Evaluating a Potential Area-wide IPM Strategy for Managing Hemlock Woolly Adelgid in the Eastern United States

Kenton Lucas Sumpter

Thesis submitted to the faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science in Life Sciences

In

Entomology

Scott M. Salom, Chair

Carlyle C. Brewster, Co-Chair

Albert E. Mayfield III

Troy D. Anderson

Keywords: *Adelges tsugae*, *Laricobius nigrinus*, IPM, forest health, HWA, imidacloprid, biological control

Evaluating a Potential Area-wide IPM Strategy for Managing Hemlock Woolly Adelgid in the Eastern United States

Kenton Lucas Sumpter

Abstract (Academic)

Use of imidacloprid has been found to be highly effective in suppressing hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) is a predator released as a biological control in the eastern U.S. This project was designed to develop a pest management strategy that utilizes both tactics concurrently within the same site. It will assess the efficacy of this strategy in reducing HWA populations and improving the health of hemlock forests. The project was started in 2010 and data have been collected annually through 2016. The project spanned three sites in three different states. Results showed that tree health has generally declined across all sites for each year. HWA population index values are highly variable and are more strongly influenced by the occurrence of low winter temperatures than by treatment effect. Cross-correlation analysis of tree health and HWA population, revealed characteristics of their temporal relationship. In two of the three sites, tree health lagged up to three years behind changes in HWA population and HWA populations lagged approximately one year behind changes in tree health. *L. nigrinus* did not establish at any site as of 2016. The lack of sustained recovery of the biological control may be attributable to the occurrence of polar vortices during the winters of 2014 and 2015, which produced subsequent crashes in the HWA population at two of the three sites. In TN, the *L. nigrinus* population may have never established due to a decline in the HWA population shortly after release.

Evaluating a Potential Area-wide IPM Strategy for Managing Hemlock Woolly Adelgid in the Eastern United States

Kenton Lucas Sumpter

Abstract (Public)

The insecticide imidacloprid, has been found to be highly effective in suppressing hemlock woolly adelgid, *Adelges tsugae*. *Laricobius nigrinus* is a predatory beetle released as a biological control of adelgids in the eastern U.S. This project was designed to develop a pest management strategy that utilizes both tactics concurrently within the same site. It will assess the efficiency of this strategy in reducing HWA populations and improving the health of hemlock forests. The project was started in 2010 and data were collected annually through 2016. The project spanned three sites in three different states (KY, WV, and TN). Results show that tree health has generally declined across all sites for each year. HWA population index values are highly variable and are more strongly influenced by the occurrence of low winter temperatures than by treatment effect. Cross-correlation analysis of tree health and HWA population, revealed characteristics of their temporal relationship. In two of the three sites, tree health lagged up to three years behind changes in HWA population, and HWA populations lagged approximately one year behind changes in tree health. *L. nigrinus* did not establish at any site as of 2016. The lack of sustained recovery of the beetle may be attributable to the occurrence of extremely cold temperatures during the winters of 2014 and 2015 which produced subsequent crashes in the HWA population at two of the three sites. In TN, the *L. nigrinus* population may have never established due to a decline in the HWA population shortly after release.

Acknowledgements

This project could not have been completed without the dedication and hard work of multiple individuals. I would like to thank my committee first and foremost for providing the insight and technical know-how for me to be able to tackle my first big project. They were always available whether I needed a point clarified or a new draft critiqued. I would also like to extend my thanks to the Salom lab as a whole for the assistance they rendered in getting me to my field sites and helping me in data collection. A special thanks to Tom McAvoy for accompanying (and if I'm being honest, leading) me on all of my field excursions. A big thanks to the site managers Brent Galloway, Scott Durham and Michael Froelich who allowed me and my colleagues to work on their property. Thank you to the administrative staff at Price Hall, Sarah and Kathy. You guys had to deal with some goofy lodging receipts and for that I apologize! I would also like to recognize the USDA Forest Service, Forest Health Protection for their financial support through cooperative agreements 09-CA-11420004-144 and 14-CA-1142-0004-037. Lastly, I would like to acknowledge my girlfriend Kat who kept the home fires burning while I was away.

Table of Contents

Abstract (Academic)

Abstract (Public)

Acknowledgements i
Chapter 1: Introduction 1
1.1 Biology of the Hemlock Woolly Adelgid 1
1.2 Ecological Impact of Eastern Hemlock Loss 4
1.3 Economic Impact of Eastern Hemlock Loss 6
1.4 Overview of HWA Management Strategies 6
1.5 Chemical Controls for Managing HWA 7
1.6 HWA Biological Management 9
1.7 Research Rationale 11
1.8 Research Objectives 13
Chapter 2 Effects of biological and chemical treatments on hemlock woolly adelgid populations and eastern hemlock health on an area-wide level 14
Abstract 14
2.1 Introduction 15
2.2: Methods 17
2.2.1 Study Site Organization 17
2.2.2 Treatment Plot Organization 18
2.2.3 Chemical Treatment 18
2.2.4 Biological Treatment 19
2.2.5 Biological and Chemical Treatment 19
2.2.6 Untreated Control 19
2.2.7 Data Collected from All Plots 19
2.2.8 Weather Data 21
2.2.9 Statistical Analysis 21
2.3 Results 22
2.3.1 Kentucky Ridge State Forest, Kentucky 22
2.2.2 Twin Falls State Park, West Virginia 24
2.2.3 Coal Creek, Tennessee 25

2.2.5 *L. nigrinus* Recovery and Winter Weather 26

2.4 Discussion 27

References 40

Appendices 51

Chapter 1: Introduction

1.1 Biology of the Hemlock Woolly Adelgid

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), is a very small insect (< 1.5 mm), closely related to insects in the families Aphididae and Phylloxeridae. Adelgids can be distinguished from these two families by their lack of siphunculi (abdominal tubes common to aphids) and the retention of oviparity in all its generations (Havill and Foottit 2007). HWA is indigenous to Asia and the temperate, coniferous rainforests and interior coniferous forests of western North America (Ward et al. 2004). *A. tsugae* is associated with hemlock tree (*Tsuga* spp.) species worldwide with the exception of eastern (*T. canadensis* Carriére) and Carolina (*T. caroliniana* Engelmann) hemlock. It is an obligate herbivore of hemlock trees, and due to its recent introduction to the eastern United States, it has become a significant pest of eastern and Carolina hemlocks (Havill et al. 2006, Havill et al. 2011).

Much like other members of the Adelgidae, HWA is parthenogenic and possesses a holocyclic life cycle in its native range (Havill and Foottit 2007). Insects with a holocyclic life cycle include a sexually reproducing generation on a primary host in addition to other parthenogenic generations on secondary hosts. Within its native range in Japan, HWA's primary host is the tiger-tail spruce (*Picea torano* Koehne), with southern Japanese hemlock (*T. sieboldii* Carr.) and northern Japanese hemlock (*T. diversifolia* Maxim.(Mast.)) as secondary hosts (Havill et al. 2006). The inverse of holocyclic is anholocyclic, i.e. no sexual generations within the life cycle (Havill and Foottit 2007). In the eastern United States, HWA has an anholocyclic life cycle. It produces two, asexual generations per year. (bivoltine). The spring generation, known as the progredientes (singular = progrediens), emerges from eggs between February and March as a "crawler". The progredientes crawlers attach themselves to the bases of needles from the previous year's growth, and from there they develop through four larval instars before becoming adults. By June, the now adult progredientes lay their eggs in a self-produced waxy, white, flocculent mass otherwise known as an ovisac (MClure 1987). In cases where the HWA population is present at very high densities, a portion of the nymphs will develop into a winged sexual stage known as the sexuparae (Ward et al. 2004, Havill et al. 2014). In HWA's native range, the sexuparae will seek out tiger-tail spruce on which to produce the sexual generation, the sexuales (Havill and Foottit 2007, Havill et al. 2014). However, in the eastern United States there are no suitable spruce hosts, thus leaving the sexuparae to die before engaging in any meaningful reproductive activity (McClure 1989b, 1991). The progeny of the progredientes are the sistentes (singular = sistens). Sistentes eggs hatch around July (MClure 1987), and the crawlers attach themselves to the base of the current year's growth where they enter aestivation as first instars for the remainder of the summer. Around October, the nymphs break aestivation and begin feeding. Feeding and development continues throughout the winter. By early spring of the next year they will have reached the adult stage and begin oviposition. Their progeny will form the next progredientes generation (MClure 1987, McClure 1989b).

HWA was first described by Annand (1924) in Oregon. It has since been determined that HWA has been endemic to western North America for some time (Havill and Foottit 2007, Havill et al. 2016). Several estimations have been made as to the time period that HWA's migration to North America could have occurred. The most current estimates place this event at roughly 14 – 57 kya (thousand years ago) (Havill et al. 2016). During that period, the Bering land bridge was periodically intact and northern boreal forests moved more closely towards it. Historic data show the presence of hemlock species and HWA. Thus, it is possible that HWA crossed the land bridge via passive wind dispersal and by crawlers and ovisacs inadvertently clinging to migrating birds (Havill et al. 2016). Estimates also stretch back to nearly 5 mya (million years ago) during the last glacial maximum (LGM) (Havill and Foottit 2007); however, this estimate is considered less valid today due to limited archaeological evidence (Havill et al. 2016). In western North America, unlike in the East, HWA populations are kept below damaging levels through a combination of evolved host tree resistance and a complex of native predators (Havill et al. 2006, Kohler et al. 2008, Havill et al. 2016).

HWA was first observed in the eastern United States in 1951 near Richmond, Virginia. This was likely the result of an earlier, accidental introduction from Japan (Havill et al. 2006, Havill and Foottit 2007). It is unknown who is responsible for introducing HWA; however, it is hypothesized that it arrived on Japanese hemlocks that were imported for an Asian garden located near Richmond, Virginia. This introduced population has been tracked back to a clade originating in the southern region of the island of Honshu, Japan (Havill et al. 2006). Since its introduction to Virginia, HWA has managed to spread at a rate of roughly 12 km/year mainly via wind dispersal and indirect animal activity (McClure 1989a, Evans and Gregoire 2007, Russo et al. 2016). Currently in the eastern U.S., HWA can be found around the Great Lakes, northeast to New England, south as far as Georgia, and as far west as Kentucky and Tennessee (Ward et al. 2004, Havill et al. 2014).

HWA feeds by inserting its stylet into the bases of hemlock needles. The stylet follows an intracellular and intercellular pathway to a feeding site within the ray parenchyma cells of the xylem (Young et al. 1995). Damage to the tree occurs through a combination of photosynthate loss, foliar desiccation, the formation of false growth-rings, and reduced springtime growth (Young et al. 1995, Miller-Pierce et al. 2010, Gonda-King et al. 2012, Oten et al. 2012, Preisser et al. 2014). Young et al. (1995) hypothesized that it was some toxin in HWA saliva that caused harm to the hemlock trees. This wound-response hypothesis has evolved over time as new evidence has come to light (Oten et al. 2012). Further research has shown that extra-oral digestion may be occurring at HWA feeding sites. This degradation of tree tissues from feeding could be a major factor in understanding the detrimental, systemic response of eastern hemlock to HWA (Oten et al. 2014). Trees affected by HWA show discolored and desiccated foliage with reduced spring-time growth (MClure 1987). Heavily infested trees can lead to extensive damage which can result in tree death (MClure 1987). However, the length of time an individual tree may survive is largely predicated upon the density of the resident HWA population and the health of the tree (MClure 1992, Eschtruth et al. 2013). McClure et al. (1991) showed that the highest population levels of HWA were attained just one year after initial introduction. In the year following its introduction, the HWA population decreased along with the availability of food. The deviation from this trend came in the third year of the study when the previous year's low HWA population allowed an increased spring growth by the hemlocks, followed by a surge in HWA as the insects discovered the rejuvenated food source. This phenomenon is important when a management system plans to incorporate chemical and biological treatments because the improved health of the stand from chemical treatment can allow sufficient time for regeneration while the biological control establishes (Joseph et al. 2011, Mayfield et al. 2015).

1.2 Ecological Impact of Eastern Hemlock Loss

The continued loss of eastern hemlock is of concern to ecologists because it is considered a foundational species (Ellison et al. 2005b, Daley et al. 2007, Ellison et al. 2016), and it has been shown to have little to no resistance to HWA (Orwig and Foster 1998, Orwig et al. 2002, Havill et al. 2006). According to Ellison et al. (2005) a foundational species is, "*…a single species that defines much of the structure of a community by creating locally stable conditions for other species, and by modulating and stabilizing fundamental ecosystem processes.*" Eastern hemlock is a coniferous tree that is common throughout eastern North America, from Nova Scotia to Georgia (Ward et al. 2004). Eastern hemlock is a highly shade-tolerant species, capable of surviving on as little as 5% of available ambient light. Because of this, eastern hemlocks often develop very dense and extensive canopies (Ward et al. 2004). The internal climate of most hemlock stands is moist and cool due to the shade provided by the dense canopy cover. As a result of the decomposition of its foliage, the soils of hemlock stands are often acidic with low nitrogen cycling rates, and poor nutrient levels (Jenkins et al. 1999, Ellison et al. 2005b).

Within its stands, the eastern hemlock provides a stable environment for a variety of animal species. Most notably are two avian species, the black-throated green warbler and the blue-headed vireo, which are closely associated with eastern hemlock (Tingley et al. 2002). Eastern hemlock forests also provide vital habitat for aquatic organisms. Streams bordering eastern hemlock forests were found to contain significantly more fish and macroinvertebrates than areas that were not so protected (Evans 2002).

In areas where hemlock is no longer present, changes in the balance of species has been shown to occur as new species immigrate into the area (Ellison et al. 2005a). The impact of such immigration can be difficult to predict. In stands experiencing significant mortality, deciduous hardwoods often replace hemlocks. In eastern hemlock's more northern range, the next successive tree species are often birch (*Betula* spp.) and maple (*Acer* spp.). In its more southern range, hemlock is more commonly replaced by tulip tree (*Liriodendron tulipifera* L.) and maple (*Acer* spp.) (Ellison et al. 2005b). Stands in transition from hemlock to deciduous hardwoods have been found to possess significantly different soil chemical properties, experiencing increased soil-nitrogen content and elevated pH levels (Ellison et al. 2005b).

Soil fungi are also affected by the loss of eastern hemlock. Soil ectomycorrhizal root-tip density and morphotype diversity were reduced by 63 and 27%, respectively; in eastern hemlock stands that suffered HWA infestation (Lewis et al. 2008). This has further implications for post-hemlock hardwood regeneration. Lewis et al. (2008) also found that oak seedling dry mass was significantly lower in areas where ectomycorrhizal fungi were reduced or absent, thus suggesting reduced efficiency for future forest regeneration.

Furthermore, the loss of dense hemlock canopy results in more sunlight reaching the forest floor with a coincident increase in the ambient temperature of exposed patches (Jenkins et al. 1999). This can have an especially meaningful impact upon stream ecosystems as their inhabitants often rely on well-shaded, thermally-stable riparian zones for survival (Beschta 1997). These changes in temperature, species diversity and soil chemistry may have significant implications for the composition and survivability of the species most closely associated with eastern hemlock.

1.3 Economic Impact of Eastern Hemlock Loss

The economic value of eastern hemlock is less significant than its ecological importance; however, it is still a factor that needs to be considered. Eastern hemlock is a commonly used ornamental tree with roughly 260 cultivars currently being bred (Preisser et al. 2014). It is valued for its aesthetic qualities as well as its easy management and positive response to pruning. The value of ornamental eastern hemlocks in nurseries in Tennessee and North Carolina was placed at around $34 million in 1995 (Preisser et al. 2014). Although this information is now rather out-of-date, it does give some indication of the relative value of eastern hemlock to local businesses. The eastern hemlock is also semi-valuable as a source of timber and wood-pulp (Preisser et al. 2014). Anecdotally, there is also some value in eastern hemlock as framing material for houses and some carpenters use it for making furniture. The value of the eastern hemlock has doubtlessly now declined due to HWA-related mortality. In fact, property values have been estimated to have decreased by up to $7,261 per house in areas that suffered a loss of 20-22% hemlock cover. Furthermore, losses of just 10% cover resulted in decreased property values up to $2,331 within 1 km (Holmes et al. 2005).

1.4 Overview of HWA Management Strategies

Due to eastern hemlock’s lack of innate host-resistance to HWA (Orwig and Foster 1998, Cheah et al. 2004), state and federal organizations, as well as private companies and universities, have collaborated to develop suppression methods for the insect. Although many control programs have produced individually positive results, it does not appear that a single method can provide an overall solution to the problem (Preisser et al. 2014). Since this appears to be the case, it may be feasible that a combination of tactics can be developed into an integrated pest management (IPM) strategy. An IPM-based strategy could overcome the limitations of other, single-method systems. Due to their versatility, integrated approaches have become a signature feature of insect management. A good example in a forest setting is the gypsy moth (*Lymantria dispar* L.) control program, in North America. Here, managers have combined pheromone-based mating disruption, biological insecticide sprays, pheromone trapping, and biological control agents (Lee et al. 1996, Sharov et al. 2002). The result of these strategies has been a reduction in the spread of gypsy moth by nearly 50% (Sharov et al. 2002). Preventative measures in the form of silvicultural management, improving host resistance and the creation of transgenic plants are also considered viable components of a long-term IPM strategy (Boulter 1993, Waring and O'Hara 2005). In the case of HWA, such efficacious solutions have yet to be developed. The operational control strategy for HWA has primarily employed two tactics; chemical control and biological control. These two systems are reviewed in greater detail in the following, below.

1.5 Chemical Controls for Managing HWA

One of the methods implemented for the management of HWA is the application of the neonicotinoid insecticide, imidacloprid. This chemical was first synthesized in 1985 and it is structurally similar to nicotine. Currently, imidacloprid is produced by a number of private companies under several commercial names including Merit®, Imicide®, and Silwet® (Silcox 2002). Imidacloprid has been shown to be highly effective when used against HWA. In field trials, mortality rates amongst HWA ranged between 50-100% (Silcox 2002, Cowles et al. 2006). Imidacloprid's primary mode of action is as an agonist of nicotinic acetylcholine receptors (nAChRs). Imidacloprid binds with post-synaptic nAChRs and activates them, thereby initiating nervous system stimulation. This eventually leads to neurotoxicity and death (Lind et al. 1999, Tomizawa and Casida 2005). The LC_{50} for HWA is approximately 100 – 200 ppb (parts per billion) (Benton et al. 2015). In addition to imidacloprid's direct lethal effect, one of its secondary metabolites, olefin, is even more toxic. In oral ingestion assays involving cotton whitefly (*Bemisia tabaci*), olefin was demonstrated to have a 10x greater effect upon treated populations than imidacloprid or its other metabolites (Nauen et al. 1998, Nauen et al. 1999).

There are three ways in which imidacloprid is applied. The first is soil treatment. This can be accomplished in a variety of ways including surface soil washes, subsurface injections, and the placement of subsurface tablets (Steward et al. 1998). All of these soil treatment methods achieve the same goal of forcing the roots of the tree to take up the insecticide. It is then systematized throughout the tree's tissues. Soil treatment is considered the longest lasting treatment method and is thus more widely used in HWA suppression. The typical protective period for soil treatment is roughly 4 – 5 years (Silcox 2002). Interestingly, traces of imidacloprid and its metabolites have been found up to 7 years post-treatment, although the concentration of these chemicals was below the previously-mentioned LC_{50} for HWA (Benton et al. 2015, Mayfield et al. 2015). Of note is the issue of surface water runoff, where imidacloprid can be carried into local waterways. Imidacloprid is a broad-spectrum insecticide that is capable of impacting any arthropod community it is introduced into (Eisenback et al. 2010, Benton et al. 2016). Recent studies have shown that although runoff from soil treatment does eventually find its way into adjacent waterways, the concentrations at which imidacloprid are found are well below the mandated EPA chronic and acute threat levels. Furthermore, no secondary metabolites were recovered from the waters that were tested (Benton et al. 2016).

The second method for chemical application is stem injection. This method involves the direct application of the insecticide into the cambium of the tree, usually via a large-bore needle penetrated through the bark. The period of protection for this treatment method is roughly two years (Silcox 2002).

This method is preferred when treating trees near bodies of water because there is very little chance of runoff occurring. The third application method is foliar spraying. This method is only protective for a short amount of time and thus is rarely used in forest management (Silcox 2002, Eisenback et al. 2010, 2014). Foliar sprays are also highly susceptible to precipitation. In the interest of striving for more efficient use of insecticides, the use of reduced concentrations of imidacloprid has been tested. Joseph et al. (2011) showed that trees treated with only 10% of the label rate could reduce the number of HWA ovisacs and improve tree health. Mayfield et al. (2015) showed that imidacloprid administered systemically at 25% of label rate was effective at improving crown health in eastern hemlock. This could be beneficial when considering combining imidacloprid with predatory beetles as part of an IPM plan.

1.6 HWA Biological Management

There are several classical biological control agents that have been selected in hopes that their contribution to the regulation of HWA populations. Each of these insect predators has been found to possess varying degrees of specificity to HWA (Flowers et al. 2005, Gatton et al. 2009); however, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) was found to be particularly promising due to its unique life history and close association with HWA (Zilahi-Balogh et al. 2003b, Lamb et al. 2006). The adult beetle is black-bodied, is less than 3 mm long, and is a voracious predator of HWA. In lab studies, *L. nigrinus* larvae were found to consume between 225 and 252 HWA eggs during their development and adults were shown to consume on average 4 – 5 adelgids per day (Zilahi-Balogh et al. 2003a). *L. nigrinus* adults feed primarily on HWA adults and nymphs and larvae feed on HWA eggs (Zilahi-Balogh et al. 2003a). *L. nigrinus* oviposition occurs from January through March with eggs typically laid singly in HWA ovisacs. *L. nigrinus* is univoltine and only lays 1 set of eggs per year (Zilahi-Balogh et al. 2003a). Adult beetles have been found to lay between 38 – 41 eggs each (Lamb et al. 2006). Throughout April and May, after developing through four instars, the larvae drop off the hemlock trees and pupate in the soil beneath. Larvae eclose to the adult stage within a few weeks, after which they enter aestival diapause for the remainder of the summer months (Zilahi-Balogh et al. 2003b). They then resume activity as adults in the fall. The timing of the emergence of *L. nigrinus* is one of the most important characteristics of the beetle. In October when the HWA sistentes are ending their aestival diapause, *L. nigrinus* adults also emerge from the soil and begin feeding on HWA (Zilahi-Balogh et al. 2003a). This was originally difficult to reproduce in the laboratory setting. In the lab, *L. nigrinus* adults began emerging in July and peaked in August; more than two months earlier than HWA. After study, it was determined that a longer light photoperiod and increased temperatures would delay the emergence of *L. nigrinus* (Lamb et al. 2007). Because of these discoveries, beetles mass-reared in insectaries can now be ready for release when the sistentes begin their Fall activity.

In lab studies, *L. nigrinus* has shown a much greater preference for HWA as a food source, and females have been shown to prefer HWA ovisacs for egg-laying over other host options (Zilahi-Balogh et al. 2003a). Additionally, *L. nigrinus* was found to the incapable of developing past the fourth larval instar on other adelgid species (Zilahi-Balogh et al. 2003a), indicating that it is host-specific. This is ecologically important because it means that there is a reduced risk of *L. nigrinus* harming non-target species.

Because of its beneficial characteristics, *L. nigrinus* was cleared for release in the United States in 2000, and open releases began in 2003 (Zilahi-Balogh et al. 2005a). Since then, it has successfully established at numerous locations in the eastern United States (Mausel et al. 2010, Roberts et al. 2011, Davis et al. 2012). One potential concern to ecologists was that *L. nigrinus* might compete with the native Derodontid beetle, *Laricobius rubidus* LeConte. *L. rubidus* is a predator of pine bark adelgid, *Pineus strobi* Hartig (Lawrence and Hlavac 1979). It has been found to feed and complete development on HWA (Zilahi-Balogh et al. 2005b). The fact that *L. nigrinus* can only complete its development on HWA means that both congeners should not interfere with one another on pine (Zilahi-Balogh et al. 2005b, Zilahi-Balogh et al. 2005a); however, *L. nigrinus* and *L. rubidus* have been observed mating and were found to be capable of hybridizing (Havill et al. 2012). The main concern is that the new hybrid could subsume *L. nigrinus* and/or *L. rubidus* as the dominant species in hemlock forests. Fortunately, field studies have shown that the proportion of beetle populations on eastern hemlock trees greatly favors *L. nigrinus* and the proportion of hybridized beetles stabilizes at approximately 11% introgression (Fischer et al. 2015). At this stage, entomologists are not concerned that an *L. nigrinus*/*L. rubidus* hybrid will adversely impact biological control efforts.

The combination of imidacloprid and *L. nigrinus* as a control strategy for HWA is currently under consideration (Mausel et al. 2008, Davis et al. 2012). In a recent study, Mayfield et al. (2015) showed that through a combination of insecticide treatment and predatory beetle releases, the health of a stand of trees could be improved within a few years. The application of the insecticide gives the surviving trees time to recover, thus providing time for predators to establish and reach high enough density to impact HWA. The exact meaning of “recovery” has been standardized using the USDA Forest Inventory Analysis (FIA) system (USFS FIA 2015). This system lists seven categories for tree health. These categories are rated on a percentage scale taken by a pair of observers. The system is subjective; however, the findings of both observers are averaged in order to reduce single-observer bias (USFS FIA 2015).

1.7 Research Rationale

HWA has proven itself to be a highly-lethal pest of eastern and Carolina hemlock trees in the eastern United States. There is a well-established body of literature that supports *Tsuga* spp. as being a critically important “foundational” species (Ellison et al. 2005b). The services these trees provide are far reaching and extend to habitat preservation for birds and insects, thermoregulation of forest floor and adjacent waterways and soil chemical balance (Ellison et al. 2005b). Hemlock trees have shown a reduced capacity to re-establish once HWA has infested an area (Preisser et al. 2011). Soil fungi are also suppressed thus hindering the reestablishment of any further tree species, be they deciduous or coniferous (Sullivan and Ellison 2006, Lewis et al. 2008). Although hemlock trees are not known for their high economic value, they still represent a significant investment both at the nursery level and at the home-owner level where damaged and dead trees have been found to reduce property values by many thousands of dollars (Holmes et al. 2005). With these two factors in mind, the loss of eastern and Carolina hemlocks represents an unacceptable outcome. Unfortunately, while many control methods have been implemented, they have not been coordinated in such a way as to take advantage of any mutually beneficial properties. Thus, it is the goal of this project to evaluate the integration of biological and chemical control tactics at an area-wide level. The chemical treatments rely on the neonicotinoid, imidacloprid. This chemical has been used extensively in agricultural, forest, and ornamental settings and has been shown to be highly lethal to HWA at very low concentrations (Silcox 2002, Cowles et al. 2006). In addition to its initial lethality, imidacloprid is metabolized by plants into a number of metabolites one of which, olefin, is 10x more toxic to insects than imidacoprid. A key biological control agent being used is *Laricobius nigrinus*. *L. nigrinus* was approved for release in 2000, and since then, more than 370,000 individuals released in the eastern United States (Roberts et al. 2011). *L. nigrinus* is highly specific to HWA both in terms of seasonal behavior and developmental requirements (Zilahi-Balogh et al. 2003b, Zilahi-Balogh et al. 2003a). It is the hope for this project that the implementation of a IPM-based strategy will reduce the HWA population and improve tree health at each study site. The success of this program will serve as a basis for similar treatments in the future.

1.8 Research Objectives

Overall objective

To examine the stand-level effects of chemical and biological control treatments applied singly or in combination to hemlock forests across three different sites.

Specific objectives

1. To assess and compare the effects of chemical and biological treatments applied singly or in combination on HWA populations in hemlock tree plots. I hypothesize that the treatments, singly or combined, will decrease HWA populations compared with populations in untreated plots.
2. To assess and compare the effects of chemical and biological treatments applied singly or in combination on tree health in hemlock tree plots. I hypothesize that the treatments, singly or combined, will improve tree health compared with populations in untreated plots.
3. To assess and compare the establishment of *L. nigrinus* in chemically-treated and untreated hemlock tree plots. I hypothesize that *L. nigrinus* will establish in treated and untreated plots

Chapter 2 Effects of biological and chemical treatments on hemlock woolly adelgid populations and eastern hemlock health on an area-wide level

Abstract

Since its introduction to the East Coast, *Adelges tsugae* Annand (Hemiptera: Adelgidae) has become an important pest of eastern and Carolina hemlocks. Due to a lack of host resistance and the absence of any significant native predators, HWA now threatens large stands of hemlock trees. Hemlock trees perform a vital role at the landscape level as foundational species. They maintain forest and stream temperatures as well as soil-chemical interactions, and they provide habitat to local fauna. In order to suppress HWA, chemical and biological control methods have been employed. This project tests combining both control methods into an integrated pest management system. The study was conducted from 2010 through 2016; across three sites in three states (Kentucky 2010, West Virginia 2011, and Tennessee 2012). Results show that in of spite treatment, tree health has generally declined across all sites over that time. However, plots involving chemical treatment do sometimes show greater tree health and smaller HWA population index values. HWA populations are highly variable and are more strongly correlated with the occurrence of low winter temperatures rather than treatment effect. Cross-correlation analysis of tree health and HWA population indicates a time-lag effect. In two of the three sites, tree health lagged 1 – 3 years behind changes in HWA population and HWA populations lagged approximately 1 year behind changes in tree health. The lack of sustained recovery of the biological control agent may be attributable to the occurrence of polar vortices during the winters of 2014 and 2015, which produced subsequent crashes in the HWA population at two of the three sites. In TN, the *L. nigrinus* population may have never established due to a decline in the HWA population shortly after release.

Keywords: *Adelges tsugae*, *Laricobius nigrinus*, IPM, forest health, HWA, imidacloprid, biological control

2.1 Introduction

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adeglidae), is an arthropod pest of most hemlock species (*Tsuga* spp.) worldwide (Havill and Foottit 2007). HWA is an obligate herbivore of hemlock tree species which it uses as a secondary host (Havill and Foottit 2007). In its native ranges, HWA rarely reaches population levels that are injurious to hemlocks because its populations are suppressed through a combination of evolved host resistance and a complex of native predators (Havill et al. 2006, Havill and Foottit 2007). The insect was first described in Oregon (Annand 1924). It was discovered on the East Coast of the United States in the early 1950's near Richmond, Virginia. It was likely introduced via infested foliage from imported Japanese hemlocks (Havill et al. 2006). Since then, HWA has established on eastern (*T. canadensis* Carriére) and Carolina hemlocks (*T. caroliniana* Engelmann). By the 1980's, HWA populations rose to outbreak levels due to a lack of host tree resistance and the absence of any significant predators (Wallace and Hain 2000, Havill and Foottit 2007).

Upon arriving at a hemlock tree, HWA adults settle at the base of the needles where they remain sessile. Subsequent juveniles, or "crawlers", have the capacity to disperse on a host tree and can situate onto new needles (Havill and Foottit 2007). HWA damages its host by inserting its stylet into xylem ray parenchyma cells where it feeds (Young et al. 1995). This results in the desiccation of needle tissue, the formation of false growth-rings, and the loss of new growth (Miller-Pierce et al. 2010, Gonda-King et al. 2012, Oten et al. 2012). Infested hemlock stands deteriorate depending on the level of infestation (McClure 1991, Eschtruth et al. 2013). HWA spreads to new trees primarily through wind-blown dispersal and secondarily by incidental transport via birds and other animals (McClure 1989a, Russo et al. 2016). Currently, HWA is established throughout the eastern seaboard; as far north as Maine and south to Georgia and, more recently, as far west as the Great Lakes (USFS 2015).

Control measures for HWA include chemical and biological methods. Chemical control programs utilize highly lethal and long-lasting insecticides to provide protection from infestation. The most commonly used chemical for treatment is imidacloprid (Silcox 2002). Imidacloprid belongs to the neonicotinoid class of insecticides. Its primary mode of action is as an agonist of nicotinic acetylcholine receptors (nAChRs). It works by binding to post-synaptic nAChRs; thereby activating them. This leads to nervous-system stimulation and generally results in death for the affected insect (Lind et al. 1999, Silcox 2002, Tomizawa and Casida 2005). Imidacloprid has shown great efficacy in field and lab trials (Silcox 2002, Cowles et al. 2006). One of the benefits of imidacloprid is the formation of secondary metabolites once it has been metabolized by its host tree. The metabolite known as olefin is of particular interest because it has been shown to be potentially 10x more toxic to insects than its parent compound (Nauen et al. 1998, Nauen et al. 1999). Imidacloprid treatment can involve a wide variety of application methods including soil drenches, stem injections, foliar sprays and soil injections (Steward et al. 1998). Of all of these methods, soil injections typically retain their efficacy the longest; usually between 3-5 years depending on the concentration used (Silcox 2002). Although residues of imidalcoprid and its metabolites have been recovered up to 7 years post-treatment, they were found at levels that were well below the LC_{50} of HWA (approx. < 120 ppb) (Cowles et al. 2006, Benton et al. 2015).

Classical biological control programs involve the importation of organisms that possess characteristics that make them able to survive in the targeted environment, unlikely to damage non-target species and voracious enough to impact the population of the invasive pest. *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) was found to be a good candidate for biological control. It is a specialist predator of HWA and can only complete its life cycle if its eggs are first implanted into HWA ovisacs (Zilahi-Balogh et al. 2003b, Zilahi-Balogh et al. 2003a). *L. nigrinus* was cleared for release in 2000 and open releases began in 2003 (Zilahi-Balogh et al. 2005a). Since then, more than 370,000 individuals have been released in the eastern U.S., with evidence of establishment (Mausel et al. 2010, Roberts et al. 2011). *L.*

nigrinus is a highly voracious predator of HWA. A single beetle is capable of destroying many hundreds of HWA in its lifetime (Zilahi-Balogh et al. 2003a). An important characteristic of *L. nigrinus* is its synchrony with the sistentes generation of HWA. The sistentes enter aestival diapause during the summer months. Similarly, in the late spring *L. nigrinus* larvae drop to the ground beneath the trees where they pupate in the soil. Following eclosion to the adult stage, they also enter aestival diapause. In the Fall, both insects resume activity (Zilahi-Balogh et al. 2003b, Havill and Foottit 2007, Lamb et al. 2007).

Each of the previously mentioned suppression tactics have characteristics that can limit their effectiveness when applied singly. Chemical treatments suffer from a short effective period relative to biological controls. This requires regular retreatment on the part of forest health workers. Otherwise, the control program would become totally ineffective after the chemical protection ended. Biological controls are difficult to implement because they often require a significant amount of time to establish, and this may be too long for infested trees that are rapidly deteriorating.

Thus, the goal of this project is to combine chemical and biological control methods across multiple sites to test the efficacy of an area-wide, integrated pest management strategy for HWA. The best characteristics of each system will be used to complement the other and counteract shortcomings in either system. Chemical treatments will provide initial protection while *L. nigrinus* establishes. Afterwards, *L. nigrinus* will provide continuing suppression. Tree health and HWA population were tracked and collections of *L. nigrinus* beetles were carried out to determine the efficacy of this IPM strategy.

2.2: Methods

2.2.1 Study Site Organization

Study sites were established in three states. The first site was established in 2010 at Kentucky Ridge State Forest (KRSF) near Middlesboro, Kentucky. It is situated within plant hardiness zone 6b (Appendix 1). The second site was established in 2011 at Twin Falls State Park (TFSP) in West Virginia.

TFSP is located in Wyoming County near the town of Mullens, and lies within plant hardiness zone 6a (Appendix 2). The third site was established in 2012 on Coal Creek Company (CC) property near Oak Ridge, TN. This site falls within plant hardiness zone 7a (Appendix 3). Plant hardiness zones define the lowest, average temperature a region may experience during the year (Appendix 4).

2.2.2 Treatment Plot Organization

Four treatment plots were established at each site and replicated three times for a total of twelve treatment plots (Appendices 5, 6 & 7). Plots were placed no closer than 200 m to each other to delay the dispersal of *L. nigrinus* into non-release plots (Davis et al. 2012). The treatments tested were 1). chemical, 2). biological, 3). biological + chemical (bio+chem) and 4). untreated control. Within each treatment plot, eighteen trees were selected. Selection was based upon observed high-density of HWA ovisacs and the presence of enough vigorous foliage to live throughout a multi-year study. Furthermore, foliage had to be accessible by at least a 5.5 m pole pruner and present in enough quantity to provide material for rearing *L. nigrinus* larvae. Tree health index values and HWA population index values were recorded for each of the eighteen trees in each plot. Of the eighteen trees, a subset was selected for treatment. The following section describes the details for each treatment plot. For timing of the treatments please see Appendix 8.

2.2.3 Chemical Treatment

In each chemical treatment plot, six trees were treated with Merit 2F (imidacloprid), applied at the label rate of 6 ml per 2.54 cm diameter at breast height (DBH). The insecticide was injected into the soil at the base of each tree using a Kioritz© injector (Steward et al. 1998). In 2013, six additional trees were added to each chemical plot at KRSF, KY for a total of twelve treated trees per plot. A similar addition was made to TFSP, WV in 2014, and at CC, TN in 2015. The additional trees were included to increase the proportion of protected trees in each treatment plot from 33 – 50%.

2.2.4 Biological Treatment

In each biological treatment plot, six trees received 125 *L. nigrinus* adults for a total of 750 beetles per plot. All of the beetles used were reared at the Virginia Tech Insectary and were started from a founding colony of beetles collected in the Seattle, WA area. Beetles were released in KRSF, KY in 2010, at TFSP, WV in 2011, and at CC, TN in 2012 (Appendix 8). Releases were made in Recovery operations were conducted two-years post-release at each site.

2.2.5 Biological and Chemical Treatment

In each combined chemical and biological treatment plot, six trees were treated chemically in the same manner as mentioned above and six different trees each received 125 adult *L. nigrinus* for a total of twelve trees receiving treatment. Beginning in KRSF, KY in 2013, six additional trees were treated chemically for a total of twelve chemical and six biologically treated trees. This process was repeated in 2014 in TFSP, WV and in 2015 in CC, TN.

2.2.6 Untreated Control

Eighteen trees received no treatment, in each untreated control plot. Tree health and HWA population were recorded each year.

2.2.7 Data Collected from All Plots

Tree crown health was recorded based upon the observation of live crown ratio, live branches, foliage density, new growth and live branch tips. Each of these measures was rated 0 – 100. The average of these five measures was then used to establish a tree crown health index of 0 – 100 (Jones et al. 2016).

HWA abundance was measured by randomly sampling ten, 30 cm branch sections from around the entire circumference of the tree. Sistentes ovisacs were counted, up to twenty ovisacs per branch section, and the total number of HWA from these branches was divided in half to arrive at a density index of 0 – 100 (adapted from McClure 1989a).

L. nigrinus recovery efforts began two-years post-release at each site. *L. nigrinus* recovery was conducted in April and May by beat-sheeting for adults and by collecting branches for use in larval rearing (Mausel et al. 2010). Beat sheeting was conducted using a 0.5 m PVC beating stick and a white, nylon cloth approximately 1 x 1 m in size supported by crossed PVC piping for collecting dislodged beetles (Appendix 9). Branches were randomly selected from around the entire circumference of each tree. Selection was based upon branch accessibility, due to the fact that many trees within the treatment plots were in poor health and possessed only a limited amount of foliage suitable for beating. Beating involved 10 – 15 strikes per branch. Any adult beetles that were potentially *Laricobius* were collected and taken back to Virginia Tech to have the species genetically identified according to the protocols established in Davis et al. (2011). Six, 30 cm branches were clipped from each beetle-release tree. Branches were randomly selected based upon the presence of a high density of HWA ovisacs and branch availability on the tree. Non-release trees within the treatment plot were also sampled if they displayed sufficiently numerous HWA. Branches were collected and taken back to the Virginia Tech Insectary in Blacksburg, VA for larval rearing, where they were placed in specially designed rearing cages (Appendix 10).

Each rearing cage was made up of a Mylar cylinder capped with a polyester mesh cover affixed to the widest opening of a galvanized metal funnel. The base of the funnel emptied into a 237 ml Mason jar. The cut branches were inserted into water-saturated floral foam and were placed into the rearing cage. The cages were kept at approximately 13 °C (±2 °C) on a timed day/night cycle that simulated environmental conditions. Once the larvae emerged from the HWA ovisacs, they dropped into the Mason jar where they were counted (Salom et al. 2012).

2.2.8 Weather Data

Weather data were collected from the National Oceanographic and Atmospheric Administration (NOAA) (retrieved from https://www.ncdc.noaa.gov/cdo-web/datasets) and the minimum daily temperature for each day on which a polar vortex occurred was recorded. The 2014 vortex occurred on January 7th and the 2015 vortex occurred on February 20th. Percent HWA mortality as a function of minimum temperature (^{o}C) was estimated based upon predictions made by McAvoy et al. (2015) (Table 2.2).

2.2.9 Statistical Analysis

Data on each of the response variables (tree health index and HWA population index values) collected at each site were analyzed separately. Statistical analyses were performed using a linear mixed model ANOVA with repeated measures (LMMARM), with a first-order autoregressive covariance structure (Littell et al. 2000, Preisser and Elkinton 2008, Jones et al. 2016) (Appendix 11). For each analysis, treatment, year, and the interaction of treatment × year were the fixed effects factors. Tree nested within treatment and plot was the random effects factor, and year and tree were the repeated measures parameter and subject, respectively. All data on tree health and HWA population were transformed in order to satisfy statistical assumptions. Tree health index values were transformed using a Box-Cox transformation, and HWA population index values were log10(y + 1) transformed (Zar 2010). The line graphs were constructed using untransformed data in standard least squares models (SLSM) followed by multiple comparisons with Student's t for assessing significant differences among the levels of the fixed effects factors. The mixed model analyses and fixed effects comparisons were carried out in JMP Pro 12.0.0® (SAS 2013) at a significance level of $\alpha = 0.05$. Line graphs were constructed in JMP Pro 12.0.0® (SAS 2013). Time-lag, cross-correlation analyses were performed using MATLAB®. Tree health was first compared against HWA population, then the order was reversed. This accounts for the use of the negative and positive values in the results. The threshold for a strong correlation was set at ≥ .50 (pers. corr. with Carlyle Brewster).

2.3 Results

2.3.1 Kentucky Ridge State Forest, Kentucky

Analysis via LMMARM for KRSF, KY HWA population index values showed an overall significant interaction for year and treatment (DF = 18, 1265.9; *F* = 4.68; *p* < 0.0001). In 2010, HWA population index values were significantly lower for the chemical and control treatment plots (30.86 ± 2.88 and 30.07 ± 2.98, respectively) than the other treatments (Fig. 2.1A). HWA population index values fell significantly in 2011 compared to 2010, with the exception of the untreated control plots. Chemical treatment plots exhibited significantly lower population index values (4.14 ± 2.83) compared with either the biological or untreated control treatments. Populations rose significantly for all treatments in 2012 compared to 2011. The biological plots (49.35 ± 3.88) contained significantly more HWA than any other treatment. In 2013, HWA populations declined significantly for the chemical and untreated control treatment plots, and did not change significantly for the biological or bio+chem treatments compared to 2012. The HWA populations for the chemical and untreated control treatment plots (23.65 ± 2.98 and 25.04 ± 3.19, respectively) were both significantly smaller than the other plots. In 2014, HWA populations in each treatment plot declined significantly compared to 2013, with no treatment displaying significantly different population size. In 2015, HWA index values did not change significantly compared to 2014, and in 2016 they were not significantly different compared to 2015. In each of these years, no treatment type was significantly different from another.

At KRSF, analysis via LMMARM showed significant interaction of year and treatment with respect to tree health (DF = 18, 1222.7; *F* = 4.27; *p* < 0.0001). At the start of the study in 2010, the chemical and bio+chem plots (71.72 ± 1.73 and 68.10 ± 1.73, respectively) had significantly greater tree health index values than the other plots (Fig. 2.1B). Biological plots showed the lowest tree health at 63.17 ± 1.73. In 2011, tree health did not change significantly for any treatment compared to 2010. In 2012, tree health for each treatment declined significantly compared to 2011. The chemical and bio+chem plots (65.97 ± 1.73 and 65.47 ± 1.73, respectively) were significantly greater tree health than the other plots. This trend continued in 2013. In 2014, the control and biological plots experienced significant decline in their HWA index values compared to 2013. The chemical and bio+chem treatments did not change significantly compared to 2013. In 2015, there was no significant change in the chemical or bio+chem plots compared to 2014. The untreated control and biological plots both continued to experience significant declines tree health compared to 2014. In 2016, every treatment plot experienced significant tree health recovery compared to 2015. Chemical and bio+chem plots (64.69 ± 1.51 and 65.96 ± 1.54, respectively) continued to maintain significantly greater health index values than the other plots.

Comparison of the relationship between tree health and HWA population in the untreated control plots for each site allowed us to characterize the inherent time-lag in this system. Cross-correlation analyses performed in MATLAB® compared lag periods in increments of one year. At KRSF, KY the strongest correlations existed for lag periods -3, -2, -1, 1, and 2 (Table 2.1). Negative values represent the number of years that tree health index lags behind changes in HWA population, while the positive values represent the number of years that HWA population lags behind tree health index. The results in Table 2.1, therefore suggests that tree health index lags approximately 1 – 3 years behind changes in the HWA population as shown by the relatively high correlations at lag periods -1, -2 and -3; similarly, changes in HWA populations appear to lag about 1 – 2 years behind changes in tree health index because of the relatively high correlations at lag periods 1 and 2.

2.2.2 Twin Falls State Park, West Virginia

Analysis via LMMARM of HWA population index values at TFSP, WV showed an overall significant interaction for year and treatment (DF = 15, 1048.6; *F* = 5.45; *p* < 0.0001). In 2011, HWA index values were significantly greater in the chemical and bio+chem plots (30.57 ± 3.23 and 38.33 ± 3.80, respectively) than in the other treatments (Fig. 2.2A). In 2012, the HWA population increased significantly for all of the treatments compared to 2011. The chemical and the biological plots (42.62 ± 3.46 and 43.35 ± 3.29, respectively) showed significantly smaller index values than the other plots. In 2013, HWA population index values in the chemical and bio+chem plots declined significantly compared to 2012. They also had lower index values (26.46 ± 3.39 and 31.32 ± 3.26, respectively) than the other two treatments. The untreated control and biological treatment plots showed no significant change compared to 2012. In 2014, the control treatments experienced significant decline compared to 2013. The other treatments showed no significant change from 2013. In 2015, all treatments experienced significant decline compared to 2014. There were no differences among treatments. In 2016, there was no significant change for any treatment compared to 2015, and there were no differences amongst treatments.

Analysis via LMMARM of tree health index values at TFSP, WV showed significant interaction for year and treatment (DF = 15, 955.7; *F* = 1.98; *p* = 0.0140). Within most years, no significant treatment effects were observed in any of the plots (Fig. 2.2B). Tree health did not significantly change between 2011 and 2012 for any treatment. In 2013, there was significant decline in tree health in the chemical, bio+chem, and biological treatments compared to 2012. In 2014, tree health declined significantly for the chemical, biological, and bio+chem plots; tree health in the untreated control plots remained unchanged compared to 2013. In 2015, the chemical treatments displayed significant recovery compared to 2014, and the other plots showed no significant changes. At this time, the chemical treatment plots did exhibit significantly greater tree health than the control plots (53.21 ± 1.32 vs 49.05

± 1.51, respectively). In 2016, there was significant recovery in tree health for all treatments compared to 2015, although no significant difference was observed among plots.

Cross-correlation of HWA population and tree health at TFSP showed the strongest correlations for lag periods -3, -2, -1, and 1. This suggests that tree health lags 1 – 3 years behind changes in HWA population and that HWA populations lag about 1 year behind changes in tree health (Table 2.1).

2.2.3 Coal Creek, Tennessee

Analysis via LMMARM at CC, TN for HWA population index values showed an overall significant interaction for year and treatment (DF = 12, 681.6; *F* = 2.51; *p* = 0.0031). In 2012, the chemical plots had significantly smaller population index values than the untreated control or bio+chem treatments (29.33 ± 3.37) (Fig. 2.3A) . In 2013, HWA population index values displayed significant decline for all treatments compared to 2012. There was no statistically significant difference amongst treatments. In 2014, there were no significant changes compared to 2013. The bio+chem and chemical index values (7.40 ± 3.37 and 7.82 ± 3.37, respectively) were significantly smaller than the control plots. In 2015, HWA index values rose significantly for all treatments compared to 2014. Chemical and bio+chem plots displayed significantly smaller population index values than the biological treatments (30.41 ± 3.62 and 26.28 ± 3.20 vs 48.65 ± 3.41, respectively). In 2016, the control and bio+chem treatments showed significant increases in their HWA index values compared to 2015. The chemical and biological plots did not show any significant changes from 2015. The chemical and the bio+chem plots (36.70 ± 3.10 and 37.84 ± 3.05, respectively) were significantly smaller than the other plots.

Analysis via LMMARM at CC, TN for tree health index values showed significant interaction for year and treatment (DF = 12, 750.2; *F* = 3.17; *p* < .0002). In 2012, tree health index values for the bio+chem treatments (71.00 ± 1.50) were significantly greater compared with the other treatment plots (Fig. 2.3B). In 2013, tree health declined significantly for all treatments compared to 2012. The bio+chem treatments (62.34 ± 1.51) displayed a significantly greater tree health index than either the chemical or the biological plots. In 2014, tree health did not change significantly for any treatment compared to 2013. The bio+chem treatments maintained a significantly greater tree health index (62.57 ± 1.50) than the chemical or the biological plots. In 2015, the biological, chemical and untreated control plots all declined significantly compared to 2014. The bio+chem treatments did not change significantly, and they again exhibited a significantly greater tree health index (59.58 ± 1.37) than the other plots. In 2016, all of the treatments experienced significant recovery compared to 2015. The bio+chem treatments had significantly greater tree health index values (69.24 ± 1.31) than the other plots, and the index values of the chemical treatments (64.99 ± 1.32) were significantly greater than the biological or the untreated control plots.

Cross-correlation of tree health and HWA population at CC showed the strongest correlations at lag periods -1, 1, 2, and 3 (Table 2.1). This represents a divergence from the lag periods established at KRSF and TFSP. The analysis of CC indicates that tree health lags roughly 1 year behind changes in HWA population and that HWA populations lag 1 – 3 years behind changes in tree health. The exact cause of this discrepancy is not known; however, it could be a result of the shorter study period at CC than at TFSP or KRSF (4 years vs. 5 and 6 years, respectively).

2.2.5 *L. nigrinus* Recovery and Winter Weather

Recovery of *L. nigrinus* began two-years post-release. Recoveries were made from KRSF, KY in 2012 and 2013 and from TFSP, WV in 2013; however, there have been no recoveries since then at any site (Table 2.3).

Minimum daily temperature for the days of the polar vortices in 2014 and 2015 were included as possible variables in HWA decline, tree health improvement and a lack of *L. nigrinus* recovery (Figs.

2.4A – C). Predicted percent HWA mortality was adapted from McAvoy et al. (2015) (Table 2.2). The polar vortex in 2014 occurred on January 7th, and the 2015 polar vortex occurred on February 20th.

2.4 Discussion

In order to conduct a proper assessment of the health of a forest and the extent of a pest invasion, it is necessary to establish sufficient scale. The juxtaposition of a small, select cohort of trees as a reliable indicator of overall forest health underrepresents the magnitude of the forest. It is therefore in the best interest of forest health professionals to develop large-scale projects that observe the long-term effects of various treatment methods on invasive insects (Eschtruth et al. 2006, Eschtruth et al. 2013). Studying on an area-wide scale is also appropriate when considering other aspects of forest management, as it relates to biological invasions (i.e. plant, avian, mammalian, fungal, etc.), to matters of pollution, timber harvest or fallout from natural disasters.

The results of this study indicate that the control methods being tested have had very little impact on HWA populations and tree health. The tree health at each of my study sites has generally declined across all years with evidence of recovery only 2016. This is likely a result of decline in the HWA population brought about by previous fluctuations in tree health and not from direct treatment effects. This will be discussed in greater detail further, below. HWA populations have displayed a great deal of variability and do not show consistent suppression by any one treatment type. First, I will address the effectiveness of the chemically treated plots. Although the efficacy of chemical treatments is well known (Silcox 2002, Cowles et al. 2006), chemically treated plots still showed declining health and fluctuating HWA numbers. The proportion of trees under chemical protection in each plot began at 33% and was raised to 50% three years later. This is a noteworthy observation because it demonstrates that chemical treatment at these proportions alone cannot suppress HWA. Next, in the case of the plots involving biological control, the predators failed to establish and thus were unable to provide protection to trees in the treatment plots (Table 2.3). This is not to imply that the treatments were entirely ineffective. Plots involving chemical treatments often exhibited reduced HWA populations and greater tree health index values, relative to those that did not (Figs. 2.1 – 2.3). Additionally, trees that received chemical treatment were often entirely clear of HWA ovisacs and had abundant, healthy foliage. The problem is that these results were not consistent. Frequently, the tree health and/or HWA population index values of chemically treated plots would not be significantly different from those of the untreated control or biological treatments. The confounding factor lies within the way the treatment plots were organized. Because they incorporated treated and untreated trees, the positive effects any treatment may have had were diluted by the untreated trees which continued to deteriorate as HWA fed; this was an intentional act. We wanted to create a simulation that was representative of a large, forest management project wherein it was not feasible to treat every single tree. It was the hope, that we could discover a proportion of trees that could be protected chemically that would be sufficient to maintain or improve stand health while the biological control agent established. After all, one of the goals of IPM is to reduce the use of chemical insecticides (Ehler 2006). The purpose of the biological control is to overcome the limitations of human-applied chemical treatments, and to act on its own to target and eliminate HWA. Ultimately, the lack of consistency indicates that other factors may have had greater impacts on tree health and HWA population than our treatment methods did. These factors are discussed further, below.

Organisms associated within an ecosystem are often organized in a way wherein a change in one does not result in the immediate change of the other (Brooks et al. 1999, Metzger et al. 2009). The interaction of HWA and eastern hemlock is one such example. McClure (1991) showed that the progress of an HWA invasion is intimately linked with the health of the trees that they feed on. His study, showed that HWA populations achieved an all-time peak in the first year. In the second year, HWA populations declined due to the reduced health of the trees, and tree health consequently improved. Then, in the third year, the HWA were able to recover their numbers by feeding on the foliage of the partially-recovered hemlocks. By the fourth year, all of the eastern hemlocks were dead. The results of this study demonstrate that this is due to a time-lag effect (Table 2.1). Results show that in KRSF, KY, changes in tree health lagged up to three years behind changes in the HWA population. I arrived at this conclusion based upon the relatively strong (≥ .50) correlation present in the cross-correlation analysis at lag periods -3, -2, and -1. Changes in the HWA population, lagged up to two years behind changes in tree health. This is supported by observation of Figure 2.1. Tree health at KRSF, KY begins to fall after 2011, and HWA populations don't begin their decline until after 2012. This is followed by steady decline in tree health and HWA population until 2015, when tree health begins to recover. A similar pattern was observed at TFSP, WV, with tree health lagging up to three years behind changes in the HWA population, and the HWA population lagging one year behind changes in tree health. The results differed at the CC, TN site. Here, tree health seems to be strongly correlated only one year behind changes in HWA population, and HWA populations show strong correlations up to three years after changes in tree health. This difference could be due to a shorter period of study, compared to KY or WV, which may have missed changes in the HWA population prior to 2012, or due to the fact that CC, TN occupies a warmer climate than KY or WV (hardiness zone 7a vs 6b and 6a, respectively). The results of this study differ from McClure (1991) in that the lags reported in that experiment were shorter than those shown in this one. In McClure's experiment, it took only four years from the initial invasion until all of his study stands were killed. However, Eschtruth et al. (2013) showed that hemlock stands are not uniform in their rate of decline and some may survive for more than a decade. The results of this study corroborate Eschtruth's conclusions, with KY hemlocks surviving six years and WV hemlocks surviving for five. The fact that trees in two of the three study sites took up to three years to fully show changes, could be due to long-term physiological responses to HWA feeding. As mentioned previously, HWA can reduce new growth, drain photosynthate, and instigate the formation of false growth-rings (Gonda-King et al. 2012, Oten et al. 2012). Each of these outcomes has an impact on the trees' ability to sequester nutrients and to maintain efficient transportation of water and food, which may delay recovery and produce long lag periods. Describing hemlock and HWA interactions in the context of a time-lagged correlation is a novel means of interpretation and one that lends itself well to studying complex ecosystem processes (Loehle and Li 1996, Metzger et al. 2009). Continued observation is needed at each site in order to gain a better understanding of the yearly flux of HWA and tree health.

In order to ensure the establishment of *L. nigrinus*, each release plot was inoculated with approximately 750 adult beetles (125 per tree x 6 trees). According to Mausel et al. (2010), the likelihood of *L. nigrinus* establishing at KY and TN was approximately 85 – 95% and at WV it was roughly 60 – 85%. These percentages were estimated based on the USDA plant hardiness zones each site occupies. However, the lack of sustained recovery of *L. nigrinus* suggests that there are factors at work which may be able to overcome the large, initial introductions. First, the winters of 2014 and 2015 saw the occurrence of polar vortex events that produced very low temperatures (Fig. 2.4). In KRSF, KY and TFSP, WV the HWA populations declined, likely as a result of these events. Coincidentally, recovery of *L. nigrinus* failed in these years continuing through 2016 (Table 2.3). One could conclude from these data that the crash in the HWA population produced a concurrent decline in *L. nigrinus*. However, due to the fact that *L. nigrinus* was successfully recovered several years prior to the polar vortices, there could be a remnant population. Observations of the sites will need to be maintained in order to see if HWA will recover and if *L. nigrinus* will also increase. At CC, TN *L. nigrinus* was introduced in 2012 and recovery attempts began in 2014. In between these operations, the HWA population declined in 2013 and remained low in 2014 (Fig. 2.3). However, unlike in KRSF or TFSP, it is possible that *L. nigrinus* was never able to establish because it did not have a sufficient initial prey population to feed on. Again, future observations may reveal the presence of survivors, although if *L. nigrinus* was unable to establish, then there may be no chance of recovery. The cold temperatures themselves may have caused mortality through freezing. The *L. nigrinus* used in this experiment were collected from a coastal population in the Pacific Northwest. Mausel et al. (2011) found that the average super cooling point for this cohort was -16.9 ± .03 ^{o}C. The temperatures achieved during the polar vortices would have been well below that point (Fig. 2.4). Next, beat-sheet sampling was used in this project to recover adult *L. nigrinus*. Beat-sheet sampling is often used to survey a large study area due to the rapidity with which vegetation can be surveyed; however, it has been criticized for creating false negatives (Mausel et al. 2010). This is due to the fact that a researcher may miss the presence of *L. nigrinus* either due to the poor timing of surveys or inexpert beating technique. In order to remedy this shortcoming, branches were collected for larval sampling. This technique has the benefit of sampling for a less mobile life stage and directly targets large concentrations of HWA ovisacs, where *L. nigrinus* larvae are likely to be. Despite surveying in this manner, a researcher can still overlook beetle nesting sites.

Although we did not intentionally focus on weather effects on the system, the occurrence of extremely cold winter temperatures during the polar vortex events of January 7th, 2014 and February 20th, 2015, led to significant HWA mortality across the East Coast (McAvoy et al. 2015). Polar vortices are rotating, low-pressure systems of Arctic air located at the poles of the Earth. The vortices can weaken or strengthen as the seasons pass and if a vortex weakens enough, mid-latitude, high-pressure airstreams can draw the polar air equatorward. Observations of minimum, daily temperatures for the days on which a polar vortex occurred showed that KRSF, KY and TFSP, WV were colder than CC, TN. Comparing these temperatures to work done in prior research, (Costa et al. 2008, Trotter and Shields 2009, McAvoy et al. 2015) shows that the HWA mortality at KRSF, KY reached approximately 66.5 and 88% each year, respectively (Table 2.2). TFSP, WV experienced approximate mortality rates of 85 and 95% each year, and CC, TN, which experienced warmer weather, resulted in mortality of approximately 48 and 59% each year. These winter temperatures are in keeping with the USDA plant hardiness zones that each site occupies. KRSF, KY resides within hardiness zone 6b where the average, annual, minimum winter temperature is between -20.6 and -17.8 ^{o}C. TFSP, WV can be found within hardiness zone 6a where the temperature range is between -23.3 and -20.6 ^{o}C, and CC, TN falls within hardiness zone 7a where minimum winter temperature range is between -17.8 and -15 ^{o}C. Due to the unique nature of a polar vortex, some sites did exceed their expected, minimum winter temperatures. Although polar vortex events are not a common occurrences, they represent variable weather that presents a further limit to the northwards range expansion and establishment of HWA (Costa et al. 2008, Trotter and Shields 2009).

While the treatment methods did not result in improved stand health as we anticipated, we were able to observe area-wide changes in eastern hemlock forest health and HWA populations over time. This helps provide insight into the cycle of decline and recovery of the trees as partly influenced by climate in the southern U.S. Continued long-term monitoring may still yield treatment effects, and we will endeavor to sample for *L. nigrinus* establishment and evaluate the treatment effects at different scales of resolution than we have currently done.

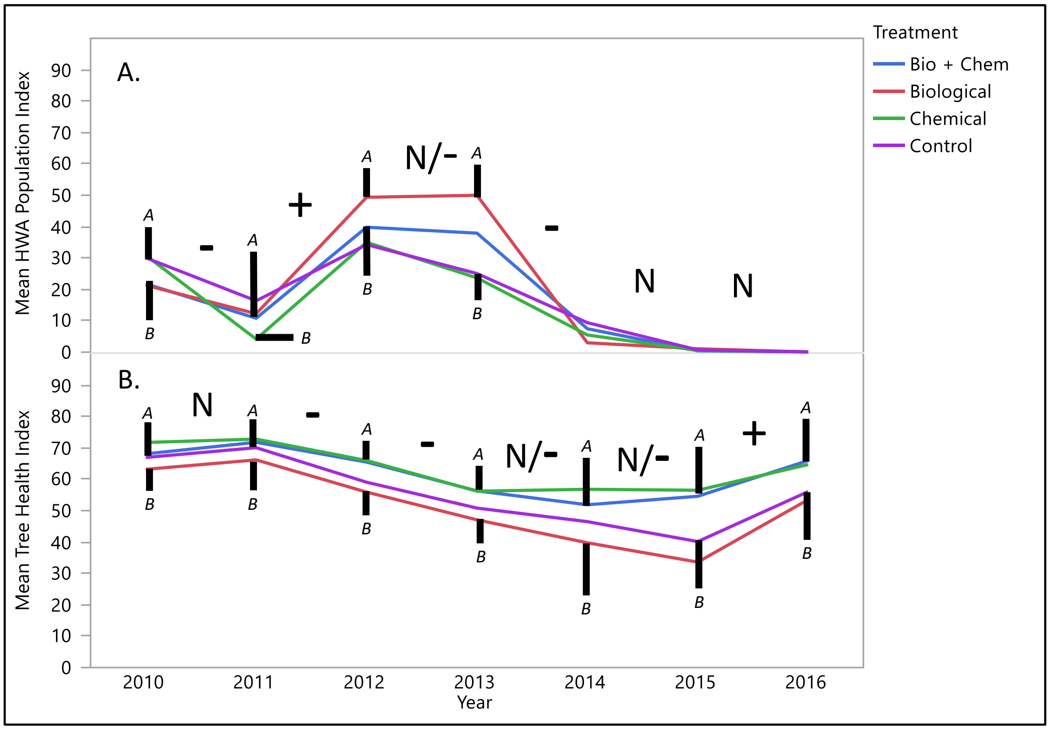


Figure 2.1 Mean HWA index (A.) and mean tree health index (B.) at Kentucky Ridge State Forest KY, 2010 - 2016. Tree health was determined by averaging five health characteristics, all rated 0 – 100 (live crown ratio, live branches, live tips, new foliage, and foliage density). HWA population was determined by counting up to twenty ovisacs each on ten branches and dividing by two. Graphs were constructed using standard least square means models. Effects compared via Student's t test. Letters A & B (with black bars connecting treatments) represent significant differences among treatments within each year (*p* ≤ .05). **+**, **-**, and **N** represent significant differences among years (*p* ≤ .05); **+** = increase, **-** = decrease, **N** = no change. **+**, **-** or **N** separated by a **/** represents different trends among treatments within each year.

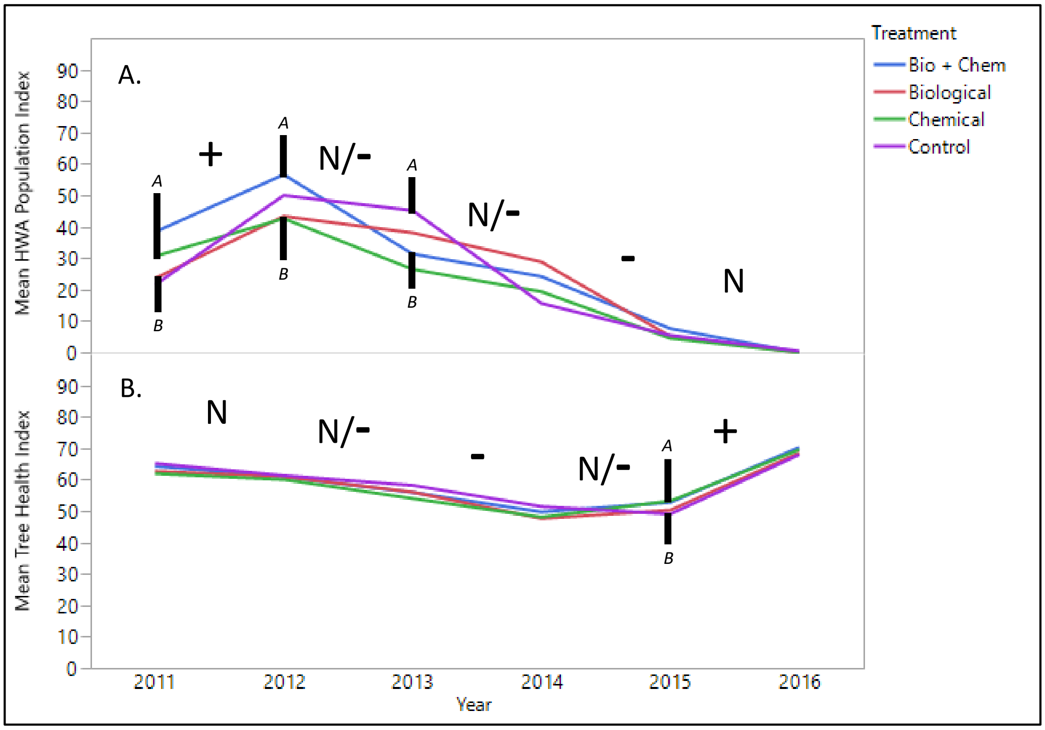
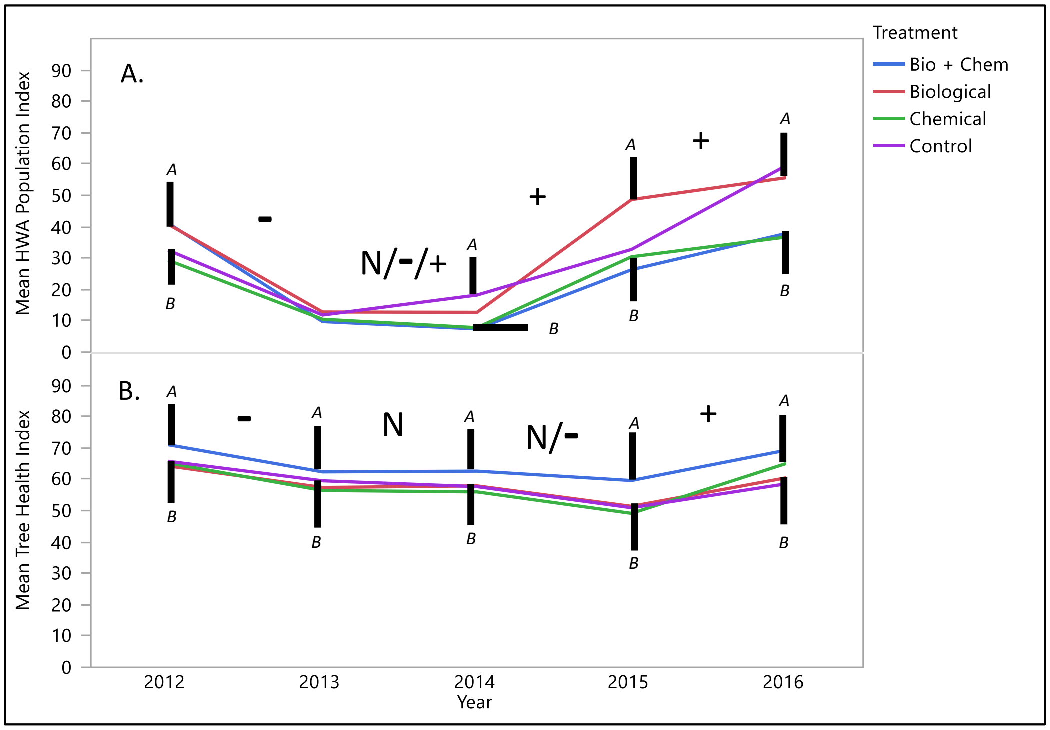


Figure 2.2 Mean HWA index (A.) and mean tree health index (B.) at Twin Falls State Park WV, 2011 - 2016. Tree health was determined by averaging five health characteristics, all rated 0 – 100 (live crown ratio, live branches, live tips, new foliage, and foliage density). HWA population was determined by counting up to twenty ovisacs each on ten branches and dividing by two. Graphs were constructed using standard least square means models. Effects compared via Student's t test. Letters A & B (with black bars connecting treatments) represent significant differences among treatments within each year (*p* ≤.05). **+**, **-**, and **N** represent significant differences among years (*p* ≤ .05); **+** = increase, **-** = decrease, **N** = no change. **+**, **-** or **N** separated by a **/** represents different trends among treatments within each year.



Figures 2.3 Mean HWA index (A.) and mean tree health index (B.) at Coal Creek TN, 2012 - 2016. Tree health was determined by averaging five health characteristics, all rated 0 – 100 (live crown ratio, live branches, live tips, new foliage, and foliage density). HWA population was determined by counting up to twenty ovisacs each on ten branches and dividing by two. Graphs were constructed using standard least square means models. Effects compared via Student's t test. Letters A & B (with black bars connecting treatments) represent significant differences among treatments within each year ($p \leq .05$). **+**, **-**, and **N** represent significant differences among years ($p \leq .05$); **+** = increase, **-** = decrease, **N** = no change. **+**, **-** or **N** separated by a **/** represents different trends among treatments within each year.

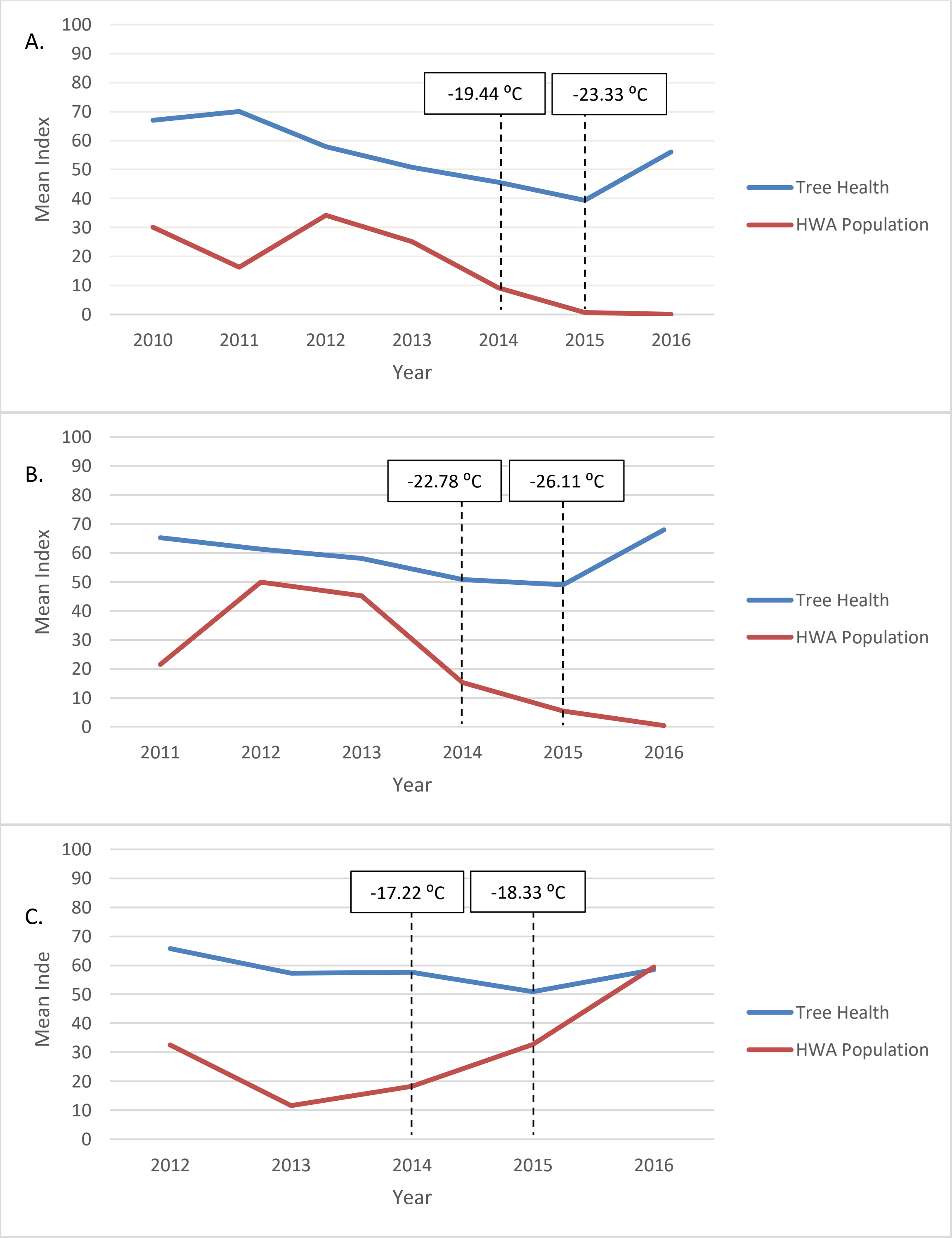


Figure 2.4 These figures display the relationship between tree health and HWA population for the untreated control plots for each study site (KY top, WV, TN bottom). Vertical dashed lines represent the minimum winter temperature during the day of each polar vortex event. The 2014 vortex occurred January 7th, and the 2015 vortex occurred February 20th.

Table 2.1 Results of cross-correlation tests of control plots at all study sites. Red rows denote strong correlations (> .50). Leftmost columns represent 1-year lag periods. Negative lag periods represent tree health lagged behind changes in HWA population. Positive lag-periods represent HWA population lagged behind changes in tree health. Rightmost columns represent correlation strength. Correlation strength scales from 0 to 1. Analyses carried out via MATLAB©.

KRSF, KY		TFSP, WV		CC, TN	
Lag period	Correlation	Lag period	Correlation	Lag period	Correlation
-6	.21	-5	0.14	-4	0.17
-5	.26	-4	0.42	-3	0.23
-4	.48	-3	0.63	-2	0.35
-3	.61	-2	0.67	-1	0.53
-2	.69	-1	0.73	0	0.88
-1	.77	0	0.78	1	0.66
0	.84	1	0.69	2	0.64
1	.66	2	0.40	3	0.55
2	.56	3	0.13	4	.38
3	.29	4	0.04		
4	.08	5	.002		
5	.01				
6	.001				

Table 2.2 Predicted percent mortality of HWA based upon minimum winter temperature. Adapted from McAvoy et al. (2015).

Minimum Winter Temperature ^{o}C	Predicted % Mortality
-16	38
-18	59
-20	74
-22	81
-24	91
-26	95
-28	97

Table 2.3 Recovery of *L. nigrinus* adults and larvae for each site, separated by year. Recovery began two-years post-release.

Site	2012	2013	2014	2015	2016
KRSF, KY	1 adult, 15 larvae	1 adult, 11 larvae	0 recovered	0 recovered	0 recovered
TFSP, WV	No Collection	0 adult, 38 larvae	0 recovered	0 recovered	0 recovered
CC, TN	No Collection	No Collection	0 recovered	0 recovered	0 recovered

References

Annand, P. 1924. A new species of *Adelges* (Hemiptera, Phylloxeridae). Pan-Pacific Entomologist 1: 79-82.

Benton, E., J. Grant, R. Webster, R. Nichols, R. Cowles, A. Lagalante, and C. Coots. 2015. Assessment of imidacloprid and its metabolites in foliage of eastern hemlock multiple years following treatment for hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), in forested conditions. Journal of Economic Entomology 108: 2672-2682.

Benton, E. P., J. Grant, T. Mueller, R. Webster, and R. Nichols. 2016. Consequences of imidacloprid treatments for hemlock woolly adelgid on stream water quality in the southern Appalachians. Forest Ecology and Management 360: 152-158.

Beschta, R. L. 1997. Riparian shade and stream temperature: an alternative perspective. Rangelands 19: 25-28.

Boulter, D. 1993. Insect pest control by copying nature using genetically engineered crops. Phytochemistry 34: 1453-1466.

Brooks, T. M., S. L. Pimm, and J. O. Oyugi. 1999. Time lag between deforestation and bird extinction in tropical forest fragments. Conservation Biology 13: 1140-1150.

Cheah, C., M. E. Montgomery, S. Salom, B. L. Parker, S. Costa, and M. Skinner. 2004. Biological control of hemlock woolly adelgid, vol. 22. USDA Forest Service FHTET 2004-04, Morgantown, WV.

Costa, S. D., R. T. I. Trotter, M. Montgomery, and M. Fortney. 2008. Low temperature in the hemlock woolly adelgid system, pp. 47-52. *In* B. Onken and R. Reardon (eds.), Fourth Symposium on Hemlock Woolly Adelgid in the Eastern United States. USDA Forest Service FHTET 2008-01, WV.

Cowles, R., M. Montgomery, and C.-J. Cheah. 2006. Activity and residues of imidacloprid applied to soil and tree trunks to control hemlock woolly adelgid (Hemiptera: Adelgidae) in forests. Journal of Economic Entomology 99: 1258-1267.

Daley, M. J., N. G. Phillips, C. Pettijohn, and J. L. Hadley. 2007. Water use by eastern hemlock (*Tsuga canadensis*) and black birch (*Betula lenta*): implications of effects of the hemlock woolly adelgid. Canadian Journal of Forest Research 37: 2031-2040.

Davis, G. A., S. M. Salom, C. C. Brewster, B. P. Onken, and L. T. Kok. 2012. Spatiotemporal distribution of the hemlock woolly adelgid predator *Laricobius nigrinus* after release in eastern hemlock forests. Agricultural and Forest Entomology 14: 408-418.

Ehler, L. E. 2006. Integrated pest management (IPM): definition, historical development and implementation, and the other IPM. Pest Management Science 62: 787-789.

Eisenback, B. M., S. M. Salom, L. T. Kok, and A. F. Lagalante. 2010. Lethal and sublethal effects of imidacloprid on hemlock woolly adelgid (Hemiptera: Adelgidae) and two introduced predator species. Journal of Economic Entomology 103: 1222-1234.

Eisenback, B. M., S. M. Salom, L. T. Kok, and A. F. Lagalante. 2014. Impacts of trunk and soil injections of low rates of imidacloprid on hemlock woolly adelgid (Hemiptera: Adelgidae) and eastern hemlock (Pinales: Pinaceae) health. Journal of Economic Entomology 107: 250-258.

Ellison, A. M., A. A. B. Plotkin, and S. Khalid. 2016. Foundation species loss and biodiversity of the herbaceous layer in New England forests. Forests 7: 1-9.

Ellison, A. M., J. Chen, D. Díaz, C. Kammerer-Burnham, and M. Lau. 2005a. Changes in ant community structure and composition associated with hemlock decline in New England, pp. 294-289. *In* B. Onken and R. Reardon (eds.), Proceedings of the Third Symposium on Hemlock Woolly Adelgid in the Eastern United States. USDA Forest Service FHTET 2005-01.

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

Eschtruth, A. K., R. A. Evans, and J. J. Battles. 2013. Patterns and predictors of survival in *Tsuga canadensis* populations infested by the exotic pest *Adelges tsugae*: 20 years of monitoring. Forest Ecology and Management 305: 195-203.

Eschtruth, A. K., N. L. Cleavitt, J. J. Battles, R. A. Evans, and T. J. Fahey. 2006. Vegetation dynamics in declining eastern hemlock stands: 9 years of forest response to hemlock woolly adelgid infestation. Canadian Journal of Forest Research 36: 1435-1450.

Evans, A. M., and T. G. Gregoire. 2007. The tree crown distribution of hemlock woolly adelgid, *Adelges tsugae* (Hem., Adelgidae) from randomized branch sampling. Journal of Applied Entomology 131: 26-33.

Evans, R. A. 2002. An ecosystem unraveling?, pp. 23-33. *In* B. Onken, R. Reardon and J. Lashomb (eds.), Proceedings of the Symposium on Hemlock Woolly Adelgid in the Eastern North America. New Jersey Agricultural Experiment Station and Rutgers University, East Brunswick NJ.

Fischer, M. J., N. P. Havill, C. C. Brewster, G. A. Davis, S. M. Salom, and L. T. Kok. 2015. Field assessment of hybridization between *Laricobius nigrinus* and *L. rubidus*, predators of Adelgidae. Biological Control 82: 1-6.

Flowers, R., S. Salom, and L. Kok. 2005. Competitive interactions among two specialist predators and a generalist predator of hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae), in the laboratory. Environmental Entomology 34: 664-675.

Gatton, H., G. Zilahi-Balogh, S. Salom, and L. Kok. 2009. Biology and development of *Laricobius kangdingensis* Zilahi-Balogh and Jelínek, a newly discovered predator of *Adelges tsugae*. Biological Control 54: 219-228.

Gonda-King, L., L. Radville, and E. L. Preisser. 2012. False ring formation in eastern hemlock branches: Impacts of hemlock woolly adelgid and elongate hemlock scale. Environmental Entomology 41: 523-31.

Havill, N. P., and R. G. Foottit. 2007. Biology and evolution of Adelgidae. Annual Review of Entomology 52: 325-349.

Havill, N. P., M. E. Montgomery, and M. Keena. 2011. Hemlock woolly adelgid and its hemlock hosts: a global perspective, pp. 3-14. *In* B. P. Onken and R. Reardon (eds.), Implementation and Status of Biological Control of the Hemlock Woolly Adelgid. USDA Forest Service FHTET 2011-04, Morgantown, WV.

Havill, N. P., L. C. Vieira, and S. M. Salom. 2014. Biology and control of hemlock woolly adelgid 2ed, vol. USDA Forest Service FHTET 2014-05, Morgantown, WV.

Havill, N. P., M. E. Montgomery, G. Yu, A. Caccone, and S. Shiyake. 2006. Mitochondrial DNA from Hemlock Woolly Adelgid (Hemiptera: Adelgidae) Suggests Cryptic Speciation and Pinpoints the Source of the Introduction to Eastern North America. Annals of the Entomological Society of America 99: 195-203.

Havill, N. P., G. Davis, D. L. Mausel, J. Klein, R. McDonald, C. Jones, M. Fischer, S. Salom, and A. Caccone. 2012. Hybridization between a native and introduced predator of Adelgidae: An unintended result of classical biological control. Biological Control 63: 359-369.

Havill, N. P., S. Shiyake, A. Lamb Galloway, R. G. Foottit, G. Yu, A. Paradis, J. Elkinton, M. E. Montgomery, M. Sano, and A. Caccone. 2016. Ancient and modern colonization of North

America by hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), an invasive insect from East Asia. Molecular ecology 25: 2065-2080.

Holmes, T. P., E. A. Murphy, and D. D. Royle. 2005. The economic impacts of hemlock woolly adelgid on residential landscape values: Sparta, New Jersey case study, pp. 15-24. *In* B. Onken and R. Reardon (eds.), Proceedings of the Third Symposium on Hemlock Woolly Adelgid in the Eastern United States. USDA Forest Service FHTET 2005-01.

Jenkins, J. C., J. D. Aber, and C. D. Canham. 1999. Hemlock woolly adelgid impacts on community structure and N cycling rates in eastern hemlock forests. Canadian Journal of Forest Research 29: 630-645.

Jones, A. C., D. E. Mullins, C. Brewster, J. P. Rhea, and S. M. Salom. 2016. Fitness and physiology of *Adelges tsugae* (Hemiptera: Adelgidae) in relation to the health of the eastern hemlock. Insect Science 23: 843–853.

Joseph, S. V., J. L. Hanula, S. K. Braman, and F. J. Byrne. 2011. Effects of fertilizer and low rates of imidacloprid on *Adelges tsugae* (Hemiptera: Adelgidae). Journal of Economic Entomology 104: 868-878.

Kohler, G. R., V. L. Stiefel, K. F. Wallin, and D. W. Ross. 2008. Predators associated with the hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. Environmental Entomology 37: 494-504.

Lamb, A., S. Salom, and L. Kok. 2007. Factors influencing aestivation in *Laricobius nigrinus* (Coleoptera: Derodontidae), a predator of *Adelges tsugae* (Hemiptera: Adelgidae). The Canadian Entomologist 139: 576-586.

Lamb, A., S. Salom, L. Kok, and D. Mausel. 2006. Confined field release of *Laricobius nigrinus* (Coleoptera: Derodontidae), a predator of the hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), in Virginia. Canadian Journal of Forest Research 36: 369-375.

Lawrence, J. F., and T. Hlavac. 1979. Review of the Derodontidae (Coleoptera: Polyphaga) with new species from North America and Chile. The Coleopterists' Bulletin: 369-414.

Lee, M. K., A. Curtiss, E. Alcantara, and D. Dean. 1996. Synergistic effect of the *Bacillus thuringiensis* toxins CryIAa and CryIAc on the gypsy moth, *Lymantria dispar*. Applied and Environmental Microbiology 62: 583-586.

Lewis, J. D., J. Licitra, A. R. Tuininga, A. Sirulnik, G. D. Turner, and J. Johnson. 2008. Oak seedling growth and ectomycorrhizal colonization are less in eastern hemlock stands infested with hemlock woolly adelgid than in adjacent oak stands. Tree Physiology 28: 629-636.

Lind, R. J., M. S. Clough, F. G. P. Earley, S. Wonnacott, and S. E. Reynolds. 1999. Characterisation of the relationship between binding sites for imidacloprid and other nicotinic ligands in insects. Pesticide Science 55: 1029-1031.

Littell, R. C., J. Pendergast, and R. Natarajan. 2000. Tutorial in biostatistics: modelling covariance structure in the analysis of repeated measures data. Statistics in Medicine 19: 1819.

Loehle, C., and B.-L. Li. 1996. Habitat destruction and the extinction debt revisited. Ecological Applications 6: 784-789.

Mausel, D., S. Salom, L. Kok, and J. Fidgen. 2008. Propagation, synchrony, and impact of introduced and native *Laricobius* spp. (Coleoptera: Derodontidae) on hemlock woolly adelgid in Virginia. Environmental Entomology 37: 1498-1507.

Mausel, D. L., R. G. Van Driesche, and J. S. Elkinton. 2011. Comparative cold tolerance and climate matching of coastal and inland *Laricobius nigrinus* (Coleoptera: Derodontidae), a biological control agent of hemlock woolly adelgid. Biological Control 58: 96-102.

Mausel, D. L., S. M. Salom, L. T. Kok, and G. A. Davis. 2010. Establishment of the hemlock woolly adelgid predator, *Laricobius nigrinus* (Coleoptera: Derodontidae), in the Eastern United States. Environmental Entomology 39: 440-448.

Mayfield, A. E., B. C. Reynolds, C. I. Coots, N. P. Havill, C. Brownie, A. R. Tait, J. L. Hanula, S. V. Joseph, and A. B. Galloway. 2015. Establishment, hybridization and impact of *Laricobius* predators on insecticide-treated hemlocks: Exploring integrated management of the hemlock woolly adelgid. Forest Ecology and Management 335: 1-10.

McAvoy, T. J., E. A. Roberts, N. G. Johnson, N. F. Schneeberger, and S. M. Salom. 2015. Hemlock woolly adelgid sistens mortality and progrediens recovery during the winter of 2014 - 2015 (unpublished report), pp. 1-9. Virginia Polytechinc and State University, Blacksburg, VA.

McClure, M. S. 1989a. Importance of weather to the distribution and abundance of introduced adelgid and scale insects. Agricultural and Forest Meteorology 47: 291-302.

McClure, M. S. 1989b. Evidence of a polymorphic life cycle in the hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). Annals of the Entomological Society of America 82: 50-54.

McClure, M. S. 1991. Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on *Tsuga canadensis*. Environmental Entomology 20: 258-264.

MClure, M. S. 1987. Biology and Control of Hemlock Woolly Adelgid. The Connecticut Agricultural Experiment Station, New Haven 851.

MClure, M. S. 1992. Effects of implanted and injected pesticides and fertilizers on the survival of *Adelges tsugae* (Homoptera: Adelgidae) and on the growth of *Tsuga canadensis*. Journal of Economic Entomology 85: 468-472.

Metzger, J. P., A. C. Martensen, M. Dixo, L. C. Bernacci, M. C. Ribeiro, A. M. G. Teixeira, and R. Pardini. 2009. Time-lag in biological responses to landscape changes in a highly dynamic Atlantic forest region. Biological Conservation 142: 1166-1177.

Miller-Pierce, M. R., D. A. Orwig, and E. Preisser. 2010. Effects of hemlock woolly adelgid and elongate hemlock scale on eastern hemlock growth and foliar chemistry. Environmental Entomology 39: 513-519.

Nauen, R., K. Tietjen, K. Wagner, and A. Elbert. 1998. Efficacy of plant metabolites of imidacloprid against *Myzus persicae* and *Aphis gossypii* (Homoptera: Aphididae). Pesticide Science 52: 53-57.

Nauen, R., U. Reckmann, S. Armbors, H.-P. Stupp, and A. Elbert. 1999. Whitefly-active metabolites of imidacloprid: Biological efficacy and translocation in cotton plants. Pesticide Science 55: 265-271.

Orwig, D. A., and D. R. Foster. 1998. Forest response to the introduced hemlock woolly adelgid in southern New England, USA. Journal of the Torrey Botanical Society 125: 60-73.

Orwig, D. A., D. R. Foster, and D. L. Mausel. 2002. Landscape patterns of hemlock decline in New England due to the introduced hemlock woolly adelgid. Journal of Biogeography 29: 1475-1487.

Oten, K., A. Cohen, and F. Hain. 2012. Insertion site selection and feeding of the hemlock woolly adelgid: Implications for host-plant resistance. *In* R. A. Sniezko, A. D. Yankchuk, J. T. Kliejunas, K. M. Palmieri, J. M. Alexandar, S. J.Frankel and S. J (eds.), Proceedings of the Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry: Disease and Insect Resistance in Forest Trees. USDA Forest Service SWRS, Albany, CA.

Oten, L. F. K., A. C. Cohen, and F. P. Hain. 2014. Stylet bundle morphology and trophically related enzymes of the hemlock woolly adelgid (Hemiptera: Adelgidae). Annals of the Entomological Society of America 107: 680-690.

Preisser, E. L., and J. S. Elkinton. 2008. Exploitative competition between invasive herbivores benefits a native host plant. Ecology 89: 2671-2677.

Preisser, E. L., K. L. F. Oten, and F. P. Hain. 2014. Hemlock woolly adelgid in the eastern United States: What have we learned? Southeastern Naturalist 13: 1-15.

Preisser, E. L., M. R. Miller-Pierce, J. Vansant, and D. A. Orwig. 2011. Eastern hemlock (*Tsuga canadensis*) regeneration in the presence of hemlock woolly adelgid (*Adelges tsugae*) and elongate hemlock scale (*Fiorinia externa*). Canadian Journal of Forest Research 41: 2433-2439.

Roberts, A., A. Lamb, B. Onken, and S. Salom. 2011. HWA predator release and monitoring database. *In* R. Reardon and B. Onken (eds.), Implementation and Status of Biological Control of the Hemlock Woolly Adelgid. USDA Forest Service, FHTET-2011-04. http://hwa.ento.vt.edu/hwa/hwa.cgi.

Russo, N. J., C. A.-J. Cheah, and M. W. Tingley. 2016. Experimental evidence for branch-to-bird transfer as a mechanism for avian dispersal of the hemlock woolly adelgid (Hemiptera: Adelgidae). Environmental Entomology 45: 1107-1114.

Salom, S. M., L. T. Kok, A. B. Lamb, and C. Jubb. 2012. Laboratory rearing of *Laricobius nigrinus* (Coleoptera: Derodontidae): A predator of the hemlock woolly adelgid (Hemiptera: Adelgidae). Psyche: A Journal of Entomology 2012: 1-9.

Sharov, A. A., D. Leonard, A. M. Liebhold, E. A. Roberts, and W. Dickerson. 2002. "Slow the spread": a national program to contain the gypsy moth. Journal of Forestry 100: 30-36.

Silcox, C. A. 2002. Using imidacloprid to control hemlock woolly adelgid, pp. 280-287. *In* B. Onken, R. Reardon and J. Lashomb (eds.), Proceedings, Symposium: Hemlock Woolly Adelgid in the Eastern United States USDA Forest Service, East Brunswick, NJ.

Steward, V. B., G. Braness, and S. Gill. 1998. Ornamental pest management using imidacloprid applied with the Kioritz [R] soil injector. Journal of Arboriculture 24: 344-346.

Sullivan, K. A., and A. M. Ellison. 2006. The seed bank of hemlock forests: Implications for forest regeneration following hemlock decline. Journal of the Torrey Botanical Society 133: 393-402.

Tingley, M. W., D. A. Orwig, R. Field, and G. Motzkin. 2002. Avian response to removal of a forest dominant: consequences of hemlock woolly adelgid infestations. Journal of Biogeography: 1505-1516.

Tomizawa, M., and J. E. Casida. 2005. Neonicotinoid insecticide toxicology: Mechanisms of selective action, pp. 247-268. Annual Reviews, Inc, United States.

Trotter, R. T., and K. S. Shields. 2009. Variation in winter survival of the invasive hemlock woolly adelgid (Hemiptera: Adelgidae) across the eastern United States. Environmental Entomology 38: 577-587.

USFS. 2015. Forest Health Protection—Hemlock Woolly Adelgid: Northeastern Area. http://na.fs.fed.us/fhp/hwa/maps/distribution.shtm.

USFS FIA. 2015. Field Guides for Standard (Phase 2) Measurements. http://www.fia.fs.fed.us/library/field-guides-methods-proc/.

Wallace, M. S., and F. P. Hain. 2000. Field surveys and evaluation of native and established predators of the hemlock woolly adelgid (Homoptera: Adelgidae) in the southeastern United States. Environmental Entomology 29: 638-644.

Ward, J. S., M. E. Montgomery, B. P. Onken, C. A. S.-J. Cheah, and R. S. Cowles. 2004. Eastern hemlock forests: Guidelines to minimize the impacts of hemlock woolly adelgid, vol. 1. USDA Forest Service NA-TP-03-04, Morgantown, WV.

Waring, K. M., and K. L. O'Hara. 2005. Silvicultural strategies in forest ecosystems affected by introduced pests. Forest Ecology and Management 209: 27-41.

Young, R. F., K. S. Shields, and G. P. Berlyn. 1995. Hemlock woolly adelgid (Homoptera: Adelgidae): Stylet bundle insertion and feeding sites. Annals of American Entomology 88: 827-835.

Zar, J. H. 2010. Biostatistical Analysis, 5 ed, vol. 1. Pearson, Englewood Cliffs, NJ.

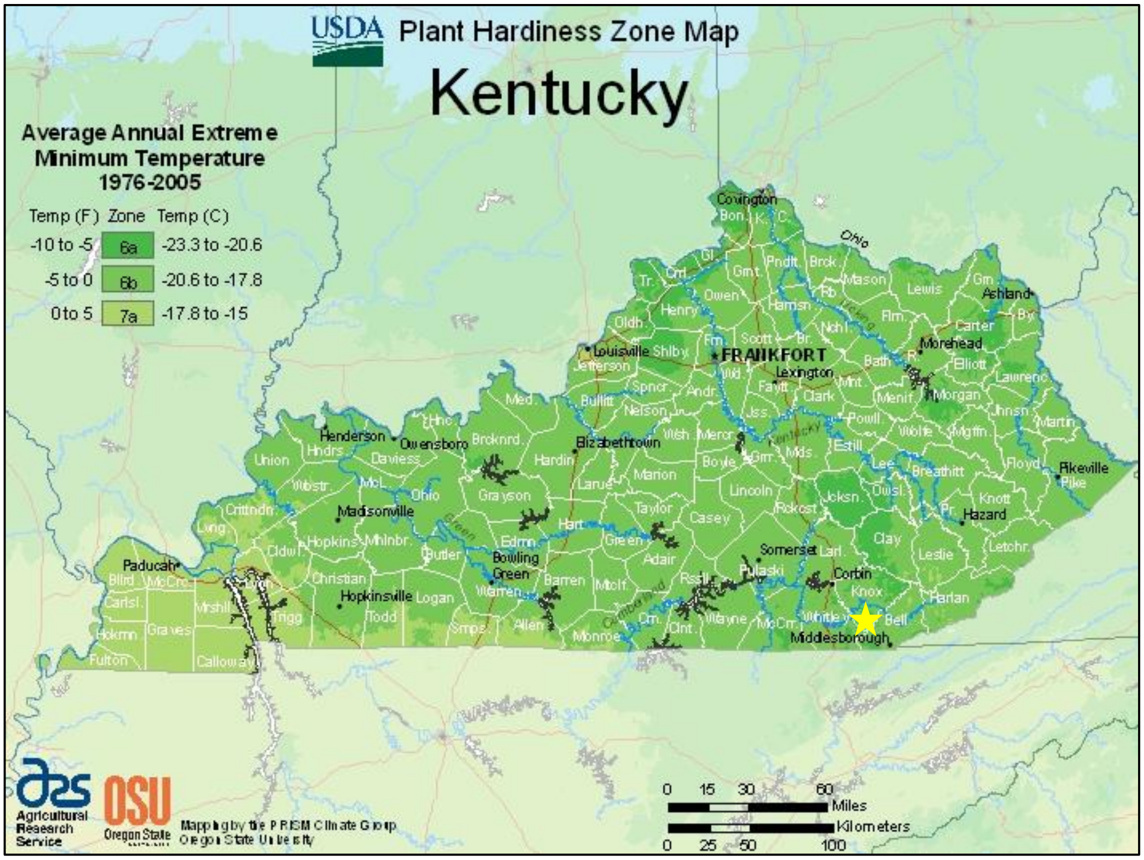
Zilahi-Balogh, G., S. Salom, and L. Kok. 2003a. Development and reproductive biology of *Laricobius nigrinus,* a potential biological control agent of *Adelges tsugae*. Biocontrol 48: 293-306.

Zilahi-Balogh, G., L. Humble, A. Lamb, S. Salom, and L. Kok. 2003b. Seasonal abundance and synchrony between *Laricobius nigrinus* (Coleoptera: Derodontidae) and its prey, the hemlock woolly adelgid (Hemiptera: Adelgidae). The Canadian Entomologist 135: 103-115.

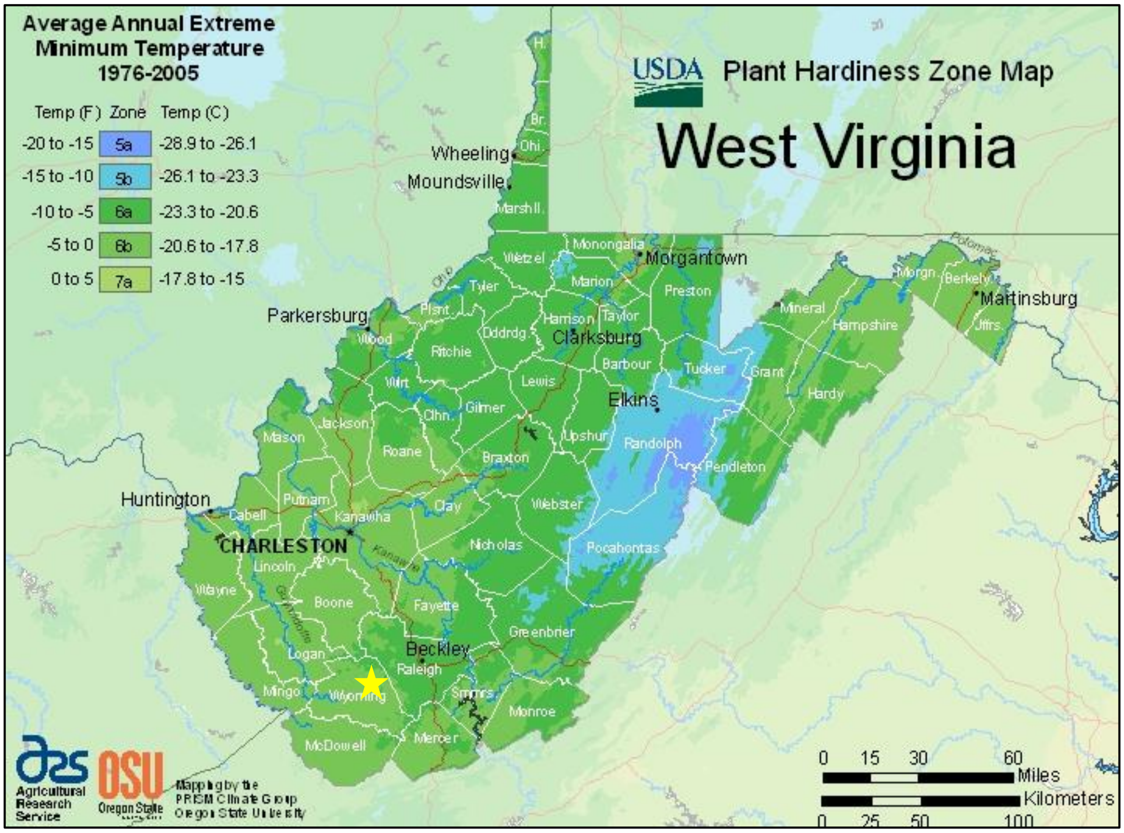
Zilahi-Balogh, G. M. G., L. T. Kok, and S. M. Salom. 2005a. A predator case history: *Laricobius nigrinus*, a derodontid beetle introduced against the hemlock woolly adelgid, pp. 651-664. *In* M. S. Hoddle (ed.), Second International Symposium on Biological Control of Arthropods. USDA Forest Service, Davos, Switzerland.

Zilahi-Balogh, G. M. G., C. D. Broeckling, L. T. Kok, and S. M. Salom. 2005b. Comparison between a native and exotic adelgid as hosts for *Laricobius rubidus* (Coleoptera: Derodontidae). Biocontrol Science and Technology 15: 165-171.

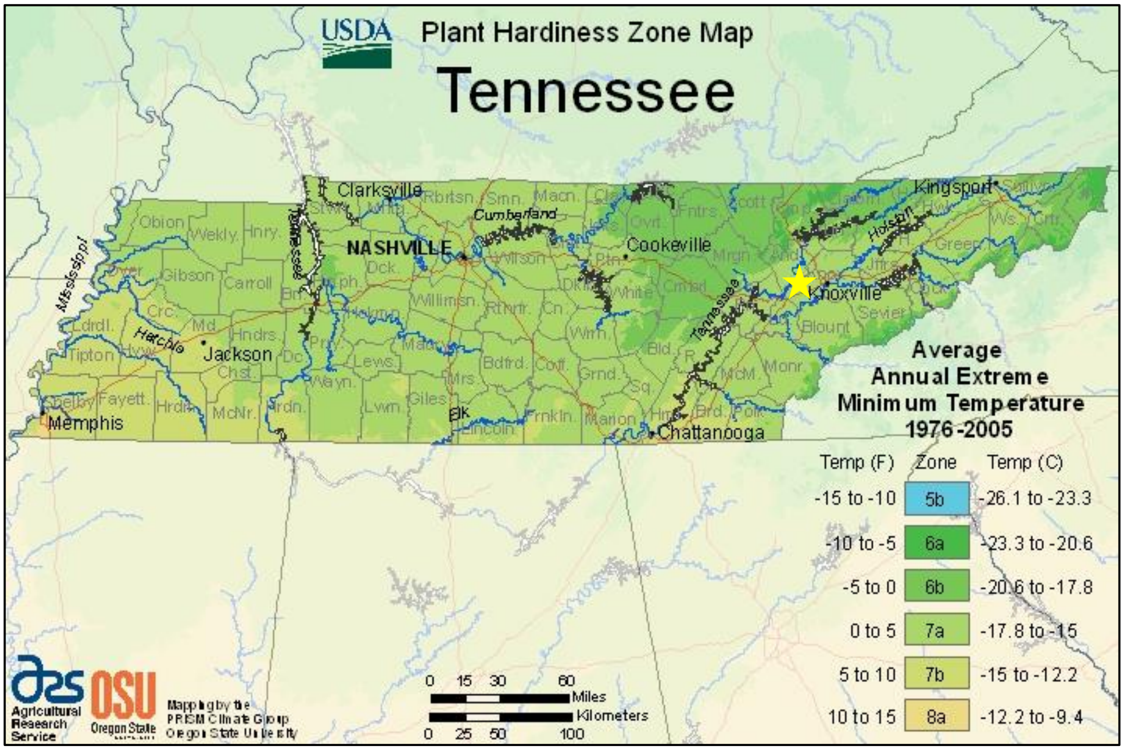
Appendices



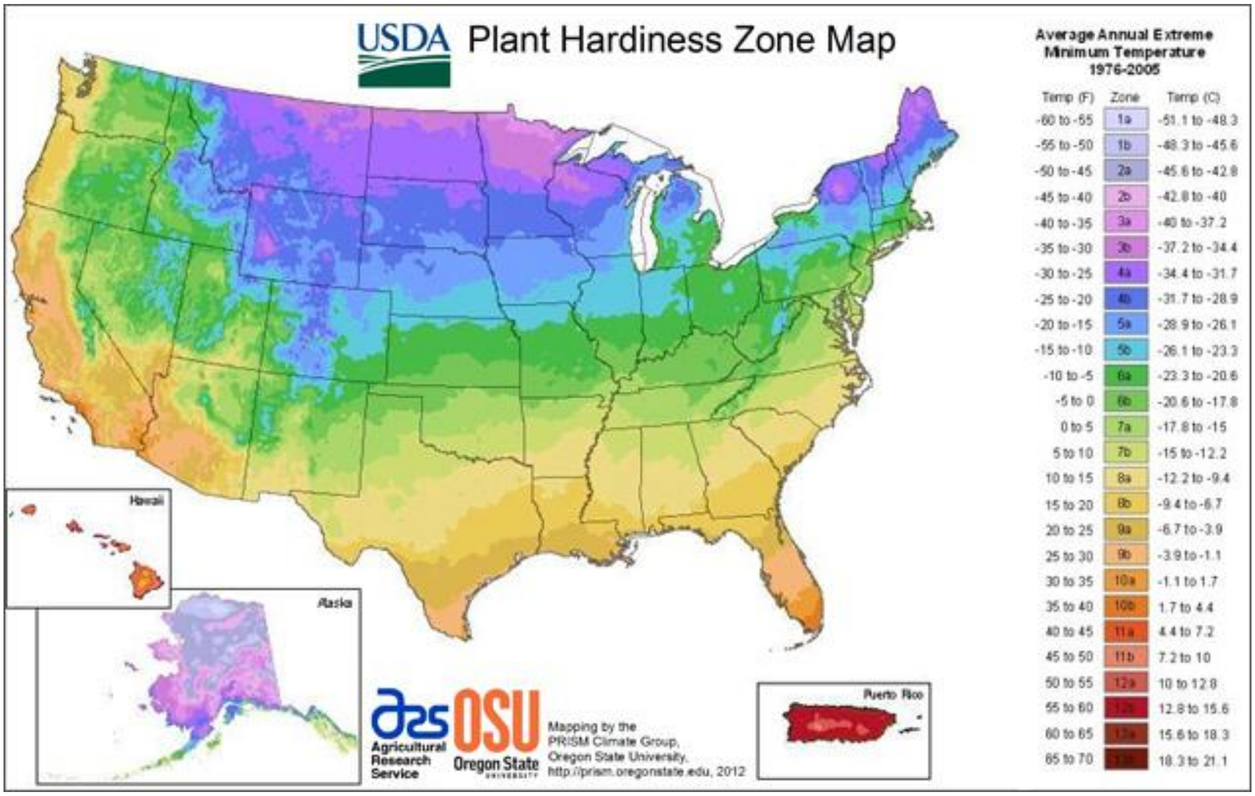
Appendix 1 USDA Plant Hardiness Zone map for KY. The study site is marked by the yellow star. Retrieved from http://planthardiness.ars.usda.gov/.



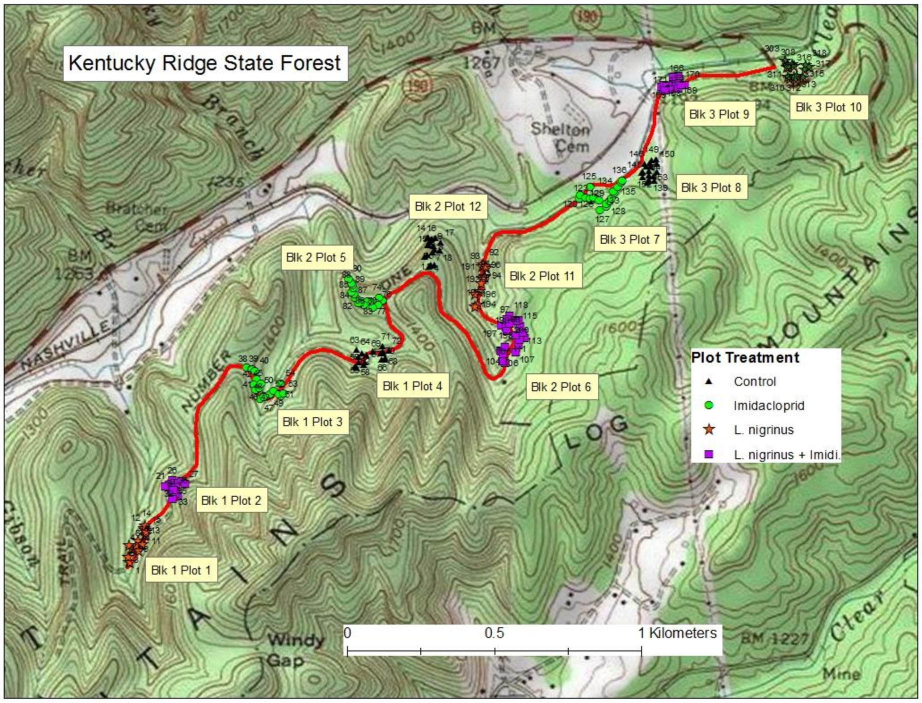
Appendix 2 USDA Plant Hardiness Zone map for WV. The study site is marked by the yellow star. Retrieved from http://planthardiness.ars.usda.gov/.



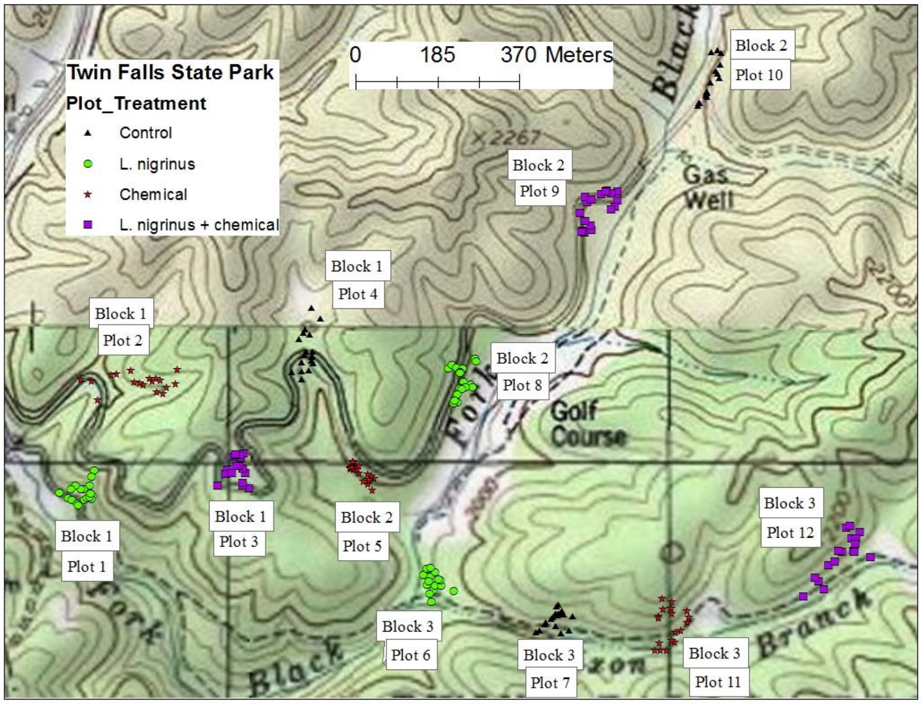
Appendix 3 USDA Plant Hardiness Zone map for TN. The study site is marked by the yellow star. Retrieved from http://planthardiness.ars.usda.gov/.



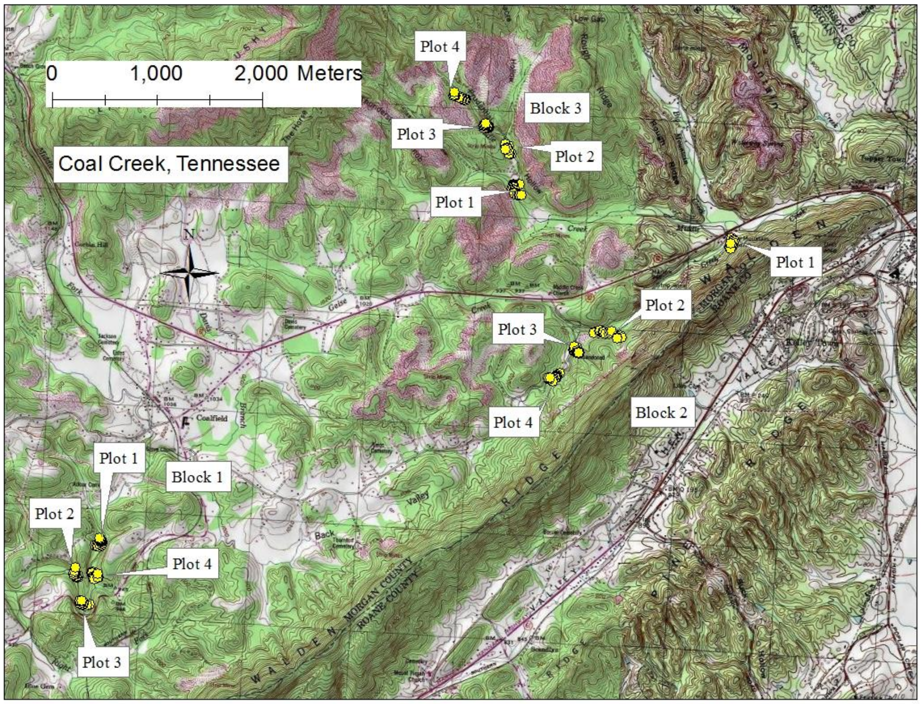
Appendix 4 USDA Plant Hardiness Zone map. Retrieved from http://planthardiness.ars.usda.gov/.



Appendix 5 Map of all treatment plots and replicates at Kentucky Ridge State Forest, Kentucky. Created by Tom McAvoy.



Appendix 6 Map of a treatment plots and replicates at Twin Falls State Park, West Virginia. Created by Tom McAvoy.



Appendix 7 Map of all treatment plots and replicates at Coal Creek, Tennessee. Created by Tom McAvoy.

Appendix 8 Schedule of field activities from 2010 – 2016.

HWA Chemical-Biological Control Integration Project Timetable

	2010						2011						2012						2013						2014						2015						2016					
Activity	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec
Plot establishment, tree selection		KY																																								
								WV																																		
												TN																														
HWA sistens counts, crown health ratings			KY						KY					KY						KY						KY						KY						KY				
								WV						WV						WV						WV						WV						WV				
														TN						TN						TN						TN						TN				
Imidacloprid treatments			KY																				KY																			
									WV																				WV													
															TN																				TN							
Laricobius release						KY																																				
												WV																														
																		TN																								
Assess *Ln* larval, adult establishment														KY						KY						KY						KY						KY				
																				WV						WV						WV						WV				
																										TN						TN						TN				

Appendix 9 Beat sheet produced by Bioquip Products, Inc. Retrieved from https://www.bioquip.com/html/view_prodpics.asp?CatalogNum=2840R&P=3.

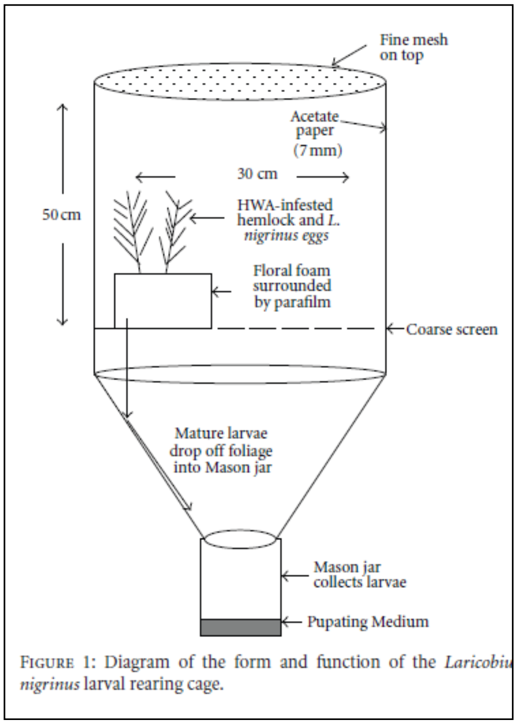
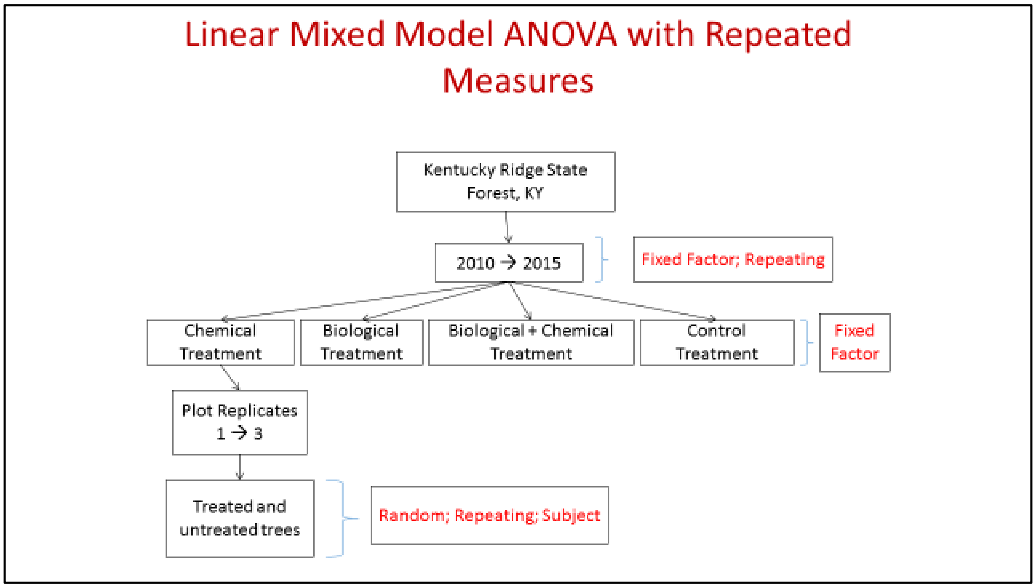


FIGURE 1: Diagram of the form and function of the *Laricobiu*
nigrinus larval rearing cage.

Appendix 10 Schematic for a larval rearing cage (Salom et al. 2012).



Linear Mixed Model ANOVA with Repeated Measures

Kentucky Ridge State Forest, KY

2010 → 2015

Fixed Factor; Repeating

Chemical Treatment

Biological Treatment

Biological + Chemical Treatment

Control Treatment

Fixed Factor

Plot Replicates 1 → 3

Treated and untreated trees

Random; Repeating; Subject

Appendix 11 Outline of components of the linear mixed model ANOVA with repeated measures. Kentucky Ridge State Forest is used as an example.