies in eastern North America is not capable of maintaining HWA populations below damaging levels. This results in widespread death of hemlock trees, which is having serious consequences for biodiversity, ecosystem functions, and ornamental and urban resources.

There are quite a few studies that have examined the effects of HWA on hemlock ecosystems. Hemlocks are the most shade tolerant of conifers (Farjon 1990) and hemlock dominated forests provide a uniquely cool and densely shaded micro-environment. Loss of hemlock because of HWA is changing forest composition and structure (Orwig and Foster 1998, Spaulding and Rieske 2010), nutrient cycling (Kizlinski et al. 2002, Stadler et al. 2006; Nuckolls et al. 2009, Albani et al. 2010, Cobb 2010), and the composition of wildlife communities (Becker et al. 2008, Allen et al. 2009).

Since the late 1980's, there has been a concerted effort to understand HWA biology, evolutionary history, host effects, ecological impacts, and natural enemies, with the goal of finding ways to control this devastating pest. Although progress has been made on all of these fronts, trees continue to die at an alarming rate. Individual trees can be protected with repeated application of insecticidal soap, horticultural oil, or systemic insecticides (Ward et al. 2004), and silvicultural thinning is being evaluated as a way to prolong the health of hemlock stands (Fajvan and Wood 2008), but these interventions are expensive and not sustainable at the landscape scale.

Manipulating hemlock resistance to HWA is another approach with potential to control adelgid populations. Researchers are searching for naturally resistant trees (Ingwell and Preisser 2011), developing resistant crosses between North American and Asian species (Montgomery et al. 2009), and establishing protected plantings to conserve hemlock genetic diversity (Jetton et al. 2011). Unfortunately, restoring forest and urban ecosystems with these trees would take many decades and there is no guarantee that this can be completed in time to safeguard hemlock's unique role in eastern forests. As a consequence, the establishment of effective biological control agents is a critical component of efforts to maintain hemlock resources in eastern North America. Predicting the safety and success of biological control is challenging because natural enemies function within a complex system of multi-species, multi-trophic interactions. In this chapter, we summarize the evolutionary history of the interaction among hemlocks and adelgids. At the end of the chapter, we discuss how this information can help in the selection and establishment of biological control agents to maximize their potential to control HWA populations and minimize undesirable non-target effects.

DIVERSITY AND DISTRIBUTION OF HEMLOCK

There are nine species of hemlock currently accepted (Farjon 1990), five are found in Asia and four in North America (Fig. 1). Hemlock trees grow naturally in cool, humid areas from sea-level to the subalpine zone, depending on the species and region. All species have a strict requirement of adequate soil-moisture throughout the growing season.

Both species of hemlock native to eastern North American are susceptible to HWA. Eastern hemlock, *T. canadensis*, has a broad distribution, spanning from New Brunswick in the north, to Alabama in the south, and west to Minnesota, with isolated disjunct populations to the south and west of its main range. Eastern hemlock is also highly valued as an ornamental, thus HWA is impacting property values throughout the eastern United States (Holmes et al. 2010). The other hemlock species in eastern North America, Carolina hemlock, *Tsuga caroliniana*, has a very limited distribution in the southern United States, and is not commonly planted as an ornamental. It is typically found in small, isolated populations in the southern Appalachians on exposed ridges and rocky outcroppings where it can escape competition from hardwoods (Jetton et al. 2008). Because of its restricted range, Carolina hemlock is at even higher risk from HWA than eastern hemlock.

A recent molecular phylogeny of *Tsuga* provided new information about the diversity, evolutionary relationships, and historical biogeography of hemlock (Havill et al. 2008). The results suggest that in addition to the nine species typically recognized, there are two endemic island species of hemlock that should probably be given species status. Hemlock from Taiwan is often treated as a variety of *T. chinensis* (Franchet) Pritzel in Diels (e.g. Farjon 1990); however, phylogenetic analyses show that this variety is not closely related to hemlocks from mainland China. It appears that Taiwanese hemlock was correctly described as a separate species, *T. formosana*, by Hayata in 1908. Hemlocks on Ullung Island, Korea, a small volcanic island in the Sea of Japan, were previously thought to be *T. sieboldii* Carrière, the southern Japanese hemlock. The Ullung Island hemlocks are actually more closely related to, but distinct from, *T. diversifolia* (Maximowicz) Masters, the northern Japanese hemlock. Work is underway to determine whether the Ullung Island hemlock is distinct enough to be considered a new species.

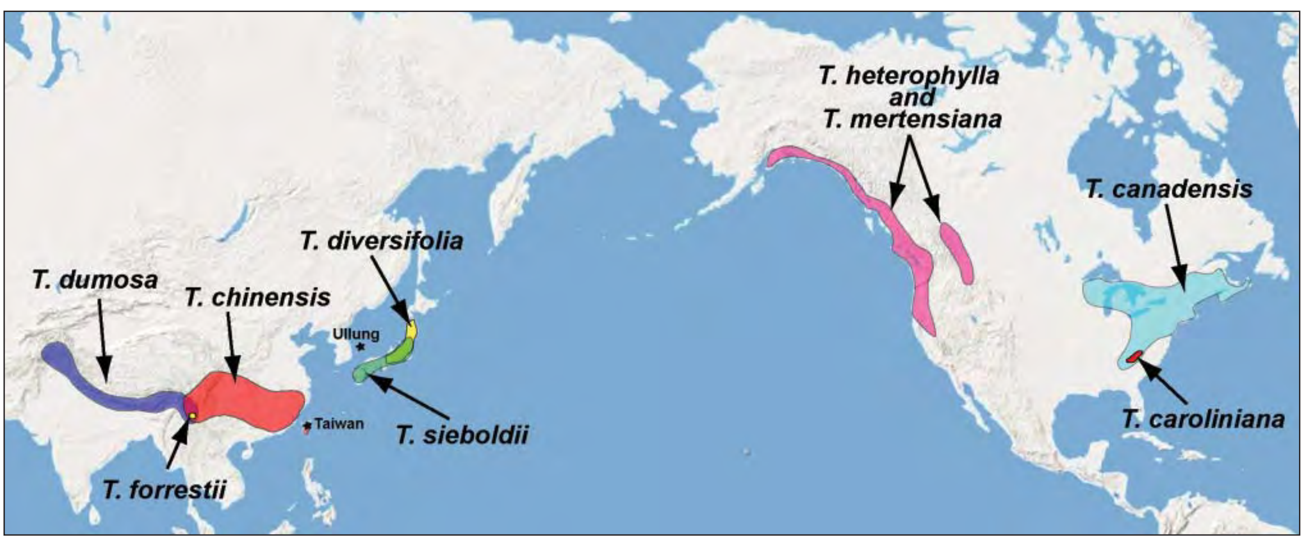

Figure 1. Map showing the distribution of hemlock species worldwide (reprinted with permission from Havill et al. 2008).

Havill et al. (2008) also showed that the two eastern North American hemlocks are not close relatives. *Tsuga canadensis* diverged from all the other hemlocks very early in the evolutionary history of the genus, but *T. caroliniana* diverged more recently and is more closely related to the Asian species. The observation that *T. caroliniana* is closely related to the Asian hemlocks is consistent with its ability to successfully hybridize with the HWA resistant Asian species, whereas attempts to cross *T. canadensis* with other species have failed (Bentz et al. 2002). Hybrids between *T. caroliniana* and *T. chinensis* are resistant to HWA and could be good replacements for *T. canadensis* in the urban environment (Montgomery et al. 2009). *Tsuga chinensis* by itself is also highly resistant to HWA and grows well in the northeast (Del Tredici and Kitajima 2004; Evans 2008; Weston and Harper 2009). Although the two western North American species appear to be resistant or tolerant to HWA, they do not survive well in the east.

Tsuga canadensis has low genetic variation compared to other hemlock species and other eastern North American conifers (Zabinsky 1992; Potter et al. 2007). This could have implications for HWA control if this pattern translates into less natural variation in resistance to HWA. According to the pollen record, there were two periods of rapid decline in eastern hemlock that occurred approximately 9,800 and 5,300 years ago (Zhao et al. 2010). The more recent decline has been attributed to insect feeding (e.g. Bhiry and Filion 1996), but it is more likely that both periods of decline were due to increased variation in temperature and drought that occurred during the early- to mid-Holocene (Foster et al. 2006, Shuman et al. 2009, Zhao et al. 2010). Among conifers, hemlocks are the most susceptible to drought (Farjon 1990), which may explain why the decline of eastern hemlock was more severe than other tree species experiencing the same environmental changes. The pattern of genetic diversity in *T. canadensis* in the southern part of its range suggests that when its range contracted, there was a refuge southeast of the Appalachians out of which the species eventually spread to re-occupy its current distribution (Potter et al. 2007). Carolina hemlock was found to have moderate levels of genetic diversity and the genetic signature of a similar glacial refuge southeast of the Appalachians (Potter et al. 2011). Interestingly, Havill et al. (2008) hypothesized that *T. caroliniana* was closely related to European hemlocks based on an analysis that took into account hemlock phylogeny, molecular dating, the fossil record, and the timing of ancient connections among the continents. Hemlock eventually recovered in eastern North America, but this was not the case in Europe. Hemlock was common throughout Europe until approximately 750,000 years ago when it went extinct due to drier climate and repeated glaciations (LePage 2003; Follieri 2010).

The combination of eastern hemlock's low genetic diversity and relatively narrow site requirements may make the search for resistant trees difficult. With this in mind, Camcore (International Tree Conservation and Domestication, N.C. State University) is collecting seeds from eastern and Carolina hemlocks throughout their ranges, placing them in long-term storage, and growing them in protected plantations to conserve their genetic diversity for future restoration (Jetton et al. 2011). If biological control of HWA is successful, these trees could be used as a source to restore hemlock to eastern forests.

DIVERSITY AND BIOGEOGRAPHY OF HEMLOCK ADELGIDS

HWA has been documented on all hemlock species including those present on Taiwan and Ullung Island (Annand 1924, Takahashi 1937, Inouye 1953, Ghosh 1975, Montgomery et al. 2000). The earliest reports of HWA in North America are from the west coast. The earliest North American specimens were collected in 1907 from South Bend, Washington (U.S. National Collection of Insects, Beltsville, Maryland). Other early records from the west include a report of damage to western hemlocks in Vancouver, British Columbia (Chrystal 1916), and specimens collected in Oregon and California used to formally describe *A. tsugae* as a new species (Annand 1924). In eastern North America, the earliest specimens were collected decades later in

Richmond, Virginia in 1951, and damage on eastern hemlocks was reported in Pennsylvania starting in 1969 (Gouger 1971). Widespread mortality of eastern hemlocks began in the Mid-Atlantic States then spread to southern New England in the mid 1980's. HWA is currently established in more than half of the range of eastern hemlock, occupying 18 states from Maine to Georgia (Fig. 2)

This sequence of records led to the incorrect assumption that HWA was first introduced into western North America in the early 20th century, and then brought to eastern North America from the west some time after that. This is understandable since HWA collected from different regions do not show any obvious morphological differences. It was only after a series of genetic analyses that *A. tsugae* was recognized as a diverse group of related insect lineages with a complex evolutionary history (Havill et al. 2006, Havill et al. 2007, Havill et al. 2009). Molecular dating methods estimated that the diversification of hemlock adelgids began approximately 30 million years ago, which corresponds to when much of the genus *Tsuga* was also diversifying. There are at least six distinct lineages of hemlock adelgids endemic to different parts of the world: one each in China, Taiwan, and western North America, and two in Japan. HWA is also found in India and Nepal, but it is not yet known how these populations relate to the others. We now know that HWA was introduced to the eastern United States directly from Japan, and that the lineage in western North America is native.

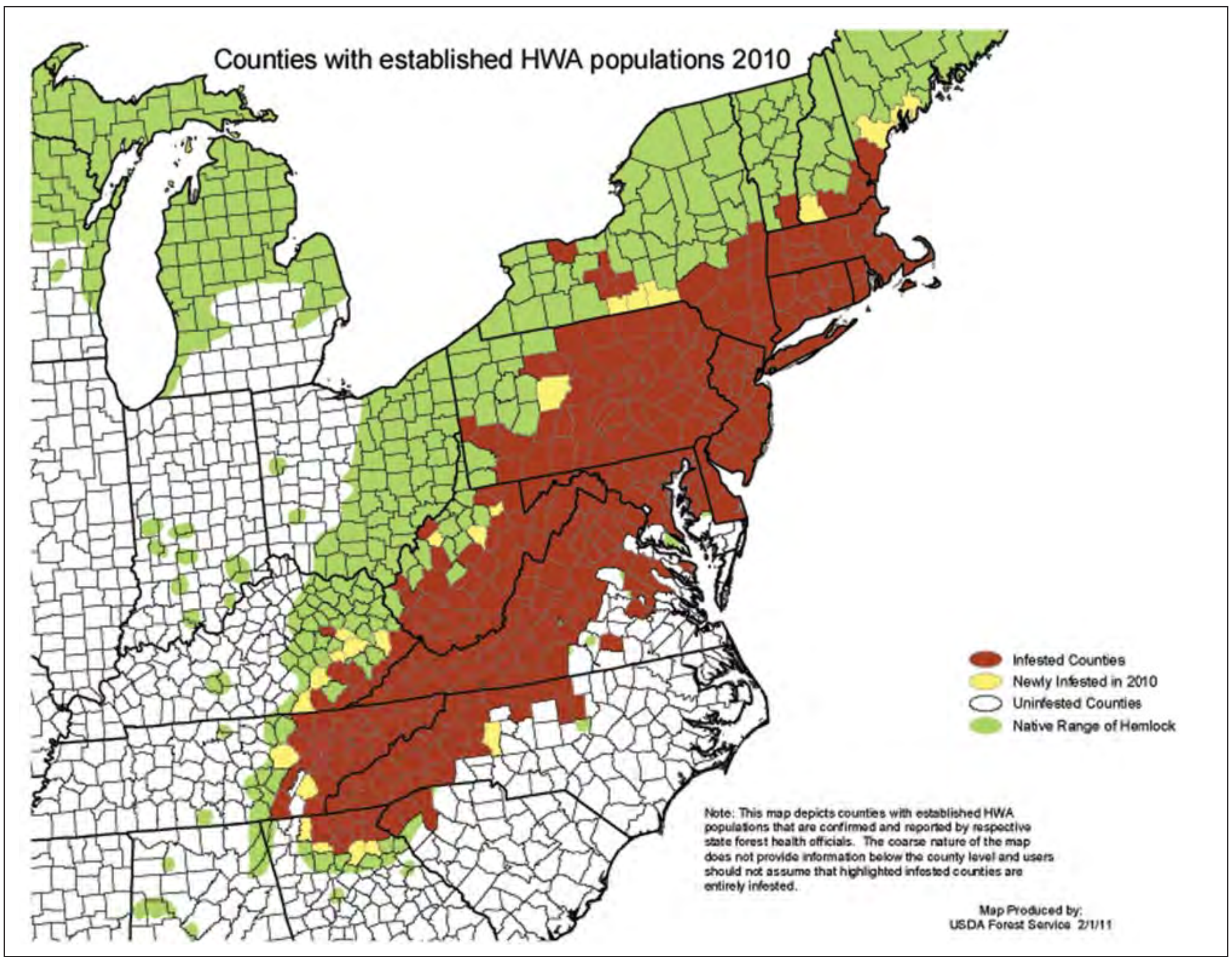

Figure 2. Native range of hemlock in the eastern United States (green) and range of hemlock woolly adelgid (brown) in 2010.

In Japan, there are two lineages of HWA that specialize on each of the Japanese hemlock species, *T. sieboldii* and *T. diversifolia*. *Tsuga sieboldii* grows at lower elevations and further south while *T. diversifolia* grows to the north at higher elevations. The geographic ranges of the two species overlap in central Honshu, but it is rare for them to grow naturally in the same stands. For example, they could be found on the same mountain, but *T. sieboldii* will grow at the base, and *T. diversifolia* will grow at the top. Extensive sampling throughout Japan has confirmed that the two Japanese HWA lineages are not exchanging genes despite the existence of areas where the two species grow in close proximity (Havill, unpublished data).

The adelgids that were introduced to the eastern United States are from the lineage that lives on *T. sieboldii*, the southern Japanese hemlock. We know this because DNA sequences from adelgids in the eastern United States are an exact match to adelgids living on *T. sieboldii* in Japan. In the eastern United States, we observe only a fraction of the genetic variation found naturally in Japan (Havill et al. 2009). This is characteristic of a recently introduced species with a single introduction. In contrast, HWA in western North America is much more genetically diverse than in the east and their DNA does not match any of the Asian lineages (Havill et al. 2009). Hemlock adelgids from China and Taiwan are conspicuously different from those in Japan and North America; perhaps enough to consider them separate species.

ADELGID BIOLOGY

Adelgids have multi-generation, complex life-cycles with many different morphological forms within a single species (Havill and Foottit 2007). The typical adelgid life-cycle involves alternation between spruce (*Picea*) primary hosts where they form galls and where there is a sexual generation, and other conifer secondary hosts where reproduction is strictly asexual.

The lineage of HWA that was introduced to the eastern United States alternates between *T. sieboldii* and tigertail spruce, *Picea torano* (K. Koch) Koehne, in Japan. The HWA gall is morphologically different than the typical "pineapple" adelgid gall. It is nearly spherical and can be quite large, up to 4 cm in diameter (Fig. 3). Tigertail spruce is a protected species in Japan where it is uncommon and patchily distributed on the Japanese landscape. Like many other adelgid species, HWA can maintain continuous asexual generations on its secondary hosts in areas where there are no suitable spruce primary hosts. In Japan, this has resulted in a patchwork of sexual and asexual populations of HWA, depending the proximity and availability of primary and secondary host trees. If both host species are present, a proportion of the HWA population migrates from hemlock to spruce where there is a sexual generation. When tigertail spruce is absent, winged migrants do not survive to reproduce and the population is limited to asexual generations on hemlock. In southwestern China, HWA alternates between *T. chinensis* and *Picea likiangensis* (Franchet) Pritzel, and *P. brachytyla* (Franchet) Pritzel (Montgomery and Havill, unpublished data). In western North America, HWA feeds on both *T. heterophylla* and *T. mertensiana* but winged migrants have not been observed and it does not alternate to spruce.

Figure 3. Hemlock woolly adelgid gall on tigertail spruce in Japan.

The ability of HWA to continue reproducing asexually on hemlock probably contributed to its successful invasion of eastern North America where there are no suitable spruce species to support the sexual generation (McClure 1989). HWA has two generations per year on hemlock in eastern North America. One generation consists of wingless sistens individuals that hatch in late spring to early summer, and quickly enter a diapause which continues through late summer and into the fall. Sistentes then overwinter as nymphs, becoming adults in early spring when they lay a large clutch of eggs. The next generation consists of both wingless progrediens and winged sexuparae. Most individuals develop as wingless progredientes in early spring, progress very quickly to the adult stage, and lay eggs in late spring and early summer. The remaining individuals of this generation develop into the winged sexuparae that would give rise to the sexual generation, though these individuals do not reproduce because suitable spruce species are not available. The phenology of the life cycle is somewhat more accelerated in the southern versus the northern areas of the introduced range because of the warmer climate (Mausel et al. 2008), and overwintering mortality is much higher in the north than in the south (Trotter and Shields 2009). The overwintering sistentes are generally more fecund than the progredientes (Fig. 4).

Population genetic analyses indicated that HWA has very little genetic variation in the eastern United States, and since it only reproduces asexually, new genotypes can only arise from mutation, not from recombination. However, its extremely high population sizes are likely to harbor enough mutations to allow for adaptation to local environmental conditions as it spreads. For example, there is evidence that it is evolving increased cold tolerance as it moves north (Butin et al. 2005).

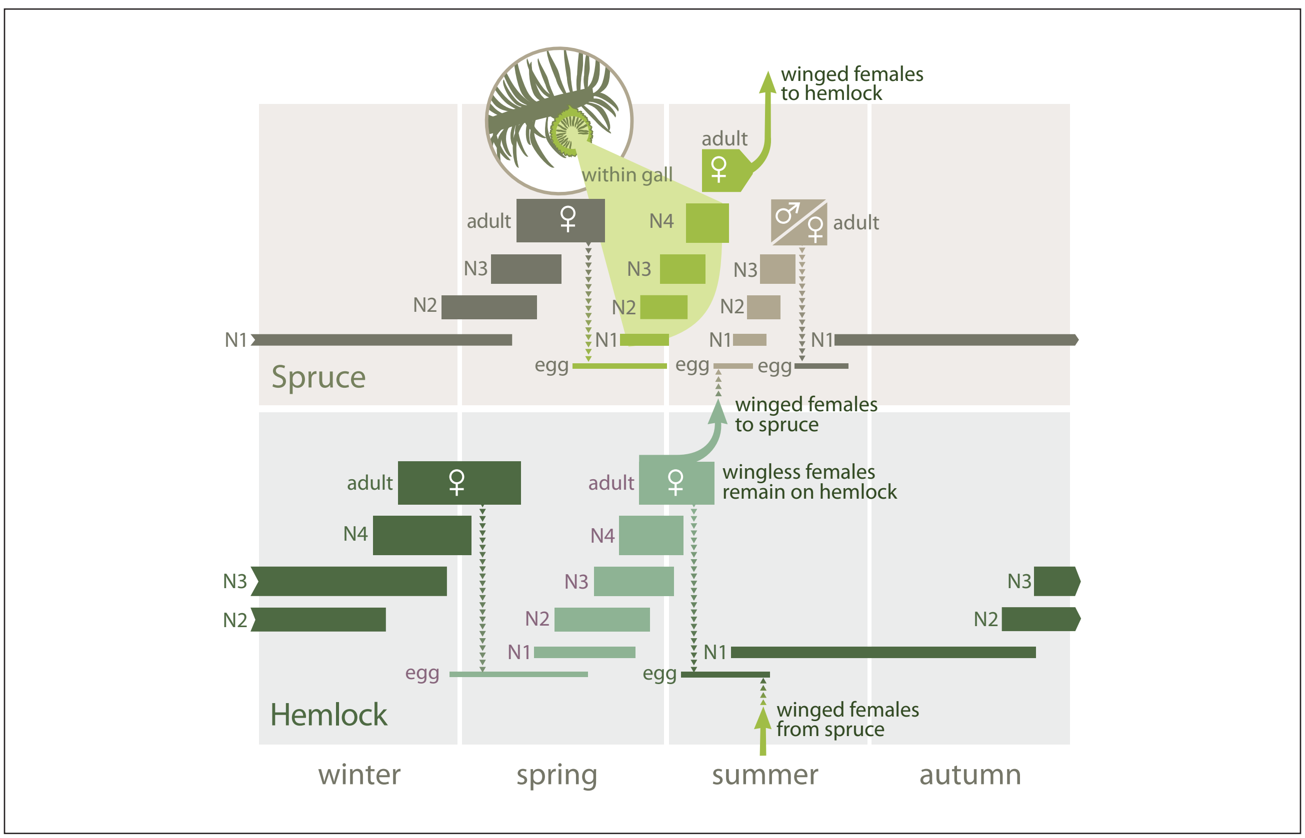

Figure 4. Hemlock woolly adelgid life cycle. In Japan, the adelgid alternates between hemlock and tigertail spruce. Tigertail spruce supports a sexual generation and gall formation. In the eastern United States there are only two generations on hemlock, because winged migrants do not find suitable spruce species on which to complete the entire life cycle. (Vince D'Amico and Nathan Havill created the artwork for this figure.)

HOST IMPACTS

HWA settles at the base of hemlock needles, primarily on the underside of branches. HWA has piercing-sucking mouth parts that extend through the leaf cushion into the ray parenchyma cells where it extracts stored plant nutrients (Young et al. 1995). Feeding causes the loss of hemlock needles, and the mortality of buds and branch tips. Loss of foliage and dieback can become apparent 2 to 4 years after infestation in many locations, and trees can die within a few years, or can survive in a weakened state for many years (Paradis et al. 2008).

Recent work suggests the damage caused by HWA may be more complicated than simple depletion of nutrients. We know that adelgids feeding on spruce induce dramatic changes in the host, as evidenced by the formation of galls. Adelgids settled at the base of spruce buds cause the developing needles to be stunted, to expand laterally, and merge together into a gall rather than form a normal shoot. Gall tissue is high in lipids and starch and low in phenolic compounds making them more suitable for adelgid feeding (reviewed in Havill and Foottit 2007). Secondary hosts such as hemlock do not respond to adelgid feeding by producing galls, but they may have similarly complex reactions to adelgid feeding. Another introduced adelgid species, *Adelges piceae* (Ratzeburg) for example, induces abnormal growth of bark and wood tissue in susceptible *Abies* species called “gout disease” or “rotholtz” (Balch 1952). HWA feeding appears to induce similar responses in hemlock including: 1) formation of abnormal xylem that limits the tree’s ability to transport water (Rivera et al. 2010); 2) increased foliar nitrogen (Stadler et al. 2005); 3) dramatic changes in amino acid concentration and composition (Gómez et al. 2011); and 4) the maintenance of high levels of starch at the feeding site (Schwartzburg and Montgomery 2011). Thus, HWA may be able to induce localized changes in both its primary and secondary hosts that favor its survival and reproduction. In eastern North America, where hemlocks did not co-evolve with adelgids, tree response to feeding appears to be hypersensitive, which coupled with the lack of population suppression by natural enemies, can result in tree death.

The terpenoid chemistry of the two eastern North American hemlock species is an intriguing example of herbivore/host co-evolution. Although *T. canadensis* and *T. caroliniana* are not phylogenetically closely related, both species have relatively high levels of isobornyl acetate (about 40%), which is twice the percentage of total terpenoids found in other hemlock species, and low levels of alpha-humulene (2-4%), which is less than half the percentage found in all other species, except *T. mertensiana* (Lagalante et al. 2003). In terms of the chemical signature of all 40 terpenoids detected, the terpenoid composition of *T. caroliniana* is more similar to the Asian species than the North American species. Since eastern North American hemlocks have evolved with more chewing insects such as the hemlock looper, *Lambdina fiscellaria* (Guenée), and fewer piercing-sucking insects, their defenses may not be effective against sucking insects such as HWA (Lagalante et al. 2007, Montgomery and Lagalante 2008). HWA infestation is also known to increase the release rate of volatile monoterpenes in eastern hemlock branches (Broeckerling and Salom 2003), but the role of terpenes in defense against HWA is not known, nor is their role in attracting natural enemies to HWA infested trees.

IMPLICATIONS FOR BIOLOGICAL CONTROL

The evolutionary history of the interaction among hemlocks, adelgids, and their natural enemies should be considered when developing a successful biological control program for HWA. We know of eleven distinct hemlock taxa that support six different HWA lineages. Each population represents a multitrophic community that is a potential source of HWA biological control agents. These communities share fundamental traits that can be traced to shared ancestry within each trophic level (i.e. within *Tsuga*, *Adelges tsugae*, *Laricobius*, Chamaemyiidae, etc.). Each community of predators, herbivores, and hosts has unique adaptations to local climatic conditions and to the community of specific species present. For example, each endemic adelgid lineage co-evolved with different host species, has adapted different variations on the life cycle to fit local ecological conditions, and contends with a different community of natural enemies. Knowledge of these systems can inform and guide the development of biological control agents. For example, because HWA was introduced from southern Japan, this region could yield biological control agents that are well adapted to feed on this specific lineage of HWA. *Laricobius osakensis* Montgomery and Shiyake, a beetle recently described from populations of HWA in Japan is therefore a promising biological control of HWA (Montgomery et al. 2011). In western North America, there is an additional assemblage of adelgid-specific natural enemies (Kohler et al. 2008) on a different lineage of HWA which, like the one in the eastern United States, does not alternate hosts. This lineage is also closely related to the one in southern Japan, and so predators of the western North American lineage of HWA may also be effective biological controls in the eastern United States. One such western predator, the beetle *Laricobius nigrinus* Fender, collected from western hemlock has been widely established as a biological control of HWA in the east (Mausel et al. 2010). Predaceous flies in the family Chamaemyiidae from the west also show potential as biological controls. In southwest China, there is a remarkably diverse assemblage of adelgid predators, especially in the lady beetle *Scymnus* (Montgomery et al. 2000). This diverse community of natural enemies could yield effective biological controls, especially if the native and non-native climates are similar, but careful attention should be paid to the prominent differences between the Chinese and Japanese lineage of HWA.

Predators of specific herbivores often use characteristic volatile chemicals released by specific plants as cues to locate their prey. The interaction between HWA and different host species could influence the ability of predators to control HWA. To examine this, Wallin et al. (2011) tested whether *L. nigrinus* was attracted to conifer hosts of different adelgid species. *Laricobius nigrinus* collected from western hemlock infested with western HWA was more attracted to western hemlock volatiles than to Ponderosa pine, Douglas fir, white spruce, or eastern hemlock volatiles. They were also attracted to western white pine. Beetles that were lab reared on eastern hemlock infested with Japanese HWA did not respond well to volatiles from either hemlock species. Put in the context of what we know about HWA in western North America, this lends further evidence that *L. nigrinus* is adapted to locating HWA in the west, and raises questions about the roles of learning and pre-conditioning in prey location.

Tsuga sieboldii, the host species with which the introduced HWA lineage co-evolved, is genetically and chemically different than the eastern North American hemlock species. The importance of a shared evolutionary history is highlighted when we recognize that *Tsuga canadensis* and *T. caroliniana* are not closely related to each other, yet both have independently evolved similar chemical signatures, perhaps because of the absence of pressure from sucking insects. For HWA control to be effective, it may be necessary to combine biological control with more resistant hemlocks. This may be difficult because *T. canadensis* has low genetic variation and does not readily hybridize with other hemlock species.

These studies are examples of why it is useful to consider adelgid biology and host interactions in the context of its evolutionary history when evaluating biological control agents. Different natural enemy species will behave differently in the context of the complex interaction between HWA and its hosts in different environments. An understanding of the diversity of hemlock species and HWA lineages in different parts of the world, and careful consideration of how natural enemies perform in their native and introduced ranges can be used to optimize the impact of biological control.

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