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Temporal and Spatial Distribution of Imidacloprid and the Arthropod Fauna Associated with Eastern Hemlock, Tsuga canadensis (L.) Carr

Richard M. Turcotte

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by

Richard M. Turcotte

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Approved by
Yong-Lak Park, Ph.D., Co-Chair
Linda Butler, Ph.D. Co-Chair
Bradley Onken, M.S.
William MacDonald, Ph.D.
James Amrine, Ph.D.
Ray Hicks, Ph.D.

Division of Plant and Soil Sciences

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ABSTRACT

Temporal and Spatial Distribution of Imidacloprid and the Arthropod Fauna Associated with Eastern Hemlock, *Tsuga canadensis* (L.) Carr.

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Eastern hemlock, *Tsuga canadensis* (L.) Carr (Pinales: Pinaceae), is an important component of both the urban and forest landscape of the eastern United States. Eastern hemlock has been heavily impacted by the introduction of the hemlock woolly adelgid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae). Two goals of this research were (1) to determine the effect of treatment timing (spring versus fall) and application method (tree injection versus soil injection) on the spatial and temporal distribution of imidacloprid the primary insecticide used to treat *A. tsugae* and (2) to assess the impact of application method and timing of imidacloprid treatments on the arthropods associated with eastern hemlock. The results of this study showed that xylem fluid concentrations of imidacloprid were significantly ($P < 0.05$) higher for spring applications than for fall applications, and for trunk injections than soil injections in the first year post treatment. A diverse group of arthropods, making up 393 species, were collected by branch beating the lower crowns of eastern hemlock. No significant ($P > 0.05$) differences in arthropod abundance were found between imidacloprid treated and control trees and application methods. An extensive literature review revealed 484 native and exotic arthropods from three different taxonomic classes and 21 different orders associated with eastern hemlock in North America. A total of five arthropod species were eastern hemlock dependent, and are likely to experience local extirpation as a result of declining and dying eastern hemlock. In addition, an assessment of the impact of application method and timing of imidacloprid treatments on the spider communities were carried out because spiders are the primary arthropod predator present in the crown of eastern hemlock. No significant ($P > 0.05$) differences in spider abundance were found between imidacloprid treated and control trees and application methods. This study provides fundamental information to aid the conservation and management of eastern hemlock and biodivisity at risk due to extensive applications of imidacloprid.

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CHAPTER 1: INTRODUCTION

Dissertation Organization

This dissertation is organized into six chapters. Chapter 1 is a general introduction to the study and a literature review. Chapter 2 presents the spatial and temporal distribution of the primary chemical treatment used to control the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). Chapter 3 addresses the potential impact of chemical treatments on the arthropod fauna associated with eastern hemlock, *Tsuga canadensis* (L.) Carr (Pinales: Pinaceae). Chapter 4 delineates arthropod species directly associated with eastern hemlock and to assess the relative risk of endangerment of these species. Chapter 5 describes the impact of chemical treatment on spiders associated with eastern hemlock. Chapter 6 provides a general conclusion for this study. This dissertation was prepared according to the publication guidelines established by the Entomological Society of America.

General Introduction

Eastern hemlock, *Tsuga canadensis* (L.) Carr (Pinales: Pinaceae), is an important component of both the urban and forest landscape of the eastern United States. It is a long-lived, shade-tolerant species that strongly influences its environment and other species. The dense evergreen canopy of this species, along with its ability to grow in nearly pure stands, creates a distinct microclimate that is important for a wide variety of plant and animal species. Eastern hemlock has been heavily impacted by the introduction of both the elongated hemlock scale *Fiorinia externa* Ferris and the hemlock woolly adelgid, *Adelges tsugae* (Annand). Chemical control is the primary method used to control both of these exotic insects. As *A.tsugae* and elongate hemlock scale continues to spread and impact eastern hemlock throughout eastern North America, we are likely to see many unforeseen effects occurring on the invertebrate and vertebrate species that utilize eastern hemlock forests ecosystems. Despite increased awareness of the arthropods associated with eastern hemlock few longterm and landscape-level studies of the arthropods associated with eastern hemlock have occurred, and it is obvious that the diversity of the arthropod fauna associated with eastern hemlock is still incompletely known.

Objectives of Study

The goals of this research were to better understand the non-target impacts on arthropods associated with the movement of imidacloprid within the crown of treated hemlocks. The objectives of this research were:

1. To determine the spatial and temporal distribution of imidacloprid within the crown of eastern hemlock (Chapter 2).
2. To determine the impact of imidacloprid treatments on canopy-dwelling arthropods associated with eastern hemlock (Chapter 3).
3. To catalogue the number of arthropod species associated with eastern hemlock; and assess the relative risk of endangerment of these species as eastern hemlock is affected by the hemlock woolly adelgid and elongate hemlock scale. (Chapter 4).
4. To determine the impact of imidacloprid treatments on canopy-dwelling Araneae associated with eastern hemlock (Chapter 5).

Literature Review

Eastern Hemlock. Eastern hemlock, *Tsuga canadensis* (L.) Carr, is an extremely shade-tolerant, monoecious, slow-growing, late successional conifer with a dense, evergreen crown and that strongly influences its environment and other species (Ward and McCormick 1982, Godman and Lancaster 1990, Evans et al. 1996, Quimby 1996, Evans 2000). Eastern hemlock has a conical crown with horizontal-to-pendulous branches (Ruth 1974) and 2-ranked needles (Dirr 1998). It exhibits relatively low branch shedding (Kenefic and Seymour 2000), and retains its needles for an average of three years (Barnes and Wagner 1981). Eastern hemlock is a relatively long lived species with a life span of over 800 years (Godman and Lancaster 1990). Seed production usually begins when trees are 20-30 years of age (Ruth 1974). It is a frequent and abundant cone producer (Crow 1996), with good crops being produced every 2 to 3 years (Frothingham 1915, Ruth 1974).

Native Range of Eastern Hemlock. Eastern hemlock is widely distributed in North America from Nova Scotia across southern Ontario to northern Minnesota, and south to Alabama along the Appalachian Mountains (Fig. 1) (Brisbin 1970, Godman and Lancaster 1990, Quimby 1996). Hemlock generally grows in areas with cool humid climates (Godman and Lancaster 1990, McWilliams and Schmidt 2000). Annual precipitation ranges from 74 cm to more than

127 cm across the range of eastern hemlock (McWilliams and Schmidt 2000). It grows at elevations from sea level to 730 m in the northeastern and northern areas, from 300 to 910 m on the Allegheny Plateau and from 610 to 2036 m in the southern part of its range (Hough 1960, Eyre 1980, Godman and Lancaster 1990).

Hemlock-Associated Forest Types. Within the eastern forest cover type, hemlock occupies a variety of sites, soil types and climatic conditions (McWilliams and Schmidt 2000). It is associated with 29 different eastern forest cover types (Eyre 1980, Godman and Lancaster 1990), and is a major component in four: white pine-hemlock (Type 22), eastern hemlock (Type 23), hemlock-yellow birch (Type 24), and tulip poplar-eastern hemlock (Type 58). It is also commonly associated with seven forest cover types: white pine-northern red oak-red maple (Type 20), eastern white pine (Type 21), red spruce-yellow birch (Type 30), red spruce-sugar maple-beech (Type 31), red spruce (Type 32), red spruce-balsam fir (Type 33), and red spruce-Fraser fir (Type 34), and can be found as a minor component in eighteen more (Godman and Lancaster 1990).

Growth of Eastern Hemlock and Associated Species. Hemlock can occur in pure stands (Eyre 1980), or mixed with other species. On favorable sites, it usually forms a climax position (Brisbin 1970) while on sites that are rich in nutrients, it can be out competed by hardwoods (Kotar 1996). In pure stands, undergrowth vegetation can be sparse (Eyre 1980) due to intraspecific allelopathy (Ward and McCormick 1982) and to the dense evergreen crown of hemlock which intercepts both light and precipitation. Because of this dense canopy in hemlock stands the microclimate is cooler than under hardwoods (Tubbs 1996). This distinct microclimate provides an important habitat for a wide variety of wildlife (Evans 2000). In the northeastern United States 96 bird and 47 mammal species have been found to be associated with eastern hemlock forests (Yamasaki et al. 2000). This includes 23 species of small mammals, 14 species of wide ranging carnivores, 10 species of amphibians, and 7 species of reptiles (Degraaf et al. 1992). Hemlock forests can also be a critical factor in the support of native brook trout populations, where it maintains cool stream temperatures and stabilizes stream flows (Evans et al. 1996, Quimby 1996). Eastern hemlock fills a unique ecohydrological role because it transpires throughout the year and it provides stable water fluxes within a watershed and high water flux patterns in the spring, reducing nutrient loss and decreasing watershed discharges (Ford and Vose 2007).

Utilization of Eastern Hemlock. In addition to hemlock being a valuable forest tree it is also an important component of eastern urban forests (Raupp et al. 2004). Hemlocks are popular for hedges, shrubbery, Christmas trees, and border trees around yards (Hough 1960). In the urban environment it provides habitat for wildlife, provides shade, and acts as both a noise absorber and wind barrier (Quimby 1996). There are 274 cultivars of eastern hemlock, making it one of the most cultivated landscape trees (Swatley 1984). Hemlock has been used for wood containers, flooring, roofing, sheathing, general millwork and furniture (Frothingham 1915, Godman and Lancaster 1990). The bark was once used as a source of tannin for the leather industry (Hough 1960). However, the wood characteristics limit its current use to low grade products: structural lumber, pallets, pulpwood and landscape mulch (Howard et al. 2000).

Susceptibility of Eastern Hemlock to Injury and Damaging Agents. Eastern hemlock has an extensive shallow root system (Quimby 1996) making it susceptible to wind throw (Mladenoff and Stearns 1993), fire, and drought (Hepting 1971, Godman and Lancaster 1990). Hemlock is a preferred browse species of white tail deer, and when deer populations are abundant all stages of hemlock can be heavily browsed (Mladenoff and Stearns 1993). It is a very tolerant tree but is subject to several fungi attacking cones, shoots, leaves, twigs and boles (Hepting 1971). One of these foliage disease, the *Fabrella* needle blight of hemlock recently causing problems in Pennsylvania, *Fabrella tsugae* (Farlow) Kirschstein (Helotiales: Hemiphacidiaceae), resulting in premature needle drop in late summer. Hemlock has no major fungal canker diseases, root diseases, or trunk rots of importance since most are weakly or nonpathogenic and seldom kill trees (Hepting 1971).

At least 24 species of insects are known to attack eastern hemlock (Godman and Lancaster 1990). The most important of these are the hemlock borer, *Melanophila fulvoguttata* (Harris) (Coleoptera: Buprestidae) which attacks stems of weakened trees, the hemlock looper, *Lambdina fiscellaria fiscellaria* (Guenée) (Lepidoptera: Geometridae), the black vine weevil, *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae), the hemlock scale, *Abgrallaspis ithacae* (Ferris) (Hemiptera: Diaspididae) which feed on the leaves (Stoelzel and Davidson 1974, Godman and Lancaster 1990), and the strawberry root weevil, *Otiorhynchus ovatus* L. (Coleoptera: Curculionidae) which attacks the roots (Godman and Lancaster 1990). Several other non-native invasive species also attack eastern hemlock including the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae) (Lovett et al. 2006), the elongate hemlock scale, *Fiorinia externa* Ferris (Hemiptera:Diaspididae) both of which feed on the leaves (McClure 1977), and the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) which is the single greatest threat to the health and sustainability of hemlock as a forest and urban resource in eastern North America (Knauer et al. 2002).

Adelges tsugae. Adelgids (Hemiptera: Adelgidae) are small, soft-bodied insects that feed on plant sap and have a complex life cycle. The family is divided into two genera: *Adelges* and *Pineus* (Montgomery 1999). The members of this family feed exclusively on Pinaceae (Havill and Foottit 2007). There are six species of *Adelges* in North America including *A. tsugae.* The Cooley spruce gall aphid, *A. cooleyi* (Gillette), the eastern spruce gall adelgid, *A. abietis* (L.), the balsam woolly adelgid, *A. piceae* (Ratz.), the larch woolly adelgid, *A. laricis* (Vallot), Douglas fir woolly aphid, *A. coweni* (Gillette), and the larch cone adelgid *A. lariciatus* (Patch); Havill and Foottit 2007). Of these, only *A. cooleyi*, *A. coweni*, and *A.lariciatus* are native to North America (Havill and Foottit 2007).

Adelges tsugae is a tiny insect (~ 2 mm) that is covered by a secreted woolly mass for most of its lifespan (McClure 1987, 1989). *A. tsugae* is native to Asia (Japan, India, Nepal, southwestern China and Taiwan) (Cheah et al. 2004) where it is frequently controlled by natural enemies and host plant resistance (McClure 1996). In eastern North America it has become a major pest of eastern hemlock and Carolina hemlock, *Tsuga caroliana* Engelmann (Pinales: Pinaceae) (Onken et al. 1999, Ward et al. 2004), both of which are considered highly susceptible to *A. tsugae* with no documented resistance (Bentz et al. 2005). Carolina hemlock is found only in a limited area of the southern Appalachian Mountains (Onken et al. 1999), where it occurs infrequently from southwestern Virginia to northern Georgia (Harrar and Harrar 1962).

Introduction and Spread of A. tsugae. *A. tsugae* was introduced into the eastern United States from Japan (Havill et al. 2006) sometime before 1951. It was first discovered on eastern hemlock trees in a municipal park that had previously been a private estate (Souto et al. 1996, Ward et al. 2004). Over the next 30 years *A. tsugae* slowly spread through the Mid-Atlantic States (Souto et al. 1996). By the late 1980s and 1990s *A. tsugae* population had expanded rapidly and was reported to be causing widespread mortality (Cheah et al. 2004). *A. tsugae* is currently established in 18 eastern States from Georgia to Maine. The adelgid appears to have the capacity to develop greater cold tolerance (Butin et al. 2005), which likely means that it will continue to spread to the north and west.

Life Cycle of A. tsugae. *A. tsugae* adelgid has a complex life cycle involving both sexual and asexual stages on both hemlock and spruce (McClure 1989). The life cycle on eastern hemlock is bivoltine including a sistens or wingless winter generation that starts in late spring and lasts for 9 to 10 months (McClure 1989)(Fig. 2) and a progredien or spring generation that starts in the early spring. The progredien generation is composed of both winged (sexuparae) and wingless offspring and lasts for about three months (Ward et al. 2004). The winged generation is the sexual migratory stage which leaves hemlock to find spruce (McClure 1987). The percentage of the population of progrediens is strongly density dependent; as the tree health declines and preferred feeding sites (new growth) are reduced the percentage of winged adults increases (McClure 1991). Because of the lack of a suitable spruce species in the eastern United States the production of the winged form results in a substantial loss of individuals from the spring generation (McClure 1989). This adelgid has a high reproductive potential with each adult producing up to 300 eggs (McClure et al. 2001). The eggs hatch into first instar mobile crawlers, which are active for one to two days, before settling or being dispersed (McClure 1987, Ward et al. 2004). Once settled the nymph inserts its stylet and feeds on the xylem ray parenchyma cells at the base of the hemlock needles (Young et al. 1995). The adelgid then develops through four instars before becoming an adult (McClure 1989).

Feeding Impact of A. tsugae. The combination of two annual generations, a high reproductive capacity and the lack of natural enemies (Van Driesche et al. 1996, Wallace and Hain 2000, Cheah et al. 2004), gives *A. tsugae* the ability to increase rapidly in numbers (McClure 1989). Feeding can quickly lead to needle loss, dieback and mortality (Cheah et al. 2004). Feeding by the adelgid restricts the uptake and movement of water (McClure 1995), which reduces the trees energy reserves (Ward et al. 2004) and can lead to tree mortality in 4-7 years (Orwig and Foster 1998, McClure et al. 2001), although some trees can last more than ten years (Souto et al. 1996, Paradis et al. 2008). All life stages of hemlock, from seedling to mature old-growth trees are fed upon (McClure 2001).

Dispersal and Spread of A. tsugae. *A. tsugae* spreads mainly as eggs and crawlers which are transported by wind, birds, deer, and other forest-dwelling mammals (McClure 1990, Cheah et al. 2004, Ward et al. 2004). It can also be moved on infested nursery stock or during logging and recreational activities (McClure 1995, Gibbs 2002, Ouellette 2002). Roads, hiking areas and riparian areas have all been implicated in the long-distance spread of the adelgid by humans and birds (Koch et al. 2006). Recent evidence suggests that the current rate of spread is between 8-16 km per year (Evans and Gregoire 2007).

Imidacloprid. Neonicotinoids represent the most effective insecticide for controlling piercing sucking insects such as aphids, leafhoppers, planthoppers, thrips, fleas and some coleopteran (e.g. leaf beetles) and selected species of lepidopteran and dipteran pests (Mullins 1993, Tomizawa and Casida 2005, Elbert et al. 2008). Neonicotinoids comprise seven different commercially available products: acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam (Tomizawa and Casida 2005, Elbert et al. 2008) and have been the only new class of insecticides developed since the 1970s (Tomizawa and Casida 2005). The name neonicotinoids was adopted to show the structural and mode of action differences from nicotine and nicotine-related compounds (Matsuda et al. 2009). The factors that contribute to the success of this class of insecticides is their plant systemicity (Elbert et al. 2008), and mode of action, which offers no cross-resistance to other conventional long-established insecticides (Jeschke and Nauen 2008).

Imidacloprid, 1-[(6-chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine, is a broad spectrum neonicotinoid insecticide with low to moderate mammalian toxicity (Mullins 1993), high insecticidal potency (Lansdell and Millar 2000, Tomizawa and Casida 2005), and a good environmental and toxicological profile (Silcox 2002). As a result it has become one of the world's most widely used insecticides (Silcox 2002, Jeschke and Nauen 2008). It is both a systemic and contact insecticide (Mullins 1993) and has become the preferred pesticide for controlling *A. tsugae* (Smith and Lewis 2005, Eisenback et al. 2008).

Imidacloprid was first synthesized by Nihon Bayer Agrochem in 1985 (Elbert et al. 1998, Figure 3), and first registered in the United States under the tradename Merit® in 1994 (Silcox 2002). It is classified in toxicity classes II (moderately toxic) and III (slightly toxic) by the Environmental Protection Agency. Imidacloprid is sold under a variety of tradenames: Admire®, Advantage®, Gaucho®, Premise®, and Touchstone®. In 2006, imidacloprid came off patent and became generic (Jeschke and Nauen 2008).

Mode of Action of Imidacloprid. Imidacloprid has a mode of action similar to that of the botanical product nicotine, functioning as a fast-acting insect neurotoxicant (Schroeder and Flattum 1984) that binds to the post-synaptic nicotinic acetylcholine receptors (nAChRs) of the insects' central nervous system (Jeschke and Nauen 2008). Imidacloprid mimics the action of acetylcholine, and thereby heightens, then blocks the firing of the postsynaptic receptors with increasing doses (Schroeder and Flattum 1984, Felsot 2001). Acetylcholine is the major excitatory neurotransmitter of insect's central nervous system (Lansdell and Millar 2000, Tomizawa and Casida 2003); it binds and then is degraded by the inactivating enzyme acetylcholine esterase (Breer and Sattelle 1987). Because imidacloprid is not removed by acetylcholine esterase, it causes substantial disorder within the nervous system leading to tremors, paralysis and in most cases death (Mullins 1993, Smith and Krischik 1999). Toxicity studies have demonstrated that this insecticide is neither carcinogenic nor teratogenic (Mullins 1993).

Translocation of Imidacloprid in Plants. Translocation experiments from a number of vascular plants (e.g. corn, cotton, and eggplant) have shown that imidacloprid has good translaminar movement (Elbert et al. 2008) and excellent xylem mobility to shoots and leaves and poor phloem mobility to storage organs, roots and fruits; as a result the highest residues are expected in the older leaf portions of the plant (Sur and Stork 2003). The systemic properties of imidacloprid are a function of its physical properties, mainly its high water solubility (Cox et al. 1997, Oi 1999), low n-octanol/water partition coefficient ($K_{o/w}$) (Nemeth-Konda et al. 2002), low vapor pressure (Lagalante and Greenbacker 2007) and dissociation coefficients (K_d) (Sur and Stork 2003).

Metabolism of imidacloprid in Plants. Most of the imidacloprid administered to plants is metabolized, with little of the parent compound imidacloprid remaining (Nauen et al. 1998). The known metabolic pathways of imidacloprid (Placke and Gustin 1993) are presented in Figure 4. The metabolites formed are dependent on the method of application (Nauen et al. 1998) and the species of plant treated (Sur and Stork 2003). Because of the variety of functional groups present in the imidacloprid molecule (Figure 3), it undergoes degradation by a number of different pathways and creates a number of different metabolites (Table 1) (Tomizawa and Casida 2003). Metabolites vary in their biological activity against certain insect species (Nauen et al. 1998, Nauen et al. 1999, Nauen et al. 2001), with some being active against mammalians and deactivated against insects (Tomizawa et al. 2000).

Metabolism of Imidacloprid in Soil. Under field application conditions only a small amount of the applied pesticide ever reaches the target; the majority is released into the soil, and must be degraded photochemically, abiotically and biologically (Wamhoff and Schneider 1999).

For imidacloprid, sorption-desorption processes along with photodegradation and hydrolysis determine the distribution and fate in the soil-water environment (Cox et al. 1997). Imidacloprid undergoes various physio-chemical processes when applied to the soil (Nemeth-Konda et al. 2002).

As with the metabolism in plants, imidacloprid and its metabolites are affected by application method and soil properties (e.g. pH and clay content), with different metabolites having different sorption rates based on the amount of organic carbon present (Cox et al. 1997) and the length of time in the soil (Oi 1999). Insecticides that are sorbed to soil particles are not bioavailable, so they first must be desorbed from the soil into solution to be bioavailable (Koskinen et al. 2001). Desorption for imidacloprid and its metabolites has been shown to be hysteric (Cox et al. 1997). Hysteric desorption indicates that there is a higher desorption coefficient than sorption for some of the metabolites (Oi 1999), making it more difficult for these molecules to reach the target (tree roots) (Cox et al. 1997). The half-life of imidacloprid in soil is between 48-190 days, depending on the formulation, application rate and amount of ground cover (Scholz and Spiteller 1992). In neutral or acidic water, imidacloprid is stable and slowly hydrolyzed (Liu et al. 2006).

Methods of Imidacloprid Application. In each of the application methods used to treat *A. tsugae*, tree health has been shown to be an important factor in successful treatment (McClure 1992, Fidgen et al. 2006). This is especially true for the systemic methods, soil injection and trunk injection. In each of these cases the tree must be healthy enough to move the insecticide through the vascular system (McClure 1995).

Imidacloprid used for *A. tsugae* control can be applied as a contact foliar application or as a systemic soil treatment and trunk injection (Silcox 2002). The foliar application is sprayed directly on the tree (to the point of runoff) and works as a contact insecticide. It can be applied any time of the year either with a backpack, garden hose or hydraulic sprayer (McClure 1995). This treatment method provides rapid activity with a short residual time (Silcox 2002). Foliar applications have been shown to be effective in controlling *A. tsugae* populations (Rhea 1996, Cowles and Cheah 2002). Some factors preclude the use of foliar treatments, including the difficulty in treating very tall trees, areas inaccessible to spraying equipment (McClure 1987) and the potential for non-target impacts related to spray drift (Tattar et al. 1998).

Imidacloprid can be applied by soil injection, soil drench, or tablet application and all application methods have been shown to be effective in controlling *A. tsugae* (Steward and Horner 1994, Fidgen et al. 2002, Webb et al. 2003, Cowles et al. 2006, Doccola et al. 2007, Cowles and Lagalante 2009, Dilling et al. 2010). Soil treatments provide the longest duration of control of *A. tsugae*, but they also are the slowest acting (Silcox 2002). Soil drenching is a technique of applying imidacloprid to the soil surface to the root zone at the base of the tree (Silcox 2002). Soil injection is a technique in which imidacloprid is hydraulically injected into the soil using either high-volume hydraulic sprayers (McClure 1995) or handheld low-volume soil injectors (Steward et al. 1998). Three different applications patterns are recommended for soil injections: (1) Grid System, in which injection sites are spaced on 76 cm and arranged in a grid pattern extending to the drip line of the tree (Silcox 2002, Cowles et al. 2006), (2) Circle System, in which injection sites are evenly spaced in concentric circles out to the drip line of the tree (Silcox 2002), and (3) Basal System, evenly spaced injections are made 10-20 cm away from the base of the tree (Fidgen et al. 2002, Silcox 2002). The use of the tablet application is the newest imidacloprid application method. This is a time-released formulation which involves burying (or pushing) individual tablets into the soil surface. This can be applied in any of the soil injection patterns or in a shallow trench at the base of the tree. In either case the tablets should be covered by soil or leaf litter. Several factors need to be considered before using soil applied imidacloprid. Applying this broad spectrum insecticide to the soil poses a risk to soil organisms (Kreutzweiser et al. 2008) and the potential for contamination of surface and groundwater by runoff and leaching (Cox et al. 1997).

Imidacloprid injected into the trunk of trees has been shown to be effective in controlling *A. tsugae* (Cowles et al. 2006, Doccola et al. 2007, Cowles and Lagalante 2009, Dilling et al. 2010). Trunk injection is a technique in which imidacloprid is injected directly into the trunk of the tree. Trunk injection appears to work more quickly than soil injection (Tattar et al. 1998, Silcox 2002, Cowles et al. 2006). Several different formulations and trunk injection equipment are available for trunk injections. In all cases a small, shallow hole is drilled into the root flare near the base of the tree and inserted into these holes (McClure 1995), are the injection systems. This method damages the tree and creates a possible entry wound for disease (Steward and Horner 1994, McClure 1995, Smith and Lewis 2005).

Potential Non-Target Effects of Imidacloprid. Due to the systemic properties of imidacloprid the potential for non-target effects on arthropods may be expected. Imidacloprid is highly mobile and depending on treatment (e.g. drench and soil application) movement to other non-target plants in the treatment area should be expected. As mentioned previously, imidacloprid has high insecticidal potency and works through activation of the nicotinic acetylcholine receptors, causing paralysis and eventually death. Therefore any arthropods (beneficial or otherwise) that ingest plant material (e.g. foliage, sap, seeds, and propolis) or are exposed to a foliar application in a treatment area are likely to demonstrate lethal or sub-lethal effects.

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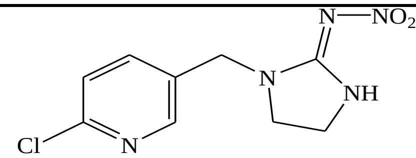
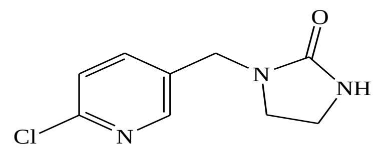
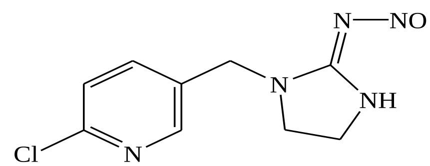
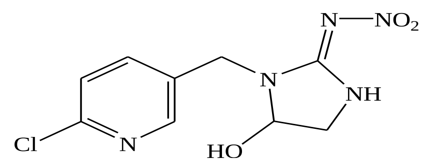
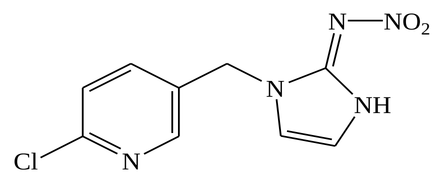
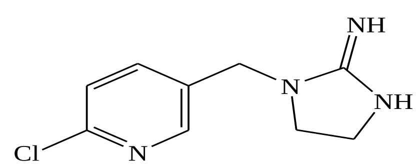
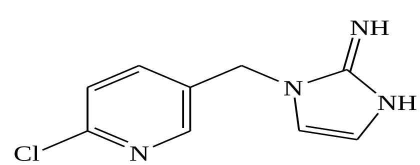
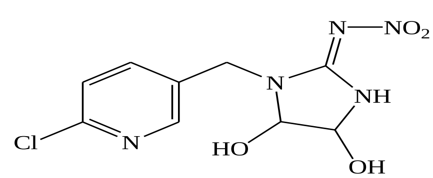
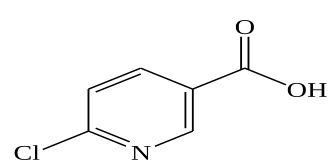
Table 1. Names and chemical structures of imidacloprid metabolites (Lagalante and Greenbacker 2007)

Imidacloprid 1-(6-chloro-3-pyridylmethyl)-*N*-nitro-imidazolidin-2-ylideneamine	
Urea 1-(6-chloro-3-pyridylmethyl)-imidazolidin-2-one	
***N*-nitroso** 1-(6-chloro-3-pyridylmethyl)-*N*-nitroso-imidazolidin-2-ylideneamine	
5-hydroxy 1-(6-chloro-3-pyridylmethyl)-2-(nitroimino)imidazolidin-5-ol	
Olefin 1-(6-chloro-3-pyridylmethyl)-*N*-nitro-1,3-dihydro-imidazol-2-ylideneamine	
des nitro 1-(6-chloro-3-pyridylmethyl)-imidazolidin-2-ylideneamine	
des nitro-olefin 1-(6-chloro-3-pyridylmethyl)-1,3-dihydro-imidazol-2-ylideneamine	
dihydroxy 1-(6-chloro-3-pyridylmethyl)-2-(nitroimino)imidazolidin-4,5-diol	
6-chloro-nicotinic acid	

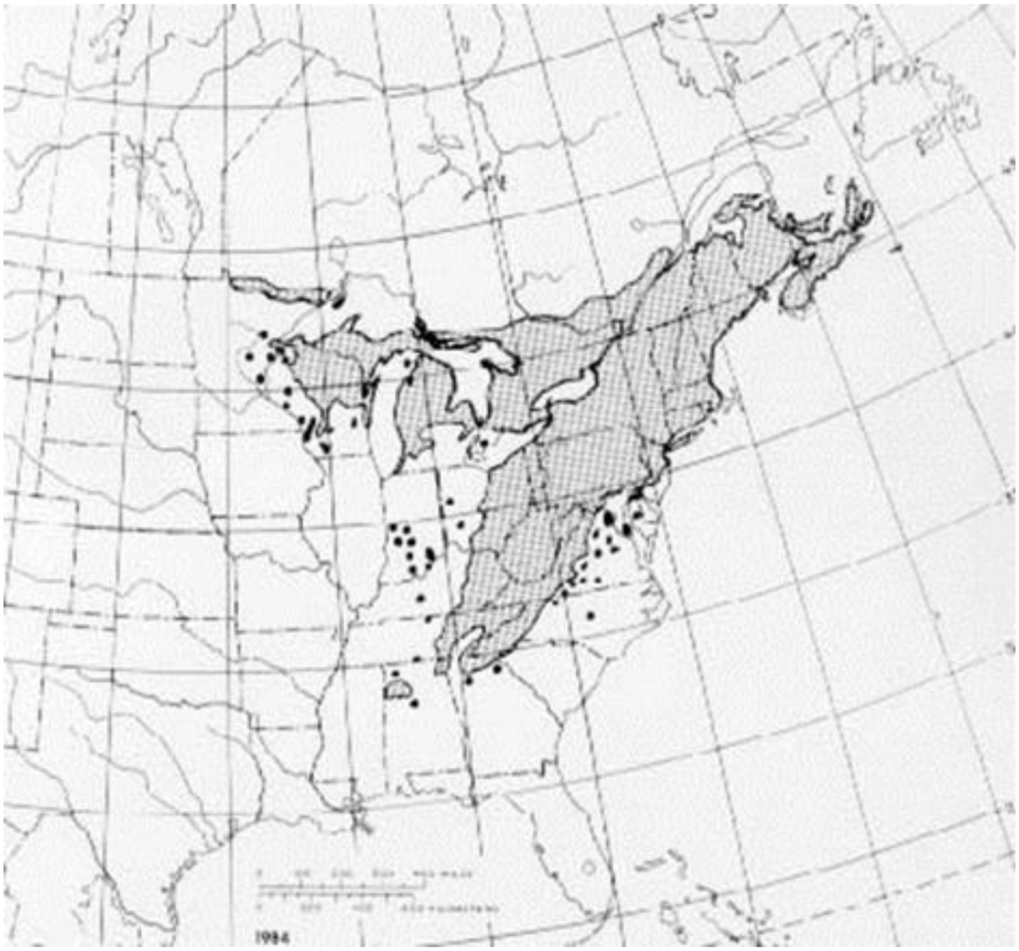


Figure 1. Native range of eastern hemlock in North America (Godman and Lancaster 2003).

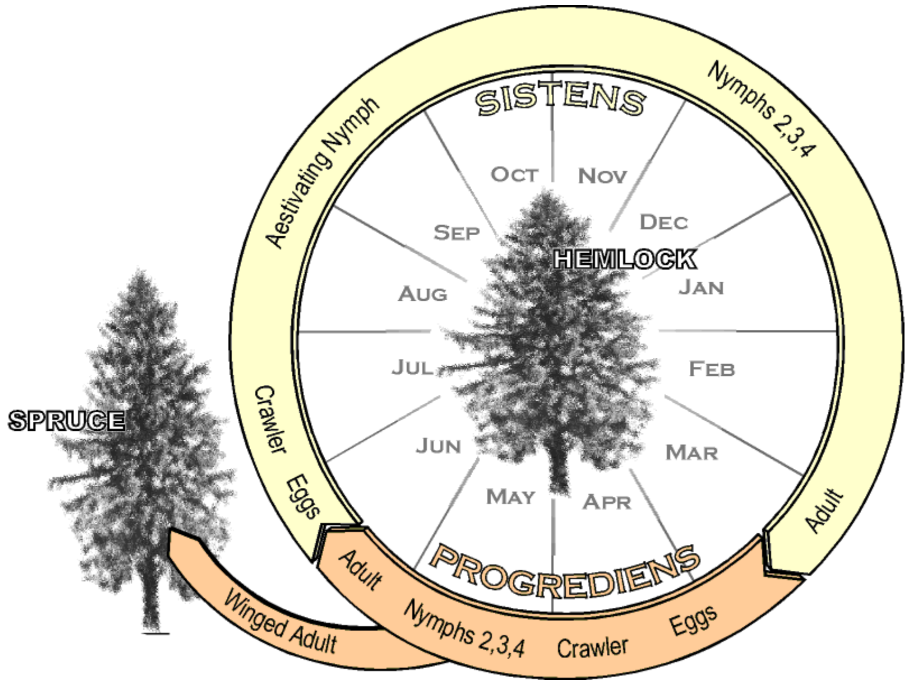


Figure 2. Hemlock woolly adelgid annual life cycle on hemlock in North America (Ward et al. 2004).

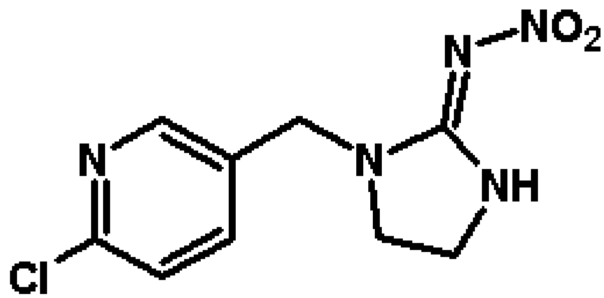


Figure 2. Structure of imidacloprid (Buckingham et al. 1997).

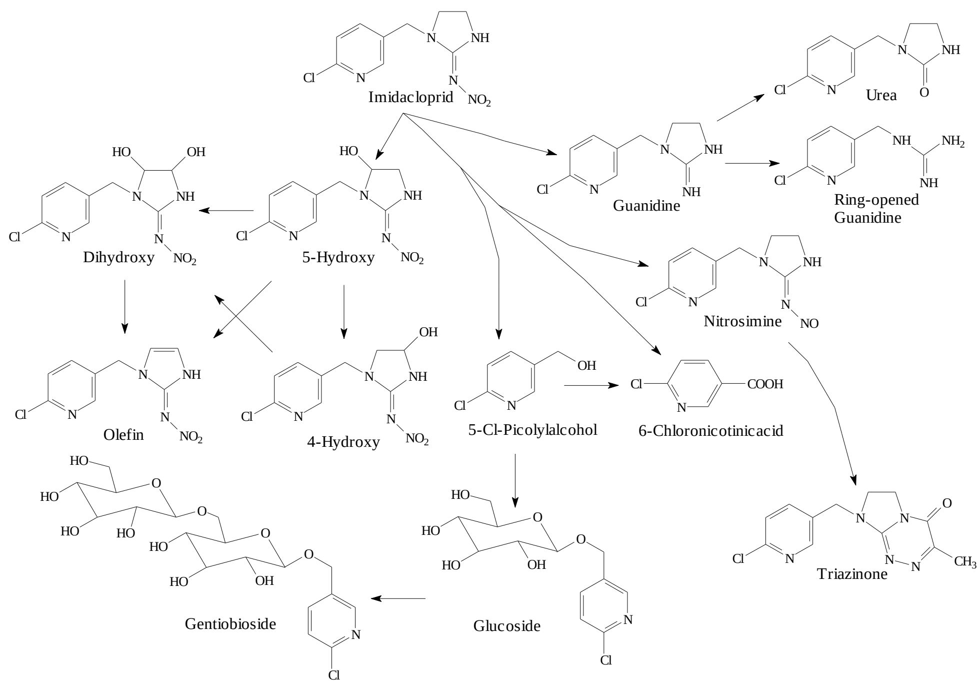


Figure 3. Imidacloprid metabolic pathways (Placke and Gustin 1993).

CHAPTER 2: Spatial and Temporal Distribution of Imidacloprid within the Crown of Eastern Hemlock

Abstract. Imidacloprid is the most widely used insecticide to control the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), an exotic pest of eastern hemlock, *Tsuga canadensis* (L.) Carr (Pinales: Pinaceae). The objectives of this study were to: (1) determine the effect of treatment timing (spring versus fall) and application method (tree injection versus soil injection) on the spatial and temporal distribution of imidacloprid within the crown of *A. tsugae*-free eastern hemlock using a competitive enzyme-linked immunosorbent assay (ELISA), (2) compare ELISA to gas chromatography-mass spectrometry (GC/MS) for the detection of imidacloprid in xylem fluid, and (3) determine the concentration of imidacloprid in leaf tissue using high performance liquid chromatography with tandem mass spectrometric (LC/MS/MS) detection methods. Xylem fluid concentrations of imidacloprid were quantified using a competitive ELISA and were found to be significantly higher for spring applications than for fall applications, and for trunk injections than soil injections in the first year post treatment. As a comparison to the ELISA samples, a random subset of 125 samples was analyzed by using derivatization GC/MS. For the samples examined, 69% of the samples analyzed by ELISA showed higher concentrations of imidacloprid than those found by GC/MS, leading to evidence of a significant matrix effect and overestimation of imidacloprid in xylem fluid by ELISA. Additionally, a comparison of the presence of imidacloprid with xylem fluid and in leaf tissue on the same branch showed significant differences, suggesting that imidacloprid is moving intermittently within the crown of eastern hemlock.

Keywords: Eastern hemlock, hemlock woolly adelgid, imidacloprid, ELISA, insecticide distribution

Eastern hemlock, *Tsuga canadensis* (L.) Carr (Pinales: Pinaceae), is an important component of both the urban and forest landscape of the eastern United States. It is a long-lived, shade-tolerant species that strongly influences its environment and other species. The dense evergreen canopy along with its ability to grow in nearly pure stands creates a distinct microclimate that is important for a wide variety of plant and animal species.

The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is the single greatest threat to the health and sustainability of hemlock as an urban and forest resource in eastern North America (Knauer et al. 2002). This exotic insect is currently established in 18 eastern States in the U.S.A. (USDA, 2014), where it causes tree decline and mortality. *A. tsugae* is a bivoltine insect with three stages of development (i.e. egg, four nymphal instars, and adult) and reproduces parthenogenetically on hemlock (McClure 1989). This adelgid (~ 1 mm) settles on young twigs at the base of the hemlock needle and feeds on the parenchyma cells of the xylem rays that transfer and store nutrients (Young et al. 1995, McClure et al. 2001). All ages of hemlock, from seedling-to-mature and old-growth trees, are fed upon. Feeding from *A. tsugae* can kill a mature tree in about 5–7 years (McClure et al. 2001).

Imidacloprid (1-[6-chloro-3-pyridinyl) methyl]-*N*-nitro-2-imidazolidinimine), a systemic pesticide, is effective against a wide variety of sap-sucking insect pests on a wide variety of crops. It has a mode of action similar to that of the botanical product nicotine, functioning as a fast-acting insect neurotoxicant that binds to the nicotinergic receptor sites in the postsynaptic membrane of the insect's nerves, mimicking the action of acetylcholine. As a result, the heightening, then blocking of the firing of postsynaptic receptors occurs with increasing doses (Schroeder and Flattum 1984). Because imidacloprid is slowly degraded in the insect, it causes substantial disorder within the nervous system, leading in most cases to death (Mullins 1993, Smith and Krischik 1999).). As a result the chemical has become one of the world's most widely used insecticides (Silcox 2002, Jeschke and Nauen 2008). Imidacloprid is sold under a variety of tradenames (e.g. Admire®, Advantage®, Gaucho®, Premise®, and Touchstone®) and has 140 crop uses (Jeschke et al. 2010). It is both a systemic and contact insecticide (Mullins 1993) and has become the preferred pesticide for controlling *A. tsugae* (Smith and Lewis 2005, Eisenback et al. 2009).

Several methods have been developed for quantifying the amount of imidacloprid present in treated plants. Enzyme-Linked Immunosorbent Assay (ELISA) is a common and relatively inexpensive method (Cowles et al. 2006) used to detect imidacloprid in eastern hemlock sap and tissue (Cowles et al. 2006, Eisenback et al. 2009, Dilling et al. 2010). In this assay, imidacloprid residues in a sample compete with enzyme (horseradish peroxidase)-labeled imidacloprid for a limited number of antibody binding sites on the inside of the test well (EnviroLogix 2004). The levels of bound conjugate are determined spectrophotometrically and the sample concentrations are inversely proportional to the color development. A micro-titer plate reader and software are then used to measure end-point absorbance at 450 nanometers (nm) to determine the level of insecticide. Other analytical techniques such as gas chromatography and mass spectrometry analysis (GC/MS) have been found to be selective and sensitive for determining imidacloprid in soil and plant tissue (Li and Li 2000, Di Muccio et al. 2006, Cook 2008).

The effect of application method (Tattar et al. 1998, Cowles et al. 2006), season, and the movement of imidacloprid throughout the wood and needle tissue of the crown of *A. tsugae* -infested eastern hemlock have been examined in other studies (Eisenback et al. 2009, Dilling et al. 2010). Due to the difficulty in detecting *A. tsugae* at low densities (Evans and Gregoire 2007), imidacloprid is sometime used as a preventative treatment measure for high value trees and stands that are at risk from *A. tsugae*.

Induced plant responses to insect feeding are well documented and can have a significant impact on the physical and biochemical systems of plants (Haukioja 1991, Nykanen and Koricheva 2004, Karban and Baldwin 2007, Radville 2011). *A. tsugae* feeding can quickly lead to needle loss, resulting in dieback (Cheah et al. 2004), restrictions in the uptake and movement of water (McClure 1995), and reduced new growth (McClure 1991). These plant responses are likely affect the movement and distribution of imidacloprid. Previous studies (Eisenback et al. 2009, Dilling et al. 2010) investigated the effect of application method (Tattar et al. 1998, Cowles et al. 2006), season, and the movement of imidacloprid throughout the wood and needle tissue of the crown of *A. tsugae*-infested eastern hemlock. However, none of the previous studies investigated the movement of imidacloprid on *A. tsugae*-free eastern hemlock. Therefore, investigating the movement of imidacloprid on *A. tsugae*-free hemlock with similar live crown ratios allows one to investigate the temporal and spatial distribution of imidacloprid without the confounding factors of the presence of *A. tsugae* and the size of crowns.

This study was conducted to: (1) determine the effect of treatment timing (spring versus fall) and application method (tree injection versus soil injection) on the spatial and temporal distribution of imidacloprid within the crown of uninfested eastern hemlock using a competitive ELISA, (2) compare ELISA to GC/MS for the detection of imidacloprid in xylem fluid, and (3) determine the concentration of imidacloprid in leaf tissue by LC/MS/MS.

Materials and methods

Study Sites. This study was conducted at two *A. tsugae*-free sites located in Monongalia County, West Virginia, USA, in 2005 and 2006. Site A was located at the West Virginia University Forest (39° 39′ 22.80″ N, 79° 45′ 04.33″ W) within a 13-ha stand of eastern hemlock, and Site B at a 16-ha stand located at the West Virginia Botanic Garden (39° 37′ 41.50″ N, 79° 51′ 52.45″ W). A total of 32 single-stem hemlock trees were randomly selected from the hemlock stands with live crown ratio (LCR) of > 80% at each site. The minimum distance between trees was 9.1 m. For each tree, DBH (diameter at 1.37 m above the ground) and tree height were recorded. Trees were blocked by DBH so similar sized trees were present in each treatment class.

Insecticide Application Methods. At each site, eight trees were treated with Merit® 2F imidacloprid soil treatment (0.86 g a.i. in 30 ml/2.5 cm dbh) and eight trees were treated by trunk injection with an Arborjet Tree I.V.® system (IMA-jet® 5%) at label rates (Doccola et al. 2007) in the spring (2 May) and fall (10 October) of 2005. Soil treatments were made using a Kioritz applicator (Kioritz Corp., Tokyo, Japan) using the basal system (Silcox 2002, Dilling et al. 2010) with the footplate set at 12.7 cm (Turcotte et al. 2008a).

Branch Sampling and Sample Processing. To monitor the movement of imidacloprid within a tree, each tree was divided into four cardinal directions and three height sections at 2.4–4.8 m (lower), 5.1–7.3 m (middle), and 7.6–9.7 m (upper) (i.e. a total of 12 branch samples per tree). All samples were collected from the tip of the branch (ca. 61 cm in length) by using a telescoping pole pruner (Hasting HV-240, Hasting, Michigan). Branch samples were placed in polyethylene bags packed in ice, transported to the laboratory, and stored in a freezer at -18°C until the xylem fluid and leaf tissue could be extracted. The sampling was done five times at 3, 9, 15, 21, and 52 weeks post treatment. Xylem fluid from the samples was extracted using a 61-cm pressure chamber (PMS Instrument Co., Albany, Oregon). The cut end of each hemlock branch was trimmed and the cambium layer removed. This end was inserted through a rubber gasket and the entire branch was placed within the pressure chamber. The chamber was gradually pressurized with nitrogen up to 4.14 MPa. A 500–1,000 μL of expressed xylem fluid was collected with a micropipette, placed in a 1.5-mL microcentrifuge tube and refrigerated at 4°C.

Imidacloprid Concentration in Xylem Fluid. Concentration of imidacloprid within xylem fluid was determined using a competitive ELISA. Envirologix (Portland, ME) ELISA test kits (EP-006) were used according to the manufacturer's recommended procedures. In these 96-well test plates, imidacloprid residuals in samples compete with horseradish peroxidase (HRP)-labeled imidacloprid enzyme for a limited number of antibody binding sites on the inner surface of the well (Lagalante and Greenbacker 2007). The plates were washed, and the outcome of the reaction was visualized by a color development stage (Lagalante and Greenbacker 2007). In this study, a 100-μL negative control was used, and each calibrator (0.2, 1, 5, and 6 ppb) and xylem samples were added in duplicate to individual wells. To each well 100 μL of the enzyme (horseradish peroxidase)-labeled imidacloprid was then added. The plate was covered with a sheet of Parafilm and shaken at 200 rpm on an orbital plate shaker. After 1 h, the well contents were emptied, vigorously rinsed with cool water, and the well-plate was slapped on a paper towel to remove all visible water. When the plate was dry, 100 μL of substrate (hydrogen peroxide) was added to each well. The kit was covered with a new sheet of Parafilm and shaken at 200 rpm on an orbital plate shaker. After 30 minutes, 100 μL of a stop solution (1.0 M HCl) was added to each well and the optical density was read at 450 nm (600 nm reference wavelength) using a Bio-Rad Benchmark Plus (Hercules, CA) plate reader at 25°C (Jones 2007). The greater the amount of imidacloprid bound in a well, the less the optical density. A negative control is used to calculate B_0, the amount of HRP bound in the absence of imidacloprid. The percentage of B_0 value is the ratio of the optical density of each of the samples to the optical density of the negative control times 100 (Cook 2008).

The Envirologix ELISA kits used to detect imidacloprid do not distinguish between imidacloprid, its metabolites, and other chemical compounds containing similar chemical groups (Lagalante and Greenbacker 2007). To account for this effect of using ELISA on natural matrices, xylem fluid was collected from untreated trees. These samples were prepared for analysis at the following dilutions: undiluted, 10-fold, 20-fold, 50-fold and 100-fold dilutions. The results of this calibration showed that a 20-fold dilution produced an optical density that was equivalent to the negative controls (Jones 2007). However, a 20-fold dilution of hemlock xylem fluid elevated the working range and limit of detection (LOD) of the ELISA kit from 0.2–6 ppb to 4–120 ppb. If the measured imidacloprid concentration of xylem fluid sample was higher than 120 ppb, further dilutions were made (e.g. 1:10, 1:100 or 1:1000) to bring the sample into the working range of the ELISA kit (Cowles et al. 2006, Jones 2007, Eisenback et al. 2009).

As a comparison to the ELISA samples, a random subset of 125 samples was analyzed using derivatization gas chromatography-mass spectrometry (GC/MS). Concentrations of imidacloprid were determined on a Star software (Varian, Walnut Creek, CA) computer-controlled Varian 3900 gas chromatograph. The Varian 1177 injector was fitted with a Merlin Microseal septum. The injector temperature was maintained at 250 °C and a splitless injection was used. Separation was accomplished using a Varian VF-5 MS column (30 m, 0.26 mm i.d., 0.25 μm phase thickness). The column temperature program was 80°C (2 min hold) to 250°C at 20°C/min then to 320°C at 10°C/min (0.5 min hold). The helium carrier gas was electronic pressure controlled at a constant flow of 1.0 mL/min. The Varian 2100T ion-trap mass spectrometer was operated in CI+ mode (acetonitrile liquid CI reagent, multiplier 1400 V, *m/z* range of 50-450) (Jones 2007).

Imidacloprid Concentration in Leaf Tissue. To determine the concentration of imidacloprid in leaf tissue, a subsample of three trees from each of the two injection methods were selected from the spring treatment at Site B. Needles were removed from the same branches used for xylem fluid analysis. The twigs were separated by new growth and old growth (based on position) and placed in separate paper bags. The bagged samples were then air dried overnight, and dried at 60°C for a minimum of four hours in a drying oven. Once dried, the needles were separated from twigs, pulverized using a coffee grinder (Mr. Coffee, Model IDS55, Cleveland, OH) (Cowles et al. 2006), and then placed in opaque storage containers and frozen at 4°C. A 1:10 (needle: solvent) ratio was used to extract the compounds from the hemlock needles because this ratio was known to be adequate for needle extraction (Cowles et al. 2006). A total of 1.5 mL of extraction solvent was added to 0.15-g dried needles in a 2.0-mL microcentrifuge tube (Fischer Scientific, Pittsburg, PA). The microcentrifuge tubes were shaken overnight on an orbital bench shaker (Model G33, New Brunswick Scientific, Edison, NJ). The microcentrifuge tubes were then spun down on a Heraeus Instruments benchtop microcentrifuge (Biofuge 13, Heraeus Instruments, Germany) at 13,000 G for 10 min. The supernatant extraction solvent was removed by pipette and transferred to an autosampler for LC/MS/MS analysis (Cook 2008).

Statistical Analysis. We analyzed imidacloprid concentration data using a generalized linear mixed-effects model using PROC MIXED (SAS Institute 2011).

Individual trees were considered as the experimental unit, site as a blocking variable, and concentration of imidacloprid as the response variable. Tests for significance for the factors of site, application method, treatment season, height sections, quadrant, and weeks post treatment as well as the random effects of height section, quadrant and week post treatment (nested within tree) along with each two-way interaction were tested using type III F-ratios. The model used was an unstructured covariance model. A total of 90 ELISA observations were classed as outliers using studentized residuals (±3 SD from the mean) and excluded from the analysis. The conventional $\alpha = 0.05$ level of significance was used to determine variable retention in the model. Site and quadrant were found to be not significant ($P < 0.05$) and were removed from the model along with all insignificant two-way interactions. Multiple comparisons of means were conducted using Tukey-Kramer tests. We report adjusted P values that can be interpreted in a fashion similar to an experiment-wise error rate of $\alpha = 0.05$.

The association between GC/MS and ELISA was investigated by computing Pearson's correlation coefficient (r) and regression analysis using PROC CORR and PROC REG (SAS Institute, 2011), respectively. The concentrations of imidacloprid in xylem fluid analyzed by both ELISA and GC/MS were not normally distributed and consequently both were transformed using the natural logarithm (ln) of concentration. A total of 17 observations were classed as outliers (±3 SD from the mean) and excluded from the analysis. The association between ELISA and LC/MS/MS was investigated using PROC CORR (SAS Institute 2011). Because of the skewed data distributions with numerous zero values of both the ELISA and LC/MS/MS data, which violated the normality assumptions needed for Pearson correlation, Spearman's rank correlation was used. Based on the results of the ELISA and GC/MS comparison we chose to use a binary response variable (i.e. detected or undetected) to compare the imidacloprid levels in xylem fluid to the imidacloprid in leaf tissue found within the same branch. These data were analyzed using the continuity adjusted chi-square test in PROC FREQ (SAS Institute 2011).

Results

Spatial and Temporal Distribution of Imidacloprid in Xylem Fluid. Xylem fluid concentrations of imidacloprid extracted from branch samples within trees were highly variable.

Of the 3,475 xylem samples analyzed by ELISA, only 1,494 samples (43%) were positive for imidacloprid. Of the 64 trees treated in this project, 63 (98%) had detectable levels of imidacloprid in at least one sample of xylem fluid; only one fall soil-injected tree never displayed detectable levels of imidacloprid. Significant differences in imidacloprid concentration in xylem fluid were found between treatment season (F = 158.24; df = 1; P < 0.0001), application method (F = 46.31; df = 1; P < 0.0001), height (F = 4.98; df = 2; P = 0.0078; Figure 1), and weeks post treatment (F = 42.5; df = 4; P < 0.001). None of the two-way interactions were significant. Xylem fluid concentrations were significantly higher (post-hoc Tukey–Kramer test: t = -12.58, df = 2941; *Adj P* = < 0.0001) for spring than fall applications with averages of 25.49 and 7.19 µg/L (ppb), respectively. The trunk injection application method produced significantly higher (t = -6.80, df = 2941; *Adj P* = < 0.0001) concentrations of imidacloprid in xylem fluid than soil injection with averages of 25.00 and 6.61 µg/L, respectively. Mean concentration of imidacloprid was significantly lower in the bottom section of the tree crown than either the middle (t = -2.79, df = 187; *Adj P* = 0.0161) or top (t = -2.69, df = 187; *Adj P* = 0.0211) sections; no difference was found between the middle and top sections (t = 0.10; df = 187; *Adj P* = 0.9948) across all application methods and seasons. Detectable concentrations of imidacloprid were found in xylem fluid 3 wks post treatment with concentrations increasing over the weeks with the highest concentration found at 52 wks post treatment. Differences in mean concentration levels began to appear at week 3 with significant difference documented between weeks 3 and 52 (t = -11.47; df = 248; *Adj P* = < 0.0001), weeks 9 and 52 (t = -9.79; df = 248; *Adj P* = < 0.0001), weeks 15 and 52 (t = -10.33; df = 248; *Adj P* = < 0.0001), and weeks 21 and 52 (t = -9.28; df = 248; *Adj P* = < 0.0001; Table 1).

A moderate positive correlation (n = 107, r = 0.678, P < 0.0001) was found between ELISA and GC/MS imidacloprid concentrations as determined by each method (O'Rourke *et al*., 2005). The linear regression for imidacloprid concentration between GC/MS and ELISA was $y = 0.56x + 1.62$, where x is the natural log value of imidacloprid concentration determined by ELISA and y is the natural log value of imidacloprid concentration determined by GC/MS (F =89.18; df =1,105; P < 0.0001; r^2 = 0.459) (Figure 2). For the 106 samples examined, 69% of the samples analyzed by ELISA give higher concentrations of imidacloprid than those found by GC/MS, leading to evidence of a significant matrix effect and overestimation of imidacloprid in xylem fluid by ELISA. GC/MS detected imidacloprid in all 106 samples analyzed as ELISA detected imidacloprid in 100 (94%) of the samples analyzed.

Imidacloprid Concentration in Leaf Tissue. A significant positive correlation was found between the levels of imidacloprid in the xylem fluid compared to the levels in leaf tissue ($n = 235$, $r = 0.3632$, $P < 0.0001$). A significant difference in imidacloprid concentration was found between xylem fluid (ELISA) and leaf samples (LC/MS, $\chi^2 = 14.17$, df = 1, $P = 0.0002$). Of 235 samples analyzed, 36% (84 samples) had no detectable imidacloprid in either the xylem fluid or leaf samples. Detectable levels of imidacloprid were found in both xylem and leaf samples 27% (63 samples) of the time. The remaining samples had mixed results, with 14% (34 samples) of the samples having detectable imidacloprid in the xylem fluid but not in the leaf samples, and 23% of the leaf samples having detectable imidacloprid did not show detectable levels in their xylem tissue.

Discussion

Previous imidacloprid efficacy tests conducted with *A. tsugae* -infested trees have shown significant differences in imidacloprid concentration between treatment methods and within the crown of *A. tsugae*-infested eastern hemlock using ELISA (Cowles et al. 2006, Dilling et al. 2010). In our study, trees with similar sized crowns without the presence of *A. tsugae*, were examined, thus allowing us to look at the spatial and temporal distribution of imidacloprid without any confounding effects related to *A. tsugae* feeding, crown size, and tree response. The results of our study showed that ELISA detected differences by season, application method, height, and weeks post treatment; however, no significant difference for site, direction, and no two-way interactions were detected. Imidacloprid concentrations detected within xylem fluid were very similar to those found in other studies (Cowles et al. 2006, Dilling et al. 2010).

The live crown ratio is the ratio of crown length to tree height (Olivier and Larson 1996) and is a measure of a tree's foliar canopy. In our study, *A. tsugae*-free eastern hemlock with similar live crown ratios were chosen. However, no mention of crown size was made in the previous studies (Tattar et al. 1998, Cowles et al. 2006, Dilling et al. 2010) on the movement of imidacloprid in eastern hemlock. Although our results are similar to those of previous studies, it does raise the question of how the results of previous studies on the spatial and temporal distribution of imidacloprid were impacted by the crown size and presence and spatial arrangement of *A. tsugae*.

ELISA is a popular tool for imidacloprid quantification, but it also can produce false positives and overestimate imidacloprid concentrations due to matrix effects in sap (Cowles et al. 2006), needle tissue, and wood (Eisenback et al. 2009). In this study a 20-fold dilution was used to account for this effect in xylem fluid (Eisenback et al. 2009), but the dilution might not be sufficient to account for all the potential individual tree and seasonal effects of metabolism on imidacloprid and its metabolites within the tissue of eastern hemlock. In addition to ELISA, other detection methods that did not suffer from a matrix effect were used, allowing us to investigate the movement of imidacloprid within xylem fluid and leaf tissue. In nearly a quarter of the samples analyzed by both ELISA and LC/MS/MS, imidacloprid was found in the needles but not in the xylem fluid of individual branches. This points to several possibilities, two of which may be that imidacloprid was present in the xylem fluid but was below the detection of the ELISA kit, or that imidacloprid is moving intermittently within the crown and was not present at the day and time the branch was collected. Cowles et al. (2006) found concentrations of imidacloprid in new growth tissue similar to that of previous year's growth, and suggested that either remobilization or continued uptake was occurring after application. Our results support these hypotheses. Imidacloprid is a water soluble insecticide and is believed to move by mass flow in the transpiration stream (water flux) (Vite and Rudinsky 1959, Ford et al. 2010) of eastern hemlock. Numerous factors could be affecting the movement and distribution of imidacloprid. Some of these factors, such as the availability of water, season, time of day, tree condition, tree size, amount of crown, infestation levels, and local environmental factors (Ford et al. 2007), could be affecting the movement and distribution of imidacloprid within the tree.

Imidacloprid has been shown to be an effective insecticide against *A. tsugae* regardless of season and treatment method (McAvoy et al. 2005, Cowles et al. 2006, Doccola et al. 2007). Although site-specific (e.g. soil type) and tree-specific factors (e.g. amount of new growth, current tree condition, *A. tsugae* density, and live crown ratio) must be taken into account when choosing the method, dosage, and season of treatment, all of these factors are likely to affect an individual tree's ability to transport these insecticides and provide effective control of *A. tsugae*. Results from this study and field observations support the hypothesis that trees under stress from attack are less likely to move and distribute imidacloprid, suggesting that pretreatment of eastern hemlocks at high risk from *A. tsugae* can be justified, if only to allow for better spatial distribution and movement of imidacloprid within the crown of hemlock trees.

Currently the amount of systemic insecticide applied is based on tree diameter at breast height (diameter at 1.37 m above the ground) (Steward and Horner 1994, Fidgen et al. 2002, Silcox 2002, Doccola et al. 2007), with no change in dosage for differences in crown volume. Most recently, xylem water movement models (Ford et al. 2010) have been developed for eastern hemlock that show water usage (mass flow) is exponentially related to tree diameter, with smaller trees using proportionally less water than larger trees. This work has shown that the current manufacturer's recommended dose, which is based on a linear function of tree diameter, can be scaled to match water usage and still provide effective control of *A. tsugae*. The next step in this progression is to develop models that account for crown volume differences (Turcotte et al. 2008). Future research is needed to develop crown volume equations that could be used as the foundation for the development of new treatment tables based not only on tree diameter but also on the amount of live crown present (e.g. 30 cm DBH tree with 80% live crown ratio vs. a 30 cm DBH tree with 40% live crown ratio), which could reduce the cost of treating *A. tsugae*-infested eastern hemlock.

Acknowledgments

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Table 1**.** Mean imidacloprid concentration (ppb) in xylem fluid, determined by ELISA from eastern hemlock.

Weeks post treatment	Imidacloprid Concentration (ppb) ± SD
3	8.08 ± 25.75[a]
9	12.72 ± 33.03[ab]
15	12.98 ± 34.04[abc]
21	16.2 ± 38.37[abc]
52	29.25 ± 37.06[d]

*Means sharing a letter in the superscript are not significantly different at the 0.05 level according to Tukey-Kramer multiple comparisons tests.

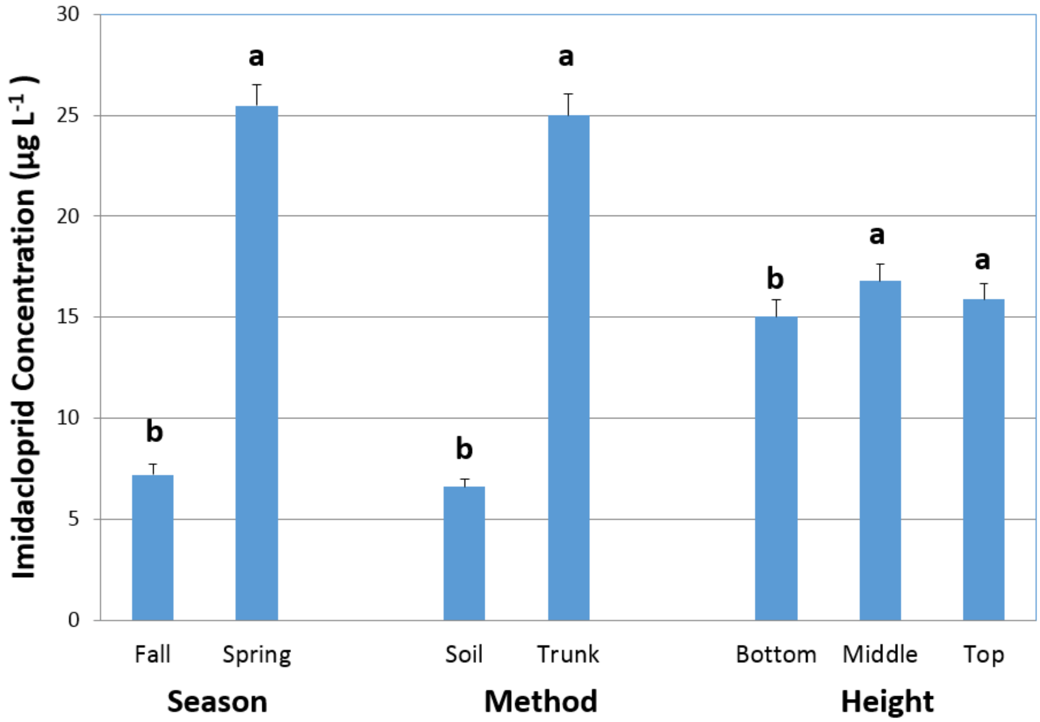

Figure 1**.** Imidacloprid xylem concentrations (mean ± SEM), determined by ELISA, by treatment season, treatment method and height section for treated eastern hemlock. Means sharing a letter in each category (i.e. season, method and height) are not significantly different at the 0.05 level according to Tukey-Kramer multiple comparisons tests.

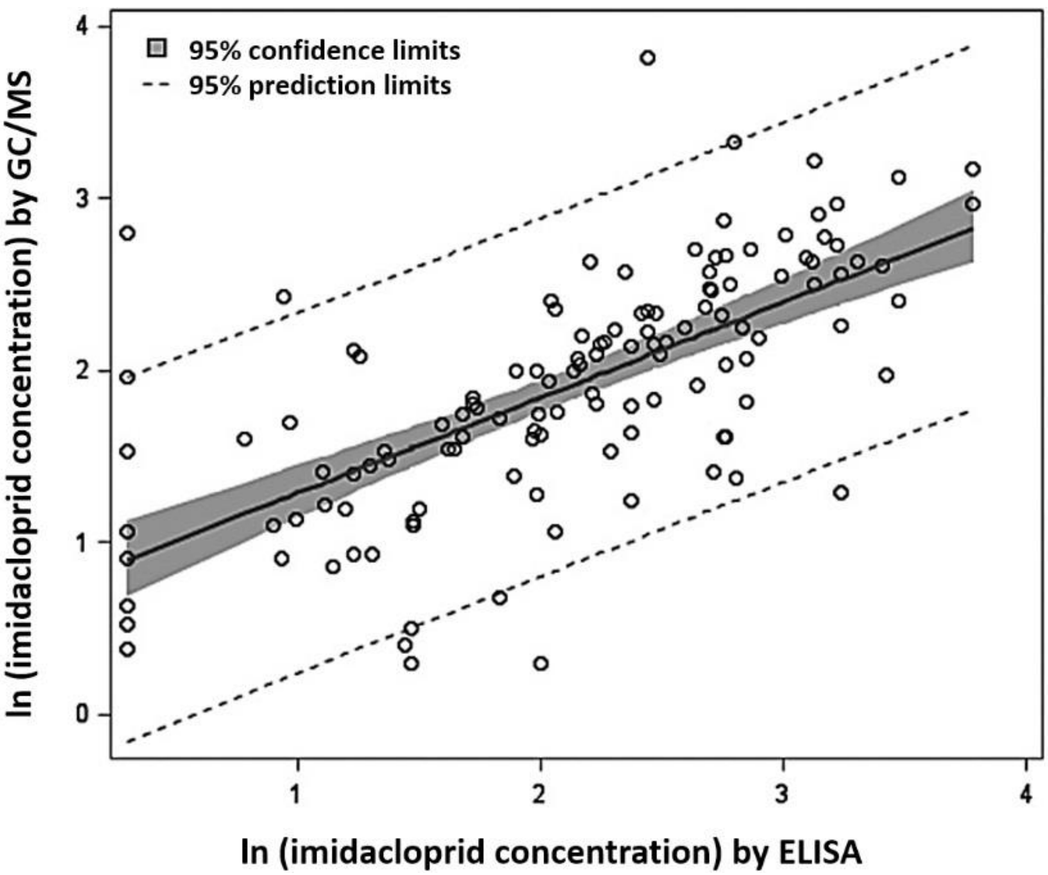

Figure 2. Comparison for imidacloprid in xylem fluid samples between ELISA and GC/MS. The regression equation was y =0.56x+1.62, where x is the ln(imidacloprid concentration) determined by ELISA and y is the ln(imidacloprid concentration), and the regression coefficient: R^2 = 0.459.

CHAPTER 3: Arthropod Community Composition in the Lower Crown of Eastern Hemlock in West Virginia

Abstract. Eastern hemlock, *Tsuga canadensis* (L.) Carriére, (Pinales: Pinaceae) has been heavily impacted by the introduction of both the elongated hemlock scale *Fiorinia externa* Ferris (Hemiptera: Diaspididae) and the hemlock woolly adelgid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae). The primary method to control these exotic insects is by chemical treatment. An assessment of the impact of application method and timing of imidacloprid treatments on the arthropods associated with eastern hemlock was carried out at two locations in northcentral West Virginia prior to the arrival of either pest. The application methods compared were near trunk soil and basal trunk injections made in spring and fall of 2005. A total of 12,423 individual arthropods, making up 393 species, were collected by branch beating the lower crowns of eastern hemlock. In addition to taxonomic grouping of arthropods, we recognized six feeding guilds in this study: detritivores, fungivores, herbivores, predators, parasitoids and tourists. No significant differences in arthropod abundance were found between imidacloprid treated and control trees and application methods. Similarly no significant differences in abundance of each feeding guild were found between the imidacloprid treated and untreated controls trees. The results of this study also showed that only about one-third of arthropods (130 of 393 species) examined are potential direct consumers of eastern hemlock. The other species utilize the unique aboreal habitat created by hemlock, and thus they are unlikely to be impacted by the use of imidacloprid applied by either trunk or soil injection.

Keywords: Eastern hemlock, hemlock woolly adelgid, imidacloprid, arboreal arthropods

Eastern hemlock, *Tsuga canadensis* (L.) Carr (Pinales: Pinaceae), is an extremely shade-tolerant, slow-growing, late successional conifer with a dense, evergreen crown and an extensive, shallow root system that strongly influences its environment and other plant and animal species (Ward and McCormick 1982, Godman and Lancaster 1990, Evans et al. 1996, Quimby 1996, Evans 2000). This ecologically important species has a conical crown with horizontal-to-pendulous branches (Ruth 1974) and 2-ranked needles (Dirr 1998). It exhibits relatively low branch shedding (Kenefic and Seymour 2000), and retains its needles for an average of three years (Barnes and Wagner 1981). The form and shape of needles and branches of eastern hemlock provide a collection surface for leaf litter, pollen, and other debris falling through the forest canopy, giving hemlocks the moniker of "trash collector of the forest" (Turcotte 2008).

Eastern hemlock is widely distributed in North America from Nova Scotia across southern Ontario to northeastern Minnesota, and south to Alabama along the Appalachian Mountains (Brisbin 1970, Godman and Lancaster 1990, Quimby 1996). Eastern hemlock forests create distinctive microclimates and provide important habitat for a wide variety of wildlife (Evans 2000). Eastern hemlock is well known as an important winter habitat for white-tailed deer, and also can be a critical factor in supporting native brook trout populations by maintaining cool stream temperatures and stable flows (Evans et al. 1996, Quimby 1996). The bark of hemlock was once a source of tannin for the leather industry; now the wood is important to the pulp and paper industry (Brisbin 1970, Godman and Lancaster 1990).

Among the nearly 400 species of arthropods that have been reported in association with eastern hemlock (Buck et al. 2005, Dilling et al. 2007, Turcotte 2008, Coots et al. 2012a) only a few are considered to be a threat to eastern hemlock. These are the hemlock woolly adelgid, *Adelges tsugae* Annand (HWA) (Hemiptera: Adelgidae) and three species of armored scales (Hemiptera: Diaspididae) such as the elongated hemlock scale, *Fiorinia externa* Ferris, the shortneedle evergreen scale, *Dynaspidiotus* (*Nuculaspis*) *tsugae* (Marlatt), and the cryptomeriae scale, *Aspidiotus cryptomeriae* Kuwana. These three scale insects feed on the needles of hemlock by sucking cell contents from the mesophyll while the adelgid settles at the base of the hemlock needle and feeds on the parenchyma cells of the xylem rays that transfer and store nutrients (Young et al. 1995, McClure et al. 2001). Of these pests *A. tsugae* is by far the most important (McClure and Fergione 1977, McClure 1985, Raupp et al. 2008). This non-native invasive species is currently established in 18 eastern states from Georgia to Maine (USDA 2014). The impact of *A. tsugae* in North America is the result of limited host resistance, lack of effective natural enemies, bivoltine life cycle, and high reproductive capacity (McClure 1992, Cheah and McClure 2000). This insect can be controlled on individual trees by systemic insecticides (Fidgen et al. 2002, Webb et al. 2003, McAvoy et al. 2005, Cowles et al. 2006, Doccola et al. 2007, Dilling et al. 2010) and foliar sprays (McClure 1987, 1988). However, many unanswered questions remain regarding the impacts of these insecticides on arthropods associated with *A. tsugae* throughout its range.

Imidacloprid (1-[6-chloro-3-pyridinyl) methyl]-*N*-nitro-2-imidazolidinimine) is the most widely used insecticide used to control *A. tsugae* (Smith and Lewis 2005, Eisenback et al. 2009). It is a systemic and contact insecticide that is effective against a wide variety of sap-sucking and mining insect pests on a wide variety of crops and is effective as a seed treatment (Matsuda et al. 2001). It has a mode of action similar to that of the botanical product nicotine, functioning as a fast-acting insect neurotoxicant (Schroeder and Flattum 1984) that binds to the nicotinergic receptor sites in the postsynaptic membrane of the insect's nerves, mimicking the action of acetylcholine. As a result, it disrupts the nervous system of the insect with lethal effect (Matsuda et al. 2001). Due to the potency and selectivity of imidacloprid this chemical has become one of the world's most widely used insecticides (Silcox 2002, Jeschke and Nauen 2008, Matsuda et al. 2001). Imidacloprid has 140 crop uses and is sold under a variety of trade names (e.g. Admire®, Advantage®, Gaucho®, Premise®, and Touchstone®) (Jeschke et al. 2010).

Numerous studies have demonstrated that *A. tsugae* feeding can lead to needle loss, dieback and a reduction of new growth, all effects which likely impact the arthropods associated with eastern hemlock (Cheah et al. 2004). Although a few previous studies investigated the arthropods associated with eastern hemlock (Buck 2004, Buck et al. 2005, Dilling et al. 2007) and the impact of insecticide treatments on non-target insects (Dilling et al. 2009), none have investigated the impact of imidacloprid in the absence of HWA. Using *A. tsugae* and scale-free trees to determine the impact of imidacloprid on arthropods allows one to investigate the impact without any of the complex and confounding factors related to *A. tsugae* and scale feeding, and intra-and inter-tree pest density and distributions issues.

The objectives of this study were to: (1) document the invertebrates associated within *A. tsugae* -free and scale-free eastern hemlock in north central West Virginia; (2) investigate the effects of application method and timing of imidacloprid on the invertebrate community associated with eastern hemlock; and, (3) determine which arthropods are at risk as a result of *A. tsugae* management.

Materials and methods

Study sites. This study was conducted at two *A. tsugae* and scale-free sites located in Monongalia County, West Virginia, USA, in 2005 and 2006. One site was located at the West Virginia University Forest (WVUF) (39° 39′ 22.80″ N, 79° 45′ 04.33″ W) within a 13-ha stand of eastern hemlock, and the other at a 16-ha stand located at the West Virginia Botanic Garden (WVBG) (39° 37′ 41.50″ N, 79° 51′ 52.45″ W). At each site I randomly selected 32 single-stem hemlock trees with a live crown ratio of > 80% (live crown length to total height). To reduce inter-tree interactions, I used a minimum between-tree distance of 9.1 m. For each tree, I recorded dbh (diameter at 1.37 m above the ground) using a diameter tape (Spenser Products Co., Seattle, Washington) and tree height using a clinometer (Suunto Co., Vantaa, Finland). Trees were blocked by dbh so similar sized trees were present in each treatment class.

Insecticide application methods. At each site, eight trees were treated with Merit® 2F imidacloprid soil treatment (0.86 g a.i. in 30 ml/2.5 cm dbh) and eight trees were treated by trunk injection in the spring (2 May) and fall (10 October) of 2005 using a Arborjet Tree I.V.® system (IMA-jet® 5%, Arborjet Inc., Woburn, Massachusetts) at label rates (Doccola et al. 2007). Soil treatments were made with a Kioritz applicator (Kioritz Corp., Tokyo, Japan) using the basal system (Silcox 2002, Dilling et al. 2010) with the footplate set at 12.7 cm (Turcotte et al. 2008).

Sampling, processing, and identifying arthropods. I sampled arthropod diversity biweekly from May-to-October in 2005 and 2006. For each tree, I sampled one randomly selected branch from one of the four cardinal directions (generally N-S-E-W). Samples were taken by branch beating (five beats) the distal 45 cm of a branch over a PVC pipe frame (84 cm by 56 cm) lined with a polyethylene bag. Samples were taken from ground level to ~ 3 m above ground. Branch beating was chosen over other collection methods (e.g passive trapping) because direct association of the collected arthropods with eastern hemlock could be inferred. While branch beating is effective at capturing flightless or weak-flying species (e.g. Acari, Psocoptera, Araneae, and Hemiptera), it is less effective at catching some strong-flying species (e.g. Diptera, and Hymenoptera) (Wardhaugh et al. 2014).

Sample bags were labeled, placed in coolers with ice packs, transported to the laboratory, and stored in a freezer at - 18°C until processed. Processing of samples was accomplished by empting the contents of each polyethylene bag into a gridded plastic tray (17.5 cm by 17.5 cm). The gridded plastic tray and bags were then examined under a zoom stereo microscope (6.7 to 45X) (SZ61, Olympus, Tokyo, Japan) with a 4.0 megapixel digital camera attached (DP21, Olympus, Tokyo, Japan). Arthropods were counted, separated, and preserved.

Guild composition. In addition to taxonomic grouping of arthropods, we recognized six feeding guilds (Moran and Southwood 1982, Stork 1987, Dilling et al. 2007) in this study: detritivores, fungivores, herbivores, predators, parasitoids, and tourists (Table 1). In this study, guild relationships were considered independently of phylogenetic relationship (Blondel 2003), with species being grouped based on how members exploit the environmental resource (Root 1967) available within the crown of eastern hemlock. I used the developmental stage of the specimen collected along with a literature review of each species feeding habits to place each arthropod within each guild (Dilling et al. 2007). In the case of the herbivores, the guild was composed of any chewing, sap-sucking, and wood-boring arthropods known to feed on eastern hemlock. The tourist guild was composed of any non-predatory species with no known association to eastern hemlock (Moran and Southwood 1982).

Statistical analysis. I analyzed arthropod count data using a generalized linear mixed-effects model using PROC GLIMMIX (SAS Institute 2011). Tests of significance for the factors of site, application method, treatment season, direction, and weeks post treatment along with each two-way interaction were tested using type III *F*-ratios. The model used an unstructured covariance structure. The conventional $\alpha = 0.05$ level of significance was used to determine variable retention in the model. As a result of the arthropod count data not being normally distributed, a poisson distribution was used in the model. Multiple comparisons of means were conducted using Tukey-Kramer tests. I report adjusted *P* values that can be interpreted in a fashion similar to an experiment-wise error rate of $\alpha = 0.05$.

Results

Taxonomic grouping. Arthropod counts between trees and sites were highly variable. A total of 12,423 individual arthropods (393 species) were collected, including insects (n = 6,715, 54.4% of arthropods collected), arachnids (n = 5,395, 43.7% of arthropods collected), and

Entognatha (n = 233, 2% of arthropods collected) (Fig. 1A). The most abundant insect orders were Psocoptera (n = 3,217, 47.9% of insects collected) followed by Diptera (n = 1,081, 16.1% of insects collected) and Hemiptera (n = 687, 10.2% of insects collected (Fig. 1B). Among the arachnid orders, Sarcoptiformes (n = 2,599, 51.2% of arachnids collected) was the most common, followed by Araneae (n = 2,240, 44.1% of arachnids collected) (Fig. 1C). Adult and immature stages accounted for 73.3% (n = 4,034) and 26.7% (n = 1,468) of the arthropods collected, respectively. All the Entognatha collected were in the order Collembola.

A significant difference was found in the number of arthropods collected between the two samples sites (F = 10.13; df = 1; P = 0.0015). No difference was found between treated and untreated control trees (F = 0.84; df = 1; P = 0.36) or between treatment methods (F = 3.56; df = 1; P = 0.06) and sample direction (F = 2.19; df = 3; P = 0.09). A significant difference was found between weeks post treatment (F = 8.52; df = 16; P < 0.0001). Significant interactions were found between site and week post treatment (F = 6.01; df = 16; P= <0.0001), treatment timing and method (F = 9.08; df = 1; P = 0.0026), treatment and week (F = 2.02; df = 16; P = 0.0092), and direction and week (F = 2.72; df = 48; P < 0.0001) (Table 2). Arthropod counts were significantly higher (post-hoc Tukey–Kramer test: t = 3.18, df = 7109; *Adj P* = 0.0015) in the WVUF site than those in WVBG site with 6,363 and 6,060 arthropods being collected at each site, respectively.

No significant differences were found for any of the main effects of treatment or sample direction for Arachnida, Insecta, and Entognatha classes. A significant difference was found between the two sites for Arachnida (F = 39.73; df = 1; P < 0.0001) and for the weeks post treatment for Insecta (F = 6.42; df = 16; P < 0.0001) and Arachnida (F = 31.78; df = 1; P < 0.0001). A difference was also found between the two treatment methods for Arachnida (F = 5.25; df = 1; P = 0.0221) and for the site by week interaction (F = 3.88; df = 16; P < 0.0001).

No significant differences in arthropod counts were found between sites for the orders Psocoptera (F = 0.06; df = 1; P = 0.8041), Diptera (F = 0.02; df = 1; P = 0.8950), Hemiptera (F = 0.05; df = 1; P = 0.8268), Hymenoptera (F = 1.05; df = 1; P = 0.3057), Lepidoptera (F = 0.24; df = 1; P = 0.6239), Coleoptera (F = 0.73; df = 1; P = 0.3944), Neuroptera (F = 0.02; df = 1; P = 0.8950), and Thysanoptera (F = 0.87; df = 1; P = 0.3552). No significant differences in arthropod counts were found between treated and untreated control trees for the orders Psocoptera (F = 1.07; df = 1; P = 0.3013), Diptera (F = 0.05; df = 1; P = 0.8183), Hemiptera (F

= 0.00; df = 1; *P* = 0.9680), Hymenoptera (*F* = 0.46; df = 1; *P* = 0.4977), Lepidoptera (*F* = 0.06; df = 1; *P* = 0.8141), Coleoptera (*F* = 0.08; df = 1; *P* = 0.7797), Neuroptera (*F* = 0.02; df = 1; *P* = 0.8950), and Thysanoptera (*F* = 1.32; df = 1; *P* = 0.2559). For the sample direction, no significant differences were found for the orders Psocoptera (*F* = 0.58; df = 3; *P* = 0.6310), Diptera (*F* = 0.41; df = 3; *P* = 0.7493), Hemiptera (*F* = 0.05 df = 3; *P* = 0.9836), Hymenoptera (*F* = 0.10; df = 3; *P* = 0.9585), Lepidoptera (*F* = 0.29; df = 3; *P* = 0.8347), Coleoptera (*F* = 0.08; df = 3; *P* = 0.9702), Neuroptera (*F* = 0.02; df = 1; *P* = 0.8950), and Thysanoptera (*F* = 2.37; df = 1; *P* = 0.0790; Fig 2 A-D).

A significant difference was found between sites for the Araneae (*F* = 3.92; df = 1; *P* = 0.0478) and Trombidiformes (*F* = 10.43; df = 1; *P* = 0.0035) and for the weeks post treatment for Psocoptera (*F* = 8.72; df = 16; *P* < 0.0001), Trombidiformes (*F* = 3.23; df = 12; *P* = 0.0065), and Sarcoptiformes (*F* = 8.50; df = 16; *P* < 0.0001). A significant difference also was found for the direction quadrant for the Sarcoptiformes (*F* = 2.99; df = 3; *P* = 0.0302) and Trombidiformes (*F* = 6.79; df = 3; *P* = 0.0017).

Guild grouping. Herbivores had the highest number of observed species (n = 130, 35.4% of arthropods collected), followed by parasitoids (n = 85, 23.2% of arthropods collected), predators (n = 58, 15.8% of arthropods collected), detritivores (n = 50, 13.6% of arthropods collected), tourists (n=21, 5.7% of arthropods collected), and fungivores (n=2, 0.5% of arthropods collected). Twenty-one species had unknown feeding habits based on a review of literature. No significant difference was found for the herbivore guild between sites (*F* = 0.49; df = 1; *P* = 0.4863), between treated and control trees (*F* = 0.51; df = 1; *P* = 0.4749), between treatment methods (*F* = 2.52; df = 1; *P* = 0.1125), sample direction (*F* = 0.02; df = 3; *P* = 0.9965), or between weeks post treatment (*F* = 1.13; df = 16; *P* = 0.3185; Fig.3). No significant difference was found for the parasitoid guild between sites (*F* = 1.56; df = 1; *P* = 0.2118), between treated and control trees (*F* = 0.45; df = 1; *P* = 0.5014), between treatment methods (*F* = 0.05; df = 1; *P* = 0.8209), sample direction (*F* = 0.17; df = 3; *P* = 0.9155), or between weeks post treatment (*F* = 0.38; df = 16; *P* = 0.9856). No significant difference was found for the predator guild between sites (*F* = 1.01; df = 1; *P* = 0.3156), between treated and control trees (*F* = 0.05; df = 1; *P* = 0.8303), between treatment methods (*F* = 0.02; df = 1; *P* = 0.8957), and sample direction (*F* = 0.60; df = 3; *P* = 0.6182). No significant difference was found for the fungivore guild between sites (*F* = 0.04; df = 1; *P* = 0.8385), between treated and control trees (*F* = 1.33; df

= 1; *P* = 0.2512), between treatment methods (*F* = 0.23; df = 1; *P* = 0.6322), sample direction (*F* = 0.74; df = 3; *P* = 0.5291), or between weeks post treatment (*F* = 0.24; df = 16; *P* = 0.9986). No significant difference was found for the tourist guild between sites (*F* = 0.12; df = 1; *P* = 0.7291), between treated and control trees (*F* = 1.75; df = 1; *P* = 0.1865), sample direction (*F* = 0.27; df = 3; *P* = 0.8499), or between weeks post treatment (*F* = 1.13; df = 16; *P* = 0.3290). No significant difference was found for the detritivore guild between sites (*F* = 1.57; df = 1; *P* = 0.2100), between treated and control trees (*F* = 0.02; df = 1; *P* = 0.8972), between treatment methods (*F* = 2.03; df = 1; *P* = 0.1542), and sample direction (*F* = 1.72; df = 3; *P* = 0.1608). A significant difference in detritivore numbers was found between weeks post treatments (*F* = 14.31; df = 16; *P* < 0.0001). A significant difference was found between treatment methods for tourists (*F* = 4.00; df = 1; *P* = 0.0466) and for the number of predators collected and the weeks post treatment (*F* = 8.47; df = 16; *P* < 0.0001) with the highest counts being collected in September (Fig. 3 A-D).

Discussion

The objectives of this project were two-fold. The first was to assess the arthropod diversity and guild assemblages associated with eastern hemlock in West Virginia before any non-native insects impacted these forest stands. The second was to assess the impact of imidacloprid on those arthropods that utilize hemlock. Among the 12,423 individual arthropods (393 species) collected in this study, one new species of arboreal Collembola, *Sminthurus turcottei* n. sp. (Richard J. Snider, personal communications, Michigan State University, August 20, 2014) and several undescribed species (Roy A. Norton, in litt.) of sarcoptiform mites were included. The number of species and the percentage of species comprising different guilds varied from those found on eastern hemlock in different areas of the range (Buck et al. 2005, Dilling et al. 2007, Dilling et al. 2009). However, these differences are likely the results of different sampling methods, intensities, forest compositions, and the presence and severity of HWA. The arthropods collected in this project were all sampled directly from the lower crown foliage of eastern hemlock (up to 3 m) and were dominated by herbivores, predators, and parasites, most of which have broad distribution and host ranges (Table 2). The most commonly collected group were sarcoptiform mites; two most common species were *Camisia segnis* (Hermann) (Sarcoptiformes: Camisiidae) and *Ceratoppia bipilis* (Hermann) (Sarcoptiformes:

Peloppiidae) (Table 3). The next most commonly collected groups were the spiders and three species of arboreal psocopterans: *Ectopsocus meridionalis* Ribaga (Pscoptera: Ectopsocidae), *Peripsocus subfasciatus* (Rambur) (Pscoptera: Peripsocidae), and *Xanthocaecilius sommermanae* (Mockford) (Pscoptera: Caeciliusidae). All of these species have wide distributions and are associated with both hardwoods and coniferous trees (Mockford 1961, 1988, 1993, Coots et al. 2012b). The pscopterans feed on microphytes (fungi, algae, pollen, and lichens) that grow on the leaves (needles) and bark of trees and shrubs (Thornton 1985). Although I collected a large number of Diptera (110 species in 22 families) and Hymenoptera (107 species in 21 families), none of the species were found in any number.

Findings in this study are consistent with previous studies (e.g. Dilling et al. 2009) that showed no significant differences between treated and untreated trees for any of the taxonomic groupings or guild groupings of collected arthropods. This may be because nearly 65% of the species collected and identified are not direct consumers of eastern hemlock. Of the remaining 35%, no species collected is reported to be a specialist feeding exclusively on eastern hemlock. This is especially true for several known hemlock feeders like *Lambdina fiscellaria* Guenée, *L. anthasaria* Walker, and *Pero morrisonaria (Henry Edwards)* (Lepidoptera: Geometridae), which were collected at both sites but are known to have wide host ranges (Maier et al. 2004). These results are supported by other studies in which no differences were observed for most polyphagous lepidopteran and psocopteran species between untreated control trees and soil-and-trunk injected trees (Dilling et al. 2009).

This study and previously published arcitles (Buck et al. 2005, Dilling et al. 2007, Dilling et al. 2009) documented the wide range of arthropods associated with eastern hemlock. Most of the arthropod species collected in this project were found not to be direct feeders of eastern hemlock, and these species are unlikely to be impacted by the use of imidacloprid applied by either trunk or soil injection. The remaining are direct feeders of eastern hemlock but were found not to be impacted by the imidacloprid treatments, but will likely be impacted by the effects of the elongated hemlock scale and *A. tsugae* as they move into these areas.

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Table 1**.** Guild assignments based on identified species and reported host range (Moran and Southwood 1982, Stork 1987).

Detritivores:	**Fungivores**:	**Herbivores:**	**Predators:**	**Parasitoids:**
Arachnida: Sarcoptiformes: Caleremaeidae, Camisiidae, Carabodidae, Cepheidae, Ceratoppiidae, Cymbaeremaeidae, Hemileiidae, Liacaridae, Nanhermanniidae, Neoliodidae, Oppiidae, Orbatulidae, Oripodidae, Parakalummidae, Phenopelopidae, Pthiracaridae, Suctobelbidae, Tegoridbatidae, **Diplopoda:** all **Entognatha:** Collembola all, **Insecta:** Diptera: Bibionidae, Cecidomyiidae, Lonchopteridae, Psychodidae, Sarcophgidae, Trichoceridae; Coleoptera: Pedilidae; Psocoptera: Ectopsocidae, Peripsocidae, Caeciliusidae;	**Insecta:** Diptera: Cecidomyiidae[3], Mycetophilidae[3]; **Tourists:** **Insecta:** Ephemeroptera[1]: all, Plecoptera[1]: all; Hemiptera: Lygaeidae, Miridae, Berytidae, Diptera: Ptychopteridae[1], Chironomidae[1], Culicidae[1,2],	**Insecta:** Coleoptera**:** Alleculidae, Anobiidae, Chrysomelidae, Melandryidae, Phalacridae, Scirtidae, Elateridae, Curculionidae, Mordellidae, Lathridiidae, Cerambycidae, Erotylidae, Scolytinae, Trogostitidae, Diptera: Cecidomyiidae[3], Sciaridae, Phoridae[3], Mycetophilidae[3], Bombyliidae, Empididae; Hemiptera: Pentatomidae[3], Cicadellidae, Aphididae, Psyllidae, Membracidae, Cercopidae, Delphacidae, Hymenoptera: Tenthredinidae, Cynipidae, Lepidoptera: Pyralidae, Notodontidae, Gracillariidae, Geometridae, Noctuidae, Arctiidae, Lymantriidae, Tineoidae, Limacodidae, Orthoptera: Tettigoniidae, Acrididae, Gryllidae; Thysanoptera: Thripidae, Phlaeothripidae, Arachnida: Eriophyidae, Tetranychidae	**Arachnida:** Pseudoscorpiones, Araneae, **Insecta:** Coleoptera: Meloidae, Carabidae, Cleridae, Staphylinidae, Coccinellidae, Lampyridae, Canthridae, Staphylinidae; Diptera: Dolichopodidae, Empididae, Rhagionidae, Asilidae, Ceratopogonidae; Hemiptera: Reduviidae, Pentatomidae[3], Miridae, Nabidae, Anthrocoridae; Hymenoptera: Formicidae, Sphecidae, Halictidae, Vespidae; Neuroptera: Coniopterygidae, Chrysopidae, Hemerobiidae; Thysanoptera: Phleothripidae;	**Arachnida:** Prostigma: Erythraeidae **Insecta:** Hymenoptera all (Parasitica); Diptera: Phoridae

[1]Adults, [2] Females,[3] Group where a few of the species belong to another guild

Table 2. GLIMMIX model for arthropod counts.

Effect	df	F	P
Site	1	10.13	0.0015
Treatment	1	0.84	0.35
Method	1	3.56	0.06
Direction	3	2.19	0.0870
Week (post treatment)	16	8.52	<0.0001
Site*Week	16	6.01	<0.0001
Treatment*Week	16	2.02	0.0092
Direction*Week	48	2.72	<0.0001

Table 3. Distribution and host range for the most common arthropod species collected during this study

Order	Family	Genus	Species	Distribution[1]	Hosts[2]
Psocoptera	Ectopsocidae	*Ectopsocus*	*meridionalis*	NA (N)	Broad-leaf trees, eastern hemlock
Psocoptera	Peripsocidae	*Peripsocus*	*subfasciatus*	NA (N)	
Psocoptera	Caeciliusidae	*Xanthocaecilius*	*sommermanae*	NA	
Sarcoptiformes	Camiisidae	*Camisia*	*segnis*	COS	
Sarcoptiformes	Peloppiidae	*Ceratoppia*	*bipilis*	COS (N)	Strict Fungivore
Sarcoptiformes	Carabodidae	*Carabodes*	*brevis*		
Hemiptera	Cicadellidae	*Gyponana*	*striata*	NA(N)	Hemlock , others
Hemiptera	Cicadellidae	*Empoasca*	*vincula*	NA(N)	Maple, Viburnum
Hemiptera	Reduviidae	*Zelus*	*luridus*	NA(N)	Predator
Lepidoptera	Geometridae	*Lambdina*	*fiscellaria*	NA(N)	Hemlock, fir, pines, spruce other conifers
Lepidoptera	Geometridae	*Pero*	*morrisonaria*	NA (N)	Hemlock, fir, pines, spruce other conifers
Lepidoptera	Geometridae	*Lambdina*	*anthasaria*	NA (N)	Hemlock, fir and spruces
Coleoptera	Curculionidae	*Cyrtepistomus*	*castaneus*	COS (I)	Broad-leaf trees
Coleoptera	Curculionidae	*Anthonomus*	*rubidus*	NA(N)	Birch and Roses
Coleoptera	Elateridae	*Athous*	*excavatus*	NA (N)	
Neuroptera	Coniopterygidae	*Conwentzia*	*pineticola*	COS	
Neuroptera	Coniopterygidae	*Semidalis*	*vicina*		
Neuroptera	Coniopterygidae	*Semidalis*	*vicina*	COS	
Collembola	Tomoceridae	*Pogonognathellus*	*elongatus*		
Collembola	Entomobryidae	*Entomobrya*	*clitellaria*		
Orthoptera	Gryllidae	*Oecanthus*	*exclamationis*	NA(N)	
Thysanoptera	Phlaeothripidae	*Liothrips*	*sp.*		
Thysanoptera	Phlaeothripidae	*Leptothrips*	*sp.*		

[1] Distributions are from (Jacot 1936, Meinander 1974, Hamilton 1982, Andre et al. 1984, Drooz 1985, Hart 1986, Mockford 1993, Stelzl and Devetak 1999, Hébert et al. 2003, Frederick and Gering 2006) NA: North America; COS: Cosmopolitan; (I): introduced; (N): native

[2]Host ranges are from (Bouchard et al. 2005, Hartenstein 1962, Maier et al. 2004, Mockford 1993, Frederick and Gering 2006)

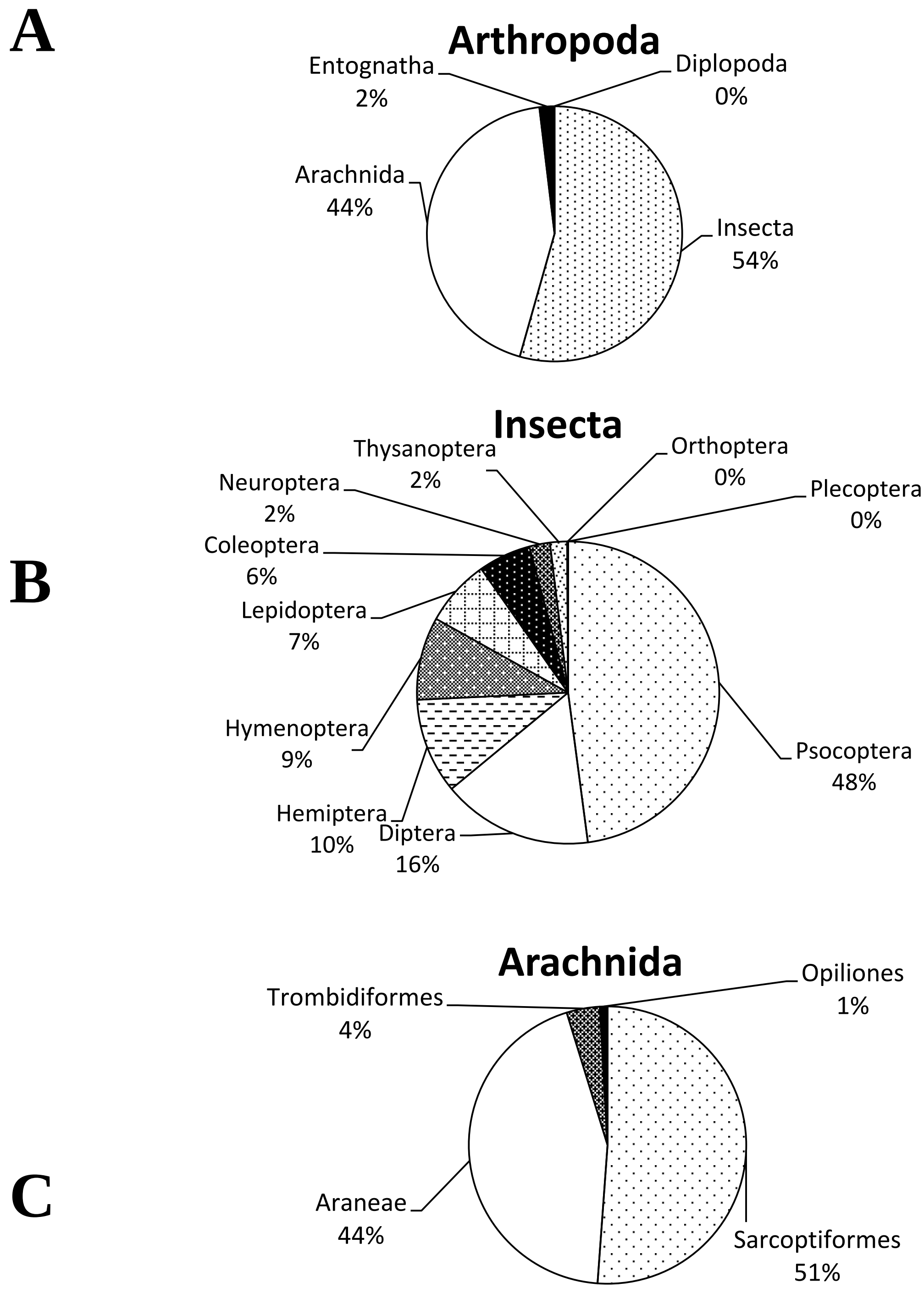

Figure 1. Pie-charts of the relative proportion of the 12,423 arthropods collected by branch beating the lower crown of eastern hemlock in West Virginia in 2005 and 2006. A, arthropods by class; B, insects by orders; C, Arachnida by order.

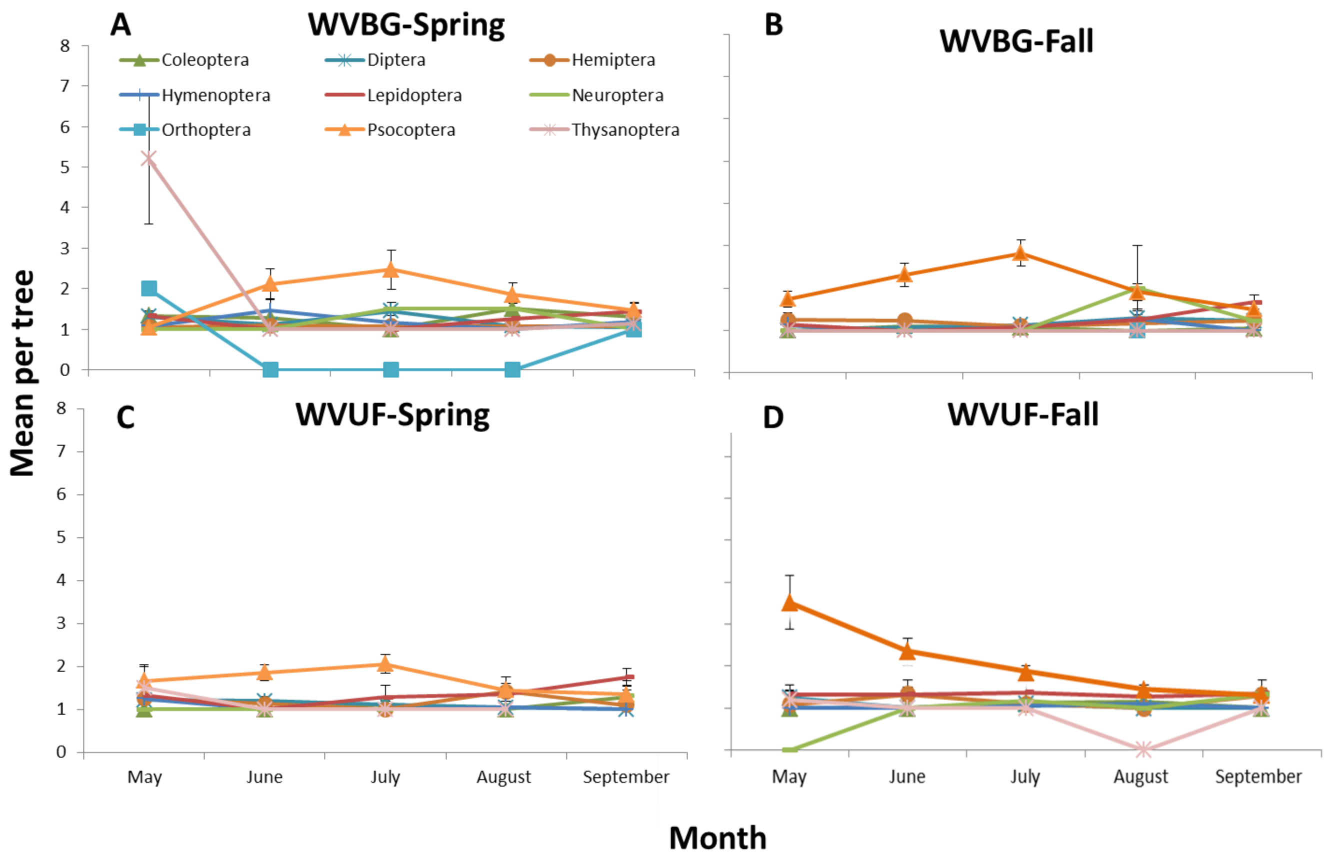

Figure. 2. Temporal patterns in the abundance (mean ± SE) of insect orders for branch beating samples of eastern hemlock at the West Virginia Botanic Garden (WVBG) and West Virginia University Forest (WVUF) for the spring (2 May 2005) and fall (10 October 2005) imidacloprid treatments.

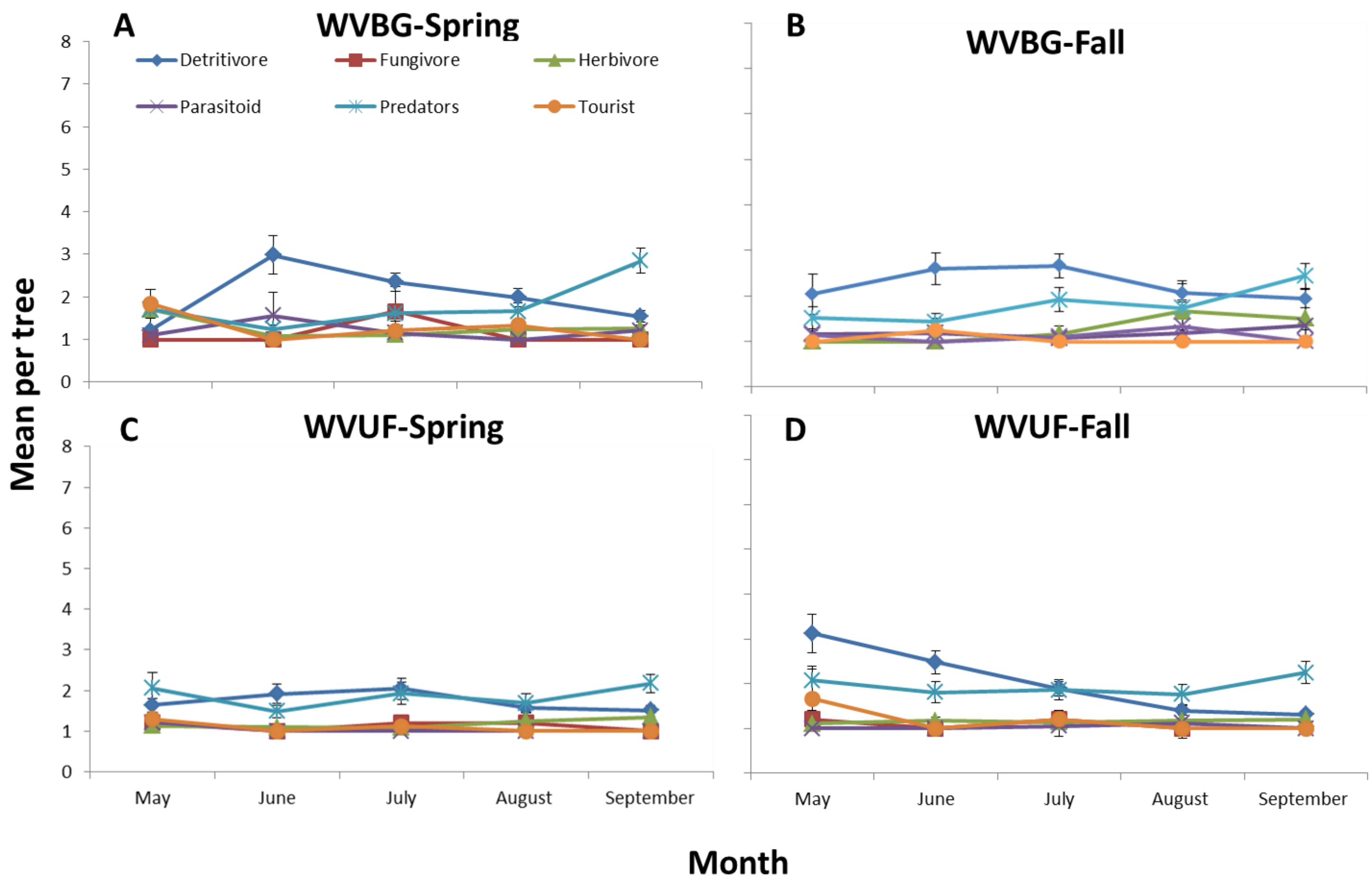

Figure 3. Temporal patterns in the abundance (mean ± SE) of feeding guilds for branch beating samples of eastern hemlock at the West Virginia Botanic Garden (WVBG) and West Virginia University Forest (WVUF) for the spring (2 May 2005) and fall (10 October 2005) imidacloprid treatments.

CHAPTER 4: Arthropods at Risk Due to Eastern Hemlock Mortality Caused by the Hemlock Woolly Adelgid (Hemiptera: Adelgidae).

Abstract. Eastern hemlock, *Tsuga canadensis* (L.) Carriére (Pinales: Pinaceae), has been impacted by the introduction of both the hemlock woolly adelgid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae) and the elongated hemlock scale *Fiorinia externa* Ferris (Hemiptera: Diaspididae). This chapter reviews the arthropod species associated with eastern hemlock to determine which species may be impacted by the loss of a major foundation species. A literature review revealed 484 native and exotic arthropods from three different taxonomic classes and 21 different orders associated with eastern hemlock in North America. A risk assessment system was developed to assess the endangerment risk of arthropod species known to be associated with eastern hemlock. Arthropods were classified into three risk categories (i.e., high, moderate and low) based on the reported host range found in the literature. A high risk rating was given to species only known to be associated with eastern hemlock; a moderate risk rating was given to species associated with only *Tsuga* or one other genus; and a low risk rating was assigned to species known to feed on *Tsuga* and more than two other host genera. This rating system identified five species at high risk. The species indentified were *Gyponana arcta* (Hemipera: Cicadellidae), *Plagiognathus tsugae* (Hemiptera: Miridae), *Megastigmus hoffmeyeri* (Hymenoptera: Torymidae), *Coleotechnites macleodi* (Lepidoptera: Gelechiidae) and *Nalepella neosuga* (Trombidiformes: Eriophyidae). It is likely that these hemlock-dwelling species will experience local extirpation as a result of declining and dying eastern hemlock. The reduction and loss of eastern hemlock as a result of these introduced species is expected to cause significant impacts on the ecological processes in the hemlock forests across the eastern United States.

Keywords: Eastern hemlock, hemlock woolly adelgid, arthropods, invasive species, dependent species

Introduced species can and do have a devastating effect on resident organisms (Wagner 2007). The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) and the elongated hemlock scale, *Fiorinia externa* Ferris (Hemiptera: Diaspididae) are two of these threats. Both were introduced into the eastern United States. *A. tsugae* was first discovered in Virginia in the 1950s (Souto et al. 1996) from southern Japan (Havill et al. 2006) and the elongate hemlock scale was found in New York in the early 1900s (Sasscer 1912). Of these two, *A. tsugae* is the greater threat to the health and sustainability of eastern hemlock as a forest resource in eastern North America (McClure 2002, Knauer et al. 2002). Currently, established in 18 eastern States from Georgia to Maine (Fig. 1; USDA 2014), *A. tsugae* has caused tree decline and mortality and is a threat to the survival of eastern hemlock.

This tiny insect (~ 1 mm) settles on young twigs at the base of the hemlock needle and feeds on the parenchyma cells of the xylem rays (cells that transfer and store nutrients) (Young et al. 1995, McClure et al. 2001). It reproduces parthenogenetically (an all-female population with asexual reproduction); has three stages of development (the egg, four nymphal instars, and the adult) and two generations a year on hemlock (McClure 1989). All ages of eastern hemlock, from seedling to mature, old-growth trees are fed upon. Both of our eastern North American hemlock (*Tsuga*) species are susceptible to *A. tsugae* (Montgomery et al. 2009). As this insect continues to spread the ecological impacts on our flora and fauna will be significant. Already extensive hemlock mortality has occurred across large areas of the eastern and southern Appalachian region (Vose et al. 2013).

Eastern hemlock, *Tsuga canadensis* (L.) Carr, is an extremely shade-tolerant, monoecious, slow-growing, late successional conifer with a dense, evergreen crown. Such characteristics of eastern hemlock strongly influences its environment and other species (Godman and Lancaster 1990, Quimby 1996, Ward and McCormick 1982, Evans et al. 1996, Evans 2000). Eastern hemlock has a conical crown with horizontal-to-pendulous branches (Ruth 1974) and 2-ranked needles (Dirr 1998). The tree retains its needles for an average of three years (Barnes et al. 1981), and exhibits relatively low branch shedding (Kenefic and Seymour 1999). It is a relatively long lived species with a life span of over 800 years (Godman and Lancaster 1990). Seed production usually begins when trees are 20-30 years of age (Ruth 1974). It is a frequent and abundant cone producer, with good crops being produced every two to three years (Frothingham 1915, Ruth 1974).

Hemlock-dominated forests comprise about 2.3 million acres in eastern North America (McWilliams and Schmidt 2000). Hemlock can occur in pure stands (Eyre 1980) or mixed with other species. Hemlock's association with other species ranges from the occasional component in broadleaf deciduous forests to a codominant role within a number of northern coniferous forests, to a dominant role in relatively pure stands (McWilliams and Schmidt 2000). Although it usually forms a climax position (Brisbin 1970) on sites which are rich in nutrients, it can be out competed by hardwoods (Kotar 1996). In pure stands, undergrowth vegetation can be sparse (Eyre 1980) due to intraspecific allelopathy (Ward and McCormick 1982) and dense evergreen crown of hemlock which intercepts both light and precipitation. Because of this dense canopy in hemlock stands the microclimate is cooler than under hardwoods (Tubbs 1996). This distinct microclimate provides an important habitat for a wide variety of wildlife (Evans 2000). In the northeastern United States 96 bird and 47 mammal species have been found to be associated with eastern hemlock forests (Yamasaki et al. 2000). This includes 23 species of small mammals, 14 species of wide ranging carnivores, 10 species of amphibians, and 7 species of reptiles (Degraaf et al. 1992). Hemlock forests can also be a critical factor in the support of native brook trout populations, where it maintains cool stream temperatures and stabilizes stream flows (Evans et al. 1996, Quimby 1996). Eastern hemlock fills a unique ecohydrological role because it transpires throughout the year and it provides stable water fluxes within a watershed and high water flux patterns in the spring, reducing nutrient loss and decreasing watershed discharges (Ford and Vose 2007). Eastern hemlock is currently listed as a near threatened species by the IUCN (International Union of Conservation of Nature) Red List database (Farjon 2013).

While the loss of eastern hemlock due to these exotic pests is occurring at a significant rate, relatively little information is available on the wide diversity of arthropods associated with eastern hemlock (Onken and Reardon 1994). Although numerous recent large-scale studies have been done using indirect methods (e.g. pit fall, panel traps, etc.) to collect arthropods (Sciascia and Pehek 1995, Buck et al. 2005, Dilling et al. 2007), few have been restricted to direct sampling of the tree (Coots et al. 2012a; Coots et al. 2012b); indirect sampling is likely to miss many of the ecological connections between faunal communities and eastern hemlock across its range.

This study was conducted to catalogue the number of arthropod species directly associated with eastern hemlock and assess the relative risk of endangerment of these species as eastern hemlock is affected by *A. tsugae* and *F. externa*.

Materials and methods

Database search. To identify arthropods associated with eastern hemlock an extensive literature search was performed on eastern hemlock. Sources searched included journal articles, book chapters, proceedings, and internet sources. Search engines and databases examined were (1) Google Scholar, (2) Scopus, (3) Agricola, (4) CAB Abstract, (5) Biosis Life Science, (6) Web of Sciences, (7) BioOne Abstracts, and (8) Entomological Abstracts. The key words "eastern hemlock" resulted in 52,400 references (Table 1) of which 5,064 remained when combined with "arthropod". The Internet search and data base searches were performed up until February 28, 2014. I am not aware of any extensive literature review of arthropods associated with eastern hemlock, although recent arthropod survey studies have been completed (Buck 2004, Buck et al. 2005, Dilling et al. 2007, Turcotte 2008, Coots et al. 2012a). Although it is clear from the available literature that some groups of arthropods are better studied than others, it is unclear if the relative proportions of information available on each arthropod reflects its relative richness or is an artifact of its range, small size, taxonomic difficulties (e.g. Acari) or the sampling methodology used (Borges et al 2000). In this study, I only included arthropods reported to feed on or have been collected directly from eastern hemlock.

Assigning Risk. There are a wide range of techniques which can be used to quantify pest risk (e.g. Zlotina 2015), conservation value (e.g. Lambeck 1997) and extinction risk (Hartley and Kunin 2003, IUCN 2014) for organisms. All of these techniques inherently contain uncertainty and largely depend on the quality and reliability of the information available (Zolotina 2015). Uncertainty is a characteristic of any risk assessment and has a profound influence on the inferences and conclusions drawn from that assessment (Wright et al. 2005). In this study I used a modified risk rating proposed by Ghandi and Herms (2010) to assess the risk of local extirpation as a result of the loss of eastern hemlock on hemlock feeding arthropods due to *A. tsugae* and *F. externa*. I assigned a risk rating to each eastern hemlock feeding species based on a rating of its known host range. A high rating was given to species only known to be associated with eastern hemlock; a moderate risk to species associated with only *Tsuga* or one other genus;

and a low rating to species known to have an association to *Tsuga* and more than two other genera (Gandhi and Herms 2010).

Assigning Feeding Guild. To further explore the arthropods associated with eastern hemlock, species were also placed into feeding guilds based on their reported feeding habits and host feeding range defined as phytophagy, zoophagy, saprophagy, or mycetophagy, which was adapted from Mahan et al. (2004) (Table 2).

Results

This literature search revealed 484 native and exotic arthropods from three different taxonomic classes and 21 different orders associated with eastern hemlock in North America (Table 3). Of these 43 species were reported to be exotic and 11 were reported only to the genera level. Many of the native insects that are associated with eastern hemlock appeared to be generalists, feeding on both conifers and hardwoods (Table 4); others were specialists feeding on only a few conifer species (Table 5). The most common taxonomic class represented was Insecta of which the most represented orders were Coleoptera (112) followed by Lepidoptera (82), Hemiptera (51) and Psocoptera (44) (Table 3). Among the species the most common feeding guild was the phytophages (222 species) followed by the zoophages (144 species), saprophages (84 species) mycetophages (31 species; Fig. 2) with two unknowns.

Of the 222 species of phytophages revealed in this search I was able to determine a risk rating for 213 species of which 5, 12 and 196 were categorized in the high (monophagous species), moderate (bi-phagous species) and low (polyphagous) risk categories, respectively (Fig 3). The five species identified to be at high risk include *Gyponana arcta* (Hemipera: Cicadellidae), *Plagiognathus tsugae* (Hemiptera: Miridae), *Megastigmus hoffmeyeri* (Hymenoptera: Torymidae), *Coleotechnites macleodi* (Lepidoptera: Gelechiidae) and *Nalepella neosuga* (Trombidiformes: Eriophyidae).

Discussion

Eastern hemlock is associated with a very diverse and complex faunal community. Among at least 484 arthropod species listed in this study, five species were identified as monophagous and rated at high risk based on their known association and host range. These species are undoubtedly threatened by the impacts of *A. tsugae*. However, it is still not known if these specialists, which are often highly sensitive to plant cues (e.g. plant secondary compounds), will be impacted by the feeding activity of *A. tsugae* that can cause a systemic hypersensitive response in eastern hemlocks (Bernays 2001, Radville et al. 2011). It is also unknown if chemical treatments used to control *A. tsugae* will impact these hemlock dependent species. Both of which will have implications for the conservation and management of these at risk arthropods.

Species rated as low risk in this review, including the hemlock looper, *Lambdina fiscellaria fiscellaria* (Guenée) (Lepidoptera: Geometridae) a polyphagous species with a wide host range which includes pines (*Pinus* spp.), spruces (*Picea* spp.), eastern larch (*Larix laricina*) and balsam fir (*Abies balsamea)*, were generalist herbivores that can feed on alternate species, assuming that they are present in the area impacted by *A. tsugae* and likely will not face the same impact as hemlock dependent species (Drooz 1985, Maier et al. 2011). *Oecanthus laricis* T.J. Walker (Orthoptera: Gryllidae), the tamarack tree cricket, is rarely collected and reported to feed specifically on eastern larch and eastern hemlock (Walker 1963, and Marshall et al 2004). This species was rated in the moderate risk category and should be considered as an extremely vulnerable species (Hoving et al. 2013). The reduction in eastern hemlock is likely to result in the local extirpation of *O. laricis* in many of the southern areas without eastern larch (Fig. 4). Of the species found to be associated with eastern hemlock, over half were found not to feed directly on eastern hemlock but were affiliated with the species, as zoophages, saprophages, mycetophages or as yet undermined associates (Table 3). For these species the impact of *A. tsugae* is unknown.

As *A. tsugae* continues to spread and impact eastern hemlock throughout eastern North America, we are likely to see further effects occurring on the invertebrate and vertebrate species that utilize eastern hemlock forests. Although a few studies have been published on the arthropods associated with eastern hemlock (Coots et al. 2012a, Coots et al. 2012b), the ecology and interactions of arthropods associated with eastern hemlock are largely unknown. Therefore, other factors in addition to feeding need to be considered to document the potential impact of the reduction and loss of eastern hemlock. To combat the impact of *A. tsugae* the USDA Forest Service in cooperation with the National Plant and the National Association of State Foresters developed a multiagency effort initiative to develop management options to reduce the spread and impact of *A. tsugae*. This Initiative started in 2001 has spent about 37 million dollars on understanding the biology, control, and impacts of *A. tsugae*. Despite increased awareness of the arthropods associated with eastern hemlock (Buck 2004, Buck et al. 2005, Dilling et al. 2007, Turcotte 2008, Coots et al. 2012a, Larcenaire 2015) few longterm and landscape-scale studies of the arthropods associated with eastern hemlock have been conducted. Therefore, it is obvous that the diversity of the arthropod fauna associated with eastern hemlock is still incompletely known. This information on the diversity and abundance of arthropods associated with hemlock-dominated and hemlock-associated forests can provide the fundamental information needed to aid in the conservation of biodiversity and planning and management of these at risk forests (Kreman et al. 1993).

Ultimately, questions such as how many trees or how big an area of eastern hemlock needs to be retained or protected to support the critical ecological functions and processes of eastern hemlock need to be answered. These important questions cannot be addressed without basic information on the biota associated with eastern hemlock. Extensive literature reviews and field studies are the keys to understanding the arthropods associated with any species. This study serves a key role to direct limited resources to better understand the impact of invasive species. Historic surveys and inventories should be repeated and future studies should be directed and focused on understanding the landscape patterns, host and geographic ranges, ecological processes and relationships of the identified arthropods at risk (e.g. high and moderate; Table 6) with their ecological important and irreplaceable tree species.

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Table 1. Summary of online databases searched for eastern hemlock and arthropods.

Database Searched	Years searched	Eastern hemlock hits	Search combined with arthropod
Google Scholar	1702-present	52,400	5,060
Scopus	1966-present	4,358	182
Agricola	1968-present	2,211	102
CAB Abstracts	1910-present	3,825	152
Biosis Life Science databases (BIOSIS)	1926-present	3,063	118
Web of Science	1955-present	685	10
BioOne Abstracts	1998-present	509	52
Entomological Abstracts	1982-present	222	5

Table 2. Terms and definitions used to describe the arthropod feeding guilds of arthropods associated with eastern hemlock (modified from Mahan et al. 2004).

Primary Guilds	Secondary Guilds
Phytophagy – feeding on flowering plants, trees, ferns, lichens, mosses (bryophytes), liverworts (hepatics,) and algae (diatoms).	Leaf chewer, leaf miner, cone feeder, gall-maker, grazer, flower feeder, pollen feeder, sap feeder, seed feeder, root feeder, woodborer, general plant feeder – feeding on multiple plant parts (generalist).
Zoophagy – feeding on other animals.	Predator – feeding on smaller or weaker animals, usually using one or more for a single meal. Living apart from their prey and seeking animals in different places for different meals.
	Parasite and parasitoid – living in or on the bodies of their hosts and live continually with their hosts during at least a part of their life cycle. Obtaining successive meals from these hosts, and their feeding is at the expense of the hosts.
	Entomophagous –feeding on insects.
	Haemophagous – feeds on blood or takes a blood meal from live animals.
Saprophagy – feeding on dead or decaying plant or animal materials, such as carrion, leaf litter, dead logs, and the like.	Detritivore – feeding on dead plant material and fragments of organic matter.
	Carrion feeder – feeding on dead animals.
	Coprophagous – feeding on feces.
	Filter feeder
Mycetophagy – feeding on fungi, mold, and yeast.	Fungivore, mold feeder, yeast feeder.

Table 3. Taxonomic distribution of arthropod species associated with eastern hemlock in different risk groups resulting from the impact of hemlock woolly adelgid and elongate hemlock scale.

Taxonomic group Class	Order	Phytophagous Risk Rating Categories High	Moderate	Low	NA*	Total number of species
Arachnida	Araneae				59	59
	Mesostigmata				15	15
	Prostigmata				7	7
	Sarcoptiformes			1	23	24
	Trombidiformes	1	1	11	1	14
Entognatha	Collembola				8	8
	Protura				3	3
Insecta	Coleoptera		2	63	47	112
	Dictyoptera				1	1
	Diptera			2	15	17
	Emphereroptera			2	1	3
	Hemiptera	2		33	16	51
	Hymenoptera	1	2	2	20	25
	Lepidoptera	1	6	74	1	82
	Mecoptera				1	1
	Megaloptera				1	1
	Neuroptera				6	6
	Orthoptera		1	5		6
	Psocoptera				44	44
	Thysanoptera			2	2	2
	Trichoptera			1		1
Totals		5	12	196	262	484

*Not applicable (non-phytophagous: e.g. zoophagous, mycetophagous, and saprophagous) and unknowns (N = 9).

Table 4. Taxonomic distribution of phytophagous insect species associated with eastern hemlock, conifers and hardwoods.

Taxonomic group		Number of species reported to feed on			
Order	Family	Eastern Hemlock	Conifers	Hardwoods	Both[1]
Coleoptera					
	Bostrichidae	1	1		
	Buprestidae	11	10	2	1
	Cerambycidae	25	22	9	5
	Chrysomelidae	4	3	2	1
	Curculionidae	11	9	9	5
	Elateridae	1	1		
	Lymexylidae	1	1	1	1
	Melandryidae	3	3		
	Mordellidae	1	1	1	1
	Oedemeridae	1	1		
	Scarabaeidae	2	2	2	2
	Scirtidae			1	
Ephemeroptera					
	Ephemerellidae	1		1	
	Leptoceridae	1		1	
Hemiptera					
	Adelgidae	1	1		
	Aphididae	1	1		
	Cercopidae	1	1	1	
	Cicadellidae	6	1	4	1
	Cicadidae	1	1	1	1
	Coccidae	3	2	2	1
	Coreidae	1	1		
	Diaspididae	13	11	3	2
	Lygaeidae	1	1	1	1
	Miridae	4	2	2	
	Pentatomidae	2		2	
Hymenoptera					
	Pamphiilidae	1	1		
	Siricidae	2	1		
	Tenthredinidae	1		1	
	Torymidae	1			
Lepidoptera					
	Arctiidae	1	1		
	Erebidae	2	2		
	Gelechiidae	3	2		
	Geometridae	39	34	16	11
	Lasiocampidae	1	1		

	Lymantriidae	5	5	3	3
	Noctuidae	12	11	3	1
	Psychidae	1	1	1	1
	Saturniidae	1	1	1	1
	Tineoidea				
	Tortricidae	13	13	4	4
Orthoptera					
	Acrididae	1	1		
	Gryllidae	2	2	1	1
	Rhaphidophoridae	1	1	1	1
	Tetrigidae	1	1	1	1
	Tettigoniidae	1		1	
Thysanoptera					
	Phlaeothripidae	2	1	2	1

[1]Both = Conifers and Hardwoods

Table 5. Taxonomic distribution of phytophagous insect species associated with different genera of conifers.

Taxonomic group		Number of species reported to feed on					
		Pines	Spruce	Larch	Cedar	Baldcypress	Douglas-fir
Order	Family	(*Pinus*)	(*Picea*)	(*Larix*)	(*Thuja*)	(*Taxodium)*	(*Pseudotsuga*)
Coleoptera							
	Bostrichidae	1					1
	Buprestidae	10	6	1		3	
	Cerambycidae	19	13	3			1
	Chrysomelidae	1	1				
	Curculionidae	9	6	3	2		1
	Elateridae	1					
	Melandryidae	1	1				
	Mordellidae		1				
	Oedemeridae	1	1		1		
	Scarabaeidae	1					
Hemiptera							
	Adelgidae		1				
	Aphididae		1		1		
	Cercopidae	1					
	Coccidae	1					
	Diaspididae	1	3		3		2
	Lygaeidae	1			1		
	Miridae	1					
Hymenoptera							
	Pamphiilidae						
	Siricidae		1	1			
Lepidoptera							
	Erebidae	2					
	Gelechiidae					1	
	Geometridae	3	7	5		1	
	Noctuidae	2	2	2			
	Tortricidae	2	5				
Orthoptera							
	Gryllidae			1			

Table 6. High and moderate risk rated arthropod species associated with eastern hemlock.

Risk Rating	Class	Order: Family	Species	Reported Host	Reference Source
High	Arachnida	Trombidiformes: Eriophyidae	*Nalepella neotsuga*	*Tsuga canadensis*	Domes 2003
	Insecta	Hemiptera: Cicadellidae	*Gyponana arcta*	*T. canadensis*	Hamilton 1982, Osborn and Knull 1947
		Miridae:	*Plagiognathus tsugae*	*T. canadensis*	Henry et al 2005, Wheeler et al 1983
		Hymenoptera: Torymidae:	*Megastigmus hoffmeyeri*	*T. canadensis*	Milliron 1949, Auger-rozenberg et al 2006, Turgeon et al 2004
		Lepidoptera: Gelechiidae	*Coleotechnites macleodi*	*T. canadensis*	Freeman 1965, Johnson and Lyons 1991, Maier et al. 2004, Maier et al. 2011
Moderate	Arachnida	Trombidiformes: Eriophyidae	*Nalepella tsugae*	*Tsuga spp.*	Lindquist et al 1996
	Insecta	Coleoptera: Cerambycidae	*Evodinus monticola*	*Tsuga spp, Pinus spp.*	Nystrom and Ochoa 2006, Vance et al. 2007, Wilson 1971
		Melandryidae	*Scotochroides antennatus*	*Tsuga spp, Pinus spp.*	Majka and Pollock 2006
		Hymenoptera Pamphiilidae	*Cephalcia distincta*	*Abies balsamea, T. canadensis*	Johnson and Lyons 1991
		Tenthredinidae	*Phymatocera racemosae*	*Polygonatum spp. Tsuga spp.*	Smith 1996
		Lepidoptera: Gelechiidae	*Coleotechnites abietisella*	*Abies spp. Tsuga spp.*	Freeman 1965,
		Geometridae	*Eupithecia albicapitata*	*Abies balsamea, Tsuga spp., Picea spp.*	Turgeon and DeGroot 1992
			Eupithecia luteata	*Tsuga spp. Larix spp.*	Schooley and Pardy 1981
			Eupithecia transcanadata	*Abies balsamea, Tsuga spp.*	Ferguson 1975
			Nepytia semiclusaria	*Pinus spp., Tsuga spp.*	Hetrick 1960, Felt 1913
		Noctuidae	*Xestia semiclusaria*	*Pinus spp., Tsuga spp.*	Tietz 1972
		Orthoptera:			

	Gryllidae	*Oecanthus laricis*	*Larix laricina, T. canadensis*	Walker 1963

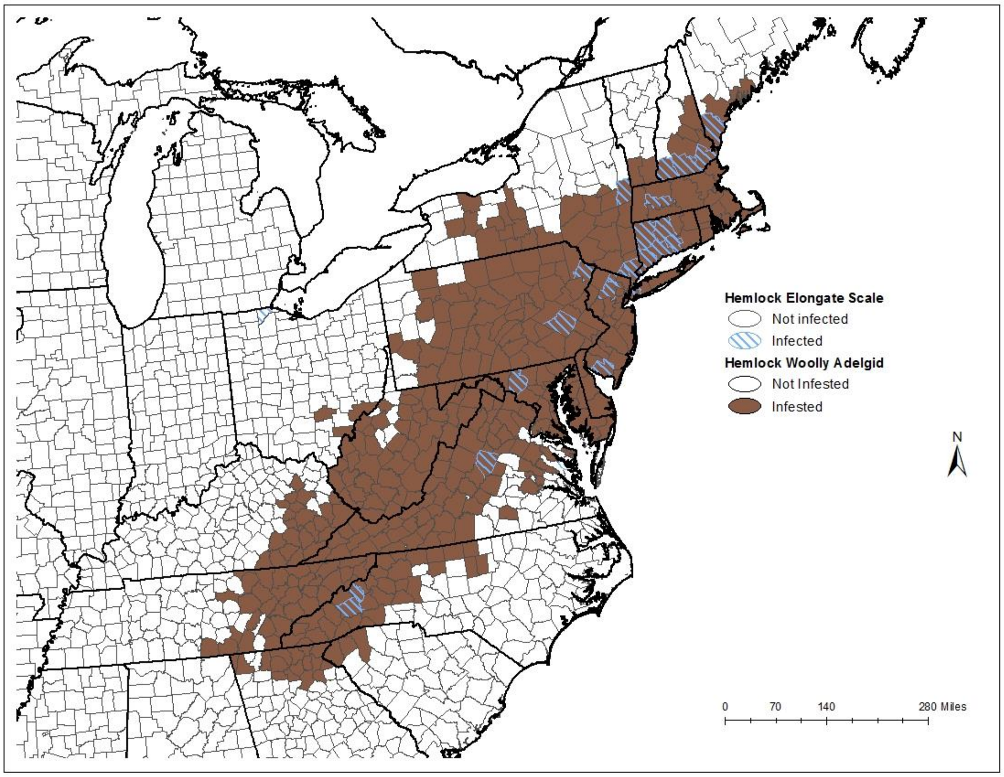

Figure 1. Current distribution of hemlock woolly adelgid and elongate hemlock scale (USDA 2014).

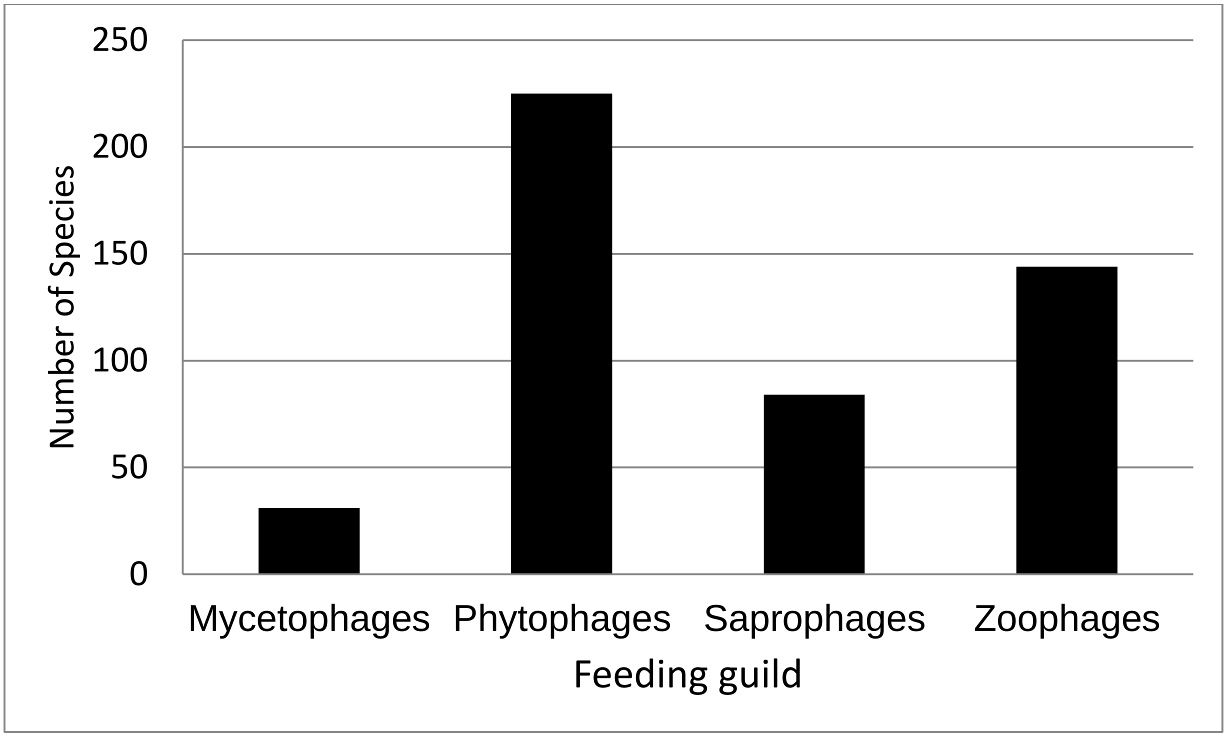

Figure 2. Feeding guild of arthropods associated with eastern hemlock.

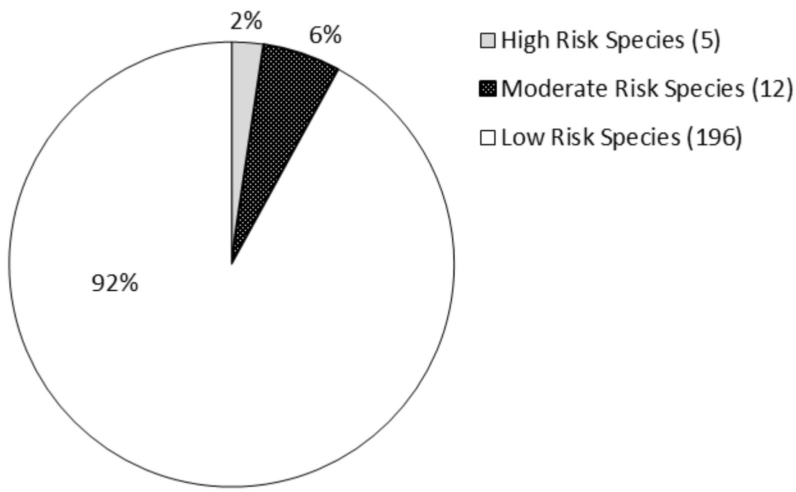

Figure 3. Percentage of arthropod species in the high (monophagous), moderate (bi-phagous) and low (polyphagous) risk catagories.

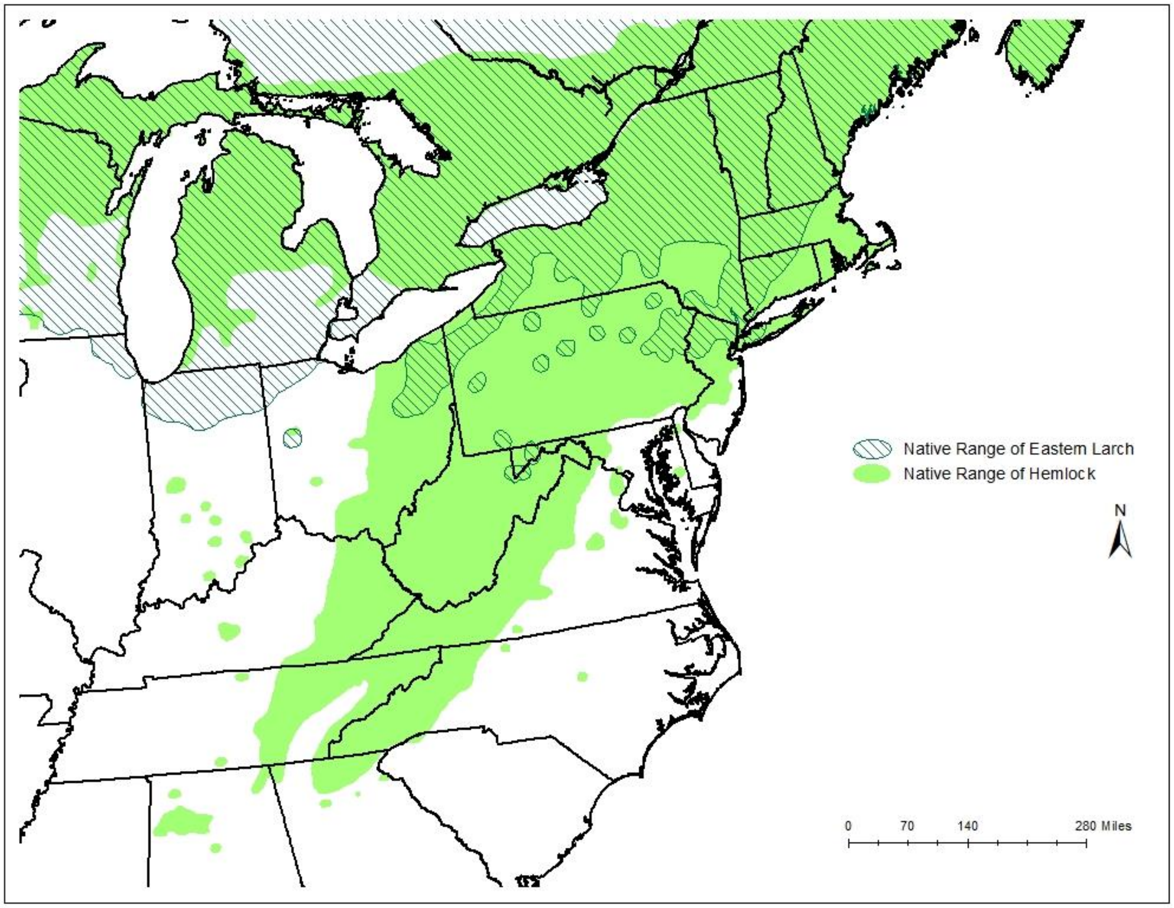

Figure 4. Native ranges of eastern hemlock and eastern larch (Burns and Honkala 1990).

CHAPTER 5: Spiders in the Lower Crown of Eastern Hemlock in West Virginia

Abstract. Eastern hemlock, *Tsuga canadensis* (L.) Carr. has been heavily impacted by the introduction of the hemlock woolly adelgid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae). The primary method to control this exotic insect has been chemical control with imidacloprid. An assessment of the impact of application method and timing of imidacloprid treatments on the spider communities associated with eastern hemlock were carried out at two locations in northcentral West Virginia prior to the arrival of this pest. The application methods compared were near trunk soil and basal trunk injections made in spring and fall of 2005. Samples were collected by branch beating the lower crowns of eastern hemlock. A total of 1,798 spiders were collected, which included ten families and 47 species of spiders. The majority of the spiders collected in this study belonged to Araneidae (N=509, 35.9%), Anyphaenidae (N=265, 18.7%) and Philodromidae (N=221, 15.6%). In addition to taxonomic grouping of spiders, I recognized three foraging guilds: web-builders (N=679, 48%), wandering spiders (N=596, 42%) and a combined guild of web and wandering spiders (N=142, 10%). No significant differences in spider abundance were found between imidacloprid treated and control trees and application methods. Similarly no significant differences in abundance of each foraging guild were found between the imidacloprid treated and untreated controls trees.

Keywords: Eastern hemlock, Imidacloprid, arboreal spiders, foraging guilds

Eastern hemlock, *Tsuga canadensis* (L.) Carr. (Pinales: Pinaceae) is a medium to tall tree that reaches up to 39 meter in height and 92 to 122 centimeters in diameter (Hough 1960). Hemlock is a extremely shade-tolerant, slow-growing, late successional native conifer with a dense, evergreen crown (Godman and Lancaster 1990, Evans et al. 1996, Quimby 1996). This ecologically important species has a wide distribution and is an important component of both the urban and forest landscape of the eastern United States (Brisbin 1970, Godman and Lancaster 1990). Unfortunately, this species is threatened by a non-native invasive species, the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae). *A. tsugae* was introduced into North America from Japan sometime before 1951 (Havill et al 2006) and has since spread to 18 eastern States from Georgia to Maine (USDA 2014). The impact of this insect in North America is the result of limited host resistance, lack of effective natural enemies, and the bivoltine life cycle and high reproductive capacity of this insect (McClure 1992; Cheah and McClure 2000).

Imidacloprid (1-[6-chloro-3-pyridinyl) methyl]-*N*-nitro-2-imidazolidinimine), a neonicotinoid insecticide, is the most commonly used insecticide against *A. tsugae* (Smith & Lewis, 2005, Eisenback et al. 2009). Imidacloprid disrupts the normal nerve impulse transmissions in insects and is effective at controlling populations of *A. tsugae* (Matsuda et al. 2001, Charles 2002, Doccola et al. 2007, Cowles et al. 2006). Soil and trunk injections are the primary control methods used for *A. tsugae* control programs on public lands (Eisenback et al. 2010). Using current label rates of imidacloprid to treat *A. tsugae* could impact non-target arthropods either through direct contact or consumption of treated plant material. Several studies have documented both lethal and sublethal effects on predators caused by prey feeding imidacloprid treated plant material (Papachristos and Milonas 2008, Eisenback et al. 2010, Szczepaniec et al. 2011).

Spiders are polyphagous predators (generalists) that feed primarily on insects and other spiders (Nyffeler 1999, Sanders et al. 2015). They play an important predatory role in agricultural and forest ecosystems (Nyffeler and Sunderland 2003, Mallis and Rieske 2011). Within forest canopies, habitat structure and prey abundance influence spider community composition (Halaj et al 1998, Halaj et al 2000, Horvath et al 2005). Numerous studies have demonstrated that *A. tsugae* feeding can lead to needle loss, dieback, and a reduction of new growth (McClure 1987, Mayer et al. 2002, Cheah et al. 2004) all of which likely impact the spiders associated with eastern hemlock.

Eastern hemlock has a complex form and shape that supports a diverse community of arthropods (Dilling et al 2007, Turcotte 2008, Kung et al. 2015). Nearly 400 species of arthropods that have been reported to be associated with eastern hemlock (Buck et al. 2005, Dilling et al. 2007, Turcotte 2008, Coots et al. 2012). Although several studies (Mallis 2007, Hakeem 2008, Mallis and Rieske 2010, Mallis and Rieske 2011) have investigated spiders associated with eastern hemlock, and the impact of *A. tsugae*, none of the studies determined the impact of imidacloprid in the absence of *A. tsugae*. Using *A. tsugae*-free trees to investigate the impact of imidacloprid on spiders allows one to determine the impact without any of the complex and confounding factors related to *A. tsugae* feeding, and intra and inter-tree density and distribution issues. The objectives of this study were to: (1) determine the spiders associated within adelgid free eastern hemlock in northern West Virginia; and, (2) determine the effects of application method and timing of imidacloprid on the spider community associated with eastern hemlock.

Material and methods

Study Sites. Spiders were collected at two *A. tsugae*-free sites in Monongalia County, West Virginia in 2005 and 2006. One site was located at the West Virginia Botanic Garden (39° 37′ 41.50″ N, 79° 51′ 52.45″ W) and the other the West Virginia University Forest (39° 39′ 22.80″ N, 79° 45′ 04.33″ W). The West Virginia University Forest (WVUF) stand is located along the west side of the Laurel Run drainage, and considered a mesic site with moderate slope. The stand is composed of a hemlock-oak overstory: eastern hemlock, white oak (*Quercus alba* L.), scarlet oak (*Q. coccinea* Muench.), and chestnut oak (*Q. prinus* L.). The stand also contains a number of other overstory species including northern red oak (*Q. rubra* L), red maple (*Acer rubrum* L.), black birch (*Betula lenta* L.), yellow-poplar (*Liriodendron tulipifera* L.), and blackgum (*Nyssa sylvatica* Marsh.). The understory is composed mostly of eastern hemlock and a mixed of red maple, black birch and blackgum.

The hemlock stand at the West Virginia Botanical Garden (WVBG) is located just east of Tibbs Run Reservoir. The site is considered to be mesic with a minimal amount of slope. The stand is similar in composition to the WVUF site in that the overstory is hemlock-oak; eastern hemlock dominated with a mix of white oak, northern red oak and yellow poplar. The understory is composed mostly of eastern hemlock and a species including, American beech (*Fagus grandifolia* Ehrh.), red maple, black birch, blackgum and sourwood (*Oxydendrum arboretum* DC).

At each site 32 single-stem hemlock trees with live crown ratio of > 80% (live crown length to total height) were randomly selected. To reduce the chances of any inter-tree interactions I used a minimum between-tree distance of 9.1 m. For each tree, I measured tree height using a clinometer (Suunto Co. Vantaa, Finland) and dbh (diameter at 1.37 m above the ground) using a diameter tape (Spenser Products Co., Seattle, Washington). Trees were blocked by dbh so similar sized trees were present in each treatment class.

***Insecticide Application Methods*.** A total of eight trees at each site were treated by trunk injection with an Arborjet Tree I.V.® system (IMA-jet® 5%, Arborjet Inc., Woburn, Massachusetts) at label rates (Doccola et al. 2007) and eight trees with Merit® 2F imidacloprid soil treatment (0.86 g a.i. in 30 ml/2.5 cm dbh) in the spring (2 May) and fall (10 October) of 2005. A Kioritz applicator (Kioritz Corp., Tokyo, Japan) was used for all soil treatments following the basal system (Silcox 2002, Dilling et al. 2010) with the footplate set at 12.7 cm (Turcotte et al. 2008).

Sampling, spider identification and guild composition. To access spider diversity one randomly selected hemlock branch was sampled biweekly from May to October in 2005 and 2006. Samples were taken from ground level to ~ 3 m, and were randomly selected from one of the four cardinal directions. Selected branches were beaten (five beats) over a PVC pipe frame (84 cm by 56 cm) lined with a polyethylene bag. Branch beating was chosen over other collection methods (e.g passive trapping) so that some level of association with eastern hemlock could be inferred.

Arthropod samples were placed in coolers with ice packs, transported to the USDA Forest Service Morgantown Field Office (Morgantown, WV), and stored in a freezer at -18°C until samples were processed. Processing of spider samples was accomplished by empting the contents of each polyethylene bag into a 17.5 x 17.5 cm gridded plastic tray. The gridded plastic tray and bags were then examined under a zoom stereo microscope (6.7 to 45X) (SZ61, Olympus, Tokyo, Japan) with a 4.0 megapixel digital camera attached. Spiders were separated, counted, and preserved in alcohol. Spiders that I could not identify were sent to specialist for determination. Spiders were classified to their foraging guilds (modified from Utez et al. 1999): web-builders, wandering spiders, and web-builders/wandering (Table 1). Representative specimens of identified species were deposited in the arthropod collection, U.S. Forest Service, Morgantown, WV.

Statistical Analysis. I analyzed spider count data using a generalized linear mixed-effects model using PROC Glimmix (SAS Institute 2011). Tests for significance for the factors of site, application method, treatment season, direction, and weeks post treatment along with each two-way interaction were tested using type III *F*-ratios. The model used an unstructured covariance structure. The conventional $\alpha = 0.05$ level of significance was used to determine variable retention in the model. As a result of the arthropod count data not being normally distributed, a poisson distribution with was used in the model. Multiple comparisons of means were conducted using Tukey-Kramer tests. We report adjusted P values that can be interpreted in a fashion similar to and experiment-wise error rate of α =0.05.

Results

Taxonomic grouping. A total of 1,798 individual spiders were collected in this study. The spiders belonged to ten families and 47 species with the families Araneidae (N=631, 35.9%), Anyphaenidae (N=335, 18.7%) and Philodromidae (N=266, 15.6%) making up the majority of the spiders collected (Fig. 1). Immature and adult stages accounted for 71.3 and 28.7% and of the spiders collected, respectively. Females accounted for 83.9% and males 16.1% of the spiders collected. A significant difference was found for the number of spiders collected between the two sites selected (F = 3.92; df = 1; P =00.0478). No difference was found between treated and control trees (F = 2.53; df = 1; P =0.1122), between treatment methods (F = 0.05; df = 1; P =0.8282) sample direction (F = 0.43; df = 3; P =0.7322) and week post treatment (F = 0.35; df = 16; P =0.9922) (Table 2). Spider counts were significantly higher (post-hoc Tukey–Kramer test: t = 1.97, df = 1456 *Adj P* = < 0.0489) for WVBG than WVUF with 991 and 807 spiders being collected at each site respectively. No significant differences in spider counts were found between treated and control trees, between treatment methods, among sample directions, and among weeks post treatment for the families Anyphaenidae, Aranidae, Linyphiidae, Oonopidae, Philodromidae, Salticidae, Tetragnathidae, Therididae, and Uloboridae (Table 1).

Guild grouping. Web building spiders were the most frequently collected (n = 679, 48% of total spiders), followed by wandering (n = 596, 42%), and web/wandering spiders (n = 142, 10%). No significant difference was found for the web-building guild between sites ($F = 2.21$; df = 1; $P = 0.1376$), between treated and control trees ($F = 0.26$; df = 1; $P = 0.6096$), between treatment methods ($F = 0.80$; df = 1; $P = 0.3727$), among sample directions ($F = 0.88$; df = 3; $P = 0.4507$), and among weeks post treatment ($F = 0.32$; df = 16; $P = 0.9952$) (Fig. 2). No significant difference was found for the wandering guild between sites ($F = 0.75$; df = 1; $P = 0.3854$), between treated and control trees ($F = 1.16$; df = 1; $P = 0.2815$), between treatment methods ($F = 0.41$; df = 1; $P = 0.5229$), among sample directions ($F = 0.01$; df = 3; $P = 0.9978$), and among weeks post treatment ($F = 0.35$; df = 16; $P = 0.9920$). No significant difference was found for the web/wandering guild between sites ($F = 0.11$; df = 1; $P = 0.7408$), between treated and control trees ($F = 0.76$; df = 1; $P = 0.3848$), between treatment methods ($F = 0.48$; df = 1; $P = 0.4891$), among sample directions ($F = 0.07$; df = 3; $P = 0.9766$), and among weeks post treatment ($F = 0.16$; df = 16; $P = 0.9999$).

Discussion

Although a couple of previous studies conducted in Kentucky and Tennessee (Hakeem 2008, Mallis and Rieske 2011) investigated spiders in eastern hemlock under *A. tsugae* infestation and chemical treatments, my study is the first assessment of spider communities and imidacloprid in the absence of *A. tsugae*. The number of species and the percentage of species comprising different families and guilds in this study varied from those found in the Kentucky and Tennesses studies. I collected a total of 1,798 individual spiders from ten families, the Kentucky study collected 4,000 spiders from 21 families while the Tennessee study collected a total of 4,332 individual spiders from 42 families. Both of these studies involved year round collections and utilized multiple sampling points/tree and in the case of Tennese study multiple sampling methods (e.g. vacuuming, and malaise traps). Similar to these and other studies we documented a numerical dominance of females which appears to be an ordinary occurrence in spider community studies in conifers (Stratton et al. 1978, Jennings and Dimond 1988, Hakeem 2008, Mallis and Rieske 2011). In the case of the Kentucky study which involved both *A.tsugae* and *A.tsugae*-free sites a significant difference was found for total spider abundance, richness and diversity between sites. The Tennesse study utilized hemlock trees with varing levels of

A.tsugae infestations and compared various chemical treatments and methods (e.g. foliar sprays, trunk injection and soil injections). This study documented a significant difference in predator abundance between imidacloprid, horticultural oil treated and control trees. Unfortunately the predator group included insects, spiders and harvestmen making it difficult to assess the impact on spider populations alone. The study also reported no difference in treatment season (fall vs spring) for predatory abundance at either project site.

Our study also documented a dominance in web-builders (48%), compared to wandering spiders (42%). This result is similar to other spider surveys in (*Pinus* spp.), spruces (*Picea* spp.), and balsam fir (*Abies balsamea)* and could be explained by the structural complexity of the needles and branches of eastern hemlock and other conifers and also by the natural history of each group (Stratton et al. 1978, Jennings and Dimond 1988). The more complex the structure the more numerous spaces are present for the construction of webs (Stratton et al. 1978).

Although we only sampled from the lower crown of eastern hemlock from May to October, other spider studies have shown that time of year, habitat complexity, tree height, form, vertical stratification and tree density can all influence spider community composition and abundance (Stratton et al. 1978, Jennings and Collins 1986, Dorcherty and Leather 1997, Mallis and Rieske 2011, Pinzon et al. 2011)

The most commonly collected species of spiders in this project were: *Eustala anastera* (Walckenaer) (Araneae: Araneidae), *Araneus gemmoides,* Chamberlin and Ivie (Araneae: Araneidae), *Mangora placida* (Hentz) (Araneae: Araneidae), *Philodromus exilis* (Araneae: Philodromidae), and *Colonus sylvanus* (Araneae: Salticidae). All are common arboreal species with wide host and geographic ranges.

It is known that *A. tsugae* is a specialist insect that feeds by inserting its stylet at the base of needles and feeds on the ray parenchyma cells (Young et al. 1995). This feeding has a significant impact on growth, causing needle drop, dieback and systemic hypersensitive responses (Cheah et al. 2004, Miller-Pierce et al. 2010, Radville et al. 2011, Gonda-King et al. 2014). It was reported in the Kentucky study that the hemlocks at the infested site were beginning to thin and that the physical impacts of the adelgid could be impacting spider communities (Mallis and Rieske 2011). I hypothesized that since *A. tsugae* infestation can have profound effects on the architecture of infested eastern hemlock trees, including changes to the live crown ratio, branch to branch contact and needle development and chemistry that these changes could have devastating impacts on spider communities. *A. tsugae* is a virulent insect capable of impacting eastern hemlock and the arthropods associated with it immediately after infestation (Dilling et al. 2010, Miller-Pierce et al. 2010, Mallis and Rieske 2011, Kung et al. 2015) and that detection of an early infestation is extremely difficult (Evans and Gregoire 2007).

When faced with decision on treating for *A. tsugae*, my results suggest that land managers should strongly consider the pretreatment of high value eastern hemlock prior to infestation by *A. tsugae*. This preventative control allows time for wide distribution of systemic insecticides in a tree and reduces the systemic changes in eastern hemlock foliar chemistry and maintains the complex architecture that supports the diverse spider community associated with eastern hemlock.

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Table 1. Spider families, total number collected (immature and adult), number of species and foraging guild classification collected by branch beating the lower crown of eastern hemlock in West Virginia in 2005 and 2006.

Family	Total number collected*	Number of Species	Guild
Anyphaenidae	335	10	Wandering spiders
Araneidae	631	12	Web-builders
Linyphiidae	168	6	Web-builders/Wandering
Oonopidae	21	2	Web-builders
Philodromidae	266	4	Wandering spiders
Salticidae	109	1	Wandering spiders
Tetragnathidae	28	2	Web-builders
Theridiidae	117	2	Web-builders
Thomisidae	8	2	Wandering spiders
Uloboridae	57	4	Web-builders
Total	1798	47	

* 58 immature spiders are currently undetermined and not included here.

Table 2. Table GLIMMIX model for spider counts.

Effect	**df**	**F**	***P***
Site	1	3.92	0.0478
Treatment	1	2.53	0.1122
Method	1	0.05	0.8282
Direction	3	0.43	0.7322
Week (post treatment)	16	0.35	0.9922

Table 3. Spider species collected and identified from eastern hemlock at at the West Virginia Botanic Garden (WVBG) and West Virginia University Forest (WVUF) from May-to-October in 2005 and 2006.

Taxa	
Clubionidae	*Linyphiidae sp. 5*
Clubiona canadensis	*Oonopidae sp. 1*
Clubiona sp.	*Oonopidae sp. 2*
Elaver excepta	*Philodromus exilis*
Elaver sp.	*Philodromidae sp. 1*
Anyphaenidae sp. 2	*Philodromidae sp. 2*
Anyphaenidae sp. 3	*Philodromidae. sp. 3*
Anyphaenidae sp. 4	*Salticidae*
Anyphaenidae sp. 5	*Colonus sylvanus*
Araneidae	*Hentzia mitrata*
Eustala anastera	*Zygoballus rufipes*
Eustala sp.	*Pelegrina sp.*
Larinioides sp.	*Tetragnathidae sp. 1*
Araneus gemmoides	*Neospintharus trigonum*
Mangora placide	*Theridiidae sp. 1*
Araneidae sp. 1	*Thomisidae sp. 2*
Araneidae sp. 2	*Thomisidae sp. 3*
Araneidae sp. 3	*Trachelas tranquilus*
Araneidae sp. 4	*Uloboridae sp. 1*
Araneidae sp. 5	*Uloboridae sp. 2*
Pithyohyphantes costatus	*Uloboridae sp. 3*
Linyphiidae sp. 1	
Linyphiidae sp. 2	
Linyphiidae sp. 3	
Linyphiidae sp. 4	

Immatures which could not be identified to genus are noted at the family level with species number.

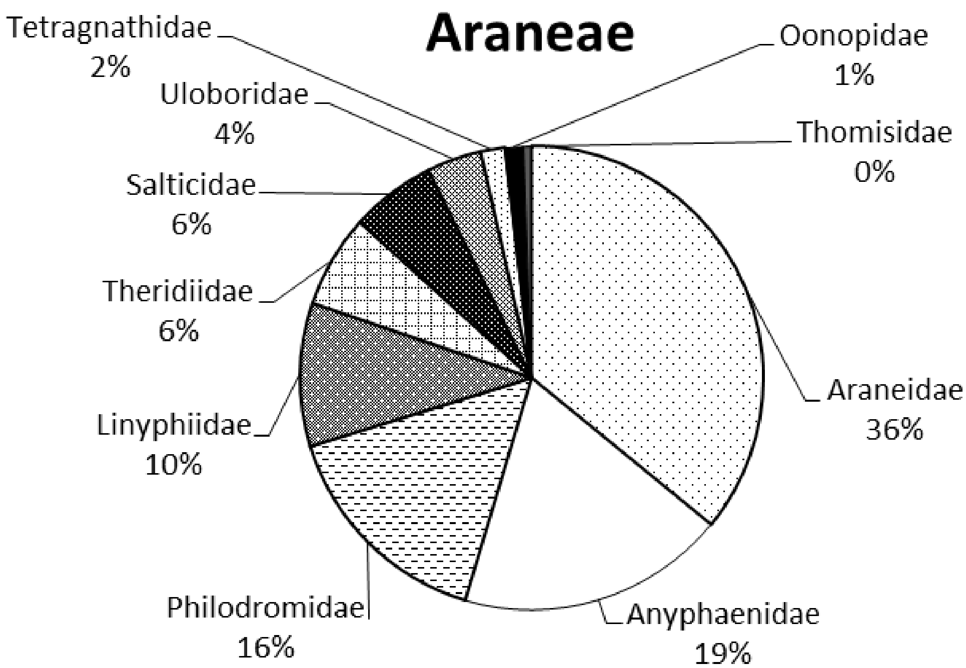

Figure. 1. Relative proportion of the 1,798 spiders by family collected by branch beating the lower crown of eastern hemlock in West Virginia in 2005 and 2006.

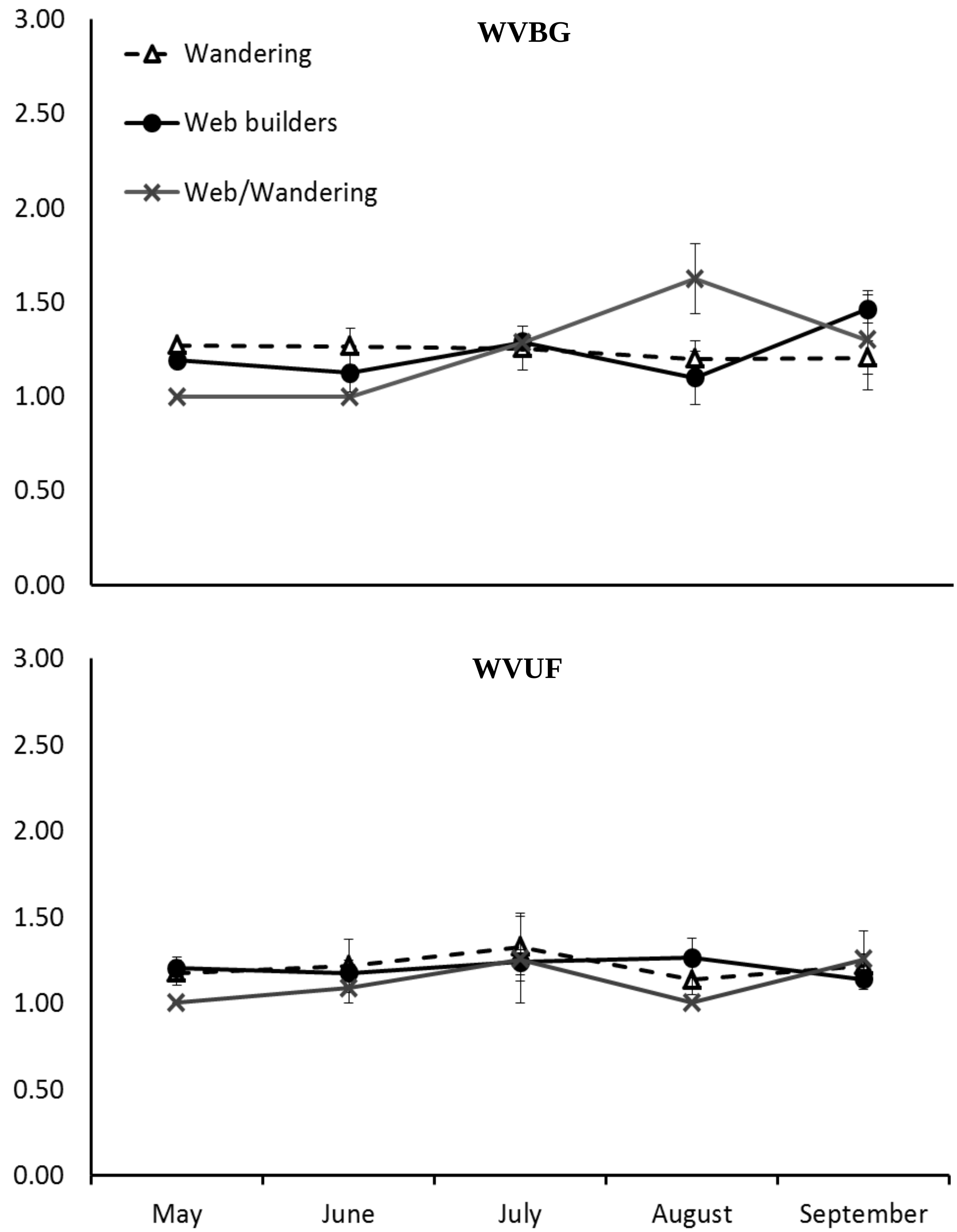

Figure 2. Temporal patterns in abundance (mean ± SE) of wandering, web builders, and web/wandering spider guilds for branch beating samples of eastern hemlock at the West Virginia Botanic Garden (WVBG) and West Virginia University Forest (WVUF).

CHAPTER 6: Conclusions

Eastern hemlock, *Tsuga canadensis* (L.) Carr (Pinales: Pinaceae), is an important component of both the urban and forest landscape of the Eastern United States. It is a long-lived, shade-tolerant species that strongly influences its environment and other species. The dense evergreen canopy of this species, along with its ability to grow in nearly pure stands, creates a distinct microclimate that is important for a wide variety of plant and animal species. Eastern hemlock has been heavily impacted by the introduction of both the elongated hemlock scale *Fiorinia externa* Ferris (Hemiptera: Diaspididae) and the hemlock woolly adelgid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae). Therefore, effective control methods are needed to help manage these exotic pests.

The results of this study (Chapter 2) demonstrated that xylem fluid concentrations of imidacloprid were significantly higher for spring applications than for fall applications, and for trunk injections than soil injections in the first year post treatment. Additionally, a comparison of the presence of imidacloprid with xylem fluid and in leaf tissue on the same branch showed significant differences, suggesting that imidacloprid is moving intermittently within the crown of eastern hemlock. These results support the hypothesis that trees under stress from attack from *A. tsugae* are less likely to move and distribute imidacloprid, suggesting that pretreatment of eastern hemlocks at high risk from *A. tsugae* may be justified, if only to allow for better spatial distribution and movement of imidacloprid within the crown of hemlock trees.

This study (Chapter 3) showed the implication of imidacloprid treatments on non-target arthropods. My results revealed that no significant differences in arthropod abundance were found between imidacloprid treated and control trees and application methods. Similarly no significant differences in abundance of each feeding guild were found between the imidacloprid treated and untreated controls trees. In addition, only about one-third, 130 of 393 species of arthropods examined were potential direct consumers of eastern hemlock. The other species utilize the unique arboreal habitat created by hemlock, and thus they are unlikely to be impacted by the use of imidacloprid applied by either trunk or soil injection.

This study (Chapter 4) reviewed the literature to determine the arthropod species associated with eastern hemlock and tried to assess which species might be impacted by the loss of a major foundation species. A literature review revealed 484 native and exotic arthropods from three different taxonomic classes and 21 different orders associated with eastern hemlock in North America. Of these five species were found to be eastern hemlock dependent. It is likely that these hemlock-dwelling species will experience local extirpation as a result of declining and dying eastern hemlock by *A. tsugae*. The reduction and loss of eastern hemlock as a result of these introduced species is expected to cause significant impacts on the ecological processes in the hemlock forests across the Eastern United States.

This final study (Chapter 5) investigated spiders as the dominant predatory group associated with the crown of eastern hemlock. My results showed no significant differences in spider abundance between imidacloprid treated and control trees and between application methods. Similarly no significant differences in abundance of each foraging guild were found between the imidacloprid treated and untreated controls trees.

In conclusion, these studies have shown two important implications. First, season and treatment method impact the spatial and temporary distribution of imidacloprid in eastern hemlock. Second, a wide diversity of arthropods utilize eastern hemlock, but they are unlikely to be impacted by the use of imidacloprid in the first year after treatment.